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Poster

PSTR312: Molecular Logic of Dendrite Patterning

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR312.01/A1

Topic: A.03. Axon and Dendrite Development

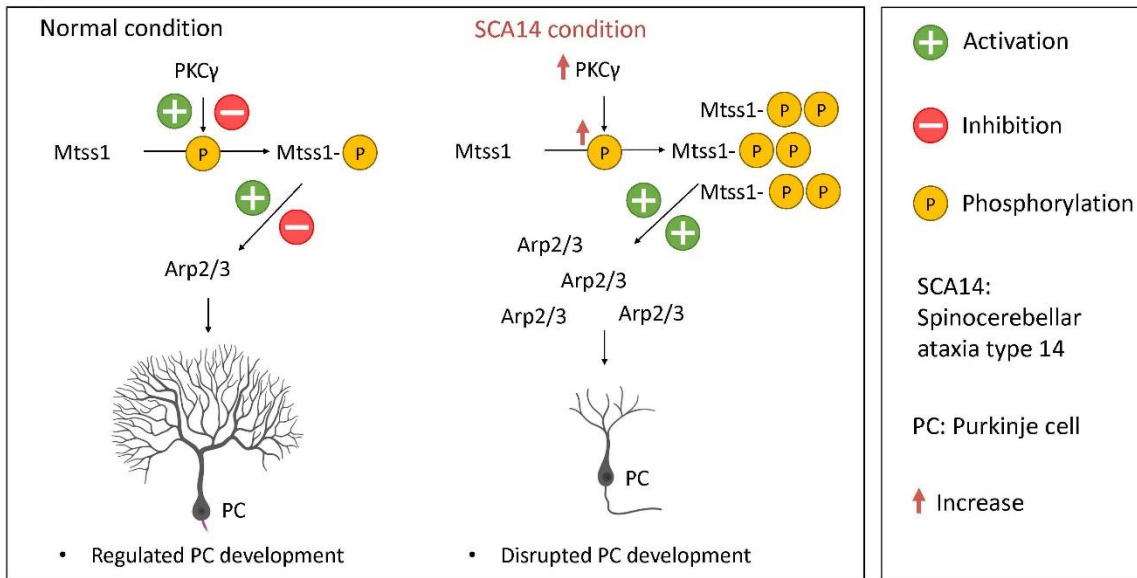
Support: SNF Grant 310030-189083

Title: PKC γ -mediated phosphorylation of Mtss1 regulates the dendritic outgrowth and spine development of cerebellar Purkinje cells

Authors: P. TORRENTS SOLÉ, Z. SZIBER, *J. P. KAPFHAMMER;
Dept. of Biomedicine, Univ. of Basel, Basel, Switzerland

Abstract: Protein kinase C gamma (PKC γ) is a signalling protein expressed in Purkinje cells (PCs) of the cerebellum. It is involved in regulating synapse formation and PC dendritic development through phosphorylation of target proteins that control cytoskeleton dynamics and PC dendritic morphology. Mutations in the protein kinase C gamma gene cause spinocerebellar ataxia 14 (SCA14), an autosomal dominant neurodegenerative disease leading to motor deficits and cognitive decline. The molecular targets of PKC γ and its interaction with cytoskeletal regulators are not yet well understood. Using a PKC γ mutant mouse line (PKC γ -A24E), which expresses a constitutively active PKC γ causing an SCA14-like phenotype, we identified Metastasis suppressor protein 1 (Mtss1) as a PKC γ molecular target involved in PC dendritic development. The two Mtss1 phospho-sites (S265 and S266) showed an increased phosphorylation in the cerebellum of PKC γ -A24E mice compared to wildtypes. Altering Mtss1-S265 and Mtss1-S266 with phospho-mimetic and phospho-defective mutations led to a loss of PC dendritic tree complexity in dissociated cerebellar cultures, with a developmental delay, a decreased dendritic branching, reduced spine density, and fewer synaptic processes, partially mimicking the PKC γ -A24E phenotype. Moreover, we found that these Mtss1 effects are partially regulated via the Arp2/3 complex. Our results demonstrate the importance of PKC γ -mediated phosphorylation of Mtss1 at S265 and S266 for PC dendritic outgrowth and suggest its contribution to PKC γ cytoskeleton signalling and the SCA14 phenotype.

Graphical abstract



Disclosures: P. Torrents Solé: None. Z. Sziber: None. J.P. Kapfhammer: None.

Poster

PSTR312: Molecular Logic of Dendrite Patterning

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR312.02/A2

Topic: A.03. Axon and Dendrite Development

Support: NIH grant MH133798
NIH grant NS132393

Title: Dendritic Localization of Fascin and Its Critical Role in Synapse Development and Plasticity

Authors: *J. Q. ZHENG¹, S. JOUBERT², Y. OGAWA³, K. HARDIN², M. N. RASBAND⁴, K. MYERS²;

¹Emory Univ. Dept. of Cell Biol., Atlanta, GA; ²Cell Biol., Emory Univ., Atlanta, GA;

³Neurosci., Baylor Col. of Med., Houston, TX; ⁴Neurosci., Baylor Col. of Med. Dept. of Neurosci., Houston, TX

Abstract: The Fascin family of actin-bundling proteins are best known for organizing tightly packed filamentous actin (F-actin) bundles in dynamic membrane protrusions such as filopodia. While fascin is known to be concentrated in axonal filopodia of developing neurons, previous reports indicated that fascin is absent from dendritic filopodia and spines, thus excluding its role in dendritic development and synaptic plasticity. Here, we present evidence that fascin is both

present and functionally essential in dendritic compartments. Using both immunocytochemistry and CRISPR-based endogenous tagging of fascin in cultured hippocampal neurons, we find that fascin localizes to developing dendritic filopodia and is enriched in mature spines, where it forms discrete nanoscale foci within spine heads. Fascin1 knockdown reduces dendritic arbor complexity, and CRISPR-mediated knockout of fascin1 abolishes synaptic potentiation following chemical long-term potentiation (cLTP) induction. Notably, fascin1 knockout impaired spine enlargement and abolished the increase in miniature excitatory postsynaptic currents after cLTP stimuli, indicating a failure to support activity-dependent synaptic strengthening. Together, our findings revise the current model of actin organization in dendritic filopodia and spines and reveal a previously unrecognized role for fascin as a critical regulator of dendritic architecture and synaptic plasticity.

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Poster

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Program #/Poster #: PSTR312.03/A3

Topic: A.03. Axon and Dendrite Development

Support: Midwestern University Intramural Funding

Title: Initial Screening of New Manzamine Compounds and their Effects on Neurite Outgrowth and Neurotoxicity

Authors: *M. L. PIERCE¹, L. RODRIGUEZ², T. POYNTON³, J. ABURAS⁴, J. DRAVES², M. HAMANN⁵, A. M. MAYER⁴;

²Chicago Col. of Osteo. Med., ³Biomed. Sci., ⁴Pharmacol., ¹Midwestern Univ., Downers Grove, IL; ⁵Med. Univ. of South Carolina, Charleston, SC

Abstract: INTRODUCTION Natural compounds have long been studied for their effect on treatment of neurological diseases. However, the complexity of neurodegenerative and neuroinflammatory diseases has prevented pharmaceutical companies from significantly investing in drug development research. For the past three decades researchers have turned to the marine environment to find more affordable alternatives for potential treatment. A particular group of marine natural products, such as the manzamine alkaloids, have demonstrated to be biologically active having potent antimalarial, antimicrobial, and anti-neuroinflammatory activity. Currently, more 100 manzamine alkaloids have been isolated from 16 different species of marine sponges. A preliminary screening of 20 new manzamine compounds determined which compounds have a potentially enhancing effect on neurite outgrowth using primary mouse neuronal cultures.

METHODS The primary mouse neuronal cultures were fixed and stained with phalloidin and

DAPI stains to enhance visualization using a Nikon Confocal Microscope. The total neurite outgrowth, number of processes and number of branches were quantified using the ImageJ and NeuronJ software. Concentrations of 1 micromolar and 10 micromolar were used to rule out potential neurotoxicity.

RESULTS The primary screening of the manzamine compounds showed that palinurinine A (M22) induced a statistically significant increase in neuronal complexity via number of processes and number of branches. However, palinurinine A did not induce a statistically significant increase in neurite outgrowth length. These findings were observed only at a concentration of 10 micromolar and not at a concentration of 1 micromolar. The screening also showed that manzamine semisynthetic compound 13 (M33) induced a statistically significant increase in neurite outgrowth length and a statistically significant increase in neuronal complexity via number of branches but not via number of processes. These findings were also only observed at a concentration of 10 micromolar and not a concentration of 1 micromolar.

CONCLUSIONS This initial screening shows that pailinurinine A and manzamine semisynthetic compound 13 induced neurite outgrowth and complexity at the higher concentration. Further studies will be completed to assess dose-dependent effects of these compounds on neurotoxicity and neurite outgrowth on live and dead cells.

Disclosures: M.L. Pierce: None. L. Rodriguez: None. T. Poynton: None. J. Aburas: None. J. Draves: None. M. Hamann: None. A.M. Mayer: None.

Poster

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Program #/Poster #: PSTR312.04/A4

Topic: A.03. Axon and Dendrite Development

Support: NIH Grant NS119512
NIH Grant NS137605

Title: Neurons rely on local lipid transporter at ER-PM contact sites to support dendrite development

Authors: *A. CHOI¹, C.-T. CHIEN², M. SHELLY²;

¹Biochem. and Cell Biol., ²Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) is a key phosphoinositide at the plasma membrane (PM) that mediates various downstream signaling, including the activation of phosphatidylinositol 3,4,5-triphosphate (PIP₃)-dependent Akt signaling, which is critical for dendrite initiation and branching. During development, neurons are perpetually exposed to extracellular neurotrophins, such as brain-derived neurotrophic factor (BDNF), that deplete PI(4,5)P₂. Therefore, the local PI(4,5)P₂ pool needs to be maintained to ensure PIP₃/Akt signaling propagation. However, the key lipid intermediates for PI(4,5)P₂ synthesis, phosphatidylinositol

(PI) and phosphatidic acid (PA), are spatially segregated at the endoplasmic reticulum (ER) and plasma membrane (PM), respectively, making efficient PI(4,5)P₂ replenishment a challenge. Recent studies have identified Nir2, a specialized lipid transport protein (LTP), which localizes to ER-PM contact sites and facilitates rapid PA/PI transport for local PI(4,5)P₂ turnover. Thus, we propose that Nir2-mediated local lipid transport is critical for neurons to maintain PI(4,5)P₂ homeostasis and support PIP₃/Akt signaling propagation and ultimately dendrite development. Using short hairpin RNA (shRNA) and *in utero* electroporation to knockdown Nir2 expression in embryonic mouse cortical neurons, we observe severe defects in dendrite growth and branching. Combining the genetically encoded PIP₃ biosensor, PH-Akt, with total internal reflection fluorescent (TIRF) microscopy, we demonstrate that PM PIP₃ availability is reduced following Nir2 knockdown (KD) in developing cortical neurons, implicating the dysregulation of PI(4,5)P₂ homeostasis. Furthermore, our biochemical data shows that Nir2 KD significantly impairs the activities of Akt and GSK-3 β , which are major signaling molecules downstream of PIP₃ production. These results suggest that Nir2 plays a critical role in dendrite development through the regulation of PI(4,5)P₂/PIP₃ pools and downstream Akt signaling. To investigate endogenous Nir2 dynamic in neurons, we used CRISPR-mediated genome editing to label Nir2 with a fluorescent tag. Upon BDNF stimulation, endogenous Nir2 robustly translocate to the PM as distinct puncta and colocalize with the ER-PM junction marker, MAPPER, indicating that Nir2 responds to PI(4,5)P₂ depletion and sits at ER-PM contact sites to facilitate local lipid exchange. Together, our study suggests that Nir2 regulates neuronal dendrite development via local PI(4,5)P₂ replenishment at ER-PM contact sites to sustain downstream PIP₃-Akt signaling propagation.

Disclosures: A. Choi: None. C. Chien: None. M. Shelly: None.

Poster

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Program #/Poster #: PSTR312.05/A5

Topic: A.03. Axon and Dendrite Development

Support: UMass Boston Proposal Development Grant
UMass Boston Undergraduate Research Fund

Title: B2 SINE is a major contributor to dendritic complexity in developing male rat hippocampal neurons

Authors: T. A. RICHTER¹, E. K. PRITCHARD², S. L. ZUP³, *R. G. HUNTER⁴;

¹Psychology, Developmental and Brain Sci., Univ. of Massachusetts, Boston Dept. of Psychology, Boston, MA; ²Psychology, Developmental and Brain Sci., Univ. of Massachusetts Boston; Dept. of Psychology, Boston, MA; ³Univ. of Massachusetts Boston, Dept. of Psychology, Boston, MA; ⁴Psychology, Developmental and Brain Sci., Univ. of Massachusetts, Boston, MA

Abstract: Though long considered “Junk”, recent research has shown that transposable elements are major contributors of regulatory non-coding RNA, especially in the brain. The brain is highly hormone-responsive and sits at the top of multiple endocrine feedback loops. The hippocampus is particularly sensitive to both circulating glucocorticoid and gonadal hormones and has been extensively studied after changes in circulating testosterone or estradiol. We and others have shown that one particular transposable element, murine B2 SINE ncRNA, is a regulatory RNA in the rodent hippocampus, especially after a psychological stressful event like acute restraint stress. It is unknown, however, if B2 SINE ncRNA is necessary for the proper development of hippocampal neurons, and furthermore, if there is a sex difference in this development. This work investigates the role of murine B2 SINE ncRNA in the development of primary hippocampal neurons. We utilized pooled locked nucleic acid (LNA) GapmeRs to knock down the expression of B2 SINE RNA and treated primary hippocampal neurons with dihydrotestosterone (DHT) to test if there is a difference in dendritic complexity. We used Sholl analysis from the simple neurite tracer package in ImageJ to quantify the branching, number of tips, and Sholl mean. We found that knocking down B2 SINE RNA in male neurons results in a reduction of dendritic complexity but not in female neurons. Taken together, this work suggests that B2 SINE RNA plays an important role in the proper development of hippocampal neurons in a sex dependent manner. This is the first evidence of the role a transposable element’s RNA has in the development of neuronal morphology and the first to show that this regulation changes based on genetic sex.

Disclosures: T.A. Richter: None. E.K. Pritchard: None. S.L. Zup: None. R.G. Hunter: None.

Poster

PSTR312: Molecular Logic of Dendrite Patterning

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Program #/Poster #: PSTR312.06/A6

Topic: A.03. Axon and Dendrite Development

Support: NIH/NEI R21 EY033874

Title: Mechanistic Insights into DSCAM-mediated neuronal self-avoidance

Authors: *R. RANA¹, A. M. GARRETT²;
²Pharmacol., ¹Wayne State Univ., Detroit, MI

Abstract: The human brain is composed of billions of neurons that must be organized into proper neural circuits for normal function. A critical step in neural circuit formation is the regulation of contact between neurons through adhesion or repulsion. A large, structurally diverse group of cell surface receptors known as cell adhesion molecules (CAMs) mediate neuronal adhesion and repulsion and keep the balance between these two forces. Down Syndrome Cell Adhesion Molecule (DSCAM), a member of the Ig superfamily of CAMs, plays a major role in various neurodevelopmental events and is associated with disorders including

Down Syndrome, autism spectrum disorder, and fragile X Syndrome. DSCAM is especially important in neuronal self-avoidance, a process which ensures branches from the same neuron avoid each other (iso-neuronal self-avoidance), and neighboring neurons of the same type contact each other without becoming entangled (homotypic self-avoidance). This phenomenon is exhibited by both dendrites and axons in vertebrates as well as invertebrates. In invertebrates like *Drosophila*, extensive alternative splicing of *Dscam1* generates more than 38,000 isoforms, which provides molecular diversity to individual neurons. This enables neurons to distinguish between 'self' and 'non-self'. Although vertebrate DSCAM lacks such alternative splicing, it can promote self-avoidance by masking excessive adhesion mediated by other CAMs. DSCAM's C-terminus is important for promoting self-avoidance in some but not all cell types. The precise molecular mechanism behind DSCAM's self-avoidance is not clear. Our research aims to elucidate this molecular mechanism in vertebrates. We hypothesize that 1) the homophilic interaction of DSCAM triggers changes in local CAMs to mask adhesion. 2) DSCAM masks diverse CAMs and masking only a subset of CAMs requires DSCAM's C-terminus. To this end, we identified CAMs expressed in different retinal cell types using a gene expression data set and RNAscope, and are testing the role of DSCAM's C-terminus in their masking *in vivo* by retinal electroporation. We are identifying mechanisms *ex vivo* and *in vitro* using retinal explants, biochemistry, and cell aggregation assays. We aim to expand our understanding of intricate molecular machinery governing self-avoidance in vertebrate neurodevelopmental processes and its role in the pathogenesis of different neurodevelopmental disorders.

Disclosures: R. Rana: None. A.M. Garrett: None.

Poster

PSTR312: Molecular Logic of Dendrite Patterning

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR312.07/A7

Topic: A.03. Axon and Dendrite Development

Support: RS-2024-00409999
RS-2024-00332875
BK21 Four Biomedical Science Program

Title: Snp8 regulates neuronal development by controlling neuronal deneddylation process

Authors: *S. LEE¹, M. KANG², J. SONG³;

²1Department of Biomed. Sci., ¹Seoul Natl. Univ., Seoul, Korea, Republic of; ³Dept. of Biomed. Sci., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Neddylolation is a cellular process in which the neural precursor cell expressed, developmentally down-regulated 8 (NEDD8) is conjugated to the lysine residue of target proteins via serial enzymatic cascades. Recently, it has been demonstrated that neddylolation is required for synaptic clustering of metabotropic glutamate receptor 7 (mGlu7) and postsynaptic

density protein 95 (PSD-95), and the inhibition of neddylation impairs neurite outgrowth and excitatory synaptic maturation. Similar to the balanced role of deubiquitylating enzymes (DUBs) in the ubiquitination process, we hypothesized that deneddylating enzymes can regulate neuronal development by counteracting the process of neddylation. We find that the SUMO peptidase family member, NEDD8 specific (SENP8) acts as a key neuronal deneddylase targeting the global neuronal substrates in primary rat cultured neurons. We demonstrate that SENP8 expression levels are developmentally regulated, peaking around the first postnatal week and gradually diminishing in mature brain and neurons. We find that SENP8 negatively regulates neurite outgrowth through multiple pathways, including actin dynamics, Wnt/ β -catenin signaling, and autophagic processes. Alterations in neurite outgrowth by SENP8 subsequently result in the impairment of excitatory synapse maturation. Our data indicate that SENP8 plays an essential role in neuronal development and is a promising therapeutic target for neurodevelopmental disorders.

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Poster

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Topic: A.03. Axon and Dendrite Development

Support: A. Ramakrishnan acknowledges the financial support received from the “WISE-PDF research grant” of WISE-KIRAN Division, Department of Science and Technology (DST), Government of India.
A. Ramakrishnan acknowledges Gargi Mandal, a PhD student at the Neuro-Electronics Lab, the Micro Nano Characterization Facility (MNCF), and the National Nanofabrication Centre (NNFC) of CeNSE, IISc, for their assistance with micropillar fabrication.

Title: How do micropillar topographies influence neuronal growth?

Authors: *A. RAMAKRISHNAN¹, V. GAUTAM²;

¹Indian Inst. of Science, Bangalore, Bangalore, India; ²Ctr. for NanoScience and Engin., Indian Inst. of Sci., Bangalore, India

Abstract: Neurons receive various signals from their extracellular matrix, including the physical cues at nanometer and micrometer scales, which guide axonal growth (1). These nano- and micro-scale patterns can be fabricated on substrates to serve as a platform for neuronal culture. When hippocampal neurons are cultured on isotopically arranged nanopillar structures, they extend their projections along the square lattice arrangement of the nanopillars (2). However, the mechanism by which neurons sense the physical cues from the nanopillar structure remains unknown. We hypothesize that these topographical structures provide physical cues in the form

of mechanical stimuli, which are transmitted to cellular signals through mechanosensitive ion channels like Piezo channel(3). This study investigates the expression and activity of the piezo channel in neurons cultured on micro-topographically patterned substrates. Polydimethylsiloxane (PDMS) has been used to create micro-pillars of varying widths (2-10 μm) and gaps (2-10 μm). Hippocampal neurons from P0-P2 rat pups were cultured on patterned and flat PDMS substrates. Piezo expression was examined with mouse recombinant piezo-1 antibody and Donkey Anti-Mouse Alexa Fluor® 488 antibody using immunocytochemistry (ICC). Whole-cell voltage clamp recordings were performed using voltage steps ranging from -80 mV to 70 mV in 10 mV increments, with a holding potential of -60 mV, to investigate the kinetics of the piezo channel. The hippocampal neurons exhibited increased branching and alignment when the pillars had a width and gap of 2-3 μm . ICC imaging revealed increased intensity of piezo channels in neurons displaying increased branching on this microtopographical structure. The neurons grown with a 2 μm width and gap showed approximately 2-fold greater piezo intensity compared to the control neurons. Moreover, electrophysiological experiments indicated a reduction in inward current and changes in the rise and decay times of neurons that grew on micro-pillar substrates. Additional details of these studies will be presented in the poster. This research uncovers fundamental insights into the expression and activation of the piezo channel during neuronal growth on micro-topographical structures. References 1.Michalska, J. M. et al. Imaging brain tissue architecture across millimeter to nanometer scales. 42(7) 1051-1064 (Springer US, 2024). 2.Gautam, V. et al. Engineering Highly Interconnected Neuronal Networks on Nanowire Scaffolds. 17(6) 3369-3375 (Nano Lett, 2017) 3.Coste, B. et al. Piezo proteins are pore-forming subunits of mechanically activated channels. 483 (7388) 176-81 (Nature, 2012).

Disclosures: A. Ramakrishnan: None. V. Gautam: None.

Poster

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Program #/Poster #: PSTR312.09/A9

Topic: A.03. Axon and Dendrite Development

Title: Map7coordinates motor recruitment to regulate transport selectivity at branch junction

Authors: *E. MOESE¹, S. TYMANSKYJ³, L. MA²;

²Neurosci., ¹Thomas Jefferson Univ., Philadelphia, PA; ³Col. of Sci. and Mathematics, Augusta Univ., Augusta, GA

Abstract: Proper development and function of neurons requires tightly regulated microtubule (MT) -based transport of various cargo from cell bodies to synaptic terminals. Improper transport is implicated in various neurological diseases. Neurons have elaborately branched axons creating complex connections. Intracellular transport at axon branch junctions requires tight regulation of cargos, as suggested by recent discovery of selective transport in cultured dorsal root ganglion (DRG) neurons. Anterograde transport of lysosomes displayed preference for longer branches or

branches with a dynamic growth cone. Furthermore, this selectivity is differentially regulated for various cargos and mediated by kinesin-3 motors. However, it is unknown what local mechanisms at branch junctions are mediating selectivity. Interestingly, MAP7 is a MT associated protein enriched at branch junctions in embryonic DRG neurons. *In vitro*, MAP7 recruits kinesin-1 to MTs but inhibits kinesin-3 from MT binding. Transport of cargos such as lysosomes when MAP7 is overexpressed displayed increased run time but decreased velocity indicating kinesin-3 MT binding is blocked. Therefore, we hypothesize that MAP7 coordinates motor recruitment and transport selectivity at branch junctions via phosphoregulation. To test this hypothesis, we first examined MAP7 knockout neurons. Preliminary data showed an increase in the anterograde transport velocity of lysosomes, consistent with the idea that MAP7 normally inhibits kinesin-3 but favors kinesin-1 transport. Currently, we are using these neurons to determine the requirement of MAP7 on transport selectivity. Next, we examined the role of MAP7 phosphorylation by mutating 13 SP/TP sites in the P-domain, a highly unstructured region that is involved in MT binding and axonal localization. Based on fluorescence recovery after photobleaching (FRAP) analysis, we found that the phospho-mimetic mutant displays decreased MT binding compared to the phospho-null mutant. Intriguingly, the phospho-mimetic mutant is not localized to the branch junctions as the wildtype or the phospho-null mutant, instead it appears in the distal axons as well as the growth cones, which are normally avoided by MAP7. These data suggest phosphorylated MAP7 is readily removed from MTs, which could in turn increase kinesin-3-mediated transport at branch junctions. To test this possible mechanism, we are currently testing the role of the phosphomutants on selective transport. By parsing out the relationship between the regulation of MAPs and intracellular transport we will begin to understand how regulated transport supports axon development and maintenance

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Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR313.01/A10

Topic: A.06. Developmental Disorders

Support: Simons Center for the Social Brain at MIT
Tan-Yang Autism Center at MIT
McGovern Institute

Title: Shank3 mutations disrupt resting-state functional connectivity in the macaque brain

Authors: *F. A. AZEVEDO¹, M. JIANG², Z. REN⁴, R. LI⁴, J. SHARMA², L. WANG⁴, G. FENG², Z. LU⁴, R. DESIMONE³;

²Brain and Cognitive Sci., ³McGovern Inst., ¹MIT, Cambridge, MA; ⁴Shenzhen Inst. of Advanced Technol., Shenzhen, China

Abstract: Mutations in the *SHANK3* gene, which encodes a synaptic scaffolding protein essential for excitatory synapse function, have been implicated in Phelan-McDermid Syndrome (PMS) and idiopathic autism spectrum disorders (ASD). While these mutations have been associated with widespread abnormalities in brain function, their impact on functional connectivity and large-scale network organization remains poorly understood. To address this, we used high-resolution resting-state fMRI in anesthetized CRISPR-induced *SHANK3*-mutant (n=7) and wild-type (n=8) cynomolgus monkeys. We applied state-of-the-art correlation-based metrics to assess local and global functional connectivity, and data-driven decomposition to identify and subsequently compare canonical resting-state networks. Preliminary results revealed group differences in local and global functional connectivity in key brain regions, suggesting that *SHANK3* deficiency may impair both circuit-level integrity and large-scale network organization.

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Poster

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Program #/Poster #: PSTR313.02/A11

Topic: A.06. Developmental Disorders

Support: SFARI Bridge to Independence Award

Title: Decoding Developmental Trajectories of Autism Using Single-Nucleus Multiome Profiling in *SHANK3*-mutant Marmoset

Authors: *Y. WANG¹, Q. ZHANG², M. DEBERARDINE¹, K. LEVANDOWSKI², G. FENG², F. M. KRIENEN¹;

¹Princeton Univ., Princeton, NJ; ²Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Autism spectrum disorder (ASD) exhibits relatively convergent molecular changes including synaptic dysfunctions across patients despite clinical heterogeneity, as shown in recent human single-cell transcriptomic studies. However, the scarcity of developing human brain tissue hinders efforts to uncover the temporal progression of ASD-related molecular pathology and to identify early biomarkers preceding symptom onset. *SHANK3* haploinsufficiency is a rare but highly-penetrant genetic risk factor for ASD and other neurodevelopmental disorders; *SHANK3* deficiency leads to profound cognitive and behavioral impairments. To assess how *SHANK3* haploinsufficiency affects the molecular properties of brain cells in a primate, we leveraged a newly developed marmoset model carrying a heterozygous *SHANK3* nonsense mutation.

We performed single-nucleus multiome sequencing (RNA + ATAC), targeting brain regions implicated in *SHANK3* function and ASD pathophysiology—specifically the prefrontal cortex,

striatum, thalamus, and bed nucleus of the stria terminalis. These datasets were mapped to existing whole-brain mouse and primate brain cell atlases to provide precise cell type annotations. By comparing mutant and control animals, we uncovered aberrant developmental trajectories marked by dysregulated gene regulatory networks inferred from both gene expression and chromatin accessibility. Notably, we identified region- and cell type-specific molecular phenotypes that diverge during the synaptogenic window (~3 months), preceding overt behavioral symptoms.

Differentially expressed gene analysis highlighted disruption in neuromodulator systems, particularly serotonin (5-HT) and dopamine pathways, which are known to modulate social behavior and cognitive flexibility. These alterations suggest a mechanistic link between early molecular disruptions and behavioral phenotypes. Together, our findings characterized the resultant molecular dysregulation following SHANK3 haploinsufficiency. Functional validation of dysregulated genes and regulatory elements newly identified in the marmoset model in mouse and in vitro systems might offer promising avenues for identifying early-intervention therapeutic targets.

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Poster

PSTR313: Autism: Genetic Models

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Program #/Poster #: PSTR313.03/A12

Topic: A.06. Developmental Disorders

Support: Hock E. Tan and K. Lisa Yang Center for Autism Research
Simons Center for the Social Brain at MIT

Title: Altered Cortical Processing of Social Information in SHANK3 Mutant Marmosets

Authors: *H. XU¹, B. ZHANG², W. MENEGAS², Q. ZHANG², F. LIANG², G. LIBERTI², C. J. VALENZUELA², G. TANG², M. RANA², C. ZHANG², G. FENG², R. DESIMONE²;
²McGovern Inst. for Brain Res., ¹MIT, Cambridge, MA

Abstract: Autism spectrum disorder (ASD) associated with Phelan-McDermid Syndrome (PMS) is caused by mutations in the SHANK3 gene and is characterized by impairments in social cognition. While studies in Shank3 mutant mice have revealed some of the neural consequences of Shank3 mutations, mouse social behavior is different from that in primates in notable ways. To address this, we investigated cortical dysfunction in SHANK3 mutant marmosets using chronic electrocorticography (ECoG) arrays implanted over visual, auditory, temporal, and prefrontal cortices. Despite exhibiting typical gaze patterns and preserved responses to low-level visual features, SHANK3 mutants showed significantly reduced cortical selectivity and decoding accuracy for social stimuli. High-gamma power (HGP) responses to faces and socially relevant

video content were diminished in mutants, particularly within lateral prefrontal cortex (PFC). Multivariate decoding revealed a specific impairment in discriminating social categories—such as the presence of a face, social valence, and monkey-monkey interactions — with these deficits primarily localized to the PFC. In contrast, decoding of object-based or non-social content remained intact across groups. Complementary wireless recordings in the home-cage environment further revealed that SHANK3 mutants lacked the increase in HGP typically seen during reciprocated gaze interactions, a response robustly present in wild-type animals. However, both groups showed similar HGP increases when the cage mate was moving versus still, suggesting a selective deficit in socially contingent neural responses. Decoding accuracy of social behavioral states—such as mutual gaze, proximity, and climbing—was significantly reduced in SHANK3 animals. Additionally, behavioral state classification from neural activity indicated a trend toward fewer deep sleep episodes in mutants, aligning with disrupted sleep architecture commonly observed in ASD. The results suggest impaired cortical encoding of social cues in Shank3 primates, with a particular vulnerability in PFC circuits and naturalistic social contexts. This work highlights SHANK3 mutant marmosets as a powerful translational model for dissecting social circuit pathology in ASD and evaluating circuit-level therapeutic interventions.

Disclosures: H. Xu: None. B. zhang: None. W. Menegas: None. Q. Zhang: None. F. Liang: None. G. Liberti: None. C.J. Valenzuela: None. G. Tang: None. M. Rana: None. C. Zhang: None. G. Feng: None. R. Desimone: None.

Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.04/A13

Topic: A.06. Developmental Disorders

Support: Hock E. Tan & K. Lisa Yang Center for Autism Research
Simons Center for the Social Brain at MIT

Title: Cognitive Deficits and Prefrontal Cortical Dysregulation in SHANK3 Mutant Marmosets

Authors: *B. ZHANG¹, H. XU³, W. MENEGAS³, Q. ZHANG⁴, F. LIANG⁸, C. J. VALENZUELA², G. LIBERTI⁵, G. TANG⁹, M. RANA⁶, C. ZHANG³, G. FENG⁷, R. DESIMONE¹⁰;

¹McGovern Inst. for Brain Res. at MIT, cambridge, MA; ²McGovern Inst. for Brain Res. at MIT, Cambridge, MA; ⁴BCS/McGovern institute for brain research, ³MIT, Cambridge, MA; ⁵MIT, Norfolk, MA; ⁶McGovern Inst. for Brain Res., ⁷Brain and Cognitive Sci., MIT, Cambridge, MA; ⁸MIT McGovern Inst. for Brain Res., Chestnut Hill, MA; ⁹Massachusetts Inst. of Technol. (McGovern Institute), Cambridge, MA; ¹⁰mcgovern institute, Mit, cambridge, MA

Abstract: Phelan-McDermid Syndrome (PMS), a syndromic form of autism spectrum disorder (ASD), is frequently caused by mutations in the SHANK3 gene and is associated with profound impairments in language, social behavior, and cognition. To explore the neural basis of these cognitive deficits, we examined SHANK3 heterozygous mutant (SHANK3^{+/-}) marmosets and wild-type (WT) controls using a combination of touchscreen-based cognitive tasks and chronic wireless electrocorticography (ECoG) targeting the prefrontal cortex (PFC).

Animals were trained in their home cages on a series of tasks increasing in cognitive demand: visual discrimination, serial reversal, and delayed match-to-sample (DMS). SHANK3^{+/-} and WT marmosets performed comparably on the simpler discrimination and reversal tasks. However, SHANK3 mutants showed a pronounced impairment on the DMS working memory task, despite extended training.

Concurrent ECoG recordings revealed that SHANK3 mutants exhibited significantly elevated alpha-band (8-12 Hz) and high gamma power (70-170 Hz) in the PFC during the delay period of the DMS task—an effect not seen during serial reversal. Notably, greater delay-period alpha and high gamma power was associated with worse behavioral performance among SHANK3 mutants, suggesting that dysregulated prefrontal activity may contribute to working memory deficits.

These results demonstrate that SHANK3 mutations disrupt prefrontal cortical dynamics in a task-specific manner and provide insight into the neural mechanisms underlying cognitive dysfunction in ASD.

Disclosures: B. zhang: None. H. Xu: None. W. Menegas: None. Q. Zhang: None. F. Liang: None. C.J. Valenzuela: None. G. Liberti: None. G. Tang: None. M. Rana: None. C. Zhang: None. G. Feng: None. R. Desimone: None.

Poster

PSTR313: Autism: Genetic Models

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Program #/Poster #: PSTR313.05/A14

Topic: A.06. Developmental Disorders

Support: Hock E. Tan & K. Lisa Yang Center for Autism Research
Simons Center for the Social Brain at MIT

Title: Title: Multi-Modal Neural Biomarkers for SHANK3 Mutant Marmosets: Social Spectral Metrics and Sleep Spindle Detection

Authors: *S. C. TUKUA, H. XU, B. ZHANG, W. MENEGAS, Q. ZHANG, F. LIANG, C. J. VALENZUELA, G. LIBERTI, G. TANG, M. RANA, C. ZHANG, G. FENG, R. DESIMONE; McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Phelan-McDermid Syndrome (PMS), a syndrome highly comorbid with autism spectrum disorder (ASD), is caused by mutations in the SHANK3 gene encoding a critical

synaptic scaffolding protein. SHANK3 mutations disrupt cortico-striatal circuits and excitatory/inhibitory balance, with individuals experiencing developmental delays, intellectual disability, and prominent sleep disturbances. Up to 90% of people with PMS exhibit sleep problems, particularly frequent nighttime awakenings and difficulty maintaining sleep. Sleep spindles, generated by thalamo-cortical circuits, provide protective gating against sensory disturbances during sleep, suggesting potential dysfunction in SHANK3 mutants. We developed a dual-biomarker approach to identify SHANK3-related neural signatures in marmosets using electrocorticography (ECoG) recordings. During wake periods, animals viewed face processing tasks while ECoG arrays recorded neural activity across visual, temporal, and prefrontal cortices. Neural responses were converted into spectrograms and characterized using 20 custom-designed metrics capturing power changes across frequency bands and temporal windows. Linear Discriminant Analysis (LDA), a supervised machine learning technique maximizing class separability, was trained to distinguish SHANK3 heterozygous mutant (SHANK3+/-) from wild-type (WT) marmosets. Additionally, we implemented automated sleep spindle detection algorithms to identify potential sleep-related biomarkers. Given SHANK3's role in cortico-striatal-thalamic pathways and the importance of these circuits in sleep spindle generation, we hypothesized that spindle characteristics might differ between groups, potentially reflecting underlying excitatory/inhibitory imbalances that manifest during both wake and sleep states. This multi-modal approach aims to establish comprehensive neural signatures that could guide future investigations into the circuit-level mechanisms underlying both cognitive and sleep dysfunction in autism-related research, as well as providing objective measures of neural patterning differences and assessing treatment efficacy.

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Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.06/A15

Topic: A.06. Developmental Disorders

Title: Sex-dependent alterations in endosomal trafficking proteins in a mouse model of Autism.

Authors: R. LITTLEHALE¹, L. GEHNER², L. FREEMAN², M. WILLIAMS², *J. L. LARIMORE²;

¹Agnes Scott Col., Agawam, MA; ²Agnes Scott Col., Decatur, GA

Abstract: Mutations in Shank3 have also been reported in the polygenetic neurodevelopmental disorder schizophrenia (SZ) (Rees et.al., 2012, Gauthier et.al., 2010) and non-syndromic autism spectrum disorder (ASD) (Peca et al., 2011). SHANK3B codes for key post synaptic density (PSD) proteins that interacts with PSD95 and Homer to physically link ionotropic NMDA

receptors to metabotropic mGlu5 receptors, a linkage necessary for inducing synaptic plasticity (Heavner et al 2021, Jiang and Ehlers 2013, Mullin et. al., 2013, Grabrucker et. al., 2011, Foss-Feig et. al., 2017). There are several endosomal proteins necessary for receptor recycling in the dendritic spines - AGAP1, BLOC1 and AP3. AGAP1 expression levels also alter the endosomal trafficking kinetics in PC12 cells and primary hippocampal neurons (Arnold et. al., 2016). The octomeric coat-associated complex biogenesis of lysosome related organelle complex 1 (BLOC1) regulates synaptic vesicle trafficking at endosomes. The heterotetrameric coat protein adaptor protein 3 (AP3) regulates coat proteins and vesicle formation specifically at the level of the early endosome, where AGAP1 and BLOC1 are localized (Cuthbert et. al., 2008, Nie et. al., 2005, Nie et. al., 2003, Nie et. al., 2002, Arnold et. al., 2016). The endosomal proteins AGAP1, AP3 and BLOC1 have been implicated in neurodevelopment disorders characterized with alterations in learning, memory and cognition: such as ASD, Rett Syndrome (RTT), and SZ, which indicates potential role of endosomal trafficking in proper neuronal development (Rudolph et. al., 2016, Arnold et. al., 2016, Wassink et. al., 2005, Chapleau et. al., 2009, Nie et. al., 2005, Talbot et. al., 2004, Larimore et. al., 2013, Larimore et. al., 2017, Jung et. al., 2013, Moretti et al 2006, Yun and Trommer 2011, Orozco et. al., 2014, Savanthrapadian et. al., 2013, Kleschevnikov et. al., 2004). To better understand the role of endosomal trafficking in neurodevelopment, we examined the protein expression via immunoblotting and immunohistochemistry of AGAP1, AP3, the BLOC1 subunit dysbindin, and an endosomal vesicle marker and SNARE complex interaction GTPase, Rab3A in a sex dependent manner in a Shank3B mouse. There are sex dependent differences in Shank3B^{+/−} mice observed by immunoblotting for AGAP1 and AP3. Additionally, the hippocampal region specific changes observed by immunohistochemistry for AGAP1, AP3, dysbindin, and Rab3A were observed. Taken together, this study furthers our understanding of sex-dependent regulation of proteins and highlights the need to analyze neurodevelopmental models in a sex-dependent manner.

Disclosures: R. Littlehale: None. L. Gehner: None. L. Freeman: None. M. Williams: None. J.L. Larimore: None.

Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR313.07/A16

Topic: A.06. Developmental Disorders

Title: Sex-dependent alterations in protein expression of E/I proteins in a mouse model of Autism

Authors: *L. GEHNER¹, R. LITTLEHALE², M. WILLIAMS¹, L. FREEMAN¹, J. L. LARIMORE³;

¹Agnes Scott Col., Decatur, GA; ²Agnes Scott Col., Agawam, MA; ³Neurosci., Agnes Scott Col., Decatur, GA

Abstract: Mutations in Shank3 have also been reported in the polygenetic neurodevelopmental disorder schizophrenia (SZ) (Rees et.al., 2012, Gauthier et.al., 2010) and non-syndromic autism spectrum disorder (ASD) (Peca et al., 2011). SHANK3B codes for key post synaptic density (PSD) proteins that interacts with PSD95 and Homer to physically link ionotropic NMDA receptors to metabotropic mGlu5 receptors, a linkage necessary for inducing synaptic plasticity (Heavner et al 2021, Jiang and Ehlers 2013, Mullin et. al., 2013, Grabrucker et. al., 2011, Foss-Feig et. al., 2017). To better understand the role of E/I balance in neurodevelopment, we examined the protein expression of calcium channel CAV1.2, sodium-potassium-chloride co-transporter NKCC1, inhibitory interneuron marker parvalbumin, and the alpha-subunit of the sodium channel SCN1A, in a sex dependent manner in a Shank3B mouse. We used immunohistochemistry to describe the protein expression of ion channels regulated by glutamate and associated with neurodevelopment in the heterozygote. The sex dependent differences in Shank3B^{+/-} mice were observed by immunoblotting for VGLUT1. Additionally, the hippocampal region specific changes observed by immunohistochemistry for CAV1.2, NKCC1, parvalbumin, SCN1A, and VGLUT1 further highlight the importance of understanding sex differences in neurodevelopment.

Disclosures: L. Gehner: None. R. Littlehale: None. M. Williams: None. L. Freeman: None. J.L. Larimore: None.

Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR313.08/A17

Topic: A.06. Developmental Disorders

Title: The gut-microbiome profile analyzed in a sex-dependent manner in a mouse model of Autism.

Authors: *M. WILLIAMS¹, L. FREEMAN², L. GEHNER², R. LITTLEHALE³, J. L. LARIMORE¹;

¹Neurosci., ²Agnes Scott Col., Decatur, GA; ³Agnes Scott Col., Agawam, MA

Abstract: The gut-brain axis is a dynamic interface that has been implicated in the pathogenesis and severity of various neurodevelopmental disorders such as schizophrenia (SZ) and autism spectrum disorders (ASD). Understanding the contributions of gut microbiota to the symptomatology and comorbidity of schizophrenia (SZ) and ASD presents promising avenues for novel therapeutic development. Alterations in gut microbiota composition have been consistently correlated with symptoms of both ASD and SZ (Cocean & Vodnar, 2024; Lin et al., 2024). Studies in both humans with ASD and a mouse model of ASD report significant changes in genera such as Akkermansia, Bacteroides, Bifidobacterium, Blautia, Clostridium, Dorea, Parabacteroides, Prevotella, and Lactobacillus (Alamoudi et al., 2022). Shank3B knockout mice not only exhibit ASD-like behaviors but demonstrate altered gastrointestinal epithelium

morphology and fecal microbiota composition. We sequenced the gut microbiome from the small intestine of 12-week-old wild type Shank3B^{+/+} or heterozygote Shank3B^{+/-} mice in a sex-dependent manner. The Shank3B^{+/-} males demonstrate an increase in Staphylococcaceae and Erysipelotricaceae. Our results indicate increased biodiversity in Shank3B^{+/-} males and reduced biodiversity in Shank3B^{+/-} females. Altogether, this data reveals sex-specific microbial signatures that may contribute to the pathogenesis of ASD thus providing potential therapeutics that target gut microbiota in neurodevelopmental disorders.

Disclosures: **M. Williams:** None. **L. Freeman:** None. **L. Gehner:** None. **R. Littlehale:** None. **J.L. Larimore:** None.

Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.09/A18

Topic: A.06. Developmental Disorders

Title: The Essential Role of NR2B Receptor in Alleviating Social Deficits in Shank3B Autism Models

Authors: ***Z. ZHOU;**
McGovern Inst. for Brain Res. (MIT), Cambridge, MA

Abstract: Autism spectrum disorder is a major neurodevelopmental condition that affects communication, behavior, and social interaction—and for core symptoms like social deficits and repetitive behaviors, there are currently no effective treatments.

In Shank3B mutant mice, a well-established autism model, resting-state fMRI has shown reduced brain activity. But with awake functional ultrasound imaging, we discovered the opposite during social interaction: widespread brain hyperexcitation, revealing a context-dependent imbalance in excitatory and inhibitory signaling that disrupts social processing. Our work targets NR2B, a subunit of the NMDA receptor, to restore circuit balance and behavioral function.

A single dose of an NR2B-selective antagonist rapidly reduced brain hyperexcitation and significantly improved both social deficits and repetitive behaviors. These effects were not only immediate but also persisted for at least four weeks. We further confirmed this through genetic knockdown, which produced similar benefits.

By modulating a single NR2B receptor with just one dose, we demonstrate a powerful, lasting intervention for core autism symptoms, opening a promising path for therapeutic development.

Disclosures: **Z. Zhou:** None.

Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.10/A19

Topic: A.06. Developmental Disorders

Support: Ariane de Rothschild Women Doctoral Program

Title: Atp synthase subunit upregulation leads to mitochondrial dysfunction in shank3 mouse model of autism

Authors: *W. HAMOUDI¹, H. AMAL^{2,3};

¹The Hebrew university of Jerusalem, Jerusalem, Israel; ²The Hebrew Univ. of Jerusalem, Jerusalem, Israel; ³Harvard Med. Sch. ,Rosamund Stone Zander Translational Neurosci. Center,, Havard Univ., Boston, MA

Abstract: Background : SHANK family proteins are multi-domain scaffolding proteins that orchestrate the postsynaptic signaling complex at glutamatergic synapses. This complex is crucial for the targeting, anchoring, and regulation of neurotransmitter receptors and signaling molecules. A strong link between the SHANK3 gene deletion and autism spectrum disorder (ASD) has been established but its molecular mechanisms remain unknown. Objectives: The aim of this study is to investigate the link between SHANK3 mutations and ASD, examining the molecular mechanism underlying this connection. We first assessed the global proteomic impact of the SHANK3 mutation, which revealed upregulation of a specific ATP synthase subunit in the Shank3^{Δex4-22} mouse model suggesting potential mitochondrial dysfunction. Consequently, we investigated how this mitochondrial impairment contributes to the synaptic and behavioral deficits observed in these mice. To translate our findings from the mouse model to humans, we analyzed blood samples from children carrying SHANK3 mutations. Methods: To achieve these objectives, Shank3^{Δex4-22} mice were used as a model to investigate the effects of SHANK3 mutations in the context of ASD. A comparative analysis of the global proteome was conducted between Shank3^{Δex4-22} and wild-type mice. To assess mitochondrial function, cortical neurons from Shank3 knockout (KO) mice were examined for proton leak and membrane potential. Additionally, an ATP synthase modulator was administered to Shank3^{Δex4-22} mice, followed by behavioral experiments to evaluate its effects. Clinical samples from children carrying SHANK3 mutations were tested. Results: The study revealed a significant increase in a specific ATP synthase subunit level in the cortex of Shank3^{Δex4-22} mice compared to their wild-type counterparts. Furthermore, cortical neurons from Shank3 knockout mice exhibited increased mitochondrial proton leak and a loss of mitochondrial membrane potential, indicating mitochondrial dysfunction. Notably, modulation of ATP synthase in Shank3^{Δex4-22} mice attenuated the ASD-like behaviors, suggesting a potential therapeutic avenue. The clinical plasma samples analysis showed also increase in this ATP synthase subunit and enrichment of several mitochondrial process. Conclusions: In conclusion, mitochondrial dysfunction appears to be implicated in the pathophysiology of ASD, possibly due to the upregulation of the specific ATP synthase subunit. This provides a potential direction for further research and therapeutic interventions.

Disclosures: W. Hamoudi: None. H. Amal: None.

Poster

PSTR313: Autism: Genetic Models

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Topic: A.06. Developmental Disorders

Support: Swedish Research Council: 2021-02891
Åke Wibergs Stiftelse: M24-0270
Åke Wibergs Stiftelse: M23-0159

Title: Targeting dopaminergic dysfunction in fragile X syndrome: effects of (-)-OSU6162 in mice

Authors: S. ALSYAH¹, D. ATANASOVSKI², J. STEEN¹, E. ERIKSSON¹, *F. LONGO¹;
²Neurosci. and Physiol., ¹Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Fragile X Syndrome (FXS), the most common monogenic cause of autism spectrum disorder (ASD), stems from a silencing mutation in the *FMR1* gene, leading to loss of FMRP and dysregulated protein synthesis. Individuals with FXS often exhibit core ASD-like features, including impaired social interaction, communication deficits, and elevated repetitive behaviors (RRB). Increasing evidence suggests that striatal dopamine (DA) dysregulation underlies these behavioral phenotypes. In this context, (-)-OSU6162, both a dopamine receptor antagonist and allosteric modulator, emerges as a compelling therapeutic candidate due to its unique stabilizing properties on DA signaling. This exploratory study tested the efficacy of (-)-OSU6162 in ameliorating behavioral and neurochemical abnormalities in adult male *Fmr1* knockout (KO) mice, a widely used preclinical model of FXS. A total of 20 mice (10 *Fmr1* KO, 10 wild-type) received intraperitoneal injections of (-)-OSU6162 (30 mg/kg) or vehicle before testing. Behavioral assays were conducted to assess locomotor activity (open field), RRB (grooming, marble burying), motor coordination (pole, drag, cylinder tests), and social behaviors (three-chamber and resident-intruder tests). Dopaminergic tone in the dorsal striatum was measured via *in vivo* microdialysis followed by HPLC. (-)-OSU6162 significantly reduced hyperactivity and RRB in *Fmr1* KO mice, with no adverse effects on motor performance in WT controls. Importantly, the drug rescued social interaction deficits in KO mice, indicating a broader impact on ASD-like phenotypes. Neurochemical analysis revealed that *Fmr1* KO mice showed a blunted DA response to (-)-OSU6162, consistent with its DA-stabilizing action in a dysregulated system. Together, these findings highlight (-)-OSU6162's potential to rebalance striatal DA signaling and alleviate core behavioral impairments in FXS. This work supports the further exploration of DA stabilization as a mechanistically informed therapeutic strategy for neurodevelopmental disorders characterized by dopaminergic dysfunction.

Disclosures: S. Alsyah: None. D. Atanasovski: None. J. Steen: None. E. Eriksson: None. F. Longo: None.

Poster

PSTR313: Autism: Genetic Models

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Program #/Poster #: PSTR313.12/A21

Topic: A.06. Developmental Disorders

Title: Investigating neurodevelopmental changes in PTEN Autism-Megalencephaly using cortical organoids

Authors: *G. BAMFONGA¹, M. CHOWDHURY², M. ANDREWS¹, H. I. KORNBLUM³; ¹Sch. of Biol. & Hlth. Systems Engin., Arizona State Univ., Tempe, AZ; ²USC, Los Angeles, CA; ³Univ. of California Los Angeles (UCLA), Los Angeles, CA.

Abstract: Heterozygous mutations in the *PTEN* gene, a critical negative regulator of the mTOR pathway, are implicated in autism spectrum disorder (ASD) co-morbid with megalencephaly. *PTEN* mutations disrupt neurodevelopmental processes through altered mTOR signaling; however, the distinct contributions of mTOR pathway complexes, specifically Complex 1 (mTORC1) and Complex 2 (mTORC2), remain incompletely understood. Our study uses a novel cohort of *PTEN* patient-derived induced pluripotent stem cells (iPSCs) carrying heterozygous point mutations in the phosphatase activity domain of *PTEN*. To dissect the molecular and cellular consequences of mTOR dysregulation during early corticogenesis, we will differentiate human cortical organoids from patient iPSC lines and track neurodevelopmental changes. We will quantify mTOR pathway activity using transcriptomic profiling and immunofluorescence during peak neurogenesis, enabling cell-type-specific analysis of mTORC1 and mTORC2 activation. Our findings reveal increased mTORC2 activity, accompanied by alterations in key, expanded populations of human progenitors, indicating altered specification dynamics. Changes in progenitor proportions lead to altered differentiation timing and profiles. During neurogenic periods, PTEN patient organoids contain fewer excitatory neurons, increased markers of inhibitory neuron fate, and enhanced cell survival, suggesting that mTORC2, rather than mTORC1, mediates neuroprotection and lineage bias in these models. These results illuminate a PTEN-mTORC2 axis as a key driver of cortical lineage imbalance and survival advantage in ASD-related megalencephaly, highlighting novel avenues for therapeutic targeting of mTORC2-specific signaling.

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Poster

PSTR313: Autism: Genetic Models

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Program #/Poster #: PSTR313.13/A22

Topic: A.06. Developmental Disorders

Support: Academia Sinica AS-IA-111-L01

Title: Sex-differential transcriptomic alterations in the Ctnnbp2 autism mouse model

Authors: *T.-L. YEN;
Academia Sinica, Taipei, Taiwan

Abstract: Autism spectrum disorders (ASD) are highly prevalent and male-biased neurodevelopmental disorders. Understanding how males are particularly susceptible to ASD, we have been investigating the related synaptic deficits, behavioral impairment, alteration of neuron circuit under ASD mouse models. The Ctnnbp2 gene encodes a neuron-specific cytoskeleton-associated protein, which controls synapse formation and dendritic arborization of neurons. Human genetic studies have indicated that CTTNBP2 is highly associated with ASD. We also validated the impact of several ASD-linked mutations in the CTTNBP2 gene on synapse formation and social behaviors. However, it is still unclear whether the ASD-linked mutations and sex-specific mechanisms affect the infralimbic area of the prefrontal cortex (ILA)-related circuits. Here, I aim to use mouse behavior assay, transcriptomic and proteomic analysis, and single-nucleus transcriptomic profiling to investigate the sex-biased responses of mutant mice carrying an ASD-associated mutation in Ctnnbp2. I further validated the involvement of specific genes and/or specific types of cell in sex-differential responses in our mouse model. The results provide insightful information about the etiology of sex-biased ASD and the complex regulation in the brain.

Disclosures: T. Yen: None.

Poster

PSTR313: Autism: Genetic Models

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Topic: A.06. Developmental Disorders

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Colorado State University

Title: The effects of autism-associated delta-catenin haploinsufficiency on behaviors

Authors: *E. HINCHLIFFE¹, V. ARAGON¹, S. ROH¹, R. ROACH², R. LEE³, S. KIM³;
²Col. of Vet. Med. and Biomed. Sci., ³Biomed. Sci., ¹Colorado State Univ., Fort Collins, CO

Abstract: δ -catenin functions as an anchor for the glutamatergic AMPA receptor (AMPA receptors) to regulate synaptic activity in excitatory synapses. Mutations in the δ -catenin gene are found in autism patients from multiple families and induce a loss of δ -catenin functions at excitatory synapses, which is thought to be the etiology of autism in people. In fact, patients heterozygous for deletions or loss-of-function variants of the δ -catenin gene exhibit a variety of features of autism. Therefore, δ -catenin heterozygous mutant is a valuable loss-of-function model to investigate autism spectrum disorder pathophysiology caused by δ -catenin haploinsufficiency. Social behavior is essential to survive for many species, and various mental disorders have social impairment as a primary symptom. Additionally, studies suggest that fear memory may be enhanced and more resistant to extinction in ASD individuals. Here, we used two mouse models of δ -catenin haploinsufficiency - δ -catenin knockout and human autism-associated δ -catenin missense mutation, a glycine 34 to serine (G34S) - to address whether δ -catenin haploinsufficiency that mimics the human condition is sufficient to alter behaviors in animals. Especially, we employed a reciprocal social interaction and contextual fear conditioning to examine social behavior and learning and memory, respectively. We found that δ -catenin haploinsufficiency alters social behavior and fear memory in mice. However, the open field test showed normal locomotor activity and anxiety levels in these mice. Therefore, our data suggest that δ -catenin haploinsufficiency is sufficient to induce ASD-associated behavioral alteration in mice.

Disclosures: E. Hinchliffe: None. V. Aragon: None. S. Roh: None. R. Roach: None. R. Lee: None. S. Kim: None.

Poster

PSTR313: Autism: Genetic Models

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Topic: A.06. Developmental Disorders

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

Title: The patient-specific FOXG1 syndrome mouse models with different genetic variation display distinct phenotypes and severities.

Authors: *D. SHIN¹, S. JEON², J. LEE¹, S.-K. LEE¹;

¹Biol. Sci., FOXG1 Res. Center, State Univ. of New York at Buffalo, Buffalo, NY; ²Dept. of Systems Pharmacol. & Translational Therapeut., Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: FOXP1 is a critical transcription factor involved in forebrain development. Mutations in the *FOXP1* gene cause a severe neurodevelopmental disorder known as FOXP1 syndrome (FS). FS patients exhibit a range of structural and developmental abnormalities in brain and behavioral deficits. The most common symptoms are microcephaly, corpus callosum agenesis, sitting and walking difficulty, mental retardation, difficulties in fine motor control, higher anxiety and deficit in social abilities, with severity varying depending on the specific type of mutation. To study the correlation of this phenotypic variability with the *FOXP1* gene mutations, we generated mouse models carrying patient-specific *Foxp1* mutations. These mouse models recapitulated the most common structural brain abnormalities observed in FS patients, such as corpus callosum agenesis and microcephaly, although the severity varied among different models. Behavioral phenotypes also differed, particularly in motor function, anxiety and sociability. In the open field test (OFT), which measures spontaneous locomotion in a novel environment, and anxiety by measuring time spent in the center zone. Q84Pfs, G161Gfs and W300X showed reduced travel distance compared to wildtype (WT) controls at postnatal day 60. Missense G216S mice exhibited an increased travel distance, and notably, their activity did not decline over time, unlike WT or the other mutant models. Additionally, Q84Pfs and G161Gfs mice spent significantly less time in the center, suggesting an elevated anxiety level, whereas G216S and W300X mice did not show significant difference from WT. All four mutant models also displayed impaired muscle strength and endurance in the wire hanging test, falling more quickly than WT mice, with Q84Pfs exhibiting the shortest hanging time. Social behavior was tested using a three-chamber sociability test. In the object vs. mouse phase, G161Gfs mice displayed an increased preference for interacting with a mouse over an object, while G216S and W300X mice showed no significant difference from WT in this measure. In the novel vs. familiar mouse phase, G216S and W300X mice failed to show the natural preference to the novel mice, indicating deficits in social recognition of novelty. These findings illustrate how distinct *FOXP1* mutations differentially affect brain development and behavior, providing valuable insights into the genotype-phenotype relationships underlying FS. The differences observed across these models suggest that the location and type of mutation play a critical role in shaping the resulting phenotypes highlighting the importance of mutation-specific approaches in the study of FS.

Disclosures: D. Shin: None. S. Jeon: None. J. Lee: None. S. Lee: None.

Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.16/A25

Topic: A.06. Developmental Disorders

Support: NIH Grant 1R56MH130599-01A1

Title: Early Life Stress Disrupts Social and Non-social Behavior in a Mouse Model of Autism

Authors: *T. L. FORD¹, S. KATTEWAR², W.-Y. KIM¹;
¹Biol. Sci., ²Kent State Univ., Kent, OH

Abstract: Although Autism Spectrum Disorder (ASD) and early life stress (ELS) are both extensively studied, their intersection is often overlooked in the field. Using an *Arid1b* haploinsufficient mouse model of ASD, we explored how ELS influences behavioral outcomes during development and into adulthood. . ELS was induced using the early life scarcity-adversity (S-A) paradigm, which involved reducing bedding in pup cages from postnatal day (P)8 to P12. We found that ELS disrupted social bonding behavior in autistic models of pups. Specifically, *Arid1b* haploinsufficient pups exposed to ELS displayed increased time spent approaching their mom, while their wild type counterparts showed no significant changes in bonding behavior. Alterations in social behavior persisted to adulthood as 4 days of ELS resulted in reduced sociability in 4-month-old *Arid1b* haploinsufficient mice. In addition to social deficits, ELS during early development also affected anxiety-like behavior and recognition memory in adulthood. We also explored whether neuroendocrine signaling is associated with ELS-induced behavioral change in control and *Arid1b* haploinsufficient mice by measuring oxytocin levels in several brain regions implicated in social behaviors such as the paraventricular nucleus, the ventral tegmental area, the bed nucleus of the stria terminalis, and the basal lateral amygdala. We found that ELS significantly altered oxytocin levels in these regions in both control and *Arid1b* haploinsufficient mice, with more profound changes observed in the mutants. Together, our data suggest that ELS is a significant contributor to both short- and long-term behavioral outcomes in ASD-related conditions and may represent a potential therapeutic target for this developmental disorder.

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Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.17/A26

Topic: A.06. Developmental Disorders

Support: Pilot Project NIH P30ES030283
DOD AR230178P1

Title: Differences in neuroinflammation, microglial activation and BBB dysfunction may underlie variability in ASD-like characteristics of murine models of autism

Authors: *E. GOZAL¹, R. JAGADAPILLAI², A. IKRAM⁴, N. NAGARAJAN⁴, G. N. BARNES³;

¹Dept of Pediatrics PRI / Univ. of Louisville, Louisville, KY; ²Pediatric Res. Inst., ³Dept Pediatrics, PRI, Neurol., Univ. of Louisville Sch. of Med., Louisville, KY; ⁴Pediatrics, PRI, Univ. of Louisville, Louisville, KY

Abstract: Preclinical research models are essential for the study of autism (ASD) pathophysiology and the development of new therapies. The BTBR mouse is a polygenic inbred mouse strain ASD model and 1-2% of ASD patients exhibit mutations in *Shank3*, encoding for a scaffold postsynaptic density protein. While both are validated as pre-clinical ASD models, their behavioral characteristics differ. We previously reported disrupted blood-brain barrier (BBB), microglial activation, and oxidative stress in the brain of a different murine ASD model, the *Sema3F* KO mouse. Additionally, compromised BBB and neuroinflammation have been identified in human ASD postmortem brain tissue. Therefore, we aim to determine whether these markers may also be consistent in *Shank3* KO and BTBR, suggesting a convergent immune dysregulation mechanism across cellular components of these pre-clinical models. To our knowledge, an in-depth study of these features has not been done in these ASD mouse models. **Methods:** Cortex and cerebellum of 3-month-old *Shank3* KO, BTBR, and control mice (C57BL/6) were dissected for western blot (WB) or perfused and sectioned for immunofluorescence (IF). To demonstrate neuroinflammation and microglial activation, DHE (superoxide) staining, IF staining of GFAP (astrocytes), Iba1 (microglia), TREM 2 (microglial activation), albumin leakage (BBB permeability) and WB of complement protein 3 (C3) and TREM2 were performed. Images were acquired using a confocal microscope (Leica). **Results:** WB demonstrated increased C3 and decreased TREM 2 expression in both *Shank3* KO and BTBR cerebellum and cortex. GFAP IF increased in *Shank3* KO to a greater extent than in BTBR while both Iba1 and DHE increased less in *Shank3* KO than in BTBR. TREM2 IF decreased in both *Shank3* KO and BTBR in both areas, confirming microglial activation. Albumin diffused throughout the cerebellar tissue of perfused *Shank3* KO and BTBR mice while minimally localizing to vasculature in C57/BL6, suggesting a compromised BBB in both ASD models. **Conclusions:** Increased glial activation, albumin leakage, and oxidative stress in brain areas of *Shank3* KO and BTBR mice, comparable with our previous findings in *Sema3F* KO, suggest that neuroinflammation and BBB disruption are common features of ASD from different etiologies. However, variability in the intensity of these markers may suggest differences of signaling pathways, neural circuits, and ASD-like behavioral traits between these models. In future studies, we aim to identify signaling pathways underlying these cellular phenomena in the broader ASD population to elaborate common therapeutic strategies in autism.

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Poster

PSTR313: Autism: Genetic Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.18/A27

Topic: A.06. Developmental Disorders

Support: SFARI award # 633450

Title: Developmental and circuit-specific plasticity deficits in the mPFC-MD pathway of shank3^{-/-} mice: mechanisms and potential for rescue

Authors: *G. DEVIENNE, G. VANTOMME, J. R. HUGUENARD;
Stanford Univ., Stanford, CA

Abstract: Shank3 is a high-confidence autism spectrum disorder (ASD) risk gene, and its loss leads to profound synaptic and behavioral impairments. The medial prefrontal cortex (mPFC)—a hub for executive function and social cognition—is especially vulnerable in Shank3 knockout (Shank3^{-/-}) models. Emerging evidence, including our recent work, points to disrupted plasticity within the mPFC and its reciprocal connections with the mediodorsal (MD) thalamus. We identified a striking developmental trajectory in Shank3^{-/-} mice, with early postnatal (~P14) hypoexcitability in the mPFC that transitions to hyperexcitability in adulthood. Additionally, we observed a hyperfunctional mPFC-MD synapse, potentially reflecting a compensatory or maladaptive mechanism that disturbs circuit homeostasis. Crucially, we found that long-term potentiation (LTP), robustly expressed in adult wild-type mPFC, is absent in adults Shank3^{-/-}. Remarkably, chronic inhibition of mPFC activity in adults via chemogenetics rescued LTP, with effects persisting days after treatment ended. These findings reveal a previously unappreciated plasticity window and highlight the potential for circuit-level interventions to reverse synaptic deficits in Shank3^{-/-} mice. Ongoing work aims to define the developmental emergence, synaptic mechanisms, and reversibility of mPFC-MD dysfunction, providing new insight into therapeutic strategies for ASD.

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Poster

PSTR313: Autism: Genetic Models

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR313.19/A28

Topic: A.06. Developmental Disorders

Support: Yang Tan Collective
Simons Center for the Social Brain

Title: Desynchronization of neural activity during naturalistic viewing of social stimuli in SHANK-3 mutant marmosets

Authors: *M. RANA¹, H. XU¹, B. ZHANG¹, K. SRINIVASAN¹, Q. ZHANG², F. LIANG¹, C. J. VALENZUELA¹, G. LIBERTI¹, C. ZHANG¹, G. FENG², R. DESIMONE¹;
¹McGovern Inst. for Brain Res., ²Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Autism Spectrum Disorder (ASD) is characterized by deficits in social behavior, but their neural mechanisms remain unknown. We investigated social visual processing in 4 wild-type (WT) and 4 SHANK3 mutant marmosets (a genetic model of ASD) using naturalistic

movie-viewing tasks featuring social (faces) and non-social (objects) stimuli. We used eye-tracking to analyze gaze patterns, while electrocorticography (ECoG) recordings were conducted in the posterior temporal lobe (PD), anterior temporal lobe (MD), and prefrontal cortex (A45). Face-selective channels in these areas were used to analyze high gamma power (70-200 Hz) response latency and inter-area coherence. Behaviorally, gaze analyses revealed no significant differences in face vs object fixation between the WT and SHANK3 marmosets. Both groups demonstrated higher fixation on faces compared to objects, with peak fixation occurring within the first second of stimulus presentation. The difference in time to peak fixation between faces versus objects for the WT and SHANK3 cohorts were not statistically different. Saccade metrics, including velocity, rate, amplitude, and inter-saccade fixation time, did not differ between the WT and SHANK3 cohorts as well. Overall, social behavior differences were not discernible between WT and SHANK3 marmosets during movie-viewing tasks from gaze analyses. When investigating neural differences, latency analysis of high gamma power with respect to stimulus onset across areas PD, MD, and A45 revealed no significant differences between the WT and SHANK3 cohorts for both the face and object stimuli. Generally, PD exhibited an earlier latency than MD and A45, consistent with its role as a relatively earlier stage in the high-level visual processing pathway. Across both cohorts, face stimuli resulted in earlier neural response latencies than object stimuli in all areas. We are also examining the coherence between the identified face channels when the WT and SHANK3 cohorts are viewing stimuli containing faces or objects. Because the SHANK3 mutation affects synaptic scaffolding and excitatory connectivity, we hypothesize that long-range cortical communication during face processing may be impaired. Since synchronized activity across visual networks supports integration during visual processing, we expect reduced coherence in SHANK3 mutants when viewing faces. This disruption may indicate altered excitatory-inhibitory balance or weakened top-down modulation, potentially contributing to impaired social perception in SHANK3 marmosets.

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Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.01/A29

Topic: A.07. Development of Neural Systems

Support: NIMH Grant R01MH134966

Title: Exploring Measurements of Low Frequency BOLD Fluctuations in Infant fMRI

Authors: E. TARAKCI¹, L. HANTZSCH², A. LABONTE⁵, C. M. SYLVESTER⁶, J. ELISON³, D. A. FAIR⁴, J. MOSER⁴;

¹Univ. of Minnesota, Twin Cities, Minneapolis, MN; ²Inst. of Child Develop., ⁴Masonic Inst. for

the Developing Brain, ³Univ. of Minnesota, Minneapolis, MN; ⁵Washington Univ. Sch. of Med., St. Louis, MO; ⁶Psychiatry, Washington Univ. Sch. of Med., St Louis, MO

Abstract: Functional magnetic resonance imaging (fMRI) is increasingly used to monitor infant brain development. Recently, the amplitude of low frequency fluctuations (ALFF) in the blood-oxygen level dependent (BOLD) fMRI signal has been proposed as a measure of sensorimotor plasticity in adults. As developing brains are highly plastic, ALFF is also a promising measure for investigating sensory and motor plasticity in neonates. However, ALFF's reliability within individual infants has yet to be tested. ALFF is a measure that requires a continuous, uninterrupted time series which can be difficult to obtain in high motion populations such as neonates where high motion frames are removed from the data as a form of noise reduction. Thus, standard practice uses interpolated frames when calculating ALFF. Our goal is to examine the reliability of ALFF within individual neonates using interpolated BOLD data. 10 infants (mean postmenstrual age: 48 weeks (range: 45-51); 6 female) were scanned over 2-4 days within 1 week during natural sleep on a 3T Siemens Prisma scanner with a 32 channel head coil, resulting in anatomical references and a minimum of 60 minutes of low motion BOLD data (FD<0.3; 11-23 runs) collected during a passive listening auditory oddball paradigm. Data was preprocessed using NiBabies 25.0.1 and post-processed with XCP-D 0.10.5 to apply motion scrubbing and a bandpass filter (.01-.08 Hz). ALFF (defined as the square root of average power between .01-.08 Hz) was calculated on the processed data and runs with less than 60% low motion data were excluded from further analysis. Within subject reliability of ALFF was calculated using a correlation matrix of ALFF per greyordinate of every run per subject. Initial results show that the within-subject correlation of ALFF had a range of $r = .46-.77$ across all subjects (mean = .56). While ALFF has potential as a reliable measure of low frequency BOLD fluctuations, further investigation is needed to parse the causes of individual variability such as the role of interpolated frames, the highly variable nature of infant fMRI data as well as motion and scan time. Moving forward, we will explore the reliability of measures such as standard deviation (SD) which do not require continuous data and their relationship to ALFF in infants. While BOLD fluctuations and their role in neuroplasticity are being investigated in both infants and adults, additional measures such as SD may provide another tool for researchers to investigate this relationship in populations where continuous low motion data is difficult to obtain.

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Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.02/A30

Topic: A.07. Development of Neural Systems

Title: Congenital Loss of Corticospinal Connectivity in Mice Reveals an Ancestral Role in Motor Control Beyond Skilled Movement

Authors: *K. P. REES¹, L. GALLAGHER¹, A. KUNWAR¹, P. IANCHULEV¹, J. K. THACKRAY², A. IQBAL¹, V. E. ABRAIRA³, V. V. SAHNI⁴;

¹Burke Neurolog. Inst., White Plains, NY; ²Rutgers Univ., New Brunswick, NJ; ³Cell Biol. and Neurosci., Rutgers, The State Univ. of New Jersey, Warren, NJ; ⁴Burke Neurolog. Inst., Weill Cornell Med., White Plains, NY

Abstract: Neocortical connectivity with the brainstem and spinal cord, via cortico-bulbar and corticospinal projections, is essential for skilled movement across mammals. This is supported by lesion studies in adult animals. In rodents, however, many resulting deficits are transient, contributing to the notion that developmental loss of this pathway has no lasting impact. In contrast, developmental damage to the corticospinal tract (CST) in humans, such as in cerebral palsy, results in lifelong motor impairment that extends beyond skilled movement. This discrepancy has limited the utility of rodents as models for developmental CST disruption. Moreover, most rodent motor assessments rely on tasks emphasizing skilled forelimb use and require prior training, making them unsuitable for evaluating the consequences of early developmental loss, when such training is not possible. As a result, it remains unclear whether congenital loss of cortical-subcerebral connectivity in mice produces lasting behavioral effects. To address this, we used *Emx1-Cre* to conditionally delete *Fezf2*, a transcription factor essential for specifying subcerebral projection neurons. *Fezf2* conditional knockout (cKO) mice completely lack cortical projections to the brainstem and spinal cord. We assessed skilled and spontaneous movement in adult mice (>3 month old) using training-independent tasks. For skilled movement, we used a balance beam assay with Deep Lab Cut, a markerless pose estimation tool, to quantify paw placement. *Fezf2* cKO mice showed persistent deficits relative to wild-type littermates, with greater impairment in hindpaw than forepaw placement. To assess spontaneous movement, we used Motion Sequencing (MoSeq), an unsupervised machine learning method that identifies sub-second behavioral motifs, or “syllables.” MoSeq revealed reduced expression of several syllables in *Fezf2* cKO mice, many involving hindlimb use. These findings challenge the notion that the CST governs only skilled motor control and instead support its broader role in spontaneous movement. Our results suggest that this function is evolutionarily conserved in mice and potentially expanded in humans. Thus, congenital CST disruption in mice leads to lasting motor deficits and provides new insight into the ancestral role of this pathway.

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Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.03/A31

Topic: A.07. Development of Neural Systems

Title: Analysis of peripheral neuromuscular activity modulation of the lower limb during gait function acquisition in toddlers.

Authors: *C. YAMASAKI¹, M. ITO¹, T. KOKUBUN²;

¹Saitama Prefectural Univ., Saitama, Japan; ²Physical Therapy, Saitama Prefectural Univ., Koshigaya-Shi, Japan

Abstract: Independent walking is normally achieved between the ages of 12-14 months. Mature gait consists of periodic joint movements and muscle activity, whereas early independent walking shows non-phasic limb movements and muscle activities. Neurological development, musculoskeletal growth, and accumulation of walking experience contribute to the maturation of walking. The peripheral neuromuscular system ultimately controls limb movements. However, few reports have focused on the developmental process of peripheral muscle activities. We analyzed the dynamic changes of motor units (MUs) activity to explore changes during gait acquisition in toddlers. Eight toddlers aged 10-24 months were assessed at three time points. Surface electromyography and a 3D motion analyzer were synchronized to record neuromuscular activity and kinematic data during gait. Target muscles were the rectus femoris (RF), biceps femoris (BF), tibialis anterior, and lateral gastrocnemius. We calculated the standard deviation (SD) of joint angles and the regression coefficient (RC) from linear regression of MU action potentials / firing frequency by age in months and months since gait acquisition. Results ordered by age, an increase in range of motion, and a decrease in SD appeared simultaneously after the age of 19 months in the hip joint. In this period, the RF increased RC from 19 to 22 months of age, and the BF increased RC from 20 to 24 months of age. Results sorted by months of walking experience, both hip and knee joints showed an increase in range of motion and a decrease in SD after 4 months of gait acquisition. The data for RF showed a decrease in RC from 4 to 8 months, and the data for BF showed a decrease in RC from 5 to 8 months. Infants have a smaller range of motion of the lower limb joints during gait and a larger SD during the gait cycle compared to adults. Therefore, the development of gait can be considered as the period when the range of motion of the lower limb joints increases and the SD decreases. The neuromuscular activity control was changed after the age of 19 months by the increase of hip extension angle during the anterior swing phase, and the decrease of muscle output related to the subsequent swing due to muscle lengthening tension in the anterior thigh. In the analysis of months since gait acquisition, a change in walking development was observed in the range of motion of the lower limb joints after 4 months of gait acquisition. The neuromuscular activity control was changed by the increase in muscle output due to the increase in lower limb load during the stance phase of development. Thus, the effects of both antigravity and gravity-assisted movement were considered to promote gait development.

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Poster

PSTR314: Development of Motor Systems

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.04/A32

Topic: A.07. Development of Neural Systems

Title: Comparative single nuclei RNA sequencing in zebra finch and human brains reveals the evolutionary history of avian vocal learning and motor control

Authors: ***M. H. DAVENPORT**¹, C. LEE², S. MARCUS³, B. N. PHAN⁴, R. GANESAN⁵, A. R. PFENNING⁶, E. D. JARVIS²;

¹Rockefeller Univ., New York City, NY; ²Rockefeller Univ., New York, NY; ³The Rockefeller Univ., New York, NY; ⁴Computat. Biol., ⁶Computat. Biol. Dept., ⁵Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Zebra finches (*t.castanotis*) learn their songs by imitating conspecifics during development. Such vocal learning behavior is rare, found in 3 clades of birds and 5 clades of mammals including humans. Across species, learned vocalization requires a series of specialized neural circuits in the pallium which ultimately project to the brainstem motor neurons for vocal organ control. Despite this anatomical similarity, the learned vocal motor circuits are not strictly homologous as vocal learning evolved independently in each lineage. However, both the human and finch pallial vocal motor circuits are embedded in far older non-vocal motor circuits; the primary motor cortex of mammals (M1C) and a distributed avian motor circuit in the dorsalventricular ridge (DVR) including the lateral intermediate arcopallium (LAI) which surrounds the robust nucleus of the arcopallium (RA), the dorso- and postero- lateral nidopallium (DLN, PLN) which surround the song region HVC (proper name), and the anterior nidopallium (AN) which surrounds the lateral nucleus of the anterior nidopallium (LMAN). The homology of these DVR motor circuits to the subdivisions of mammalian pallium is contested. Some groups attribute homology to the mammalian M1C and other groups attribute homology to the claustrum (CLA) and/or amygdala (AMY). To resolve this ambiguity, here we compare the single nucleus transcriptomes of neurons from microdissections of finch vocal- and non-vocal-motor circuits in the DVR to the single nucleus transcriptomes of human neurons from M1C, CLA, and AMY made available by the Allen Brain Institute as part of the Human Brain Atlas. We apply machine learning methods (implemented in Seurat) to assess similarity between human and finch brain single nucleus transcriptomes. We find that finch DVR motor circuits are highly M1C-like. We further find that only the arcopallium contains mammalian ET-like neurons, but IT-like neurons can be found in all regions, consistent with known avian anatomy. We provide conserved gene markers for these divisions as well as conserved marker genes for various established homologous cell types (astrocytes, oligodendrocytes, MGE and CGE interneurons, and basal ganglia neuron types).

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Poster

PSTR314: Development of Motor Systems

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Program #/Poster #: PSTR314.05/A33

Topic: A.07. Development of Neural Systems

Support: University of Oklahoma, Institute for Community and Society
Transformation

Title: Exploring the impact of free play on the brain activity related to social goal-inference in elementary school students

Authors: *F. NAGHASH, E. CASEY, B. ARLEDGE, J. E. NORRIS, E. SPIVEY, D. MAHMOUD, R. RAJALA, L. ETHRIDGE;
Univ. of Oklahoma, Norman, OK

Abstract: Play is a crucial aspect of childhood development, offering emotional, physical, and social experiences that help children engage with others and learn in a supportive setting. Engaging in free play encourages independent exploration without adult guidance and supports both behavioral and physiological growth. This confirmatory crossover study investigated the impact of free play on social and action-related neurodevelopment of elementary-aged children. A total of 225 students (1st to 5th grade) participated in the OU Free Play Lab for 45 minutes each week during the Fall or Spring semester. A randomly selected, gender-balanced subset of 50 students (10 per grade) completed EEG assessments at three timepoints: baseline, crossover, and endpoint. EEG tasks involved viewing goal-directed and non-goal-directed actions videos to probe neural responses related to social-action perception. Preliminary ANOVA analyses indicated significant differences across video types, timepoints, and treatment groups. Alpha and low beta were significantly lower during the non-goal-directed videos in comparison to the goal-directed videos consistent with differential processing of socially meaningful actions. Phase-amplitude coupling (PAC) analyses revealed higher beta-gamma coupling in temporal and frontal regions during the goal-directed videos, suggesting enhanced processing of social-action cues and others' intentions. Response to goal-directed videos differed across timepoints and treatment groups similarly to behavioral outcomes, suggesting play's role in bridging neural pathways associated with social cognition.

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Poster

PSTR314: Development of Motor Systems

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Program #/Poster #: PSTR314.06/A34

Topic: A.07. Development of Neural Systems

Support: McGovern Institute for Brain Research

Title: Validating human pose estimation for infant gait assessment

Authors: S. ABDELHADI, *N. SEETHAPATHI;
MIT, Cambridge, MA

Abstract: Quantifying infant movements in real-world settings is a key challenge for advancing our understanding of sensorimotor development and for early diagnosis of neuromotor disorders. However, infant movements are highly variable and difficult to quantify outside controlled lab settings. While computer vision-based human pose estimation tools like OpenPose and newer state of the art models are gaining traction in neuromotor assessments, their accuracy in young children remains largely unvalidated. Specifically, we don't know how well they perform on toddlers, whose movements are often much more variable, fast-changing, and frequently occluded. In this study, we tested whether these tools can accurately extract gait parameters in young children in a naturalistic setting with minimal experimental constraints by comparing their outputs to data from a ground truth marker-based motion capture system, considered the gold standard for three-dimensional motion tracking. We collected video and marker-based motion capture data from 19 children between 8 months and 3.8 years old during overground walking trials, with tasks adapted to each child's motor abilities. Running and jumping were excluded unless developmentally appropriate. Using step timing, step length, and walking speed as key metrics, we applied three pipelines: AlphaPose, MotionBERT, and a custom three-dimensional motion tracking pipeline in development to the same video trials and compared their outputs to the motion capture ground truth. This dataset may also support future work in developing infant-specific pose models. Preliminary comparisons are in progress; early observations suggest that three dimensional pose estimation is essential for neuromotor assessment of at-home infant videos. This work provides a benchmark for assessing the validity of video-based gait analysis in early childhood, with future implications for the remote screening of motor development and neuromotor developmental disorders.

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Poster

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Topic: A.07. Development of Neural Systems

Support: NIH NTRAIN/NICHD K12HD093427)
Burke Foundation
NIH R01 NS131662-01

Title: Dissecting cre line specificity in layer 5 reveals incomplete labeling of subcerebral projection neuron subtypes

Authors: *P. PATEL¹, J. KAISER², V. V. SAHNI³;

²Sahni Lab., ¹Burke Neurolog. Inst., White Plains, NY; ³Burke Neurolog. Inst., Weill Cornell Med., White Plains, NY

Abstract: Recent advances in neuroscience have led to the development of numerous transgenic knock-in Cre mouselines that show specificity of cell-type expression in the neocortex. Several of these Cre lines are purported to be specific to layer 5 neurons and have been widely used to study the development, circuit organization, and function of layer 5 extratelencephalic (ET) projecting neurons. Among them, the Rbp4-Cre line has been widely used for investigating both the development and function of layer 5 subcerebral projection neurons (SCPNs). Our ongoing work has identified molecular delineation of anatomically distinct SCPN subpopulations during development. Developing SCPN subpopulations that project to distinct segmental targets exhibit unique molecular identities. Leveraging these novel molecular delineators—**Npy** (delineates cortico-brainstem neurons in lateral sensorimotor cortex), **Cartpt** (delineates corticospinal neurons in lateral sensorimotor cortex), and **Crim1** (delineates thoraco-lumbar projecting corticospinal neurons in medial cortex)—we investigated whether Rbp4-Cre is equally expressed by these distinct SCPN subpopulations. Our findings show that Rbp4-Cre does not label all SCPN subtypes uniformly; some subpopulations are labeled sparsely or not at all. These results suggest that using Rbp4-Cre alone may limit comprehensive investigations into the development and circuit-level organization of SCPN. Importantly, our data underscore that layer 5 Cre driver lines may preferentially label certain SCPN subtypes, introducing potential biases in functional studies. We provide a valuable resource to guide the selection of appropriate Cre lines for targeting specific SCPN subpopulations, enabling more precise dissection of neocortical projection neuron development and function.

Disclosures: P. Patel: None. J. Kaiser: None. V.V. Sahni: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.08/A36

Topic: A.07. Development of Neural Systems

Support: NIH Grant R15NS091977

Title: Identifying the genetic basis of a sexually differentiated vocal circuit in the frog, *Xenopus laevis*

Authors: V. OSTERBERG¹, A. OROZCO¹, A. FERFAS¹, S. SUBRAMANIAN¹, *E. J. ZORNIK²;

²Biol., ¹Reed Col., Portland, OR

Abstract: Many neuronal circuits are functionally differentiated between males and females. While gonadal steroid hormones are known to influence brain development and function, the

mechanisms underlying these processes are poorly resolved. Vocalizations of the African clawed frog, *Xenopus laevis*, are distinct between males and females and are driven by a sexually differentiated hindbrain vocal circuit that is regulated by steroids. We used next-generation RNA sequencing (RNAseq) and hybridization chain reaction fluorescence *in situ* hybridization (HCR-FISH) to identify and image differentially expressed genes in the *X. laevis* vocal circuit. RNAseq results revealed a relatively small number of genes (<100) differentially expressed by sex. We used HCR-FISH to validate differential expression patterns of candidate genes involved in steroid response, steroid metabolism, and synaptic transmission. Imaging results revealed morphological differences between male and female vocal circuits, providing clues about the genetic origins of structural and functional sex differences.

Disclosures: V. Osterberg: None. A. Orozco: None. A. Ferfas: None. S. Subramanian: None. E.J. Zornik: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.09/A37

Topic: A.07. Development of Neural Systems

Support: NIH U01-NS128612

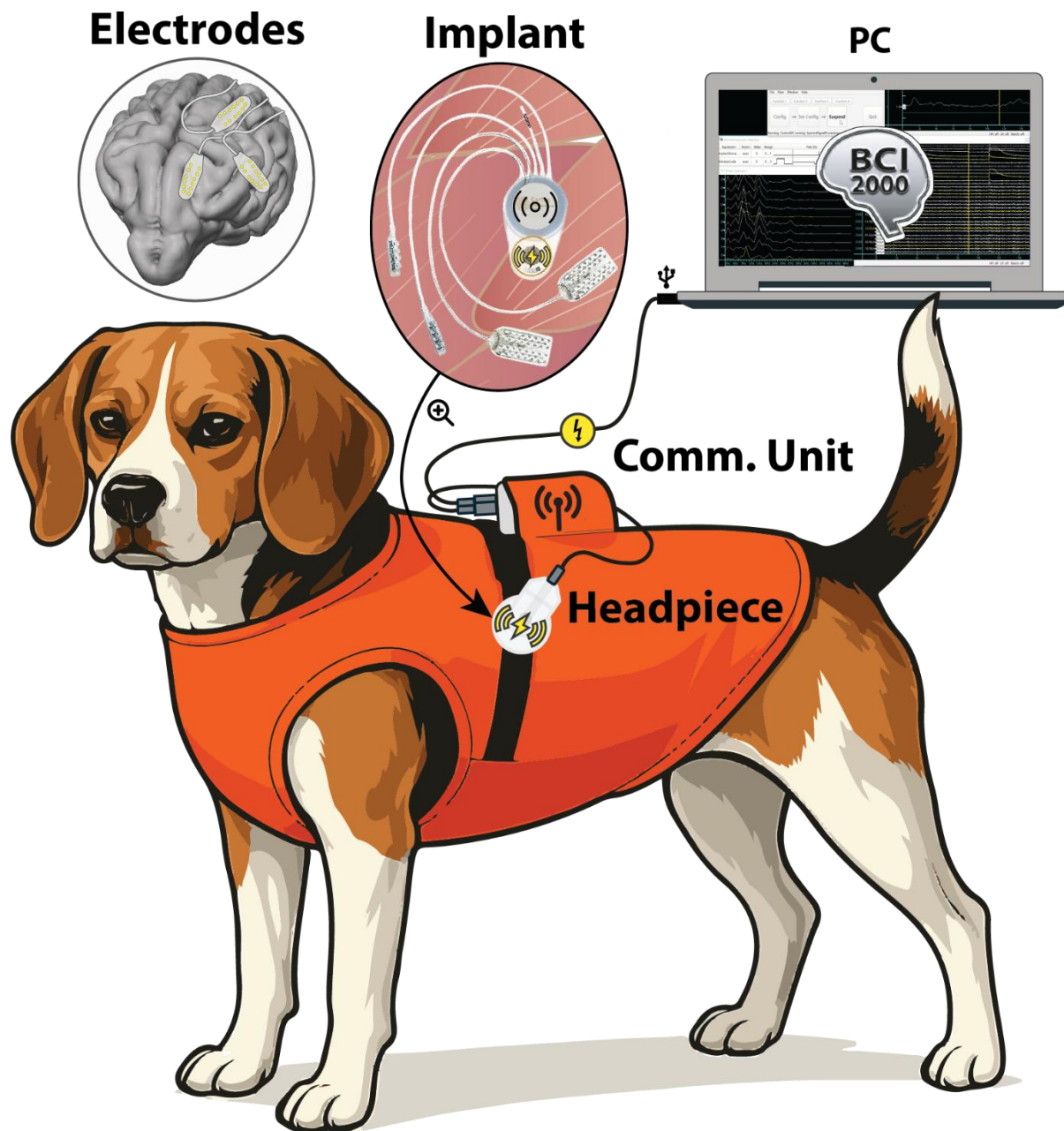
Title: Long-term evaluation of CorTec's Brain Interchange - BCI2000 ecosystem in chronically implanted canines

Authors: *F. LAMPERT¹, F. MIVALT², W. ENGELHARDT⁴, N. F. INCE³, P. BRUNNER⁵, G. A. WORRELL¹, K. J. MILLER³;

²Neurol., ³Neurosurg., ¹Mayo Clin., Rochester, MN; ⁴Washington Univ. Sch. of Med., St. Louis, MO; ⁵Dept. of Neurosurg., Washington Univ., St. Louis, MO

Abstract: Introduction: Adaptive neuromodulation and brain-computer interface (BCI) systems offer significant therapeutic potential to enhance quality of life in individuals with severe neurological disorders. However, their clinical and research applications are often hindered by the need for substantial infrastructure and specialized expertise. To address these limitations, we are developing an open-source ecosystem integrating the BCI2000 software environment with CorTec's Brain Interchange (BIC) system. This platform aims to facilitate innovation in neuromodulation therapies and reduce barriers to BCI adoption. **Methods** We implanted three canines with CorTec's BIC system, capable of sensing and stimulation across 32 channels, and paired it with BCI2000 software, enabling real-time signal visualization and analytical processing pipelines. The canines were implanted with cortical or deep brain stimulation (DBS) electrodes and were monitored on a daily basis to evaluate the capability and longevity of the ecosystem. Long-term recordings were analyzed for interictal epileptiform discharges (IEDs). We conducted functional tasks and decoded brain activity by detecting broadband spectral

changes. **Conclusion:** Canines were monitored in total for over 37 months, during which we evaluated in-vivo capabilities of the BIC, including device's noise floor, packet loss and electrode impedance. We successfully captured and decoded brain activity from the functional tasks even after two years post implant. Moreover, we detected IEDs in chronic recordings both in epileptic and non-epileptic intracranial recordings. Acquired results demonstrate the long-term stability and integrity of the BIC-BCI2000 ecosystem and underscore its potential for personalized therapies targeting patients with severe neurological disorders. **Acknowledgments:** This study is conducted under Mayo Clinic IACUC protocol A00001713-16, canines are highly socialized and available for adoption, upon the study completion. This work was supported by the NIH U01-NS128612 (KJM,GAW, PB).



Disclosures: **F. Lampert:** None. **F. Mivalt:** A. Employment/Salary (full or part-time): has received a salary support from Cadence Neuroscience Inc.. **W. Engelhardt:** None. **N.F. Ince:** None. **P. Brunner:** None. **G.A. Worrell:** None. **K.J. Miller:** None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.10/A38

Topic: A.07. Development of Neural Systems

Title: Building a gene expression atlas of developing brainstem motor neurons as a toolbox to study rare congenital neurologic disorders.

Authors: ***N. SHIROONI;**
Univ. of California, Irvine, Irvine, CA

Abstract: Ocular motor neurons (OMNs) in the brainstem mediate eye movement and are differentially affected in some rare congenital neurologic diseases. In cases such as Duane Syndrome, specific OMN subpopulations show disrupted or aberrant innervation while other subpopulations remain unaffected, but mechanisms underlying differential susceptibilities have not yet been identified. Here we generate a transcriptomic atlas to analyze unique gene expression patterns of each developing MN type as a toolbox to help study these disorders. We combined multiple mouse genetic reporter lines with intersectional temporal (embryonic days E9.5 to E18.5) and spatial transcriptomics (single cell/nuclei RNA-seq, and Slide-Seq) to isolate and compare eight distinct mouse MN populations: the three oculomotor nuclei (CN3, CN4, CN6) and the other primary MN types (CN5, CN7, CN9/10, CN12 and spinal MNs). Sample integration posed a significant “batch effect” challenge since different types of samples were acquired via multiple methods over many ages. We compared multiple benchmarked high quality integration pipelines to balance batch correction vs. bioconservation. Once cells were identified and labeled we used scDREAMER-SUP, a semi-supervised deep learning algorithm, using cell label annotations as a means to achieve further bioconservation and batch correction. We built a visualization tool to investigate integration quality. Cell clusters were mapped onto spatial transcriptomic slide-seq samples from E11.5 and E14.5 and compared with developmental mouse atlases to confirm cell identities. Candidate marker genes of each cell population were further validated via database analysis and RNA in situ hybridization. We successfully integrated a developmental time course of mouse MN gene expression from disparate sample types. The resulting atlas can be transposed to label and identify spatial structures within slide-seq datasets to identify cells in time and space. Overall, this atlas uncovers distinct developmental gene expression patterns and provides new tools to study their differential vulnerability in motor neuron related disorders.

Disclosures: **N. Shirooni:** None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.11/A39

Topic: A.07. Development of Neural Systems

Support: NIH Grant SETD1A R01 30038341

Title: Effects of SETD1A Haploinsufficiency in Human Cerebellar Organoids

Authors: *A. CHANDIA CRISTI¹, S. A. AMENT²;

¹Univ. of Maryland, Baltimore, MD; ²Dept. of Psychiatry and Inst. for Genome Sci., Univ. of Maryland, Sch. of Med., Baltimore, MD

Abstract: Abnormal cerebellar development is increasingly connected to neurodevelopmental disorders, including schizophrenia (SCZ). Several lines of evidence point to changes specifically in Purkinje cells, the output neurons of the cerebellar cortex. Anatomical studies of brain tissue from people who died with SCZ suggest that Purkinje cell soma are decreased both in size and number. Gene expression profiling of cerebella from SCZ cases vs. controls revealed down-regulated gene co-expression networks enriched for components of synapses on Purkinje cell dendrites. Cell atlases for cerebellar development revealed that many SCZ risk genes are expressed specifically in Purkinje cells during their pre- and postnatal development. However, few studies have tested the causal effects of mutations associated with risk for SCZ on the development of Purkinje cells, and almost none have investigated effects in human Purkinje cells. Rare, heterozygous loss-of-function variants in SETD1A, a histone methyltransferase, are associated with >10-fold increased risk for SCZ. Therefore, we hypothesized that SETD1A haploinsufficiency would cause defects in Purkinje cell development mirroring those observed in SCZ patients. To investigate cerebellar neurodevelopmental alterations, we standardize a protocol for generating three-dimensional human cerebellar organoids (hCerOs) from induced pluripotent stem cells (iPSCs), comparing genome-editing heterozygous loss-of-function SETD1A iPSCs to isogenic controls. We are using these resources and technologies to model the impact of SETD1A haploinsufficiency on cerebellar development.

Disclosures: A. Chandia Cristi: None. S.A. Ament: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.12/A40

Topic: A.07. Development of Neural Systems

Support: NINDS Grant R01NS131662
NTRAIN/NICHD Grant K12HD093427
NIH Grant S10OD028547
NIH Grant S10OD036432
NYSDOH Grant C39069GG
Wings for Life - Spinal Cord Injury Foundation
Craig H. Neilsen Foundation

Title: Structural plasticity of cortico-brainstem neurons and their contribution to recovery after CNS injury

Authors: J. KAISER¹, *S. FEDDE², P. PATEL¹, B. CAGLAYAN², H. RODRIGUEZ², V. V. SAHNI³;

¹Sahni Lab., ²Burke Neurolog. Inst., White Plains, NY; ³Burke Neurolog. Inst., Weill Cornell Med., White Plains, NY

Abstract: Neocortical connectivity with targets in the brainstem and spinal cord are established by both cortico-brainstem neurons (CBN) that only project to the brainstem, and corticospinal neurons (CSN) whose axons extend to the spinal cord but also collateralize in the brainstem. To date, our understanding of the potentially distinct anatomical organization and connectivity of CBN versus CSN, as well as distinct role(s) in motor control has been limited since there were no molecular tools to distinguish between these closely related populations. Furthermore, damage to these circuits result in considerable motor deficits, and therefore understanding how CBN and CSN respond to injury is imperative for finding new avenues for motor recovery after central nervous system injury. In our previous work, we identified Neuropeptide Y (Npy) as a developmentally expressed marker specific to cortico-brainstem neurons (CBN). This first known molecular delineator of CBN therefore enabled targeted investigation of their axonal projections and role in motor control, as a specific circuit that is distinct from CSN. Using DREADDs-mediated neuronal silencing, we established that Npy+ CBN modulate skilled forelimb movement. Building from these results, we are now investigating CBN axonal plasticity and role(s) in functional recovery in two distinct models of central nervous system injury: 1) a unilateral photothrombotic stroke that directly disrupts CBN projections; and 2) a cervical spinal cord injury (SCI), to test whether CBN axons show structural plasticity, even when their axons are not directly damaged by injury. CBN axonal collateralization across these different injury paradigms was analyzed using STARQ, our new machine learning-based segmentation and quantification pipeline that enables us to quantify axon collateralization across the rostrocaudal extent of the brainstem (midbrain, pons and medulla). Dependency on CBN circuits for recovery of skilled behavior was analyzed by comparing the intensity of CBN silencing-induced deficits pre- and post-injury. Through this research, we aim to elucidate the specific role of CBN circuits in adult motor control, and provide new insights into circuit mechanisms underlying motor recovery after distinct forms of central nervous system injury.

Disclosures: J. Kaiser: None. S. Fedde: None. P. Patel: None. B. Caglayan: None. H. Rodriguez: None. V.V. Sahni: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.13/Web Only

Topic: A.07. Development of Neural Systems

Support: NIH Grant 1R01HD093624-01A1
CTSA award No UM1TR004360
APTA Pediatric Physical Therapy

Title: The General Movement Optimality Score-Revised at term age in extremely born preterm infants is positively associated with motor outcomes and the Movement Optimality Score-Revised at three months of age

Authors: *S. BROWN¹, C. SPENCE², V. W. CHU³, L. THACKER⁴, A. SPITTLE⁵, S. DUSING⁶;

¹Physical Therapy, ²Dept. of Educ., ³Occup. Therapy, ⁴Dept. of Biostatistics, Virginia Commonwealth Univ., Richmond, VA; ⁵Univ. of Melbourne, Melbourne, Australia;

⁶Biokinesiology and Physical Therapy, Univ. of Southern Carolina, La Canada, CA

Abstract: Background. Infants born preterm are at risk of future cognitive and motor impairments. Currently, identifying impairments early is important for referral for early intervention. Often, early intervention community programs wait until there is an observable neurodevelopmental impairment before initiating intervention. The assessment of the detailed qualities of the General Movements, which represent an infant's developing neural integrity, may offer insight into earlier detection of future neurodevelopmental impairment.

Methods. Data were used from 83 infants participating in the Supporting Play Exploration and Early Development Intervention clinical trial. Details of the qualities of their General Movements were assessed using the optimality scoring at term age, the General Movement Optimality Score-Revised, and the optimality scoring at three months of age, the Movement Optimality Score-Revised. Scores for postural control and gross motor skills, as measured by the Test of Infant Motor Performance, were used in these analyses. Pearson's correlations and simple linear models were used to analyze the relationship between neural integrity and postural control between term and three months of age.

Results. Infant neural integrity at term age was positively associated with postural control and gross motor skills at three months of age (multiple $R^2 = 0.08$, estimated slope = 0.94, t value = 2.10, $p = 0.04$) but was not associated at term age (r-value is 0.13, CI = -0.09 - 0.35, t statistic = 1.173; df = 76; $p = 0.24$). Neural integrity at three months of age was positively associated with postural control and gross motor skills at three months of age (r-value was 0.29, 95% CI = 0.004 - 0.53, t statistic = 2.04; df = 46, $p = 0.04$). Neural integrity at term age was positively associated with neural integrity at three months of age (multiple $R^2 = 0.18$, estimated slope = 0.62, t value = 3.23, $p = 0.002$).

Conclusion. The positive associations of the General Movement optimality score at term age makes this emerging measure a potential biomarker of future neural integrity and postural control and gross motor skills at three months of age. Rather than waiting for an observable

impairment at an older age to be detected at a pediatrician's visit, the optimality score of the General Movements at term age could be used in neonatal intensive care settings to direct initiation of community therapy services after discharge from the hospital.

Disclosures: S. Brown: None. C. Spence: None. V.W. Chu: None. L. Thacker: None. A. Spittle: None. S. Dusing: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.14/A41

Topic: A.07. Development of Neural Systems

Support: Psychology Program

Title: Behavioral responses and morphological changes in zebrafish larvae exposed to microcystin-Lr at different temperatures

Authors: M. GJONBALAJ¹, *F. M. SCALZO²;
¹Psychology, ²Bard Col., Annandale-on-Hudson, NY

Abstract: Algal blooms pose significant health risks to humans and a variety aquatic species. Such freshwater blooms are exacerbated by rising water temperatures. Microcystin-LR (MCLR) is a freshwater toxin produced by algae and has been show to adversely affect growth and functional development in zebrafish larvae. The present exploration study investigated the interaction of MCLR toxicity with water temperature to determine if variations in water temperature moderated the adverse effects of MCLR on motor behavior and structural development. Larvae were exposed to 0, 50 or 100 micrograms/L at 28 degrees C or 30 degrees C from 24-96 hrs post-fertilization. Distanced moved, velocity and other movement parameters were measured using Ethovision in a series of 5 min light/dark periods of illumination. In addition, structural analyses were conducted to determine if potential alterations in motor behavior were related to gross structural malformations. The results have implications for understanding how environmental factors can modulate the neurotoxicity of naturally occurring toxins on motor systems and structural development.

Disclosures: M. Gjonbalaj: None. F.M. Scalzo: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.15/A42

Topic: A.07. Development of Neural Systems

Title: Cellular, Behavioral, and Genetic Characterization of Cerebellar Ataxia in Spastic Han-Wistar Rats

Authors: *H. BELLAFAARD¹, T. R. PELAYO², L. ZUCCHERATO³, C. G. AMORIM³, M. E. DE BELLARD⁴;

¹California State Univ. Northridge, Tustin, CA; ²Biol., ³California State University, Northridge, Northridge, CA; ⁴biology, Cal State Univ. Northridge, Northridge, CA

Abstract: Hereditary ataxia is a distinctive neurodegenerative disorder notable for its early onset, with cases affecting individuals as young as two years old. Characterized by progressive impairment of coordination and movement control, ataxia presents considerable clinical challenges. Key symptoms include gait instability, disrupted eye and speech coordination, and hindered hand movements, often associated with cerebellar atrophy. The primary pathology involves the degeneration of Purkinje cells, specialized neurons in the cerebellum, where minor alterations result in ataxia symptoms. However, the timeline and mechanisms of Purkinje cell loss in the spastic Han-Wistar rat model carrying a homozygous recessive mutation have not been fully characterized. To investigate this mutation, we employed immunohistochemistry, hematoxylin and eosin histology, cell morphometry, neuronal density quantification, whole genome and RNA sequencing to determine when Purkinje neuron loss occurs and whether degeneration begins with morphological changes in cell body size and dendritic architecture. Our histological analysis revealed a significant reduction in Purkinje cell count, indicating signs of neurodegeneration in the mutant model. Additionally, whole-genome sequencing identified potential genetic targets responsible for the observed ataxic phenotype. To validate these findings, RNA sequencing was performed to compare gene expression profiles between mutant and wildtype rats, confirming altered expression of candidate genes. The combination of morphological and genetic evidence reveals key disease mechanisms in this model. This research will contribute to a deeper understanding of ataxia genomics and its pathological progression.

Disclosures: H. Bellafard: None. T.R. Pelayo: None. L. Zuccherato: None. C. G. Amorim: None. M.E. De Bellard: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.16/A43

Topic: F.04. Voluntary Movements

Support: 25-BR-02-04
25-BR-02-06
25-BR-06-01

Title: Skeletal muscle-derived factor X enhanced motor activity in prolonged exercise-induced fatigue mice model

Authors: *S. LEE¹, H.-U. JUNG²;

¹Korea Brain Res. Inst., Daegu, Korea, Republic of; ²Korea brain research institute, Daegu, Korea, Republic of

Abstract: Prolonged and excessive exercise induces not only peripheral musculoskeletal fatigue but also central fatigue, which is closely associated with increased serotonin levels in the brain. However, the precise mechanisms underlying central fatigue remain unclear. Using a reliable secretomics approach, we identified factor X from the skeletal muscle secretome and found that its expression decreases in an age-dependent manner. We hypothesized that skeletal muscle-derived factor X is associated with central fatigue and investigated whether it could regulate this condition. Ten-week-old and thirteen-month-old male mice were administered factor X via intracerebroventricular (ICV) injection, followed by treadmill running to induce central fatigue. In behavioral tests, the running distance was significantly increased in the factor X-treated group compared to the control group, indicating that factor X enhanced physical endurance. Injection of factor X induced an increase in the level of its receptor in the cortex; a similar pattern was observed following prolonged exercise. Furthermore, immunohistochemistry revealed that in the cortex of mice subjected to prolonged exercise following administration of factor X, the receptor distribution became more localized to the cell membrane. Additionally, TPH-positive serotonergic neurons were found to be reduced by factor X. These results suggest that activation of the receptor may regulate central fatigue through modulation of TPH2-positive neurons. Finally, global proteomics analysis discovered the molecular signatures involved in factor X mediated molecular neuronal events. We investigated the underlying molecular mechanisms through subsequent experiments. These findings suggest the molecular mechanism of central fatigue and propose the factor X-related pathway as the regulatory target of central fatigue.

Disclosures: S. Lee: None. H. Jung: None.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.17/A44

Topic: A.07. Development of Neural Systems

Support: WV CTSI FWA00005078
APTA Pediatric Research Grant

Title: Quantifying head and trunk muscle control during early prone play in preterm and full-term Infants

Authors: K. INAMDAR, *V. GRITSENKO;
West Virginia Univ., Morgantown, WV

Abstract: Early motor development depends on the central nervous system's ability to coordinate multiple muscles across joints to produce complex postural and movement patterns. In preterm infants, these neural mechanisms are often disrupted, leading to delayed sensorimotor development. Such disruptions can serve as early indicators of motor impairments, particularly in regions with elevated preterm birth rates, such as West Virginia. However, the neuromechanical interactions underlying these developmental delays remain poorly understood and difficult to quantify. This pilot study evaluates a novel method for estimating muscle torques in the neck, shoulders, and back during prone play using integrated motion capture, surface electromyography (sEMG), and neuromusculoskeletal dynamic (NMD) modeling. Performance in prone play has been linked to the achievement of later developmental milestones; therefore, we selected this posture to investigate early motor control. We recorded 3D kinematics and bilateral sEMG signals from neck, back, and upper arm muscles in infants aged 2-6 months during prone play using wearable wireless sensors (PhaseSpace and Delsys). Subject-specific inverse dynamics models were applied to estimate muscle torques responsible for supporting the head and upper torso against gravity. Preliminary results reveal consistent coactivation patterns across bilateral agonist muscles and between synergistic neck and back muscles, suggesting that even early in development, infants may rely on coordinated muscle synergies to stabilize posture during prone play. These patterns varied across individuals and may reflect the influence of intersegmental dynamics that couple muscle torques through the body's kinematic chain. Distinct patterns of muscle recruitment were observed in infants of different ages and in preterm versus full-term infants, indicating that this method may be sensitive to developmental stage and neuromotor integrity. Such differences could serve as early biomarkers for differentiating typical from atypical motor control. This study provides the first kinetic analysis of early postural control and intersegmental coordination during spontaneous movements in infants. The approach offers a quantitative framework for assessing neural control in early development and may support scalable screening tools for early diagnosis of cerebral palsy and related neurodevelopmental disorders.

Disclosures: **K. Inamdar:** None. **V. Gritsenko:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurowired LLC.

Poster

PSTR314: Development of Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR314.18/A45

Topic: A.07. Development of Neural Systems

Title: Effects of dihydrotestosterone on dutasteride induced shape-change in the pond snail *Helisoma duryi*

Authors: ***D. LANGEVIN**¹, **M. LOPEZ**¹, **S. RAMAKRISHNAN**²;

²Neurosci. Program, ¹Univ. of Puget Sound, Tacoma, WA

Abstract: Despite the presence of steroidal androgen genes in gastropod mollusks, their direct effects remain unclear. Dutasteride (DUT), a dual inhibitor of 5 α -reductase types I and II, blocks the conversion of testosterone to dihydrotestosterone (DHT), and is used therapeutically to mitigate conditions linked to high testosterone levels in humans. Prior observations in *Biomphalaria* found that developmental DUT exposure altered the shape of the snail shell from helical to an uncoiled “banana” shape. Our lab previously replicated this phenotype in the closely related gastropod *Helisoma duryi* and identified a critical exposure window of 48-72 hours post-deposition. We also found DUT induced differential expression of serotonergic neurons, with DUT treatment increasing overall serotonin expression (t-test, $p < 0.001$), and increasing expression in the head/foot area relative to the mantle and shell (2-way ANOVA, $p < 0.05$). Our most recent work investigates whether treatment with 100 $\mu\text{g/L}$ DHT can rescue morphological and neurological phenotypes after treatment with 100 $\mu\text{g/L}$ DUT. We identified significant differences in rates of shell morphology across our three treatment groups (Kruskal-Wallis test, chi-squared = 10.148, $p < 0.05$), as well as specifically between DHT and combination treatment specimen and DUT and DHT specimen (multiple-comparison Dunn Test, $p < 0.05$). We observed that treatment with DHT alone induces no morphological change (36%, $n = 42$), while treatment with both DUT and DHT leads either to the banana shaped shell (11%) or arrested development (25%, $n = 55$). Interestingly, immunohistochemical analysis indicated that combination treatment decreased the number of cells expressing serotonin in all parts of the embryo relative to both DUT and DHT treatments (2-way ANOVA, $p < 0.001$, $n = 54$). It remains uncertain whether DUT is acting on the 5 α -reductase pathway in these organisms, with future work optimizing DHT dosage to determine if combination treatment can rescue either phenotype. We suggest a putative neural mechanism for DUT action on shape changes in the snail embryo - with modified catecholaminergic innervation leading to altered movement, resulting in the morphological change.

Disclosures: D. Langevin: None. M. Lopez: None. S. Ramakrishnan: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.01/A46

Topic: A.10. Schizophrenia

Support: R01DA057268

Title: Altered Resting-State Network Interactions and Psychotic-Like Experiences during Adolescence

Authors: A. TREPICCIONE, P. TUBIOLO, Y. PATEL, S. MOELLER, K. JONAS, J. X. VAN SNELLENBERG, *G. PERLMAN;
Psychiatry and Behavioral Hlth., Stony Brook Univ. Sch. of Med., Stony Brook, NY

Abstract: Functional connectivity patterns associated with psychotic-like experiences (PLEs) during adolescence are poorly understood, hindering efforts to develop effective interventions for high-risk youth. In adults, the leading circuitry-based model for hallucinations implicates sensory processing dysfunction related to aberrant input from the default mode network (DMN) and salience network (SN). However, this model cannot be assumed to generalize to PLEs during adolescence. To address this gap in knowledge, this study investigates the association between resting-state network functional connectivity in the DMN, SN, and auditory network (AN) and severity of hallucinatory experiences in 169 community-dwelling 14-17 year-olds.

The baseline visit of the Pediatric Imaging: Measuring Neuromelanin Trajectories study at Stony Brook University included two 7.5 min resting-state fMRI acquisitions using 2 mm isotropic voxels and a multiband factor of 6. PLE severity was defined as the sum of the hallucination subscale from the Specific Psychotic Experiences Questionnaire (9-items; self-report). Following preprocessing, data were mode-1000 normalized and linearly detrended. High-motion volumes were identified using the Multiband Censoring Optimization Tool and censored prior to bandpass filtering (0.009-0.08 Hz). Average time series from the DMN, AN, and cingulo-opercular network (CON), reflecting key regions of the SN, were extracted using the Cole-Anticevic Brain Network Parcellation. Resting-state functional connectivity (RSFC) was calculated as the partial Pearson correlation between networks, controlling for 24 motion parameters, as well as global signal, white matter signal, cerebrospinal fluid signal, and their respective derivatives.

Associations between RSFC and PLE severity were assessed using linear regression models, controlling for age, biological sex, and stimulant medication use.

We found that DMN-AN and DMN-CON hyperconnectivity were independently associated with PLE severity ($\beta^* = 0.16$, $p = 0.04$; and $\beta^* = 0.21$, $p = 0.007$, respectively). Additionally, a mediation analysis was conducted to determine whether DMN-CON RSFC mediates the relationship between DMN-AN RSFC and PLE severity. We found that DMN-CON RSFC fully mediated the pathway from DMN-AN to PLE severity ($\beta^* = 0.06$, $p = 0.012$).

Overall, these findings align with previous research in clinical populations, corroborating the prevalent theory that hallucinations may arise from abnormal salience attribution to internally generated stimuli. Further, this data supports the notion of a connectivity-based continuum across the psychosis spectrum.

Disclosures: A. Trepiccione: None. P. Tubiolo: None. Y. Patel: None. S. Moeller: None. K. Jonas: None. J.X. Van Snellenberg: None. G. Perlman: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.02/A47

Topic: A.10. Schizophrenia

Support: NIH IRP ZIA MH002984

Title: A role for dopamine in sculpting the development of thalamic inputs to prefrontal cortex parvalbumin interneurons

Authors: *A. Y. FAN¹, J. KLEVE², A. ROMANOWSKI², J. T. SCOTT⁴, G. J. FISHELL⁵, Y. WANG⁶, J. A. BOURNE³;

¹NIMH, Bethesda, MD; ³Section on Cell. and Cognitive Neurodevelopment, ²Natl. Inst. of Mental Hlth., Bethesda, MD; ⁴NIH/NIMH, Bethesda, DC; ⁵Harvard Med. Sch., Jamaica Plain, MA; ⁶Harvard Med. School, Blavatnik Institute,, Boston, MA

Abstract: Aberrant thalamocortical connectivity is increasingly recognized as a core feature of neurodevelopmental disorders, particularly involving the mediodorsal (MD) and medial pulvinar (PM) thalamic nuclei. These abnormalities often coincide with disrupted dopamine signaling and altered excitatory-inhibitory balance in the prefrontal cortex (PFC). While MD projections to the PFC are well-characterized, the functional role of PM remains less defined, despite its widespread early-life cortical connectivity, as revealed by developmental anterograde tracing in marmosets (PD30, n=2; adult >1.5 years, n=2). To probe thalamic input to cortical parvalbumin interneurons (PV-INs), we employed PV-specific S5E2 enhancer-driven monosynaptic rabies tracing across development (early adolescence: PM2, n=2; adult >1.5 years, n=3). We found that PM neurons robustly innervate PV-INs during early life, but this input diminishes with age. In contrast, MD to PV-IN connectivity remains stable. Using a hybridization chain reaction (HCR) approach, we detected dopamine receptor D2 (DRD2) expression in PM neurons projecting to the prefrontal cortex (PFC). Transcriptomic analyses of DRD1 and DRD2 expression (GWAS-linked dopamine genes) across four developmental stages (infancy PD0-3, juvenile PM3-5, adolescence PM8-10, adulthood >1.5 years; n=8, both sexes) revealed a transient postnatal peak in DRD2 specifically in cortical-projecting PM neurons. Tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, exhibited a similar early-life peak in PM but not in MD. Together, these findings highlight a developmentally restricted, dopaminergic-modulated PM to PFC circuit, suggesting that the PM plays a unique and time-sensitive role in assembling prefrontal networks. This transient window may represent a critical period of vulnerability relevant to the pathophysiology of neurodevelopmental disorders.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.03/A48

Topic: A.10. Schizophrenia

Support: Stanley Foundation

Title: Abnormal locomotion and striatal dopamine dynamics in the *Grin2a* mouse model of Schizophrenia

Authors: *A. HALL¹, J. JACOBY³, E. ROCHMAN², H. GANGAL¹, S. R. DATTA⁴, M. H. SHENG¹, P. S. KUNWAR¹;

¹Stanley Ctr. for Psychiatric Res., ²Broad Inst. of MIT and Harvard, Cambridge, MA; ³Harvard Med. Sch., Cambridge, MA; ⁴Harvard Med. Sch., Boston, MA.

Abstract: The dopamine hypothesis of schizophrenia (SCZ) centers on a striatal hyperdopaminergic state, yet its *in vivo* investigation has been hindered by limitations in animal models. Recent advances, driven by SCHEMA-identified SCZ risk genes like *Grin2a*, enabled the development of novel mouse models. Transcriptomic analysis in *Grin2a* knockout mice (partial, +/-; full, -/-) revealed disrupted gene sets in striatal D1 and D2 receptor-expressing cells (Farsi et al, 2023). Our behavioral screening showed *Grin2a* -/- animals exhibit hyperlocomotion, increased novel object interaction, and enhanced acoustic startle. Further analysis, using DeepLabCut and Keypoint-MoSeq, revealed abnormal behavior syllable usage in both *Grin2a* +/- and -/- mice. To investigate dopamine dynamics, we performed dopamine fiber photometry (GRAB-DA2h) in the dorsomedial striatum. *Grin2a* -/- animals showed consistent hyperlocomotion and, notably, an altered dopamine peak frequency in the open field test, along with aberrant dopaminergic responses to light presentations and novel object approaches, suggesting impaired salience processing. Our data collectively indicates a dysregulated dopamine system in the *Grin2a* model. We are now correlating dopamine signals with specific behavioral syllables via Keypoint-MoSeq and investigating cell-type specific roles of D1 and D2-possessing striatal projection neurons (SPNs) in controlling movement in *Grin2a* animals using miniscoping.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.04/A49

Topic: A.10. Schizophrenia

Title: Adolescent-onset network hyperactivity in a mouse model of psychosis: insights from hippocampal *glun1* deficiency

Authors: *M. SUBRAMANIAN¹, D. SCOTT^{5,2}, C. A. TAMMINGA^{1,2}, J. YAMAMOTO^{3,2,4},
²Peter O'Donnell Jr. Brain Inst., ³Psychiatry, ⁴Dept. of Neurosci., ¹UT Southwestern Med. Ctr., Dallas, TX; ⁵Psychiatry, UT Southwestern, Dallas, TX

Abstract: Hippocampal hyperactivity has been reported in multiple neurological conditions such as psychosis, epilepsy, and Alzheimer's disease. Post-mortem analysis of human brain tissue from individuals with psychotic schizophrenia (ScZ) has revealed reduced expression of the NMDAR subunit GluN1 specifically in the dentate gyrus (DG), a key subregion of the hippocampus (HPC). In mouse models with DG-specific GluN1 knockout, this reduction leads to downstream hyperactivity in hippocampal subfields, CA3 and CA1 as shown in molecular studies. We previously demonstrated that hyperactivity presents as hyper-synchronous events following short-term chemogenetic inhibition of the dentate gyrus (DG) during adolescence. Research studies also show that hyperactivity in the hippocampus extends to other connected regions, such as the Entorhinal Cortex (EC), the Orbitofrontal Cortex (OFC), and the Nucleus Accumbens (NAc). To characterize circuit-level changes associated with DG-specific GluN1 deletion, we performed in vivo electrophysiological recordings in freely behaving mice using two high-density Neuropixels 2.0 probes. One probe was implanted to span the HPC and EC, while the second targeted the OFC and NAc. Each probe simultaneously recorded from 384 channels, capturing activity across all targeted brain regions. Our results show that large amplitude spiking activity called Hyper-Synchronous Events (HSEs) developed first in the Hippocampus during adolescence (6 - 9 weeks of age) in DG GluN1 KO mice. Neuropixels recordings revealed a significant increase in single-cell recruitment coinciding with HSE onset, followed by a transient suppression or refractory period across recorded units. Simultaneous recordings from other regions showed that HSEs propagated to the OFC and NAc by approximately 10 weeks of age (young adulthood), though with reduced amplitude and frequency. Over time, HSEs increased in occurrence and evolve into sustained bursting activity. Spatiotemporal analysis confirms that HSEs originate within the hippocampus and subsequently propagate to downstream regions. HSEs were detected not only in the hippocampus but also in the entorhinal cortex, orbitofrontal cortex, and Nucleus Accumbens of DG-GluN1 KO mice, with distinct regional differences in amplitude and timing. These findings suggest that DG inhibition in ScZ highlights a developmental window during adolescence where the hippocampus is particularly susceptible. Our results underscore the importance of early intervention strategies targeting hippocampal circuitry in the treatment of psychosis.

Disclosures: M. Subramanian: None. D. Scott: None. C.A. Tamminga: None. J. Yamamoto: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

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Program #/Poster #: PSTR315.05/A50

Topic: A.10. Schizophrenia

Support: NIH ROIMH135792

Title: Reduced RNA Editing of the GluA2 AMPA Receptor Subunit in the Prefrontal Cortex in Schizophrenia.

Authors: *M. MCCARTHY¹, G. C. BRISTOW², T. BUCK³, M. S. SODHI³;
¹Pharmacol. and Neurosci., Loyola, Maywood, IL; ²Univ. of Bradford, Bradford, United Kingdom; ³Mol. Pharmacol. and Neurosci., Loyola Univ. Chicago, Maywood, IL

Abstract: Abnormalities in synaptic transmission and glutamatergic function are thought to contribute to progressive cognitive decline in individuals with schizophrenia. The proper functioning of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors is crucial for synaptic maturation, learning, and memory. AMPAR subunits undergo post-transcriptional modifications, including alternative splicing and RNA editing, which can have significant functional implications. A key modification is reduced RNA editing of the GluA2 AMPAR subunit, which leads to increased synaptic expression of gated AMPARs and altered kinetics of the AMPAR ion channel. Previous studies have reported reduced RNA editing of AMPAR subunits in schizophrenia, but there have been conflicting data. In this study, we tested the hypothesis that RNA editing of the GluA2 subunit is disrupted in schizophrenia. RNA was extracted from the dorsolateral prefrontal cortex (DLPFC) of post-mortem subjects with schizophrenia (n=17) and a comparison group of neurologically healthy individuals (n=46). We performed multiple transcriptional analyses of these subjects: GluA2 RNA editing, transcription of RNA editing enzymes ADARs1-3, and transcription of AMPAR subunits (GluA1-4). Our results show that individuals with schizophrenia had lower GluA2 editing (71%) compared to the neurologically healthy group (74%, $p = 0.05$). Using receiver operating characteristic (ROC) analysis, we found that combining GluA2 editing with ADAR1 enzyme transcription had an area under the curve (AUC) of 0.76 (0.64-0.88), suggesting a 76% accuracy in predicting schizophrenia. Moreover, the correlation between ADAR enzyme transcription and GluA2 R/G editing was significantly stronger in schizophrenia patients than in controls, with ADAR1 showing the largest difference. Therefore, we add to the accumulating evidence indicating the importance of GluA2 RNA editing in schizophrenia and demonstrate that GluA2 RNA editing may be useful as a biomarker.

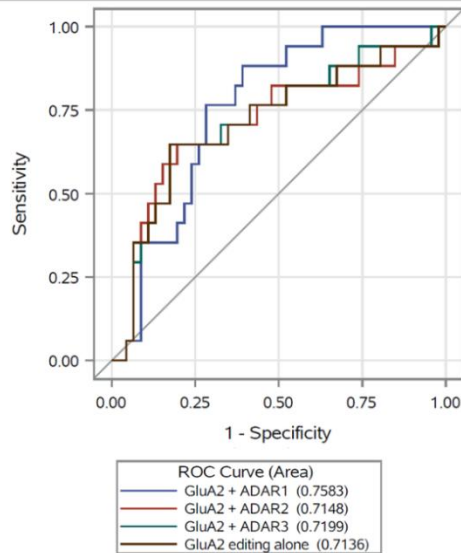
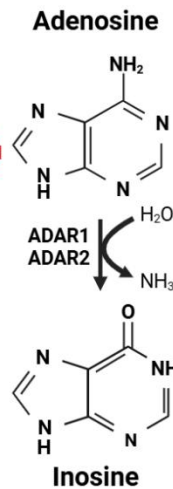
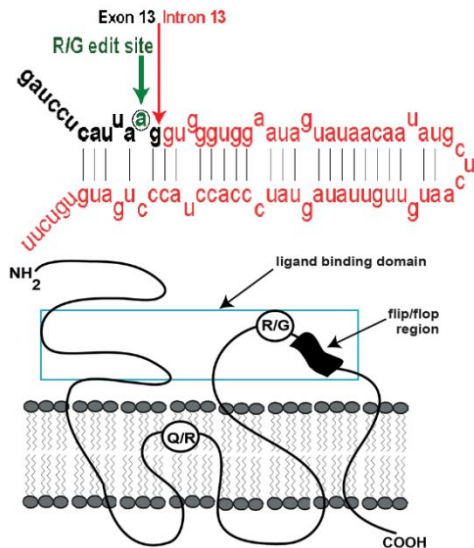
Postmortem DLPFC Samples



**Neuropsychiatric
Controls**
n = 46



**Schizophrenic
Patients**
n = 17



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Poster

PSTR315: Schizophrenia

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Program #/Poster #: PSTR315.06/A51

Topic: A.10. Schizophrenia

Support: NIH R21MH087829 to U. R.
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The Brain Foundation grant to M. K.

Title: Reversal of phenotypes in mouse models of neurodevelopmental disorders by targeting mitochondrial function with metformin

Authors: *M. KAMBALI¹, M. WANG^{1,2}, R. NAGARAJAN¹, J. LYU^{1,2}, U. RUDOLPH^{1,2,3};
¹Comparative Biosciences, Col. of Vet. Medicine, Univ. of Illinois, Urbana-Champaign, Urbana, IL; ²Univ. of Illinois, Urbana-Champaign, Neurosci. Grad. Program, Urbana, IL; ³Carl R. Woese Inst. for Genomic Biol., Urbana, IL

Abstract: Metformin is widely used as an antidiabetic drug. A retrospective analysis has revealed that patients with psychotic disorders who have been prescribed metformin have fewer admissions to psychiatric hospitals and are less prone to self-harm. Metformin has been described as a mild inhibitor of mitochondrial complex I, and mild inhibition of mitochondrial complex I may result in increased mitochondrial biogenesis, mediated in part by PGC1 α . We previously described biochemical and behavioral deficits in Gluc triplcation mice, a mouse model for schizophrenia with glutamatergic hypofunction in the dentate gyrus. Remarkably, these mice also showed deficits in mitochondrial respiration in the Seahorse assay. We investigated whether metformin is able to reverse behavioral and biochemical deficits in Gluc triplcation mice. Initial dose-finding studies revealed that metformin had dose-dependent effects in a Y maze alternation test, with 300 mg/kg being more effective than lower doses, so this dose was used in subsequent studies. A single dose had no effect on working memory deficits, but a 30-day chronic treatment in the drinking water reversed working memory deficits in the Y maze as well as startle habituation deficits, while restoring social novelty preference deficits but not sociability deficits in a three-chamber social interaction test. At the molecular level, the expression of the master regulator of mitochondrial biogenesis, PGC1 α , and of brain-derived neurotrophic factor (BDNF), which were reduced in the Gluc triplcation mice, were restored in the hippocampus. In addition, chronic metformin treatment restored the reduced expression of mitochondrial genes (mtCo-I and mt-Atp6 genes) involved in ATP production in these mice. We plan to extend these studies to other genetic mouse models of neurodevelopmental disorders to evaluate whether the potentially beneficial effects of metformin occur across a range of genetic models and may be more generalizable. Our studies are starting to provide insights into mitochondrial deficits in mouse models for neurodevelopmental disorders and their reversal by targeting mitochondrial biogenesis and function. They support a therapeutic potential of metformin.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.07/A52

Topic: A.10. Schizophrenia

Title: Comparative activity of muscarinic agonists ML-007 and xanomeline in preclinical models of psychosis and cognitive impairment

Authors: S. CHATTERJEE, M. SORIA, N. NAVARRO, *K. R. THOMPSON, J. LILLIE, A. C. KREITZER, M. W. WOOD;
MapLight Therapeut., Redwood City, CA

Abstract: Muscarinic activators represent a new class of treatments for psychosis with a distinctly different mechanism of action than typical and atypical antipsychotics. Based on the pattern of expression in human brain circuitry, M₁ and M₄ receptors have been considered relevant for the treatment of psychosis and cognitive deficits. Here we characterize a novel investigational M₁/M₄ agonist, ML-007, that is under development for schizophrenia and Alzheimer's disease psychosis. We present a comparative study with xanomeline, the first-in-class muscarinic to receive approval for schizophrenia. GTPγS assays demonstrate that ML-007 has stronger intrinsic agonist activity at both M₁ and M₄ receptors compared to xanomeline. Both xanomeline and ML-007 show robust antipsychotic activity across a range of preclinical models for schizophrenia, including amphetamine-induced hyperlocomotion, PCP-induced hyperlocomotion, and conditioned avoidance response. However, dose-response experiments in M₁ and M₄ knockout mice reveal that at low doses, the activity of xanomeline is more sensitive to loss of M₄ receptors, whereas ML-007 is more sensitive to loss of M₁ receptors. Importantly, in a transgenic mouse model of Alzheimer's disease, we find that ML-007, but not xanomeline, improves hippocampal-dependent spatial memory task performance. Taken together, our data suggest that both M₁ and M₄ receptors contribute to antipsychotic activity in preclinical rodent models of psychosis, whereas strong agonism at M₁ receptors may be required for treating cognitive impairment.

Disclosures: **S. Chatterjee:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. **M. soria:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. **N. Navarro:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. **K.R. Thompson:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. **J. Lillie:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. **A.C. Kreitzer:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. **M.W. Wood:** A. Employment/Salary (full or part-time): MapLight Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.08/A53

Topic: A.10. Schizophrenia

Title: Efficacy of SUVN-2206043 a Dual Acting 5-HT_{1A} Agonist and 5-HT_{2A} Antagonist Exhibiting Antipsychotic and Antidepressant Like Effect in Various Animal Models

Authors: *V. CHINNEDDULA, S. PETLU, R. SUBRAMANIAN, R. MEDAPATI, P. JAYARAJAN, V. BENADE, R. BADANGE, K. BOJJA, A. MOHAMMED, A. K. SHINDE, R. NIROGI;
SUVEN LIFE SCIENCES, Hyderabad, India

Abstract: Depression and psychosis are complex neuropsychiatric disorders characterized by dysregulation of serotonergic neurotransmission, the treatment of which is accompanied by side effects. The serotonergic 1A (5-HT_{1A}) receptors are a subtype of serotonin receptor that is predominantly involved in the regulation of mood, anxiety, and stress responses. The serotonergic 2A (5-HT_{2A}) receptors are another subtype of serotonin receptors, involved in regulating cognition, mood, and sensory perception, and are notably involved in the pathophysiology of both depression and psychosis. SUVN-2206043 is a ligand that acts on both the 5-HT_{1A} & 5-HT_{2A} receptors. The in-vitro binding and functional activities of SUVN-2206043 were evaluated using both radioligand binding assay and GTPγS cell-based functional assays. The pharmacokinetics of SUVN-2206043 was assessed in Wistar rats and Beagle dogs. Receptor occupancy of SUVN-2206043 at 5-HT_{1A}, 5-HT_{2A} and dopaminergic D₂ receptors was evaluated in Wistar rats using non-radiolabeled tracers. The efficacy of SUVN-2206043 to reverse amphetamine and MK-801 induced increase in locomotor activity in Wistar rats was assessed in an open field test. SUVN-2206043 was assessed in an animal model of visual hallucination using 2,5-Dimethoxy-4-iodoamphetamine. Here, the numbers of head twitches in this assay were recorded. Anti-depressant like activity of SUVN-2206043 was assessed by using mice forced swim test. SUVN-2206043 was assessed for its potential to induce motor impediment in Wistar rats using the catalepsy test. The latency to correct the posture was recorded using a stopwatch. SUVN-2206043 was found to have an affinity towards serotonin 5HT_{1A} and 5-HT_{2A} receptors. Functional studies conclude that SUVN-2206043 is an agonist at 5-HT_{1A} and an antagonist at 5-HT_{2A} receptors. SUVN-2206043 was found to have favourable pharmacokinetic parameters with an oral bioavailability of 43 ± 18 % in rats and 64 ± 13 % in dogs. SUVN-2206043 antagonized the amphetamine as well as MK-801 induced hyper-locomotion in open field assay. SUVN-2206043 did not induce catalepsy at the tested doses. SUVN-2206043 at the tested doses decreased the duration of immobility in mice forced swim test. In conclusion, SUVN-2206043 is a dual serotonin 5-HT_{2A} receptor antagonist and a serotonin 5-HT_{1A} receptor agonist exhibiting antipsychotic and antidepressant-like effects while targeting the serotonergic system. SUVN-2206043 was found to be devoid of motor impediments associated with common antipsychotics.

Disclosures: V. Chinneddula: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. S. Petlu: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. R. Subramanian: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. R. Medapati: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. P. Jayarajan: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. V. Benade: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. R. Badange: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. K. Bojja: A.

Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **A. Mohammed:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **A.K. Shinde:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **R. Nirogi:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.09/A54

Topic: A.10. Schizophrenia

Title: Modeling persistent cognitive deficit in a mouse model of schizophrenia: role of Phencyclidine (PCP) dosing frequency and reversal by clinically relevant compounds

Authors: *E. ANDRIAMBELOSON, C. DUCHEMIN, L. PETER, C. PINAULT, F. LAUGA, A. KIENTZI, E. POIRAUD, B. HUYARD, S. WAGNER; NEUROFIT, ILLKIRCH, France

Abstract: Schizophrenia is a complex psychiatric disorder with positive, negative, and cognitive symptoms. Cognitive impairments are a core feature, appearing early and strongly linked to long-term functional outcomes. Unlike positive symptoms, these deficits are largely unresponsive to current treatments, representing a major unmet need. This study aimed to develop a mouse model of schizophrenia focused on cognitive impairment using repeated administration of phencyclidine (PCP), an NMDA receptor antagonist, over ~10 days. Dosing protocols varied in PCP dose (0.2-10 mg/kg) and injection frequency (once or twice daily). Cognitive function was assessed post-withdrawal using the T-maze spontaneous alternation task, which evaluates spatial working memory, attention, and executive function. A single dose of 1 mg/kg PCP did not induce impairment at 30 min post-dose (acute effect), while 3 mg/kg induced deficits. Once-daily dosing (1 or 3 mg/kg/d for 10 days) caused significant acute effect that did not persist at 1 or 3 days post-withdrawal, even at 10 mg/kg/d (cumulative dose of 100 mg/kg over 10 days). This suggests that while acute cognitive impairment requires a minimum PCP dose, even high repeated dosing fails to produce persistent effects, indicating that dose alone is insufficient for lasting deficits. In contrast, twice-daily dosing of 0.2 mg/kg over 8 or 12 days (3.2 or 4.8 mg/kg cumulative) produced lasting deficits up to 3 days post-withdrawal. Increasing the dose tenfold (2 mg/kg b.i.d. for 12 days) did not exacerbate the deficit. These results indicate that administration frequency, not cumulative dose, is critical for inducing persistent cognitive deficits. Given PCP's short half-life in rodents (~2-3 h), it is unlikely that the drug remains active at 1-3 days post-treatment, suggesting observed persistent effects are due to lasting neurobiological changes. Acute administration of vortioxetine or modafinil, used in the clinic as adjunct therapy in schizophrenia, reversed these persistent deficits in a dose-dependent manner without affecting PCP-induced hyperlocomotion or behavioral despair, implying a selective enhancement of cognitive performance without influencing non-cognitive symptoms. The

persistence of deficits beyond the drug's clearance window, combined with their responsiveness to clinically used cognitive adjuncts, reinforces the model's translational relevance and utility for investigating long-lasting impairments and screening targeted therapies to improve functional outcomes in schizophrenic patients.

Disclosures: E. Andriambeloson: None. C. Duchemin: None. L. Peter: None. C. Pinault: None. F. Lauga: None. A. Kientzi: None. E. Poiraud: None. B. Huyard: None. S. Wagner: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.10/A55

Topic: A.10. Schizophrenia

Title: Acceleration of 40 Hz Gamma oscillatory phase resetting in the prefrontal cortex by Clozapine and Risperidone: role for 5HT_{2A}?

Authors: *D. GAUTAM¹, S. KASSA², C. JONES², A. HENSLEY², C. CHAPMAN², M. PHILLIPS², H. RUTHERFORD², O. EGUA VOEN², S. V. DIGAVALLI³;

¹East Tennessee State Univ., Johnson City, TN; ²East Tennessee State Univ., Johnson City, TN;

³Dept. of Pharmaceut. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: Gamma oscillations (~ 40 Hz) are essential for sensory perception, attention, and working memory. Disruptions in gamma synchrony are well-documented in schizophrenia and other psychiatric disorders. The 40 Hz auditory steady-state response (ASSR) is an EEG measure of cortical entrainment to a 40 Hz click train. Given its sensitivity to key neurotransmitter systems as well as a modest correlation with cognitive function, the 40 Hz ASSR is increasingly employed as a biomarker in neuropsychiatric drug development for bench to bedside translation. Our previous work showed that clozapine, an atypical antipsychotic with broad receptor affinities (serotonergic, dopaminergic, muscarinic), but not haloperidol, a high affinity D₂ antagonist, robustly accelerated gamma oscillations through phase resetting in the prefrontal cortex (PFC). We have now investigated risperidone, another atypical antipsychotic with selective serotonergic and dopaminergic affinity, for its effect on the 40 Hz ASSR. Sprague-Dawley rats (M&F, N=15) were implanted with an epidural prefrontal electrode and referred to cerebellar electrodes. Post-recovery, they received vehicle, clozapine (5 mg/kg), or risperidone (0.5 or 1 mg/kg) in a crossover design anchored to clinically relevant drug exposure. EEG (Signal 8.02; CED1401 Micro 3) was recorded during 40 Hz click trains (~1 ms, ~65 dB SPL) played over house speakers. Each 3 s EEG sweep included a 1 s click train, with 75 trials/rat. Sixty minutes post-treatment, EEG entrainment at 40 Hz was observed in the PFC across all groups. Relative to vehicle, risperidone dose-dependently accelerated as well as enhanced gamma synchrony, with the higher dose producing effects comparable to clozapine. The robust increases in phase synchrony happened often with little change in evoked power, implicating

gamma phase resetting. Since haloperidol (high D2 affinity) does not enhance gamma synchrony and risperidone lacks significant muscarinic (M1/M4) activity, we speculate that serotonergic (5HT2A) inverse agonism could be mediating these effects. Future studies will further explore receptor mechanisms, improving our understanding of how atypical antipsychotics enhance gamma synchrony and cognitive performance in psychiatric disorders.

Disclosures: D. Gautam: None. S. Kassa: None. C. Jones: None. A. Hensley: None. C. Chapman: None. M. Phillips: None. H. Rutherford: None. O. Eguavoen: None. S.V. Digavalli: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.11/A56

Topic: A.10. Schizophrenia

Title: Suvn-l3307032, a muscarinic subtype receptor 4 (m4) positive allosteric modulator (pam) for the treatment of behavioral and cognitive deficits pertinent to schizophrenia and alzheimer's disease

Authors: *R. BADANGE, V. GOURA, R. KALLEPALLI, R. ABRAHAM, A. SHAIKH, K. BOJJA, K. BITRA, N. GUDURU, A. DAS, P. NISSANKARARAO MARY, K. MUKKOLLU, H. BOBBILI, S. PETLU, R. SUBRAMANIAN, K. JOSHI, S. CHORAMALE, R. NIROGI; Suven Life Sci. Ltd, Hyderabad, India

Abstract: The muscarinic receptor (M4) has gained interest as a target to treat the behavioral and cognitive disturbances associated with schizophrenia and Alzheimer's disease. Currently, research is mainly focused on developing M4 positive allosteric modulators (M4 PAMs) that target the allosteric site instead of the highly conserved orthosteric binding site to improve selectivity over other muscarinic subtypes and minimize cholinergic side effects. SUVN-L3307032 is an in-house developed new chemical entity. A cell-based reporter gene assay was used to identify the affinity towards the allosteric site of the M4 receptor. The pharmacokinetic properties of SUVN-L3307032 were assessed in both Wistar rats and Beagle dogs. The receptor occupancy of SUVN-L3307032 was evaluated in Wistar rats. The efficacy of SUVN-L3307032 to reverse amphetamine induced behavioral and cognitive deficits was investigated in open field task and contextual fear conditioning task using male Wistar rats. SUVN-L3307032 was assessed for pro-cognitive effects using social recognition task in rats as well. SUVN-L3307032 was further assessed for cholinergic side effects in rats. In addition, preliminary toxicity studies were performed in Wistar rats and Beagle dogs. SUVN-L3307032 demonstrated affinity towards muscarinic M4 receptor as a positive allosteric modulator. SUVN-L3307032 demonstrated favorable pharmacokinetic properties such as oral bioavailability in rats and dogs, and brain penetration in rats. SUVN-L3307032 showed dose-dependent receptor occupancy at 3, 10, 30 and 60 mg/kg in Wistar rats. SUVN-L3307032 attenuated amphetamine induced

hyperlocomotion. Amphetamine decreased freezing response in a fear conditioning task. This was reversed by SUVN-L3307032 at 30 and 60 mg/kg. SUVN-L3307032 treated rats spent significantly more time investigating novel juvenile rats compared to familiar juvenile rats in the social recognition task. SUVN-L3307032 was found to be devoid of cholinergic side effects in the salivation assay. Exploratory toxicity studies in Wistar rats and Beagle dogs did not show any concern for development. Overall, preclinical data of SUVN-L3307032 paves the way for new treatments for schizophrenia and Alzheimer's disease.

Disclosures: **R. Badange:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Goura:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Kallepalli:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Abraham:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **A. Shaikh:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **K. Bojja:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **K. Bitra:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **N. Guduru:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **A. Das:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **P. Nissankararao Mary:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **K. Mukkollu:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **H. Bobbili:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **S. Petlu:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Subramanian:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **K. Joshi:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **S. Choramale:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.12/Web Only

Topic: A.10. Schizophrenia

Title: Phenotyping the DISC1 rat model of Schizophrenia using PPI, amphetamine-induced hyperlocomotion, sleep-EEG and 40Hz ASSR

Authors: *J. R. HUXTER¹, **A. M. BERNARDO**², L. DLUGOSZ², S. THEVARKUNNEL², C. KORTH⁴, G. A. HIGGINS³;

¹Transpharmation Ltd. UK, London, United Kingdom; ³Neurosci., ²Transpharmation Ltd. Canada, Fergus, ON, Canada; ⁴Heinrich Heine Univ. Dusseldorf, Dusseldorf, Germany

Abstract: Schizophrenia is a devastating lifelong psychiatric condition for which there is currently no cure, and symptomatic treatments with varying degrees of efficacy and side-effects. Presence of the Disrupted in Schizophrenia 1 (DISC1) protein is a known risk factor for schizophrenia, and in the tgDISC1 rat, overexpression of the *DISC1* transgene produces neuronal

DISC1 aggregates and a number of phenotypes consistent with the human condition. Here we describe additional phenotyping of the model using behaviour and electrophysiology. Nine male tgDISC1 rats and seven male littermate controls were tested in pre-pulse inhibition (PPI), sleep-EEG, 40 Hz auditory steady-state response (ASSR), and amphetamine-induced hyperlocomotion (0.3mg/kg IP). An atypical antipsychotic (risperidone 0.3 mg/kg s.c.) was used as a reversal agent. In PPI, we observed an increased startle response ($F_{1,15}=17.58$; $p=0.0003$) and impaired PPI ($F_{1,15}=7.508$; $p=0.0232$) in the transgenics, which was reversed with risperidone ($F_{1,25}=21.87$; $p=0.0046$). In sleep-EEG the tgDISC1 rats had faster theta oscillations during wakefulness ($F_{4.45,62.33}=21.49$ $p<.001$) which was fully reversed by modafinil ($F_{2.63,21.06}=16.97$ $p<.001$). Sleep architecture, body temperature and spontaneous behaviour in the sleep-EEG assay did not differ from the littermate controls. We observed no genotype differences in ASSR, or in the locomotor response to amphetamine. The tgDISC1 rat has a relatively mild phenotype on many of the measures used here, consistent with previously observations. However, while an absence of amphetamine sensitivity was unexpected, the reversal by risperidone of both impaired PPI and increased theta-frequency indicates the potential for the tgDISC1 rat in drug discovery for schizophrenia.

Disclosures: J.R. Huxter: None. A.M. Bernardo: None. L. Dlugosz: None. S. Thevarkunnel: None. C. Korth: None. G.A. Higgins: None.

Poster

PSTR315: Schizophrenia

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Topic: A.10. Schizophrenia

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Dokkyo International Medical Education and Research Foundation
Astellas Foundation for Research on Metabolic Disorders

Title: Prefrontal parvalbumin interneuron activation improves working memory deficit in a mouse model of schizophrenia: Toward mechanistic insights

Authors: *Y. ARIME¹, Y. SAITOH¹, M. ISHIKAWA¹, C. KAMIYOSHIHARA², N. OHKAWA¹;

¹Lab. for Memory and Cognitive Function, Dokkyo Med. Univ., Tochigi, Japan; ²Lab. for Memory and Cognitive Function, Dokkyo Medical Univ., Tochigi, Japan

Abstract: Cognitive impairments in schizophrenia remain a major unmet medical need, and its underlying neural circuitry is still poorly understood. Notably, most preclinical models overlook the clinical situation that patients typically remain under antipsychotic medication. Recently, we developed a method for continuous antipsychotic drugs (haloperidol and olanzapine) that maintain dopamine D2 receptor occupancy levels (60-80%) in the striatum for several weeks in mice using osmotic minipump. We observed synaptic alterations in layer 2-3 of the prelimbic cortex (PL), including disrupted excitatory/inhibitory balance involving VGLUT1+ excitatory terminals and parvalbumin (PV) positive interneurons, in a chronic phencyclidine (PCP) mouse model of schizophrenia. Continuous olanzapine alone did not reverse these changes nor improve working memory performance. However, chemogenetic activation of PV positive interneurons in the PL significantly ameliorated working memory deficits. This ameliorative effect was also exerted under olanzapine treatment. We further demonstrated that this chemogenetic manipulation increased PV positive interneuron activity in the PL. These findings suggest that targeting prefrontal PV positive interneurons could offer a circuit-based strategy to restore cognitive impairments in schizophrenia, even in patients undergoing antipsychotic treatment.

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Poster

PSTR315: Schizophrenia

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Program #/Poster #: PSTR315.14/A58

Topic: A.10. Schizophrenia

Support: Italian Ministry of Health Grant RF-2019-12368646

Title: Kynurenine pathway metabolites are dysregulated in human brains in a region-specific fashion and exert antipsychotic effects in experimental models of schizophrenia

Authors: *R. GINERETE^{1,2}, A. CECCHERELLI¹, L. LIONETTO¹, G. D'ERRICO², M. ALBORGHETTI^{1,2}, D. DE BERNARDINI¹, G. COSTANZI¹, D. ERBUTO¹, A. COMPARELLI¹, V. BRUNO^{1,2}, M. SIMMACO¹, M. POMPILI¹, F. NICOLETTI^{1,2}, G. BATTAGLIA^{1,2};

¹Univ. Sapienza, Roma, Italy; ²I.R.C.C.S. Neuromed, Pozzilli, Italy

Abstract: Kynurenine pathway metabolites (KPM) are emerging as key elements in the pathophysiology of schizophrenia (SZ). We have shown that cinnabarinic acid is reduced in the dorsolateral prefrontal cortex (DLPFC) of patients affected by SZ and its systemic administration induces antipsychotic-like activity in animals (Ulivieri et al, Schizophr Bull, 2020). Moreover, several KPM are significantly increased in the DLPFC of patients affected by SZ (Antenucci et al, Schizophrenia, 2024). Here we used experimental pharmacological (acute administration of MK-801) and neurodevelopmental murine models (methylazoxymethanol (MAM)

administration to dams, or prenatal restraint stress (PRS)) of SZ to test the potential antipsychotic activity of low doses of 3-hydroxyanthranilic acid (3-HANA, 0.125-0.5 mg/kg). MK-801 induced a reduction of the novel object recognition index which was attenuated by systemic acute injection of 3-HANA. Pre-treatment with 3-HANA also attenuated MK-801-induced pre-pulse inhibition disruption. These effects were lost in mGlu4^{-/-} mice. 3-HANA rescued the recognition index in MAM mice, the sociability index in PRS mice, and attenuated MK-801-induced hyperactivity in control, MAM and PRS mice. We also analyzed KPM levels in DLPFC (Brodman area 10), insular cortex (Brodmann area 44) of 37 autopsic samples of SZ subjects (24 males and 13 females) and healthy controls (HC) (31 males and 6 females), age between 40 and 64 years, kindly provided by the NIH-NeuroBiobank, and serum of 33 enrolled subjects with diagnosis of SZ (15 drug-naïve and 18 treated with antipsychotics) and 37 enrolled HC. Results showed a significant reduction of tryptophan/kynurenine ratio, indicative of the activation of the KP, both in BA-10 and BA-44 of SZ subjects. Kynurenine acid levels were significantly higher in BA-10 and BA-44 of SZ subjects, whereas quinolinic acid levels were increased only in BA-44 of male SZ subjects. Anthranilic acid levels were significantly lower in BA-44 of HC respect to BA-10. This difference was no longer observed in male SZ patients. Levels of 3-HANA were not different in BA-10 and BA-44 of HC, whereas SZ subjects showed higher levels in BA-10. Kynurenine levels were increased in blood of SZ patients, whereas kynurenine acid, anthranilic acid and xanthurenine acid were decreased, regardless of antipsychotic treatments. These data suggest that 3-HANA acts as a potent antipsychotic against cognitive and negative symptoms. Human data suggest a region-specific dysregulation of KP in brain areas crucial for SZ, and that these changes do not reflect peripheral alterations.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.15/A59

Topic: A.10. Schizophrenia

Support: Wellcome Discovery Award 302077/Z/23/Z

Title: Chronic administration of olanzapine, a second-generation antipsychotic, modifies the synaptome architecture in a Dlg2^{+/-} mouse model of schizophrenia

Authors: Ü. GÜNAR^{1,2}, *S. C. GILLARD^{1,2}, D. KOUKAROUDI¹, Z. QIU^{1,3}, N. KOMIYAMA^{1,2}, S. G. N. GRANT^{1,2};

¹Ctr. for Clin. Brain Sci., ²Simons Initiative for the Developing Brain (SIDB), Ctr. for Discovery

Brain Sci., Univ. of Edinburgh, Edinburgh, United Kingdom; ³Sch. of Sci. and Engin., Univ. of Dundee, Dundee, United Kingdom

Abstract: Excitatory synapses are highly diverse, differing in their proteome composition, nanoscale organisation, and protein turnover. This enables the classification of synapses into types and subtypes, each with a distinct spatial distribution, thus creating the synaptome architecture. Genetic studies have identified synaptic dysfunction as a hallmark of schizophrenia, a chronic mental illness that affects approximately 1% of the population worldwide. However, the impact of schizophrenia risk genes as well as the influence of antipsychotic drug treatment on excitatory synapses are poorly characterized. We hypothesised that schizophrenia risk genes could alter the excitatory synaptome architecture and that olanzapine, a second-generation antipsychotic widely used to treat schizophrenia, could modify these changes. To test this, we administered olanzapine by oral gavage for 28 days to adult mice (9.5-11.5 months) carrying a heterozygous knockout mutation in *Dlg2*, a schizophrenia risk gene. We examined four experimental groups: vehicle-treated wild-type mice (n=10, 3F), vehicle-treated *Dlg2*^{+/-} mice (n=9, 5F), olanzapine-treated wild-type mice (n=10, 3F), and olanzapine-treated *Dlg2*^{+/-} mice (n=10, 7F). For each experimental group, we mapped the synaptome architecture of PSD95 and SAP102, two postsynaptic scaffolding proteins widely expressed in excitatory synapses, at single-synapse resolution across 118 brain regions. We observed global alterations to synapse subtype densities in *Dlg2*^{+/-} mice. Olanzapine partially reversed these changes in a region-dependent manner. In addition, the synaptome architecture of wild-type and *Dlg2*^{+/-} mice underwent differential changes in response to chronic olanzapine treatment. While olanzapine-treated wild-type mice exhibited increased PSD95 and SAP102 puncta densities in the globus pallidus and substantia nigra, olanzapine-treated *Dlg2*^{+/-} mutants showed decreases in these markers. This indicates divergent synaptic responses in brain regions associated with schizophrenia pathology and dopamine D2 receptor-mediated action. These results provide the first evidence of pharmaceutical modulation of the synaptome architecture and demonstrate the potential to detect and reverse synaptic pathology in models of psychiatric disorders.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

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Topic: A.10. Schizophrenia

Support: Swedish Medical Research Council
Swedish Brain Foundation
Åhléns-stiftelsen

Stockholm County Council
Karolinska Institutet
Center for Psychiatry Research
Knut och Alice Wallenbergs Stiftelse

Title: Kynurenine aminotransferase III as the key enzyme driving immune-induced kynurenic acid synthesis in vitro and in vivo: a novel target for cognitive dysfunction and psychotic disorders

Authors: *V. LOUVROU^{1,2}, A. FAKA SCHULTE², F. ORHAN², M. GOMEZ-GALAN², X. LI², P. FRIDH², A. LINDQVIST³, Å. WIMAN², G. C. ENGBERG², Y. GRAVENFORS⁴, L. SCHWIELER², S. ERHARDT²;

²Dept. of Physiol. and Pharmacol., ¹Karolinska Institutet, Stockholm, Sweden; ³Dept. of Pharm., Uppsala Univ., Uppsala, Sweden; ⁴Dept. of Biochem. and Biophysics, Stockholm Univ., Stockholm, Sweden

Abstract: Increased levels of kynurenic acid (KYNA) in the human brain are associated with psychosis and cognitive deficits. Kynurenine aminotransferase (KAT) II is the main enzyme responsible for KYNA production. KAT II knockout (KO) mice have reduced KYNA levels in the brain and exhibit improved cognitive function. KAT II inhibitors are developed as a new therapeutic approach for psychotic disorders and cognitive impairment. In this study, we show that repeated injections of lipopolysaccharide (LPS, $2 \times 0.83\text{mg/kg}$ or $2 \times 0.5\text{mg/kg}$), or a single injection of, the KYNA precursor, kynurenine (20mg/kg , 40mg/kg or 100mg/kg) increase brain KYNA levels in FVB/N wild-type, KAT II KO mice and in Sprague-Dawley rats. Gene expression of KAT III was upregulated in the brains of WT and KAT II KO mice following repeated injections of LPS. In rats, both KAT II and III mRNA levels were upregulated. No change in gene expression of KAT enzymes (KAT I-IV) was reported following kynurenine administration. In human-derived monocytes, an increase in KYNA levels upon TLR-3 (Poly (I:C), $10\text{ }\mu\text{g/ml}$) stimulation, together with upregulation of KAT III, is reported. Additionally, induced gene expression of KAT III was also observed in human epidermoid carcinoma (A-431) cells after pro-inflammatory cytokine ($20\text{ ng/ml IFN}\gamma + 10\text{ ng/ml IL-1}\beta$) stimulation, which result in significantly higher KYNA synthesis. Using the PF-04859989 compound, at doses selective for KAT II inhibition and at doses achieving pan-KAT inhibition, we uncover a role of KAT III for KYNA synthesis during immune-induced conditions in both cells and rodents. The above evidence suggests KAT III as a potential target for antipsychotic treatment and cognitive impairment.

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Poster

PSTR315: Schizophrenia

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.17/A61

Topic: A.10. Schizophrenia

Support: VR

Title: Altered Kynurenine aminotransferase III (KAT III) Gene Expression in Postmortem Human Brain Tissue Under Inflammatory and Infectious Conditions

Authors: *E. C. JOHNSON¹, S. IMBEAULT², S. MALWADE², V. LOUVROU², M. GÓMEZ-GALÁN², L. SCHWIELER², C. SELLGREN², G. C. ENGBERG², S. K. ERHARDT²;
¹Karolinska Inst., Ranchos de Taos, NM; ²Karolinska Institutet, Stockholm, Sweden

Abstract: Increased levels of kynurenic acid (KYNA) in the human brain are associated with psychosis and cognitive deficits. Kynurenine aminotransferase (KAT) II is the main enzyme responsible for the KYNA production. KAT II knockout (KO) mice have reduced KYNA levels in the brain and exhibit improved cognitive function. KAT II inhibitors are developed as a new therapeutic approach for psychotic disorders and cognitive impairments resulting from neuroinflammation. Here, we investigated cell type-specific transcriptomic changes of genes encoding kynurenine pathway metabolites and enzymes in a previously published postmortem COVID-19 study and observed an upregulation of KAT III in Oligodendrocyte progenitor cells (OPCs) (Log2FC = 0.264), oligodendrocytes (Log2FC = 0.0755), endothelial cells (Log2FC = 0.351), and a subset of neurons (Log2FC = 0.256) (Adjusted-p value < 0.05) in the frontal cortex of COVID-19 patients relative to healthy controls. In addition, postmortem human caudal cingulate samples from individuals with schizophrenia, major depressive disorder, bipolar disorder and healthy individuals were subset into high and low inflammation groups depending on IL-1 β , IL-6, IL-8 and SERPINA3 expression levels. Greater KAT III gene expression (P<0.05) was observed in the high inflammation group, as compared to the low inflammation group. Together, these data suggest KAT III as a target for antipsychotic treatment and cognitive enhancement caused by neuroinflammation.

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Poster

PSTR315: Schizophrenia

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Topic: A.10. Schizophrenia

Support: Åhlen-stiftelsen
Stiftelsen Clas Groschinskys Minnesfond
The Swedish Research Council

Title: A 3-Dimensional Astrocyte Spheroid Model for Investigating Inflammatory Kynurenic Acid Regulation and Selective Targeting of Kynurenine Aminotransferase III

Authors: P. FRIDH¹, G. C. ENGBERG¹, S. K. ERHARDT¹, *F. ORHAN²;

¹Karolinska Institutet, Dept of Physiol. & Pharmacology, Stockholm, Sweden, ²Karolinska Institutet, Stockholm, Sweden

Abstract: Kynurenic acid (KYNA), a neuroactive metabolite primarily produced by astrocytes, is elevated in the brains of individuals with neuropsychiatric disorders such as schizophrenia, particularly under inflammatory conditions. KYNA is synthesized via four kynurenine aminotransferases (KATs), with KAT II recognized as the dominant enzyme under physiological conditions. However, emerging evidence from our group implicates KAT III as a key contributor during immune activation. To enable detailed investigation of KYNA regulation under such conditions, we developed a robust and scalable 3-dimensional (3D) human astrocyte spheroid model capable of generating over 300 spheroids per well in a 24-well plate. Upon stimulation with IFN- γ and IL-1 β in the presence of tryptophan, KYNA levels increased from ~3.9 to ~25.6 nmol/L, a ~6.5-fold rise, demonstrating a wide dynamic range suitable for drug testing. Application of PF-04859989, a KAT II-selective inhibitor that also inhibits other KATs at higher concentrations, resulted in a dose-dependent reduction in KYNA levels. This was consistent with its published IC₅₀ values for KAT II (22 nM) and KAT III (11 μ M), suggesting progressive inhibition across isoforms. Immunocytochemical analysis confirmed expression of both KAT II and KAT III in the spheroids. These findings establish our 3D astrocyte spheroid model as a physiologically relevant and scalable platform to study KYNA regulation during neuroinflammation. This model also enables screening for isoform-selective KAT inhibitors, particularly those targeting KAT III.

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Poster

PSTR315: Schizophrenia

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Topic: A.10. Schizophrenia

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Center for Psychiatry Research
Åhlén-stiftelsen
Knut and Alice Wallenberg Proof-of-Concept Grant
Karolinska Institutet

Title: Targeting kynurenine aminotransferase III: Discovery of a selective and brain-penetrant inhibitor for synthesis of immune-induced kynurenic acid.

Authors: *P. FRIDH¹, Y. GRAVENFORS^{2,3}, L. SCHWIELER¹, M. GÓMEZ-GALÁN¹, V. LOUVROU¹, Å. WIMAN¹, X. LI¹, R. CARABALLO^{2,3}, A. LINDQVIST^{2,4}, J. LARSSON^{2,5}, L. DAHLLUND^{2,7}, C. BENGTSSON^{2,3}, H. NORDSTRÖM^{2,6}, G. C. ENGBERG¹, E. C. JOHNSON¹, S. ERHARDT¹;

¹Dept. of Physiol. and Pharmacol., Karolinska Institutet, Stockholm, Sweden; ²SciLifeLab DDD Platform, Sci. for Life Lab., Stockholm, Sweden; ³Dept. of Biochem. & Biophysics, Stockholm Univ., Stockholm, Sweden; ⁴Drug Optimization & Pharmaceutical Profiling (UDOPP), Dept. of Pharm., ⁵Dept. of Medicinal Chem., ⁶Dept. of Chemistry-BMC, Uppsala Univ., Uppsala, Sweden; ⁷Dept. of Protein Sci., KTH Royal Inst. Of Technol., Stockholm, Sweden

Abstract: Kynurenic acid (KYNA) is a neuroactive metabolite produced via the kynurenine pathway. It acts as an endogenous *N*-methyl-D-aspartic acid (NMDA) receptor antagonist and is proposed to antagonize the $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ nAChR). Elevated KYNA levels are observed in several conditions, including psychotic disorders and infectious diseases, where interference with glutamatergic and dopaminergic neurotransmission contributes to psychosis and cognitive decline. Under physiological conditions, kynurenine aminotransferase II (KAT II) is the main enzyme responsible for KYNA synthesis in the brain and has been the primary target of drug development. However, our recent findings demonstrate that immune activation induces KAT III expression, shifting KYNA synthesis predominantly to this isoform and establishing KAT III as the principal contributor to immune-induced KYNA production, for which no pharmaceutical inhibitors currently exist. We therefore set out to develop a potent, selective, and brain-penetrant inhibitor of human KAT III. To initiate this effort, we developed a high-throughput screening assay for KAT III to identify candidate compounds. Structure-activity relationship (SAR) studies identified key positions amenable to chemical modifications, generating a portfolio of KAT III inhibitors. Our lead compound, SLL-9183, exhibits nanomolar potency against human and rat KAT III, and nanomolar affinity for both species determined by surface plasmon resonance (SPR), with over 100-fold selectivity against the other KAT isoforms. The X-ray crystal structure of our inhibitor in complex with KAT III revealed no interaction with the enzyme's cofactor, pyridoxal phosphate (PLP). Pharmacokinetic evaluation in rodents confirmed brain exposure, with free brain concentrations of SLL-9183 exceeding 10 times its K_d value measured by SPR. Preliminary data show that systemic administration of our inhibitor consistently and robustly suppresses immune-induced KYNA synthesis in mice, with reductions observed across several distinct brain regions, including the hippocampus (-51%), frontal cortex (-56%), and striatum (-78%).

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.20/B2

Topic: A.10. Schizophrenia

Title: Kr232: a differentiated antipsychotic for the treatment of both positive and cognitive symptoms of schizophrenia

Authors: *R. WU^{1,2}, L. PENG², Z. TAI², N. YAN², S. YAN²;

¹Jiangxi Kvvit Pharmaceut. Co., Ltd, Shanghai, China; ²Jiangxi Kvvit Pharmaceut. Co., Ltd., Shanghai, China

Abstract: *Here, we report KR232, a highly potent and selective D2/D3 partial agonist with well balanced 5-HT1A/5-HT2A activity for the treatment of both positive and cognitive symptoms of schizophrenia.*

Schizophrenia is a severe mental disorder characterized by hallucinations and delusions (positive symptoms), disorganized thinking and behavior (cognitive symptoms), and flat or inappropriate affect (negative symptoms). Current medications effectively alleviate positive symptoms but have limited impact on cognitive and negative symptoms. Accumulating evidence indicates that D3R partial agonisms enhance cognitive function by maintaining optimal dopamine levels in brain regions that regulate cognitive performance. Meanwhile, activation of 5-HT1AR may reverse cognitive disturbances and produce antidepressant effects. In an effort to develop new agents for treating both positive and cognitive symptoms of schizophrenia, we have designed a novel compound, KR232, which exhibits enhanced activity at D3R and 5-HT1AR, while retaining potent activity at D2R and 5-HT2A receptors. KR232 demonstrates a more balanced activity across multiple targets, resulting in greater efficacy and minimal side effects in preclinical studies, thereby indicating a broader therapeutic index. In *in vitro* studies, KR232 is a highly potent and selective partial agonist at both D2R and D3R, with an EC₅₀ value of sub-nanomolar range. It also exhibits partial agonism at 5-HT1AR and antagonism at 5-HT2AR, both with single-digit nanomolar potency. Moreover, it did not significantly affect other targets at the micromolar level, which are often associated with weight gain and blood pressure disturbance. In animal models of schizophrenia, KR232 demonstrated significant and persistent reductions in MK-801-induced hyperlocomotion at a dose as low as 0.05 mg/kg, mitigated cognitive deficits caused by sub-chronic PCP administration at 0.1mg/kg, reversed MK-801-induced deficits in prepulse inhibition (PPI) at 0.15 mg/kg. Meanwhile, KR232 combined with SSRIs significantly reduced immobility in the forced swimming test. These results suggest that KR232 has strong potential therapeutic applications for both schizophrenia and depression. Particularly, KR232 exhibited a favorable safety profile. It did not induce catalepsy or impair rotarod performance, or change body temperature at doses up to 40 mg/kg. In addition, KR232 demonstrated a robust pharmacokinetic (PK) profile. Overall, KR232 shows remarkable potential as one of the next-generation antipsychotics that meets urgent clinical needs. Further exploration and examination are warranted to strengthen this potential.

Disclosures: R. Wu: None. L. Peng: None. Z. tai: None. N. Yan: None. S. Yan: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.21/B3

Topic: A.10. Schizophrenia

Support: R01 MH119346

Title: Memory deficiency in *Plxna2* knockout mice is associated with reduced excitability and over-inhibition of granule cells in the dentate gyrus

Authors: *W.-C. CHANG¹, K. STANGIS⁴, R. PARENT⁴, G. VOLLMAR⁵, X.-F. ZHAO², R. J. GIGER^{7,6}, G. G. MURPHY^{3,5};

¹Mol. & Integrative Physiol., ²Cell and Developmental Biol., ³Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI; ⁴Michigan Neurosci. Inst., ⁵Mol. & Integrative Physiol., ⁶Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI; ⁷Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Genome-wide association studies have linked the semaphorin receptor Plexin A2 with several psychiatric diseases, including schizophrenia. In mice, deletion of *Plxna2* interrupts granule cell progenitor migration in the dentate migratory stream, connecting the neuroepithelium of the lateral ventricle with the hippocampal fissure. In *Plxna2*^{-/-} mice, this results in the redistribution of granule cells and a neuroanatomically distorted dentate gyrus; in addition, mossy fibers also show aberrant laminar targeting within CA3. Importantly, *Plxna2*^{-/-} mice showed a deficiency in consolidating fear memory and sensorimotor gating, as assessed by prepulse inhibition, behavioral phenotypes with links to schizophrenia. In this study, we further examined the impact of *Plxna2* deletion on hippocampal function using the Morris water maze. While *Plxna2* knockout mice exhibited intact acquisition during training, long-term memory of the platform location was impaired. In addition, using whole-cell current clamp recording we examined intrinsic membrane properties in the *Plxna2*^{-/-} mice. Deletion of *Plxna2* did not alter the intrinsic properties of the granule cells in the dentate gyrus but reduced the number of evoked action potentials in response to current injection. Finally, whole-cell voltage clamp recordings revealed that deletion of *Plxna2* enhanced the number and amplitude of inhibitory post-synaptic currents in the granule cells of the infrapyramidal blade of the dentate gyrus. Thus, we conclude that the diminished excitability and upregulated inhibition in the dentate gyrus contribute to the memory deficits observed in the *Plxna2*^{-/-} mice.

Disclosures: W. Chang: None. K. Stangis: None. R. Parent: None. G. Vollmar: None. X. Zhao: None. R.J. Giger: None. G.G. Murphy: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

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Program #/Poster #: PSTR315.22/B4

Topic: A.10. Schizophrenia

Support: NIH grant R01MH119346
Dr. Miriam and Sheldon G Adelson Medical Research Foundation
NWO Gravitation program BRAINSCAPES: Roadmap from
Neurogenetics to Neurobiology (NWO: 024.004.012)

Title: Diverse and Location-Specific Roles of PlexinA2, PlexinA4, and NCAM in Developing Hippocampal Mossy Fibers

Authors: *X.-F. ZHAO¹, R. KOHEN², E. VAN BATTUM³, Y. ZENG², X. ZHANG², C. JOHNSON², K. WANG⁴, B. LIM⁵, J. OSES-PRIETO⁶, J. RASBAND⁵, R. J. PASTERKAMP³, M. N. RASBAND⁵, R. J. GIGER⁷;

¹Univ. of Michigan, Ann Arbor, Ann Arbor, MI; ²Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI; ³Translational Neurosci., Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; ⁴Cell and Developmental Biol., Univ. of Mich, Ann Arbor, MI; ⁵Neurosci., Baylor Col. of Med., Houston, TX; ⁶Pharmaceut. Chem., Univ. of California San Francisco, San Francisco, CA; ⁷Neurology/Cell and Developmental Biol., Univ. of Mchigan, Ann Arbor, MI

Abstract: Mossy fibers (MFs) originate from dentate granule cells and innervate area CA3 of the hippocampus. Upon entry of CA3, MFs partition into two prominent axon bundles, the suprapyramidal tract (SPT) and infrapyramidal tract (IPT) and form lamina specific synaptic contacts in the stratum lucidum (SL) and stratum oriens (SO), respectively. Here we employed new mouse lines to dissect the function of *Sema6A* and its receptors, PlexinA2 (PlxnA2) and PlxnA4, in developing MFs. In *Sema6a*^{-/-} mice, MF partitioning into SPT and IPT bundles is incomplete and IPT axons in the SO are overextended, while the SPT correctly innervates the SL. Loss of neuronal *Sema6a* results in defective MF patterning and we show that this involves *Sema6A* reverse signaling. *Plxna4* controls MF partitioning, SPT axon bundling and laminar targeting to the SL, as well as IPT length. Many of these defects are replicated in mice deficient for PlxnA4 GAP catalytic activity, underscoring the importance of this GAP domain. MFs are tightly fasciculated in *Plxna2*^{-/-} mice and fail to separate into SPT and IPT bundles, and defects are significantly reduced in PlxnA2 GAP mutants, highlighting the involvement of GAP-independent signaling events. To further explore the molecular basis of aberrant axon fasciculation, we employed anti-PlxnA2 dependent proximity biotinylation and identified several PlxnA2-associated Ig-CAM family members. We observed a genetic interaction between *Plxna2* and *Ncam1*, but not *Plxna4* and *Ncam1*, for SPT and IPT formation and positioning in CA3. Together, our studies provide insights into the multifaceted and overlapping, yet distinct, functions of PlxnA family members in orchestrating specific guidance decisions in developing MFs.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.23/B5

Topic: A.10. Schizophrenia

Support: R01 MH119346

Title: Deletion of *Plxna2* Impaired Neuronal Network Synchrony in Mouse Hippocampal Slices

Authors: *G. VOLLMAR¹, W.-C. CHANG¹, X.-F. ZHAO², R. J. GIGER^{3,2}, G. G. MURPHY^{4,1};

¹Mol. & Integrative Physiol., ²Cell and Developmental Biol., ³Neurol., ⁴Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: The semaphorin receptor Plexin A2 modulates the repulsive guidance of axonal growth during development. Genome-wide association studies have linked *PLXNA2* to psychiatric illness such as schizophrenia. In mice where *Plxna2* has been genetically ablated (*Plxna2*^{-/-} mice), migration of granule cell progenitors during development is interrupted, and the hippocampal dentate gyrus is deformed. These mice presented behaviors linked to schizophrenia and impaired learning and memory, but the mechanisms behind these dysfunctions are unknown. We investigated spontaneous neuronal network activity as a functional marker to understand how the hippocampus function is disturbed by *Plxna2* deletion. Using 0 Mg²⁺, 6 mM K⁺ artificial cerebrospinal fluid, neuronal network activity was induced in horizontal hippocampal brain slices (400 µm) prepared from adult *Plxna2*^{+/+} and *Plxna2*^{-/-} mice. Electrodes were placed in the cellular layers of dentate gyrus (DG), CA1 and CA3 regions of the hippocampus, and layer V of the entorhinal cortex (EC) to record neuronal network activity. In the slices (n = 9) from *Plxna2*^{+/+} mice, two types of synchronous network patterns were observed with a CA3-driven propagation (68.6 ± 8.0%) and an entorhinal cortex-driven propagation (32.4 ± 8.0%). While the activity in slices (n = 9) prepared from *Plxna2*^{-/-} mice also exhibited two patterns in which the activity appeared to originate in either the hippocampus or the EC, the observed activity was quite different. In the cases where activity originated in the hippocampus (98.2 ± 1.1%;) it appeared to originate in CA1 and spread to CA3 and the EC, while the DG remained more or less silent. In the rare case in which the EC activity appeared to drive subsequent activity (1.8 ± 1.1%), only CA1 was significantly activated. In both cases the EC exhibited what appears to be ictal-like activity, regardless of the region from which the activity originated. These experiments suggest that the disrupted migration of progenitor cells during development altered network synchrony between the hippocampus and the entorhinal cortex.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

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Program #/Poster #: PSTR315.24/B6

Topic: A.10. Schizophrenia

Support: UC Career Development Award 2025
UC Research Innovation Award 2025(a)
UC Research Innovation Award 2025(b)

Title: Molecular and cellular mechanisms of antipsychotic resistance in a rodent model

Authors: A. KRUYER¹, A. ANGELIS², L. WITTENKELLER¹, N. KOORNDYK¹, *D. AMATO¹;

¹Univ. of Cincinnati, Cincinnati, OH; ²Med. Col. of South Carolina, Charleston, SC

Abstract: Introduction: Antipsychotics are used to treat various psychiatric symptoms, including psychosis, yet symptoms persist in one-third of patients with first-episode psychosis due to antipsychotic resistance (AR). These patients require second-line agents like clozapine, which may cause serious side effects. The molecular basis of AR remains unknown, limiting development of safer alternatives. Use of addictive drugs, including psychostimulants, is a known risk factor for AR. We propose that reduced expression of dopamine and glutamate transporters (DAT, GLT-1), key regulators of striatal neurotransmission, may predispose individuals to AR. **Methods:** Male and female Long Evans rats (200-250 g) were trained to self-administer cocaine or sucrose. Antipsychotic efficacy was measured as the ability to inhibit locomotor activity induced by dopamine-based tail pinch assay and by low-dose ketamine (30 mg/kg i.p.), known to induce positive and negative-like schizophrenia symptoms in humans. Clinically relevant doses of haloperidol (0.2 mg/kg) or clozapine (15 mg/mg) were given before testing. To test causality, rats received nucleus accumbens core (NAcore) infusions of control or morpholino oligomers targeting DAT or GLT-1 for 3 days. After incubation, behavioral and protein analyses were conducted. Blinding and replication were included. To probe the neurophysiology of clozapine's efficacy despite transporter loss, we examined astrocytic regulation of glutamate and dopamine synapses in the NAcore. **Results:** Haloperidol suppressed hyperlocomotion in control rats, but was ineffective after chronic cocaine use ($p < 0.001$), while clozapine remained effective. Cocaine reduced DAT and GLT-1 expression in the NAcore without altering D2 receptor levels. Knockdown of either transporter reproduced the antipsychotic-resistant phenotype ($p < 0.001$), confirming their role in haloperidol efficacy. While both antipsychotics increased astrocyte insulation of glutamatergic synapses ($p < 0.05$), only clozapine increased GLT-1 surface expression ($p < 0.05$), potentially overcoming its psychostimulant-induced downregulation. **Conclusions:** We present a novel, mechanistically-grounded model of antipsychotic resistance driven by psychostimulant-induced downregulation of DAT and GLT-1. These findings implicate transporter regulation and astrocyte morphological plasticity in clozapine efficacy and suggest new therapeutic targets for psychosis.

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Poster

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.25/B7

Topic: A.10. Schizophrenia

Support: Boehringer Ingelheim Stiftung

Title: Maternofetal transfer of human NMDAR-antibodies leads to cortical network defect in adulthood

Authors: *S. ALTAHINI^{1,2}, J. M. DOERING³, H. BACKHAUS², H. PRUESS^{4,5}, A. STROH^{6,2};

¹Inst. of Physiol. I, Univ. Hosp. Muenster (UKM), Münster, Germany; ²Leibniz Inst. for Resilience Res., Mainz, Germany; ³Dept. of Psychosomatic Med., Charité – Universitätsmedizin Berlin, Berlin, Germany; ⁴Dept. of Neurol. and Exptl. Neurol., Charite Berlin, Berlin, Germany; ⁵German Ctr. for Neurodegenerative Dis. (DZNE), Berlin, Germany; ⁶Inst. of Physiol. I, Univ. Hosp. Muenster (UKM), Münster, Germany

Abstract: Anti-NMDA receptor encephalitis (NMDARE) is an autoimmune brain inflammation caused by IgG antibodies that bind to the N-methyl-D-aspartate receptors. In addition to patients with acute anti-NMDARE, human anti-NMDAR antibodies can be detected in up to 1% of the healthy population. A considerable subgroup of pregnant women might be at risk of transferring anti-NMDAR antibodies to the fetus during early stages of pregnancy. Here, we asked whether in utero exposure impacts functional brain health even in early adulthood. We used a mouse model of maternofetally transferred anti-NMDA receptor antibodies. At P51, corresponding to adolescence in humans, we recorded cortical microcircuit activity with cellular resolution in layer II/III in visual cortex by two-photon calcium imaging in awake behaving mice. Microcircuits in NMDAR-Ab exposed mice exhibit a lower spontaneous activity signature compared to healthy control mice. Antibody exposure led to a bursty neuronal firing with reduced temporal stability of network states. Notably, upon visual stimulation, neurons in NMDAR-Ab exposed mice had a higher orientation selectivity. In NMDAR-AB exposed mice, a large fraction of those neurons active upon visual stimulation were inactive during spontaneous - ongoing - activity and vice versa. A dissociation between ongoing and stimulus-evoked activity is in sharp contrast to the operational principles in healthy networks and has been hypothesized to contribute to the development of psychosis.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.26/B8

Topic: I.04. Executive Functions

Support: ERC starting grant MESO-AG 101040378
Hebrew University start-up grant

Title: Brain-wide neuronal networks in a schizophrenia mouse model

Authors: *S. D. SHERER, R. TERNER-ROTHKOFF, A. GILAD;
Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Schizophrenia is a serious mental health disorder affecting millions, characterized by positive symptoms like delusions, negative symptoms such as blunted emotions, and cognitive deficits. Currently, schizophrenia remains without a truly effective treatment, especially for negative symptoms. Although schizophrenia is known to involve abnormal activity across brain regions, the underlying network dysfunction, especially related to positive and negative symptoms, remains largely unclear. This study utilizes a specialized schizophrenia mouse model treated with MK-801 via mini osmotic pumps (SZ), designed to replicate specific aspects of the disorder and provide insight into its neurobiological mechanisms. We use advanced calcium imaging with 24 implanted optic fibers targeting regions such as the amygdala, hippocampus, and nucleus accumbens to compare brain-wide neuronal activity in freely moving wild-type (WT) and SZ mice. Brain dynamics were measured during behavioral tasks assessing positive symptoms (e.g., hyperlocomotion in open field), social interaction (3-chamber test), negative symptoms (e.g., blunted emotional affect in elevated plus maze), and cognitive deficits (e.g., reduced novel object recognition). Our research goal is to investigate brain-wide deficits linked to positive and negative symptoms in schizophrenia. We found that SZ mice exhibit significant positive and negative symptoms, as well as cognitive deficits, compared to WT. Additionally, SZ symptoms vary within and between mice, with some SZ mice showing days of behavior similar to WT. Regarding brain dynamics, during negative symptoms (e.g., EPM), several brain areas show decreased activity, and specific brain areas such as the DG and PFC even flip activity patterns (compared to WT), more active in closed arms as opposed to open arms in EPM. Interestingly, the same SZ mice during the normal behaving session displayed WT-like activity patterns. Notably, during behaviors demonstrating cognitive deficit, SZ mice displayed higher activity in frontal areas (e.g., PFC) compared to WT. SZ mice also showed increased frontal activity during the 3-chamber test. In addition, we observed a lower correlation between brain activity and locomotion in SZ mice compared to WT in the OF test. Our behavioral and neuronal pipeline allows testing the effects of various treatments on brain-wide dynamics underlying schizophrenia. These findings aim to shed light on how schizophrenia alters brain-wide neuronal dynamics, advancing our understanding of its neurobiological impact.

Disclosures: S.D. Sherer: None. R. Turner-Rothkoff: None. A. Gilad: None.

Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR315.27/B9

Topic: I.05. Working Memory

Support: NIH Grant R01

Title: Identification of circuit-level phenotypes of Schizophrenia in rodent models using multi-electrode array recording and machine learning

Authors: *Z. CHEN¹, D. SONG⁴, W. CHOI¹, M. MCHUGH², X. ZHANG¹, S. SIEGEL¹, R. E. FEATHERSTONE³;

¹USC, Los Angeles, CA; ²USC, Rancho Palos Verdes, CA; ³Psychiatry and Behavioral Sci., USC, Los Angeles, CA; ⁴Biomed. Engin., Univ. of Southern California, Los Angeles, CA

Abstract: Cognitive impairments in memory are core features of schizophrenia, often linked to circuit-level disruptions involving the hippocampus and prefrontal cortex. The Dysbindin-Src-GluN2A signaling pathway, implicated in synaptic plasticity and memory function, has been associated with schizophrenia pathophysiology. This study investigates local field potential (LFP) alterations in mouse models carrying mutations in this pathway to identify electrophysiological correlates of circuit dysfunction.

Neuropixels probes were used to record LFPs from anesthetized wild-type (WT), Src heterozygous (Src-Het), and Dysbindin knockout (Dys-ko) mice, targeting hippocampal CA3/CA1 regions and the prefrontal cortex. Signals were low-pass filtered (<300 Hz), segmented into 10-second epochs, and analyzed using Welch's method to extract power spectral density across frequency bands. Cross-frequency coupling (CFC) metrics, including modulation index and phase-locking value, were computed via Hilbert transform. Z-scored spectral and CFC features were used to train supervised classifiers (SVM, decision tree, random forest) with 5-fold cross-validation. One-way ANOVA tested for group-level differences.

Multiple phenotypic difference were observed between the mutant and WT animals. Notably, spectral frequency band density features were significantly distinct across classes ($f > 1e3$, $p < 1e-12$): theta power, gamma power, and the theta/beta ratio were the most discriminative features. Dys-ko mice exhibited elevated theta-gamma coupling, while other two did not.

Machine learning classifiers reliably distinguished phenotypes within subjects, although cross-subject performance declined in WT mice, likely due to inter-animal variability and limited sample size. These findings indicate that schizophrenia-associated mutations produce distinct, classifiable LFP signatures, which may serve as novel circuit-level biomarkers for diagnosis and therapeutic targeting in schizophrenia.

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Poster

PSTR315: Schizophrenia

Location: SDCC Halls B-H

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Program #/Poster #: PSTR315.28/B10

Topic: I.04. Executive Functions

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National Science and Technology Council, Taiwan: NSTC 111-2634-F-A49-014
National Science and Technology Council, Taiwan: NSTC 112-2634-F-A49-003

Title: Fiber-specific characterization of white matter alterations in schizophrenia: a fixel-based analysis by age of onset and illness duration

Authors: *Y. HSU¹, A.-C. YANG^{2,3,4,5}, C.-H. YEH⁶;

¹Inst. of Brain Sci., ²Sch. of Med., Natl. Yang Ming Univ., Taipei, Taiwan; ³Dept. of Med. Res., ⁴Dept. of Psychiatry, Veterans Gen. Hosp., Taipei, Taiwan; ⁵Digital Med. and Smart Healthcare Res. Ctr., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; ⁶Dept. of Med. Imaging and Radiological Sci., Chang Gung Univ., Taoyuan, Taiwan

Abstract: Background: Schizophrenia is a complex psychiatric disorder marked by progressive neurobiological alterations. Conventional diffusion MRI studies often rely on voxel-based methods, which are limited in resolving crossing fibers. Fixel-based analysis (FBA), an advanced diffusion MRI approach, enables fiber-specific evaluation of white matter microstructure by generating three key metrics: fiber density (FD), fiber-bundle cross-section (FC), and their combination (FDC). This method offers improved anatomical specificity and sensitivity in detecting white matter changes in schizophrenia. **Methods:** We analyzed diffusion MRI data from 519 individuals obtained from the Taiwan Aging and Mental Illness database, including 301 healthy controls and 218 patients with schizophrenia. Illness duration ranged from below 1 year to above 40 years, and patients were stratified into seven groups at five-year intervals. Preprocessing was performed using MRtrix3, including denoising, motion and distortion correction, and bias field correction. Single-shell 3-tissue constrained spherical deconvolution was applied to reconstruct fiber orientation distributions. A population FOD template was generated, and fixel-wise metrics (FD, FC, FDC) were computed and analyzed using connectivity-based fixel enhancement with non-parametric permutation testing. **Results:** Compared to healthy controls, the schizophrenia group showed reduced FD in major white

matter tracts, including the corticospinal tract and superior corona radiata, as well as FC reductions in cerebellar peduncles. In subgroup analyses based on illness duration, progressive alterations in logFC were observed across white matter regions, reflecting increasing macrostructural disruption over time. In contrast, FD reductions appeared more localized, primarily affecting the corpus callosum and fornix, suggesting early involvement of interhemispheric and limbic pathways. **Conclusions:** These findings emphasize the progressive nature of white matter degeneration in schizophrenia and demonstrate the sensitivity of FBA in capturing subtle, fiber-specific alterations. Stratifying patients by illness duration further reveals dynamic neurobiological trajectories that may inform future staging models and intervention strategies.

Disclosures: Y. Hsu: None. A. Yang: None. C. Yeh: None.

Poster

PSTR316: Voltage-Gated Sodium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR316.01/B11

Topic: B.03. Ion Channels

Title: Targeting SCN9A and Nav1.7 with siRNA: A novel approach treating neuropathic pain

Authors: M. LUO¹, C. DUAN², K. ZHANG³, W. ZHANG¹, X. HUANG¹, *Z. LI¹;

¹Neuro3 Therapeutics, Inc., Cambridge, MA; ²Anhui Deze Preclinical Res. Labs., Xuancheng, China; ³New Vision World LLC, Aliso Viejo, CA

Abstract: Targeting SCN9A and Nav1.7 with siRNA: A novel approach treating neuropathic pain Min Luo¹, Chun Duan², Kaiming Zhan³, Wandong Zhang¹, Xinyan Huang¹, Zheng Li¹ ¹Neuro3 Therapeutics, Inc., Cambridge, MA, USA; ²Anhui Deze Preclinical Research Laboratories Inc., Xuancheng, Anhui, P. R. China; ³New Vision World, LLC, Aliso Viejo, CA The *SCN9A* gene encodes Nav1.7, a voltage-gated sodium ion channel critical in nociceptive signaling. Mutations in *SCN9A* are associated with a spectrum of human pain disorders, including congenital insensitivity to pain and inherited erythromelalgia, therefore, making Nav1.7 a high-value target for pain therapeutics.¹ RNA interference (RNAi) targeting *SCN9A* offers a promising therapeutic strategy, but effective delivery of small interfering RNAs (siRNAs) into the nervous system remains a major hurdle.² To address this, we developed an optimized siRNA platform incorporating proprietary lipid conjugation, backbone, and terminal phosphate modifications to enhance neuronal delivery and gene silencing. A panel of over 20 *SCN9A*-targeting siRNAs identified and exhibited potent knockdown ($\geq 70\%$) of *SCN9A* mRNA in cells at concentrations as low as 0.1nM. Six lead candidates achieved robust *SCN9A* mRNA silencing via free uptake in cells and human iPSC-derived sensory neurons. Furthermore, intrathecal administration of modified siRNAs in rhesus monkeys (*Macaca mulatta*, 60 mg per animal) resulted in approximately 50% *SCN9A* mRNA reduction in the dorsal root ganglion (DRG), spinal cord and cortex within two weeks. Ongoing behavioral studies will further

evaluate the functional consequences of *SCN9A* mRNA knockdown *in vivo*. These results support the potential use of siRNAs as a viable therapeutic modality for pain disorders and provide a novel, non-opioid pain therapeutic approach targeting *SCN9A*. 1. Dib-Hajj, S. D., Yang, Y., Black, J. A. and Waxman, S. G. *Nat. Rev. Neurosci.*, 2013, 14, 49-62. 2. Brown, K. M., Nair, J. K., Janas, M. M., Anglero-Rodriguez, Y. I., Dang, L. T. H., Peng, H., Theile, C. S., Castellanos-Rizaldos, E., Brown, C., Foster, D. et al. *Nat. Biotechnol.*, 2022, 40, 1500-1508.

Disclosures: **M. Luo:** A. Employment/Salary (full or part-time): Neuro3 Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuro3 Therapeutics. **C. Duan:** 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.; Anhui Deze Preclinical Research Laboratories. **K. Zhang:** F. Consulting Fees (e.g., advisory boards); Neuro3 Therapeutics. **W. Zhang:** F. Consulting Fees (e.g., advisory boards); Neuro3 Therapeutics. **X. Huang:** A. Employment/Salary (full or part-time): Neuro3 Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuro3 Therapeutics, Inc. **Z. Li:** A. Employment/Salary (full or part-time): Neuro3 Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuro3 Therapeutics, Inc..

Poster

PSTR316: Voltage-Gated Sodium Channels

Location: SDCC Halls B-H

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Program #/Poster #: PSTR316.02/B12

Topic: B.03. Ion Channels

Support: NIH 1R43NS125749
NIH 5R44MH119842

Title: State-dependent drug block of wildtype and mutant NaV 1.7 on action potential behavior in an in silico neuron modelled using a dynamic clamp system interfaced with NEURON

Authors: ***M. W. NOWAK**¹, L. KORBEL¹, L. NILSSON¹, B. K. PANAMA², H. GUIGUI¹, H. OLIVER¹, S. ACHARYA¹, M. L. HINES³, N. T. CARNEVALE³, R. L. RASMUSSEN^{2,1}, G. C. L. BETT^{2,1};

¹Cytocybernetics, Buffalo, NY; ²Jacobs Sch. of Med. and Biomed. Sci., SUNY at Buffalo, Buffalo, NY; ³Neurosci., Yale Univ., New Haven, CT

Abstract: Neurological disorders such as epilepsy and chronic pain are often caused by increased neuronal activity resulting from aberrant Na⁺ channel behavior. Conventional patch-clamp characterization of drug effects on Na⁺ channels is not always predictive of neuronal

activity or drug potency. Identifying effective drugs requires understanding which states (closed, open, inactive) are blocked. Furthermore, neurons with Na⁺ channel mutations that underly disorders may respond differently to drugs than do cells with wildtype (WT) channels. We used the Cybercye dynamic clamp system interfaced to NEURON in real-time to address these issues by computational modeling of *in silico* neurons with NaV1.7 WT and I848T mutant channels. We represented NaV1.7 channel gating with Markov models expanded from a conventional Hodgkin-Huxley formulation, and drug binding ($k_{on}=0.0001 \text{ nM}^{-1} \text{ ms}^{-1}$, $k_{off}=0.001 \text{ ms}^{-1}$, $K_D=10 \text{ nM}$) was implemented to the I1 (inactivated, fully-closed), I4 (inactivated, open) or O (open) state. AP firing was initiated with a constant current stimulus and a simulated stochastic synaptic current. For the NaV 1.7 WT model neuron, $I_{stim} = 15.6 \text{ pA}$ elicited spiking at $5.5\pm0.2 \text{ Hz}$, and I1, I4 or O state drug block decreased firing with IC_{50} values of 1.5 ± 0.2 , 28 ± 3 and $2962\pm304 \text{ nM}$, respectively. For the model neuron with the NaV 1.7 I848T mutation, which shifts activation toward hyperpolarized potentials, the same stimulus produced a $37.2\pm0.03 \text{ Hz}$ firing rate. To compare the effects of state-dependent drug block on NaV 1.7 WT and I848T neurons, a 5.5 pA stimulus applied to the NaV 1.7 I848T model neuron produced spiking at $4.6\pm0.2 \text{ Hz}$, and I1, I4 or O state drug block decreased AP firing with IC_{50} values of 0.17 ± 0.05 , 4.7 ± 0.5 and $182\pm34 \text{ nM}$, respectively. For each of the tested states, the drug effect on spiking was more potent for I848T mutant neurons than for WT neurons. This study demonstrates that drug block of an ion channel mutation can show different potencies when compared to the wildtype ion channel, based on kinetic changes in binding site availability. Evaluating drugs targeting mutant Na⁺ channels with altered kinetic behavior could be made patient-specific using this approach. Using dynamic clamp-generated *in silico* neurons can facilitate predictive screening of cloned patient-specific ion channel mutations expressed in real cells in order to identify desired effects on AP behavior, particularly in conjunction with higher throughput methods such as automated patch clamp. This work was funded by NIH R43NS125749, NIH R44MH119842 and Cytocybernetics.

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Poster

PSTR316: Voltage-Gated Sodium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR316.03/B13

Topic: B.03. Ion Channels

Support: Israel Science Foundation (grant No. 1384/19)

Title: Gamma frequency tunes Na⁺ channel availability and increases dendritic excitability in cortical pyramidal neurons.

Authors: *M. J. GUTNICK¹, N. ASTMAN², O. KOTLER³, Y. KHRAPUNSKY⁴, I. A. FLEIDERVISH⁵;

¹Koret Sch. of Vet Med, Hebrew Univ. Jerusalem, Rehovot, Israel; ²Physiol. and Cell Biol., Ben Gurion University of the Negev, Beersheva, Israel; ³Physiol. and Cell Biol., Ben Gurion Univ. of the Negev, Beer Sheva, Israel; ⁴Physiol. and Cell Biol., Ben Gurion Univ. of the Negev, Beersheva, Israel; ⁵Physiol. and Cell Biol., Ben-Gurion Univ. of the Negev, Beer Sheva, Israel

Abstract: Cortical waves in the gamma frequency range are associated with cognitive functioning, learning, memory, and information processing. Because gamma reflects oscillation of neuronal membrane potential, we asked how it relates to availability of voltage-dependent Na⁺ channels, which play a key role in determining cortical neuronal excitability. In models, the amplitude of the Hodgkin-Huxley inactivation parameter (h) is influenced by both the amplitude of the V_m oscillation and the local steepness of the $h_\infty - V_m$ relationship. If the time constant of channel inactivation (τ_h) is very fast, the value of h will closely follow the oscillating V_m waveform, with a 180° phase shift. However, measured values of τ_h are shown to be relatively slow. In this case, Na⁺ channel availability is expected to become sensitive to the frequency of voltage oscillations, particularly when the oscillation period approaches the duration of τ_h , since the instantaneous h value asymptotically approaches the steady-state h_∞ value corresponding to the midpoint of the voltage oscillation, and channel availability is increased at the positive peak of the wave. To experimentally test the functional consequences of these theoretical predictions, we used the cell-attached configuration of the patch-in-slice technique to measure the relevant Na⁺ channel parameters in somata of neocortical Layer 5 cells. The number of “ready-to-open” channels during oscillation was measured by applying sine wave voltage commands of constant amplitude (± 5 mV from V_{rest}) and varying frequencies (5-100 Hz), superimposed by brief depolarizing test pulses. At physiological temperature, Na⁺ channel availability was lowest at 5 Hz, and it increased sharply as a function of frequency, reaching a maximum at ~ 40 Hz. This frequency dependence of channel availability was associated with an increase in backpropagation of action potentials (AP) into dendrites, as measured by applying brief antidromic stimuli near the axon during the positive peaks of the oscillations. We found that Ca²⁺ transients in the apical dendrite were significantly enhanced ($\sim 54\%$) during 40 Hz oscillations as compared to 5 Hz. We conclude that enhanced membrane excitability associated with the high-pass filtering properties of Na⁺ channels renders the gamma frequency optimal for AP backpropagation.

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Poster

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Graduate School System scholarship

Title: Functional characterization of Costa Rican scorpion toxins targeting Nav1.7 and Kv1.2 channels: Evidence from oocytes to neurons

Authors: *G. AKERMAN SÁNCHEZ¹, C. DÍAZ^{2,3}, J. TYTGAT⁴, O. BRENES¹;
¹Dept. of Physiol., ²Dept. of Biochem., Univ. of Costa Rica, San José, Costa Rica; ³Clodomiro Picado Inst., San José, Costa Rica; ⁴Dept. of Pharmaceut. and Pharmacol. Sci., KU Leuven, Leuven, Belgium

Abstract: Scorpion neurotoxins are small peptides with high bioactive potential, acting on ion channels and offering opportunities for novel pharmacological tools. To investigate the functional diversity of venom components from the Costa Rican scorpion *Tityus championi*, we first evaluated the venom effects on mammalian Nav and Kv channels heterologously expressed in *Xenopus laevis* oocytes. This allowed us to identify specific channel isoforms targeted by the whole venom and by individual toxins purified via reverse-phase HPLC. To validate the physiological relevance of these findings, we conducted whole-cell patch-clamp recordings in the C1 neuron of *Helix aspersa*, a model known to endogenously express Nav1.7-like channels, using both voltage and current-clamp configurations. Mammalian channels exhibited distinct responses to the venom. Nav1.6 showed partial inhibition of inactivation, a hallmark of α -toxin activity, while Nav1.7 displayed a reduction in peak amplitude, upon whole venom exposure. After isolation, the toxin Tch3 reproduced the inhibition on peak current observed in Nav1.7, while having no detectable effect on Nav1.6, suggesting isoform-specific action of Tch3. When the venom was applied to the C1 neuron, it induced a decrease in peak amplitude and neuronal excitability. These findings suggest a potential role for Tch3 in modulating the activity of peripheral nociceptors that rely on Nav1.7 for excitability. For Kv channels, venom application led to a strong suppression of Kv1.2-mediated currents. Subsequent HPLC fractionation and mass spectrometry revealed that this inhibition was mediated by two toxins: the previously reported TchKTx3 and a newly identified peptide, TchKTx7, functionally described for the first time. Both toxins showed potent inhibitory activity on Kv1.2, with TchKTx7 representing a novel addition to the KTx family. These findings expand the known molecular diversity of *Tityus championi* venom and provide new candidates for the selective modulation of Kv channels. These results provide better insights into the functional role of the *T. championi*'s venom for the first time, and the complex interplay between venom components and ion channels.

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Poster

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Title: Creatine kinase B as a novel component of voltage-gated sodium channel 1.6 interactome: implications for medium spiny neuron plasticity

Authors: *N. A. GOODE¹, T. BAUMGARTNER⁵, A. K. SINGH⁸, Z. HAGHIGHIJOO², J. DI RE⁹, L. KOFF¹⁰, Y. MARMOL CONTRERAS⁶, J. SINGH¹¹, S. MOHANTY³, G. GOLOVKO¹², L. PALMER⁴, W. RUSSELL³, F. LAEZZA⁷;

¹Pharmacol. & Toxicology, Univ. of Texas Med. Br. at Galveston, Houston, TX; ²Pharmacol. & Toxicology, ³Univ. of Texas Med. Br. at Galveston, Galveston, TX; ⁴Univ. of Texas Med. Br. at Galveston, GALVESTON, TX; ⁵Pharmacol. & Toxicology, ⁶Pharmacol. and Toxicology, ⁷Dept. of Pharmacol. & Toxicology, UTMB, Galveston, TX; ⁸Pharmacol. & Toxicology, Univ. of Texas Med. Br. (UTMB), Galveston, TX; ⁹Pharmacol. & Toxicology, Univ. of Texas Med. Br., Galveston, TX; ¹⁰Pharmacology/toxicology, Univ. of Texas Med. Br., Texas City, TX; ¹¹The Univ. of Texas Med. Br. (UTMB), Galveston, TX; ¹²Pharmacol., Univesity of Texas Med. Br., GALVESTON, TX

Abstract: Neuropsychiatric disorders (NDs) are complex and debilitating conditions whose global prevalence and incidence continue to rise. While genetic, environmental, and epigenetic influences have been linked to the development and progression of these disorders, the underlying molecular mechanisms remain poorly understood. One key factor in determining susceptibility or resilience to NDs is neuronal plasticity. Emerging research highlights ion channels as critical regulators of neuronal plasticity, functioning as cellular hubs that integrate various transient and stable interactions with auxiliary proteins. These interactions can modulate how ion channels influence neuronal resilience or vulnerability. Among these ion channels is the voltage-gated sodium channel Nav1.6, which plays a pivotal role in initiating and propagating action potentials in excitatory neurons. Prior studies have linked Nav1.6 and its associated proteins to neuroplastic changes observed in models of resilience and vulnerability. Building on this, we recently investigated the Nav1.6 protein interactome under two distinct environmental conditions: enriched (EC) and isolated (IC), to identify auxiliary proteins that may confer resilience or vulnerability to NDs in the striatum. To determine the impact of the EC/IC behavioral paradigm on the composition of the Nav1.6 channel macromolecular complex, we housed rats (n=24) in EC and IC for 30 days. We used immunoprecipitation and nanoLC/MS/MS to investigate the proteome of Nav1.6 in these rats. Through this approach, we identified creatine kinase B (CKB) as an interactor of Nav1.6, which shows differential expression under EC versus IC environmental conditioning. Using split-luciferase complementation assay and confocal imaging, we orthogonally validated the interaction between Nav1.6 and CKB. Additionally, we used voltage-clamp electrophysiology to investigate the functional contribution of CKB to Nav1.6 channel activity *in vitro*. Our data show that CKB overexpression significantly reduces the Nav1.6 peak current density, long-term inactivation, and use dependence of fast inactivation (n = 14, p < 0.01). Furthermore, using current-clamp *ex vivo* electrophysiology, we identified genetic alterations of CKB protein expression levels that bi-directionally regulate medium spiny neuron excitability. Finally, our new findings also show that CKB overexpression prevents

hyperexcitability in medium spiny neurons of socially isolated rats. The results of this study offer valuable information on a new molecular target with potential for the development of novel neurotherapeutics.

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Poster

PSTR316: Voltage-Gated Sodium Channels

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Title: Bdnf/trkb signaling confers susceptibility to msns by fine-tuning nav1.6 channel macromolecular complex

Authors: *A. K. SINGH, N. A. GOODE, T. BAUMGARTNER, P. A. WADSWORTH, J. SINGH, F. LAEZZA;
Dept. of Pharmacol. & Toxicology, Univ. of Texas Med. Br. (UTMB), Galveston, TX

Abstract: The nucleus accumbens (NAc), a central component of the brain's reward circuitry, has been implicated in a wide range of reward-motivated behaviors. Neuroadaptive changes in intrinsic firing of medium spiny neurons (MSNs) in the NAc are a form of structural and functional plasticity that depends on brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB) signaling. Prior studies have shown that subtle changes in BDNF/TrkB signaling in MSNs promote a resilient or susceptibility phenotype. Yet, how these changes are mediated at the molecular level is still poorly understood. Previous studies identified glycogen-synthase kinase 3 β (GSK3 β), a downstream effector of BDNF/TrkB signaling, and the voltage-gated Na⁺ channel Nav1.6 as regulators of neuroplasticity induced by environmentally enriched (EC) or isolated (IC) conditions-models of resilience and vulnerability. Here, we sought to determine whether Nav1.6 and its accessory protein fibroblast growth factor 14 (FGF14) are converging sites for BDNF/TrkB signaling via GSK3 β downstream regulation. We used the split-luciferase complementation assay (LCA) to screen the Broad and UT kinase libraries of >1,400 kinase inhibitors against 3 pairwise LCA complexes (FGF14/Nav1.6, GSK3 β /Nav1.6 and GSK3 β /FGF14) under 3 conditions (no BDNF, 10 ng/ml and 75 ng/ml BDNF) in TrkB stably expressing HEK293 cells (each condition tested in duplicate). We observed that compounds

targeting the PI3K/Akt pathway reversed luminescence signals from cells treated with low BDNF, while compounds targeting Src and GSK3 β blocked the effect of high BDNF. We also used immunohistochemistry combined with laser scanning confocal imaging to characterize the expression pattern of Nav1.6, FGF14 and GSK3 β in the NAc in response to BDNF stimulation. Our results showed that a 2-hour treatment with high BDNF (100 ng/mL) significantly increased the fluorescence intensity of Nav1.6 channels in both the soma and the axon initial segment (AIS), the site of action potential initiation. In contrast, fluorescence of FGF14 increased only in the AIS of medium spiny neurons (MSNs), leading to a higher FGF14/Nav1.6 complex ratio specifically at the AIS. Preliminary patch-clamp electrophysiology studies demonstrated that this treatment also shifted the action potential initiation site to a more distal location compared to vehicle-treated cells. Our results support the existence of mechanisms downstream of BDNF/TrkB that converge on the Nav1.6 channel macromolecular complex and may confer resilience and vulnerability via changes in MSN firing as part of structural and functional plasticity.

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Poster

PSTR316: Voltage-Gated Sodium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR316.07/B17

Topic: B.03. Ion Channels

Title: Generation of a Nav1.9 cell-line for screening compounds using automated patch-clamp

Authors: *R. BURLEY¹, R. ABDELAZIZ², T. CHOWDHURY², K. KUAN², A. SIENERTH², S. STABILINI², H. GAUNT²;

¹Charles River Labs., Little Chesterford, United Kingdom; ²Charles River Labs., Chesterford Research Park, United Kingdom

Abstract: Nav1.9, is a voltage-gated sodium channel involved in pain signaling in the primary afferent sensory pathway. Genetic and pharmacological studies highlight the central role of Nav1.9 in chronic and inflammatory pain, making it a promising non-opioid analgesic target. High-throughput screening (HTS) assays are critical for discovering potent inhibitors of sodium channels and developing robust HTS assays enables the rapid identification of desirable selective inhibitors, accelerating the path to novel pain therapeutics with fewer side effects. Discovery efforts to screen against Nav1.9 have been somewhat held back due to the unusually slow activation and inactivation kinetics of the channel, difficulties to establish robust overexpression

in heterologous systems and the slightly more nuanced role of Nav1.9 in pain signaling compared to other sodium channels. We discuss here the development of a HEK293 Nav1.9 overexpressing cell line, its characterization and its validation for implementation in a drug discovery screening cascade. Electrophysiological data generated on the Sophion Qube 384 well automated system show that the biophysical properties and pharmacological profile of the recorded currents closely resemble those expected from Nav1.9. Pilot study data shows that the assay is robust, stable and scalable to enable its use for HTS. We also discuss the development of a secondary assay to examine Nav1.9 state dependency. The pharmacological profile of selected compounds show that the assay is suitable for examining compound potency, and is able to show preferential block of the inactivated state for compounds that show state-dependent pharmacology. By providing the cellular reagents and methodology required for HTS we can begin to unlock the potential of selective Nav1.9 inhibitors and allow the development of next-generation analgesics, offering hope for patients suffering from debilitating pain conditions.

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Poster

PSTR316: Voltage-Gated Sodium Channels

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Title: Stress-induced *Scn3a* upregulation increases ventral tegmental area dopaminergic neuron excitability and shapes stress resilience

Authors: *Q. HUANG¹, L. LI², Y. SHU²;

¹Fudan Univ., Shanghai City, China; ²Inst. for Translational Brain Res., Fudan Univ., Shanghai, China

Abstract: Chronic social stress such as bullying and subordination may lead to impaired reward processing and social withdrawal, contributing to pathogenesis of major depressive disorder. Dopaminergic neurons (DANs) in the ventral tegmental area (VTA) respond to motivational and social cues. While stress induces lasting changes in the spiking activity of VTA DANs, the underlying ionic mechanisms remain incompletely understood. Voltage-gated sodium channels (Nav) are essential for the generation of action potentials (APs), and thus regulating neuronal excitability. Using RNAscope combined with immunohistochemistry, we observed increased expression and nuclear localization of *Scn3a* (encoding Nav1.3) in adult VTA DANs compared

to neonatal ones, despite its typically progressive reduction during development in other types of neurons in the central nervous system. Following chronic social defeat stress (CSDS), *Scn3a* expression was upregulated in VTA DANs, particular in susceptible (SUS) mice compared to resilient (RES) ones. Correspondingly, patch-clamp recordings revealed increases in Na⁺ currents, the amplitude and the depolarizing rate of APs in VTA DANs of SUS but not RES mice. CRISPR/Cas9-induced *Scn3a* deletion in VTA DANs reduced neuronal excitability, as reflected by decreases in the frequency of both spontaneous firing and evoked APs. Following the loss of *Scn3a*, we observed decreases in the AP amplitude and its depolarizing rate, but we also detected a reduction in the AP repolarizing rate and an increase in the AP duration. Selective *Scn3a* deletion in VTA DANs also altered susceptibility to CSDS. Together, our results identify *Scn3a* as a stress-responsive gene in VTA DANs, crucial for the regulation of neuronal excitability and behavioral resilience following social stress.

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Poster

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FamiliesSCN2A

Title: Utilizing biophysically accurate computational models of Nav1.2 to simulate mutations and therapeutics in neonatal and adult neurons and neuronal networks

Authors: *T. A. FENTON¹, G. YAZDANI², M. LEITNER³, R. BARAVALLE⁴, S. M. Wafa⁵, K. CHAKRAVARTHULA², C. H. THOMPSON⁶, A. L. GEORGE, JR⁷, K. J. BENDER⁸, S. DURA-BERNAL⁴, R. BEN-SHALOM⁹;

¹Neurol., ²Univ. of California Davis, Davis, CA; ⁴Physiol. and Pharmacol., ³SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; ⁵Pharmacol., ⁶Dept. of Pharmacol., Northwestern Univ., Chicago, IL; ⁷Pharmacol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ⁸Univ. of California, San Francisco, San Francisco, CA; ⁹Neurol., UC Davis Med. Ctr., Sacramento, CA

Abstract: Dysfunction of *SCN2A*, the gene encoding Nav1.2, is implicated in a spectrum of severe neurological disorders including early infantile epileptic encephalopathies, autism spectrum disorder, and intellectual disability. Given the high number of unique patient-specific *SCN2A* mutations, the traditional approach of developing biological models for each individual variant to conduct drug discovery is not feasible due to excessive time and cost. Therefore, we leveraged *in silico* modeling to simulate a variety of *SCN2A* mutations and systematically apply drug simulations that would be prohibitive in a conventional wet laboratory setting. We utilized Hodgkin-Huxley equations to mathematically represent channel function to construct numerical

models of various *SCN2A* mutations. These channel simulations were integrated into multi-compartment reconstructions of layer 5 pyramidal neurons, with highly complex morphological features that abstract neuronal function as electrical circuits. We improved and validated multiple biophysical parameters, including the distribution of ion channels in the dendrites and the axon initial segment (AIS), ensuring they accurately reflect empirically obtained data. To understand $\text{Na}_v1.2$ dysfunction at the system level, our neuron model was incorporated into a network model that simulates a cortical column within the primary motor cortex (M1). This network comprises over 10,000 interconnected neurons with more than 30 million simulated synapses, within the NetPyNE simulation environment. Simulating the network activity and firing patterns within this circuit architecture allows us to investigate how pathological changes in $\text{Na}_v1.2$ alter network dynamics and provides information useful for predicting therapeutic targets. Some $\text{Na}_v1.2$ mutations can exhibit different properties in their neonatal versus adult isoforms. To address this limitation of our adult neuron model we constructed an immature neuron model. This model simulates a neuron undergoing early development, featuring a smaller morphology with fewer dendritic branches and different distribution of ion channels compared to the adult neuron. The $\text{Na}_v1.6$ channel, which is enriched in the distal AIS of adult neurons and plays a critical role in action potential initiation, is notably absent in developing neurons. Consequently, mutations in $\text{Na}_v1.2$ can exert an increased impact on neuronal firing patterns during the critical periods of early maturation. Using our computational models, we aim to accurately predict disease states that can be altered using therapeutic approaches personalized to each child's genetic diagnosis.

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Poster

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Title: Autism-associated SCN2A deficiency disrupts neuronal development and synaptic integration in human brain organoids

Authors: *X. CHEN, J. ZHANG, J. WU, M. S. HALURKAR, C. SWARTZ, Y. SHU, B. A. DEMING, Z. ZHANG, Y. YANG;
Purdue Univ., West Lafayette, IN

Abstract: *SCN2A* is one of the most strongly implicated genes in autism spectrum disorder (ASD), with protein-truncating variants (PTVs) frequently linked to profound neurodevelopmental impairments. To investigate the functional impact of *SCN2A* haploinsufficiency, we generated human striatal organoids (hStrOs) from CRISPR/Cas9-edited hiPSCs harboring a *de novo* nonsense mutation (*C959X*). Compared with controls, *SCN2A*-mutant organoids exhibited disrupted neurodevelopmental trajectories and significant neuronal deficits. Electrophysiological recordings and calcium imaging revealed elevated intrinsic excitability and aberrant network activity in *SCN2A*-deficient neurons. Further structural and synaptic analyses revealed shortened axon initial segments (AIS) and reduced dendritic spine density, alongside impaired excitatory and inhibitory synaptic transmission, as indicated by decreased sEPSC and sIPSC frequencies. Immunostaining confirmed these synaptic deficits, showing reduced colocalization of PSD95/Syn1 and VGAT/Gephyrin. Finally, transcriptomic profiling by bulk and single-cell RNA sequencing uncovered broad gene expression changes affecting neuronal function and connectivity. Collectively, these findings demonstrate that *SCN2A* deficiency disrupts human neuronal excitability and synaptic integration, establishing a robust organoid model for studying ASD-related dysfunction.

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Poster

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Title: Autism-associated *Scn2a* controls social information encoding

Authors: *J. ZHANG, M. EATON, X. CHEN, Y. ZHAO, S. KANT, B. A. DEMING, Y. SHU, J. WU, K. W. WETTSCHURACK, Z. ZHANG, M. S. HALURKAR, Y. YANG;
Purdue Univ., West Lafayette, IN

Abstract: Social behavior, essential for survival and adaptation, is severely disrupted in autism spectrum disorders (ASD). Social withdrawal linked to information overload, possibly arising from excessive background neuronal activity ("basal noise"), is hypothesized as a disease mechanism but remains experimentally unverified. Loss-of-function mutations in *SCN2A*, encoding the voltage-gated sodium channel Nav1.2, represent a leading monogenic cause of profound ASD. Here, we show that *Scn2a* deficiency results in robust social impairments in mice, with neurons displaying an enhanced excitation-inhibition (E/I) balance, elevated basal neuronal noise, and diminished signal-to-noise ratio (SNR) during social interactions, effects that are rescuable by restoring *Scn2a* expression even in adulthood. Notably, we found that the sociability could be dynamically tuned by *Scn2a* restoration and re-suppression. Moreover, by balancing the E/I and reducing basal neuronal firing, the GABA_A receptor-positive allosteric modulator (PAM) improves sociability in *Scn2a*-deficient mice and normalizes neuronal activities in translationally relevant human brain organoids carrying autism-associated *SCN2A* nonsense mutation. Additionally, we are working on clinically relevant genetic interventions. Collectively, our findings revealed a critical role of the Nav1.2 channel in regulation of social behaviors and uncovered molecular and circuit mechanisms underlying *SCN2A*-associated disorders.

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Poster

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Topic: B.03. Ion Channels

Support: NIH Grant R01NS117585
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FamilieSCN2A Foundation for the Hodgkin-Huxley Research Award
AES Postdoctoral Research Fellowship

Title: Microglia in human assembloids harboring SCN2A mutation and human-mouse chimeric brain

Authors: *J. WU¹, X. CHEN², J. ZHANG², K. WETTSCHURACK², M. ROBINSON², Y. ZHAO², Y.-E. YOO², B. DEMING², Z. QUE³, W. SKARNES⁴, J.-C. ROCHET², L.-J. WU⁵, Y. YANG²;

¹Prudue Univ., West Lafayette, IN; ²MCMP, Purdue Univ., West Lafayette, IN; ³Eli Lilly and Co., boston, MA; ⁴Cell. Engin., The Jackson Lab. for Genomic Med., Farmington, CT; ⁵Neurol., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Microglia critically shape neuronal circuit development and function, yet their region-specific properties and roles in distinct circuits of the human brain remain poorly understood. In this study, we generated region-specific brain organoids (cortical, striatal, and midbrain), each integrated with human microglia, to fill this critical gap. Single-cell RNA sequencing uncovered six distinct microglial subtypes exhibiting unique regional signatures, including a subtype highly enriched for the GABA_B receptor gene within striatal organoids. To investigate the contributions of microglia to neural circuitry, we created microglia-incorporated midbrain-striatal assembloids, modeling a core circuit node for many neuropsychiatric disorders, including autism. Using chemogenetics to activate this midbrain-striatal circuit, we observed increased calcium signaling in microglia involving GABA_B receptors. In assembloids harboring the autism-associated *SCN2A* nonsense mutation (C959X), microglia exhibited heightened calcium responses to neuronal hyperactivity and excessive synaptic pruning, which were reversed by pharmacological inhibition of microglial GABA_B receptors. To further validate these interactions in vivo, we transplanted human microglia into chimeric mouse brains and are currently assessing their dynamic functional integration within intact neural circuits. Collectively, our work establishes an advanced, multi-model platform to investigate human cell-centric neuroimmune dynamics, highlighting key microglial mechanisms in neural circuits.

Disclosures: J. Wu: None. X. Chen: None. J. Zhang: None. K. Wettschurack: None. M. Robinson: None. Y. Zhao: None. Y. Yoo: None. B. Deming: None. Z. Que: None. W. Skarnes: None. J. Rochet: None. L. Wu: None. Y. Yang: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.01/B23

Topic: B.04. Synaptic Transmission

Support: Funded by the European Union - Next Generation EU -NRRP M6C2 - Investment 2.1 Enhancement and strengthening of biomedical research in the NHS –Project PNRR-MAD-2022-12376126

Title: Unveiling the role of microglial dopamine D2 receptor in synaptic excitotoxicity in experimental multiple sclerosis

Authors: *S. BALLETTA¹, A. TARTACCA², F. PALMERIO^{2,3}, F. MARIANI^{2,4}, D. FRESEGNA^{3,5}, F. DE VITO¹, S. VELLUCCI³, A. MUSELLA^{3,6}, D. CENTONZE^{1,7}, G. MANDOLESI^{3,5};

¹Unit of Neurol., IRCCS Neuromed, (Pozzilli), Italy; ²Dept. of Systems Med., Univ. of Rome Tor Vergata, Ph.D. Program in Neurosci., Roma, Italy; ³Synaptic Immunopathology Lab, IRCCS San Raffaele, Roma, Italy; ⁴Synaptic Immunopathology Lab, IRCCS San Raffaele Roma Rome, Italy; ⁵Dept. of Human Sci. and Quality of Life Promotion, Univ. of Rome San

Raffaele, Roma, Italy; ⁶Dept. of Human Sci. and Quality of Life Promotion, Univ. of Rome San Raffaele, Italy, Roma, Italy; ⁷Dept. of Systems Med., Tor Vergata Univ., Roma, Italy

Abstract: Multiple Sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS), marked by progressive demyelination, synaptic dysfunction, and neuronal damage, leading to disability. Increasing evidence suggests that inflammatory synaptic dysfunction (synaptopathy) contribute to excitotoxicity and neurodegeneration in both MS patients and experimental models, such as Experimental Autoimmune Encephalomyelitis (EAE). In this context, beyond lymphocyte infiltration, reactive microglia are emerging as key mediators of synaptic pathology through the release of pro-inflammatory cytokines that disrupt synaptic homeostasis. Among the modulatory systems involved in MS pathogenesis, catecholamines—particularly dopamine (DA)—are gaining attention. Dopamine receptors (DRs), expressed on microglia, can exert pro- or anti-inflammatory actions depending on receptor subtype and disease context. However, the specific contribution of dopamine D2 receptors (D2R) in microglia to MS-related neuronal damage remains unclear. Here, we explored if microglial D2R signaling could modulate immune responses and synaptopathy in EAE. To this aim, we generated conditional knockout mice (CX3CR1^{CreERT2}/wt⁺; DRD2^{fl/fl}, micDR2^{-/-} N=15) and wild type littermates (micDR2^{+/+}, N=15). DRD2 deletion in microglia was induced via tamoxifen (75 mg/kg, i.p., for 5 days) at 4-5 weeks of age. EAE was induced with MOG₃₅₋₅₅ peptide at 9-10 weeks. Behavioral assessments were conducted in the presymptomatic phase (7-10 dpi) and disability score was monitored until 21-28 dpi. At these time points, patch-clamp recordings were performed in corticostriatal slices, and striata were dissected for immunofluorescence/confocal microscopy and molecular analysis to evaluate inflammatory synaptopathy. CFA-injected control mice (no MOG) showed no genotype-related differences. Preliminary data revealed that although clinical EAE scores were similar between groups, micDR2^{-/-} mice exhibited reduced anxiety-like behavior (Open Field, Elevated Plus Maze, and Forced Swim Tests). Importantly, these mice showed normalization of sEPSC kinetic abnormalities typically observed in EAE, suggesting a protective effect on synaptic function. These results reveal that microglial D2R plays a key role in regulating synaptic integrity under neuroinflammation. Although further experiments are needed to elucidate the underlying molecular pathways, our findings indicate that dopaminergic signaling represents a promising target for modulating microglia-driven synaptopathy in MS, opening new avenues for therapeutic intervention.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.02/B24

Topic: B.04. Synaptic Transmission

Support: Funded by the European Union - Next Generation EU - NRRP M6C2 - Investment 2.1 Enhancement and strengthening of biomedical research in the NHS – Project PNRR-MAD-2022-12376126

Title: Peripheral and central T-lymphocyte modulation via catecholamine receptors in advanced chimeric ex-vivo models of multiple sclerosis

Authors: S. BALLETTA¹, A. TARTACCA², F. PALMERIO^{3,4}, F. MARIANI^{6,7}, S. VELLUCCI⁸, A. FIORENZA⁹, E. C. LATAGLIATA^{10,9}, C. PROCACCINI^{11,12}, A. BRUNO¹³, G. LAURITANO¹³, T. MICILLO¹⁴, F. DE VITO¹, A. MUSELLA^{15,4}, D. CENTONZE^{13,17}, *G. MANDOLESI^{5,16};

¹IRCCS Neuromed, Pozzilli, Italy; ²Dept. of Systems Med., Ph.D. Program in Neuroscience, Univ. of Rome Tor Vergata, Rome, Italy, Roma, Italy; ³Dept. of Syst. Med., Ph.D. Program in Neuroscience, Univ. di Tor Vergata, Rome, Rome, Italy; ⁴Synaptic Immunopathology Lab, IRCCS San Raffaele Roma, Roma, Italy; ⁵Synaptic Immunopathology Lab, IRCCS San Raffaele Roma, Rome, Italy; ⁶Dept. of Systems Med., Ph.D. Program in Neuroscience, Univ. di Roma Tor Vergata, Roma, Italy; ⁷IRCCS San Raffaele, Rome, Italy; ⁸Synaptic Immunopathology Lab, IRCCS San Raffaele Roma, Rome, Italy, Roma, Italy; ⁹Fac. of Psychology, Uninettuno Telematic Intl. University, Rome, Italy, Roma, Italy; ¹⁰Santa Lucia Fndn., Roma, Italy; ¹¹Lab. of Immunology, Inst. for Endocrinol. and Exptl. Oncology “Gaetano Salvatore”, Dept. of Biomed. Sciences, Natl. Res. Council (CNR), Naples, Italy, Naples, Italy; ¹²Unit of Neuroimmunology, IRCCS - Fondazione Santa Lucia, Rome 00143, Italy, Roma, Italy; ¹³Unit of Neurology, IRCCS Neuromed, Pozzilli (Is), Italy, Pozzilli, Italy; ¹⁴Treg Cell Lab, Dept. of Mol. Med. and Med. Biotechnology, Univ. of Naples “Federico II”, Naples, Italy, Naples, Italy; ¹⁵Dept. of Human Sci. and Quality of Life Promotion, Univ. of Rome San Raffaele, Rome, Italy; ¹⁶Univ. of Rome San Raffaele, Roma, Italy; ¹⁷Dept. of Systems Medicine, Tor Vergata University, Rome, Italy, Roma, Italy

Abstract: Catecholamines (CAs) such as dopamine, noradrenaline, and adrenaline, are not only crucial neurotransmitters in the nervous system but also potent immunomodulators. Through their respective receptors (adrenergic receptors, ARs; and dopamine receptors, DRs) expressed on immune cells, CAs regulate both central and peripheral immune responses. Dysfunctional CA signalling is implicated in the pathogenesis and progression of Multiple Sclerosis (MS) and its animal model, Experimental Autoimmune Encephalomyelitis (EAE). Emerging evidence from our research highlighted inflammation-driven synaptic abnormalities as pathogenic mechanism contributing to excitotoxic damage in MS and EAE, with infiltrating T lymphocytes identified as crucial players. We recently developed a novel translational chimeric MS ex vivo model. In this model, corticostriatal slices from the brain of healthy donor mice were co-incubated with T lymphocytes (5×10^6 CD3+ cells for 1 hour) isolated from either the spleen or CNS of EAE mice (homologous chimeric model), or from the blood of progressive MS patients (heterologous chimeric model). This model accurately recapitulates the altered glutamatergic synaptic transmission observed in the striatum of EAE mice. By utilizing this advanced ex vivo system, we have investigated the potential effect of CA receptor-mediated modulation of T cell-driven synaptopathy. We have characterized the expression and function of CAs receptors (CASRs) on T lymphocytes from both peripheral (spleen) and CNS (infiltrating T cell) compartments of EAE mice using cytofluorimetry and electrophysiological recordings in the chimeric MS model. Pharmacological stimulation of DR2 with quinpirole of T cell derived from both EAE spleen

(1 μ M, overnight, N=4 mice) and brain (1-hour 1 μ M, N=6-8 mice) partially mitigated the glutamatergic alterations mediated by vehicle EAE T cells, in the striatum of healthy mice. Similarly, treating infiltrating T cells with the AR β 2 agonist, salbutamol (50 μ M, 1h treatment), resulted in a partial rescue of the T cell-driven synaptopathy. Similar experiments conducted in the heterologous (human - mouse) MS chimeric model are evaluating if the modulation of DR2 and AR β 2 on progressive MS patient (N=5) T lymphocytes ameliorate PMS-T cell mediated synaptopathy. While further studies are needed to fully dissect the underlying mechanisms, our data suggest a significant role for catecholamine receptors on T cells in modulating T cell-mediated synaptopathy in EAE and likely in MS by applying an advanced translational ex-vivo model of MS.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.03/B25

Topic: B.04. Synaptic Transmission

Support: GR-2018-12366154
CNR FOE 2020 and 2021 project 'Nuovi Biomarker Diagnostici e Terapeutici delle Malattie Neurodegenerative
Italian Ministry of Health, Ricerca Corrente IRCCS San Raffaele

Title: Physical activity alleviates immune-mediated synaptic dysfunction in a T cell-based ex vivo chimeric model of Multiple Sclerosis and in the EAE mouse model via vagal pathways

Authors: *F. MARIANI¹, A. MUSELLA^{2,3}, A. GENTILE⁴, C. PROCACCINI⁵, S. BALLETTA⁶, F. DE VITO⁶, A. TARTACCA¹, F. PALMERIO¹, A. BRUNO⁶, G. MANDOLESI^{2,3}, D. CENTONZE^{1,6};

¹Medicina dei sistemi, Univ. di Roma Tor Vergata, Roma, Italy; ²Synaptic Immunopathology Lab, IRCCS San Raffaele Roma, Rome, Italy; ³Dept. of Human Sci. and Quality of Life Promotion, Univ. of Rome San Raffaele, Rome, Italy; ⁴Dept. of Biomedicine and Prevention, Tor Vergata Univ., Rome, Italy; ⁵Natl. Res. Council (CNR), Naples, Italy; ⁶IRCCS Neuromed, Pozzilli, Italy

Abstract: Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease that primarily affects young adults. Autoreactive T and B cells infiltrate the central nervous system (CNS) following disruption of the blood-brain barrier, leading to inflammation, demyelination, axonal and synaptic damage, and progressive neurodegeneration. Physical exercise (PE) is

increasingly recommended—even in progressive MS(PMS)—to alleviate symptoms and improve quality of life, possibly by modulating immune and neuroinflammatory responses, although the underlying mechanisms remain unclear. Our hypothesis is that PE can ameliorate immune-mediated synaptopathy, a key pathogenic mechanism recently emerged in MS patients and in its animal model, experimental autoimmune encephalomyelitis (EAE). This study aimed to investigate whether PE modulates immunometabolism and immune-mediated synaptic dysfunction in MS, using both a chimeric human-mouse ex vivo MS model and the MOG₃₅₋₅₅ EAE model. We also explored vagal pathways as a potential neuroimmune mechanism mediating the protective effects of PE. PMS patients underwent a PE program, with clinical and neuropsychological assessments conducted before and after the intervention. Peripheral blood mononuclear cells (PBMCs) were isolated for immunometabolic profiling. In parallel, PMS T cells were incubated on murine cortico-striatal slices, and spontaneous glutamatergic transmission was recorded using patch-clamp technique (chimeric MS model). Furthermore, MOG₃₅₋₅₅ EAE was induced in female mice, which were subjected to voluntary PE (running wheels for 21 days). Flow cytometry analysis and mitochondrial metabolism on T cells were performed. qPCR and electrophysiology were conducted on the EAE striatum. To examine the involvement of neuroimmune pathways, a group of mice underwent cervical vagotomy. PE improved neuropsychological outcomes in PMS patients and partially restored mitochondrial function in their PBMCs. Pre-exercise T cells induced synaptic hyperexcitability, which was abolished after PE. In EAE mice, PE reduced T cell activation and proliferation, improving disease course and synaptic dysfunction. Cervical vagotomy diminished the protective effects of PE on both CNS inflammation and synaptic physiology. These findings suggest a vagus nerve-mediated neuroimmune mechanism underlying PE-induced neuroprotection. PE exerts neuroprotective effects in PMS by reshaping T cell immunometabolism and reducing synaptotoxicity. The involvement of the vagus nerve reveals a neuroimmune axis linking peripheral immunity to central synaptic health, offering novel therapeutic targets.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.04/B26

Topic: B.04. Synaptic Transmission

Support: Harmony Biosciences

Title: The effect of pitolisant on neurons of the ventrolateral preoptic area

Authors: *R. DE LUCA¹, S. NARDONE², T. E. SCAMMELL³, E. ARRIGONI⁴;

¹Beth Israel Deaconess Med. Ctr. - Harvard Med., Boston, MA; ²Bar Ilan Univ. Fac. of Med.,

Safed, Israel; ³Neurol., Beth Israel Deaconess Med. Ctr., Wellesley, MA; ⁴Neurol., BIDMC and Harvard Med. Sch., Boston, MA

Abstract: Pitolisant is an H3 receptor antagonist/inverse agonist indicated for the treatment of excessive daytime sleepiness in people with narcolepsy. It is currently believed to promote wakefulness by directly activating the cortex and other brain regions through release of histamine (HIS), noradrenaline (NA), and other neurotransmitters. However, wakefulness requires both the activation of arousal-promoting neurons and the inhibition of sleep-promoting neurons, including those in the ventrolateral preoptic (VLPO) nucleus. Sleep-active, sleep-promoting neurons in the VLPO express GABA and the neuropeptide galanin (VLPO^{Gal}), and these VLPO^{Gal} neurons are both necessary and sufficient for normal sleep. In this study, we tested the hypothesis that pitolisant may also promote wakefulness by inhibiting VLPO^{Gal} sleep-promoting neurons. To investigate the effects of pitolisant on VLPO neuronal circuitry, we performed whole-cell patch-clamp recordings in mouse brain slices. We found that pitolisant inhibited firing and hyperpolarized VLPO^{Gal} neurons ($n = 5$; from baseline: -6.24 ± 0.63 mV), although this effect was likely indirect as VLPO^{Gal} neurons do not express H3 receptors. Accordingly, the effect of pitolisant on VLPO^{Gal} neurons was significantly reduced in the presence of the GABA_A receptor antagonist bicuculline ($n = 6$; -3.19 ± 0.54 mV), indicating that GABA signaling contributes to pitolisant's effect on VLPO^{Gal} neurons. Pitolisant indirectly inhibits VLPO^{Gal} neurons by activating local GABAergic interneurons, which in turn inhibit VLPO^{Gal} neuron activity ($n = 4$). These interneurons express H3 receptors and inhibit VLPO^{Gal} neurons through local axonal collaterals. Moreover, pitolisant increases local NA levels, which directly inhibits VLPO^{Gal} neurons via $\alpha 2$ -adrenergic receptors, and indirectly via $\alpha 1$ -adrenergic receptors by activating the same GABAergic interneurons targeted by pitolisant. Accordingly, the $\alpha 2$ -adrenergic receptor antagonist yohimbine plus bicuculline, nearly abolished pitolisant's inhibitory effect on VLPO^{Gal} neurons ($n = 6$; -0.76 ± 0.38 mV). These results suggest that, in addition to direct activation of wake-promoting regions, pitolisant also promotes arousal by inhibiting sleep-promoting neurons in the VLPO. Pitolisant's actions in the VLPO involve activation of local GABAergic circuits via H3 receptors plus and the release of NA, both of which contribute to the inhibition of VLPO^{Gal} neurons.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.05/B27

Topic: B.04. Synaptic Transmission

Support: NIH Grant 5R01NS134128-23

Title: Igf-1r modulation of spontaneous neurotransmitter release

Authors: *B. CIMEN^{1,2}, E. T. KAVALALI^{1,2};

¹Dept. of Pharmacology, Vanderbilt University, Nashville, TN, Nashville, TN; ²Vanderbilt Brain Institute, Vanderbilt Univ., Nashville, TN

Abstract: The insulin-like growth factor 1 receptor (IGF-1R) is a receptor tyrosine kinase known to regulate neuronal survival, development, and excitability through diverse intracellular signaling cascades. While its role in activity-dependent plasticity is well established, its role in regulation of spontaneous synaptic transmission remains poorly understood. To examine the influence of IGF-1R on spontaneous neurotransmission, we performed whole cell recordings and live imaging in hippocampal neurons. Pharmacological inhibition of IGF-1R with AG1024 (1 μ M, 15-minute acute application) resulted in ~3-fold increase in mEPSC frequency and a ~2-fold increase in mIPSC frequency, suggesting that IGF-1R activity exerts a tonic inhibitory effect on basal spontaneous release. These findings are unexpected in the context of IGF-1R's canonical excitatory and neurotrophic functions. Importantly, this response provides a unique functional readout that can be leveraged as a tool to dissect how IGF-1R-dependent signaling pathways interface with the presynaptic release machinery. By mapping the mechanistic links between growth factor signaling and spontaneous neurotransmission, this approach will inform the development of novel therapeutic strategies targeting synaptic imbalance in neurological disorders.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.06/B28

Topic: B.04. Synaptic Transmission

Support: NSFC

Title: Inhibitory presynaptic cholinergic modulation on synaptic transmission at the Calyx of Held Synapses

Authors: *J. SUN¹, P. CHEN², Y. ZHENG³;

¹Shenzhen Univ. of Advanced Technol., Shenzhen City, China; ²Shenzhen Institutes of Advanced

Technology, Chinese Acad. of Sci., Shenzhen City, China; ³Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Acetylcholine (ACh) is considered as an excitatory neurotransmitter in the peripheral and central nervous systems and significantly functions in sleep, wakefulness, attention, learning, memory, and sensory information processing. This study investigates the presynaptic regulation of ACh at the Calyx of Held synapse in medial nucleus of trapezoid body of the mice auditory brainstem. It was shown that application of ACh or nicotine induced depolarization at calyceal terminals, indicating the existence of presynaptic nicotinic ACh receptors (nAChR) in the calyx of Held synapses. Postsynaptic recording showed that the application of ACh or nicotine could increase the frequency of miniature excitatory postsynaptic currents (mEPSC) but significantly reduce the amplitude and total charge of excitatory postsynaptic currents (EPSC). Furthermore, paired-pulse ratio (PPR) was found increased, displaying the presynaptic inhibitory effect of ACh on the synaptic transmission. By taking advantage of the giant size of presynaptic terminal of the Calyx of Held synapses, we observe the action potentials at presynaptic terminals with and without ACh application. It was found that ACh-induced depolarization significantly reduced the action potential amplitude at the presynaptic terminals and thus decreased calcium influx to cause the down-regulation of the glutamatergic vesicle release from the calyceal terminals. Our study suggests that ACh can play an inhibitory role on presynaptic transmitter release via axo-axonic nicotinic synapse although nAChRs are traditionally considered to mediate excitatory signaling.

Disclosures: J. Sun: None. P. Chen: None. Y. Zheng: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.07/B29

Topic: B.04. Synaptic Transmission

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NIH 5T32DA007234-37

Title: Astrocytes respond to serotonin and regulate serotonin-induced synaptic transmission in the amygdala

Authors: *J. A. NOEKER, R. FALCON MOYA, P. KOFUJI, A. ARAQUE;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Serotonin is a neuromodulator involved in many physiological processes, including cognition and emotional responses, and it has been implicated in psychiatric diseases such as anxiety and PTSD. While the investigation into serotonin signaling has focused on neurons, the

effects of serotonin on astrocyte activity remain largely unknown. Using two-photon Ca^{2+} imaging, electrophysiology, pharmacology, chemogenetic, and optogenetic approaches in amygdala brain slices, we have investigated whether astrocytes in the amygdala respond to serotonin and the consequent neuromodulatory effects of serotonin-mediated astrocyte activation. We have found that astrocytes respond to exogenous and synaptically released serotonin with intracellular Ca^{2+} elevations. This serotonin-evoked astrocyte Ca^{2+} signal is associated with the regulation of excitatory synaptic transmission. These effects were dependent on astrocyte calcium activity, as this was mediated by IP3R2 conditional knockout mice. We conclude that astrocytes in the amygdala respond to serotonin with intracellular Ca^{2+} elevations, which stimulate the release of the gliotransmitters that regulate excitatory synaptic transmission. Therefore, astrocytes respond to serotonin and regulate synaptic transmission in the amygdala.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

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Program #/Poster #: PSTR317.08/B30

Topic: B.04. Synaptic Transmission

Support: NINDS R00 (CEV) N5119783

Title: Comparative analysis of synaptic dynamics in the ventrolateral and dorsomedial columns of the periaqueductal gray

Authors: *A. CASTRO ROMERO¹, K. A. MCCABE², J. N. CARROLL¹, C. E. VAAGA¹;
¹Biomed. Sci., ²Colorado State Univ., Fort Collins, CO

Abstract: The midbrain periaqueductal gray (PAG) is divided into anatomically and functionally distinct columns involved in threat assessment and organization of fear behavior. The PAG has traditionally been viewed primarily as a relay center, with distinct columns differentially driving active (dorsomedial, dmPAG) and passive (ventrolateral, vlPAG) coping behaviors in mice in response to threatening stimuli, such as fleeing and freezing, respectively. However, recent in vivo studies have begun to challenge this view, highlighting the PAG as an important center for sensorimotor integration, particularly in the context of fear processing; underscoring the need to discern how each column processes incoming information. Thus, this project aims to understand the synaptic dynamics across the ventrolateral (vl) and dorsomedial (dm) columns of the PAG, using slice electrophysiology to elucidate potential differences in synaptic integration across columns. We demonstrate that in both columns, spontaneous inhibition is stronger than excitation. Furthermore, both columns show remarkably stable presynaptic release probability, with paired-pulse ratios near 1 across interstimulus intervals. These findings suggest that both columns engage in frequency-invariant coding strategies for information processing. In contrast, high-frequency stimulation reveals a significant difference in synaptic dynamics across columns.

More specifically, in the dmPAG, high-frequency stimulation results in a pronounced slow current during the stimulation train with an extended relaxation which leads to persistent excitation and an extended window of elevated firing. Taken together, these results suggest that while both PAG columns engage in similar frequency-invariant synaptic coding, they show distinct synaptic dynamics in response to high-frequency stimulation that could differentially impact synaptic integration within each column of the PAG.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

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Topic: B.04. Synaptic Transmission

Support: NIAAA R01 AA028566
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F31 AA-032198-01
P50 AA017823

Title: Prenatal alcohol effects on acute alcohol regulation of GABAergic transmission onto CeM CRFR1+ and CRFR1- neurons in adults

Authors: *S. WINCHESTER¹, M. R. DIAZ²;
¹Behavioral Neurosci., State Univ. of New York, Binghamton, Binghamton, NY; ²Psychology, Binghamton Univ., Binghamton, NY

Abstract: Prenatal alcohol exposure (PAE) can lead to a wide range of adverse outcomes. In particular, individual's exposed to alcohol *in utero* are at a significantly higher risk for developing alcohol use disorder (AUD) later in life. The corticotropin releasing factor (CRF) system is highly implicated in the development of AUD. CRF and its receptor (CRFR1), in part, mediate the effects of alcohol (EtOH) on GABAergic transmission in the central amygdala (CeA) of adult male rodents. Specifically, EtOH (44mM) increases GABA release in the CeA which is blocked by CRFR1 antagonism. Interestingly, our lab has shown a sex-specific disruption in CRFR1 function and expression in medial nucleus of the CeA (CeM) following *moderate* PAE in adolescent animals. It is unclear if PAE alterations to the CRF system persist into adulthood or alter the actions of acute EtOH on GABAergic transmission. The aim of this study was to investigate how mPAE may alter acute EtOH modulation of GABAergic neurotransmission in CRFR1+ and CRFR1- CeM neurons in adult offspring (P80-105) rats. To test this, wildtype females were bred with CRFR1-Cre-^{td}Tomato males. Pregnant dams were exposed to vaporized ethanol or room air (control) on gestational day 12 (G12) for 6 hours and whole-cell electrophysiology was performed in the CeM to assess the actions of acute EtOH (44,

66, & 88 mM) on GABAergic transmission onto CRFR1+ and CRFR1- neurons. In control males, we found acute EtOH increased GABA release onto CRFR1+ cells at the highest EtOH concentration (88 mM), an effect not present in PAE males; however, GABA release was decreased in PAE males at the lowest concentration (44 mM). Similar to PAE males, GABA release was decreased onto CRFR1+ neurons in PAE females at 44mM, with control females appearing to be insensitive to the effects of acute EtOH. Additionally, GABA release was decreased onto CRFR1- in control males at the middle concentration (66 mM), with no effect of acute EtOH in PAE males. Finally, PAE females exhibited a decrease of GABA release onto CRFR1+ at the lowest and middle EtOH concentrations (44 and 66mM), while control females were insensitive to acute EtOH. This study contributes to the expanding body of research exploring the effects of PAE and how a single exposure can impact neurophysiological mechanisms in brain regions associated with AUD development. Ongoing studies are investigating how PAE may interact with the actions of acute EtOH to alter synaptic function in a downstream target of the CeM, the bed nucleus of the stria terminalis (BNST).

Disclosures: S. Winchester: None. M.R. Diaz: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.10/B32

Topic: B.04. Synaptic Transmission

Title: Involvement of Toll-like receptors 7/8 on the neurophysiological imbalances at the perforant path dentate granule cell synapses in a model of maternal immune activation

Authors: *D. LAVERDE ESTRADA, E. J. GALVAN;
Farmacobiologia, CINVESTAV SUR, Mexico City, Mexico

Abstract: Prenatal Stress induced by Maternal Immune Activation (PS- MIA) is a risk factor linked to neurodevelopmental alterations and the onset of psychiatric disorders in the offspring, including autism and schizophrenia. During pregnancy, PS-MIA triggered by bacterial infections typically activates innate immune signaling via Toll-like receptor 4 (TLR4), whereas double-stranded viral infections predominantly activate the TLR3 pathway. However, single-stranded viruses, such as influenza, Rubella, and SARS-CoV-2 viruses, primarily stimulate TLR7/8 signaling, which is considered a key immunological mechanism driving neurodevelopmental dysregulation. In this study, we investigated the effects of PS-MIA triggered by i.p. administration of the TLR7/8 selective agonist, resiquimod (RQ 2mg/kg), on gestational day 15 in pregnant dams and evaluated them in the offspring. Acute hippocampal slices were prepared from the offspring at postnatal days 30±2, and extracellular recordings were conducted at the lateral perforant path (LPP) - dentate gyrus (DG) granule cell synapses to assess changes in the synaptic strength and the synchronized firing discharge (population spike, PS). The fEPSP was depressed in the presence of L-AP4 (10 µM), whereas the PS was blocked in the presence of

TTX. These criteria were systematically used to accept or reject evoked responses. I-O curves at the LPP-DG synapse revealed a $\approx$ two-fold increase in the fEPSP, accompanied by increased paired-pulse ratio (ISI: 60 ms) and desynchronization between the presynaptic fiber volley and the fEPSP ratio, indicating presynaptic alterations in response to RQ. Analysis of the temporal summation of LPP inputs to granule cells (10 fEPSPs elicited at 5, 20, and 50 Hz) uncovered a dynamic dysregulation in the filtering mechanism of DG in response to RQ. At the somatic level, the population spikes exhibited increased amplitude, accompanied by changes in the onset latency of the spikes. These experiments show that prenatal activation of TLR7/8 in the pregnant dam alters the synaptic inputs from the entorhinal cortex and affects the integration capabilities of the granule cells in the dentate gyrus.

Disclosures: D. Laverde Estrada: None. E.J. Galvan: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.11/B33

Topic: B.04. Synaptic Transmission

Support: UG3 DA048385/DA/NIDA grant

Title: The G-protein coupled receptor GPR151 in the medial habenula regulates synaptic plasticity and nicotine and opioid responses

Authors: *K. PARK¹, B. ANTOLIN-FONTES¹, K. LI¹, J. L. ABLES^{2,1}, N. HEINTZ¹, P. J. KENNY², I. IBANEZ-TALLON¹;

¹The Rockefeller Univ., New York, NY; ²Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The medial habenula (MHb)-interpeduncular nucleus (IPN) circuit is a central pathway implicated in the regulation of nicotine and opioid addiction. The orphan G-protein coupled receptor 151 (GPR151) is selectively enriched in the MHb, but its functional role in this addiction-relevant circuit remains unclear. Here, we investigated the impact of GPR151 on synaptic transmission and drug-related behaviors. Using immunohistochemistry, mass spectrometry, and electrophysiological recordings, we confirmed that GPR151 is highly localized to MHb neurons and their projections to the IPN. We found that GPR151 couples to the Gαo1 protein, leading to reduced cAMP levels and modulation of presynaptic vesicle release. In *Gpr151* knockout mice, we observed altered synaptic plasticity within the MHb-IPN circuit, including increased miniature excitatory postsynaptic current (mEPSC) frequency, decreased spontaneous EPSC (sEPSC) frequency, and reduced evoked EPSC (eEPSC) amplitude compared to wildtype controls. Behaviorally, *Gpr151* knockout mice exhibited diminished responses to both nicotine and opioids, including increased drug self-administration and blunted synaptic adaptations typically associated with these substances. Our findings identify GPR151 as a key regulator of synaptic function in the MHb-IPN pathway and demonstrate its critical role in

modulating vulnerability to nicotine and opioid addiction. These results suggest that targeting GPR151 may offer a novel therapeutic strategy for substance use disorders involving this shared neural circuit.

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Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.12/B34

Topic: B.04. Synaptic Transmission

Support: NSF IOS 157474
NIH R01 NS 128713

Title: Characterizing a synaptic connection between BLA and TRN

Authors: ***M. C. BAUER**¹, M. J. VAUGHN², E. FORREST⁴, Z. LASWICK⁵, M. N. GEFFEN⁶, J. S. HAAS³;

¹Lehigh Univ. Biol. Sci., Bethlehem, PA; ³Dept. of Biol. Sci., ²Lehigh Univ., Bethlehem, PA;

⁴Harvard Med. Sch., Cambridge, MA; ⁵Biomed. Engin., Northwestern Univ., Cook County, IL;

⁶Otorhinolaryngology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Detecting and attending to threatening stimuli is an essential mechanism for survival that has been evolutionarily conserved and is acquired through rapid learning. Activity of the basolateral amygdala (BLA) is thought to be responsible for the development of fear responses through bidirectional plasticity during fear learning paradigms (Gründemann, 2020). Although the BLA itself does not appear to gate attention to the sensory surround, a handful of results indicate that the BLA innervates the thalamic reticular nucleus (TRN), an area tasked with such a feat. TRN neurons are the main source of inhibitory input to thalamocortical relay and thereby act as an inhibitory interface between the sensory environment and cortex. Our recent work showed that optogenetic activation of BLA amplifies tone responses in auditory thalamus and cortex, acting through TRN (Aizenberg et al., 2019). Here we used viral tracing, optogenetics, and electrophysiological techniques to identify the synaptic foundation of this connection. After injecting channelrhodopsin into BLA, we recorded from single neurons in brain slices containing TRN. Optogenetic stimulation of BLA terminals drove a low latency multi-component excitatory response in TRN neurons, with both fast and slow components, as well as spikelets and dendritic calcium spikes. Responses were also sensitive to NBQX and AP-5. Our data lead us to conclude that this synapse is monosynaptic, glutamatergic and mediated by both AMPA and NMDA receptors. Observations of spikelets implies that BLA stimulation drives spiking in electrically coupled TRN neighbors. Anterograde and retrograde viral tracing, in PV and SOM Cre and Flp lines, revealed that BLA projections to TRN are not cell-type specific but are sparse, consistent

with the sparsity of responses in our recordings. We hypothesize that this link between the BLA and TRN could provide a circuit mechanism for directing cortical attention towards emotionally salient sensory stimuli during fear learning.

Disclosures: M.C. Bauer: None. M.J. Vaughn: None. E. Forrest: None. Z. Laswick: None. M.N. Geffen: None. J.S. Haas: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.13/B35

Topic: B.04. Synaptic Transmission

Title: Evaluating CaMKII β 's role in synaptic transmission and homeostatic plasticity

Authors: *A. WEIT^{1,2}, E. T. KAVALALI^{1,2}, L. M. MONTEGGIA^{1,2};

¹Vanderbilt Brain Inst., Nashville, TN; ²Dept. of Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: Changes in synaptic strength (i.e. plasticity) are fundamental processes that allow adaptation to environmental influences. Long-term potentiation (LTP) and depression (LTD) are types of Hebbian plasticity, in which activity strengthens specific synaptic connections. Calcium/calmodulin-dependent protein kinase II (CaMKII) is a kinase that phosphorylates serine/threonines in target proteins and is an important regulator of synaptic plasticity and learning and memory. CaMKII has two isoforms, α and β , that are highly expressed in the hippocampus. CaMKII α through its kinase function is a critical sensor of activity-driven synaptic transmission and regulator of LTP. In contrast, CaMKII β supportively traffics CaMKII α independently of its kinase function. Previous studies suggest that CaMKII β may be involved in homeostatic plasticity, a different form of plasticity, which counters long-term global changes in activity to maintain a homeostatic environment. CaMKII β , in contrast to CaMKII α , is also sensitive to low amounts of calcium/calmodulin and its protein expression is enriched in the absence of activity (i.e. tetrodotoxin (TTX) is used to block action potentials). These findings led us to hypothesize that CaMKII β may be a key regulator in mechanisms of spontaneous transmission-mediated homeostatic upscaling. To test this hypothesis, we have been evaluating the impact of CaMKII β expression and enzymatic activity in hippocampal neurons using whole cell patch clamp electrophysiology on basal spontaneous neurotransmission and upscaling in postsynaptic homeostatic mechanisms. We are also using confocal microscopy to investigate the understudied basal function of CaMKII β in the presynapse. Together, we seek to provide novel insight into the role of CaMKII β in synaptic transmission and synaptic homeostasis.

Disclosures: A. Weit: None. E.T. Kavalali: None. L.M. Monteggia: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.14/B36

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH112729
NIH Grant AA027023

Title: Role for Rem2 in dopaminergic regulation of transmitter release

Authors: ***R. P. D. ALEXANDER**, K. J. BENDER;
UCSF, San Francisco, CA

Abstract: Neuromodulators fine-tune the activity of neural circuits by affecting the strength of synaptic transmission. Suppression of voltage-gated calcium channels (Cav) decreases the probability of transmitter release from presynaptic terminals, which often affects short-term plasticity (STP) of subsequent responses. Previous work from our lab found that type 1 dopamine (D1/D5) receptor signaling suppressed glutamate release at prefrontal synapses without the expected changes in STP, owing to distinctive regulation of presynaptic Cav gating behavior. This signaling mode allows strong, input-specific modulation of transmission, even during high frequency activity, and may help explain previous apparently paradoxical findings at other synapses. However, the specifics of channel regulation following receptor activation remain mysterious. Here we investigated noncanonical D1/D5 receptor modulation at another central synapse and uncovered the underlying molecular mechanism of its distinct Cav regulation. In subiculum pyramidal neurons, application of dopamine and D1/D5-selective agonists reduced excitatory currents by decreasing presynaptic release probability with no observed changes to STP. During trains of repetitive stimuli, response depression was consistent throughout each bout regardless of input frequency. Hippocampal knockout of the Ras-related RGK GTPase, Rem2, prevented dopaminergic modulation, indicating its necessity for downstream inhibition of presynaptic Cav, which is reminiscent of RGK-dependent adrenergic pathways in cardiac tissue. Elimination of presynaptic Rem2 also significantly attenuated D1/D5 receptor modulation in L5 prefrontal cortex, demonstrating a common role across excitatory circuits. Rem2 is expressed throughout the brain and may be a molecular signature for a synapse's propensity for presynaptic gain control, representing a new kind of input-driven neuromodulation of hippocampal and cortical circuits.

Disclosures: **R.P.D. Alexander:** None. **K.J. Bender:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Regel Tx, BioMarin Pharmaceutical.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.15/B37

Topic: B.04. Synaptic Transmission

Title: Dopaminergic D2 receptor modulation of NMDA receptor signaling in the nucleus accumbens

Authors: *J. ADLER¹, S. GONG², C. FORD³;

¹Pharmacol., Univ. of Colorado, Anschutz Med. Neurosci. Grad. Training Program, Aurora, CO;

²Janelia Res. Campus, Ashburn, VA; ³Univ. of Colorado, Aurora, CO

Abstract: Striatal dopamine signaling critically regulates neuronal excitability and synaptic plasticity underlying motivation-based decision making and reward learning. Midbrain dopamine neurons project to the nucleus accumbens (NAc) region of the ventral striatum, where both dopamine and glutamate synaptic inputs converge at medium spiny neurons (MSNs). Here, dopamine modulates glutamatergic signaling of post-synaptic NMDA receptors (NMDARs) to regulate neuronal activity and cellular excitability. Here, we examine the cellular and synaptic mechanisms of D2-type dopamine receptors (D2Rs) in modulating NMDAR current in D2-MSNs. Using ex-vivo slice electrophysiology in combination with optogenetics and iontophoresis, we found NAc D2Rs inhibit NMDA-type glutamate receptors (NMDARs) via a putative direct interaction with GluN2B containing subunits, which was independent of canonical intracellular second messenger pathways, and could be potentiated following chronic administration of cocaine. This modulation occurred in a synapse-specific manner which was set by the amount of glutamate spillover and played an important role in controlling the extent of striatal synaptic plasticity and active avoidance aversive learning. We propose that NMDAR modulation by D2Rs in the NAc occurs through non-canonical, direct dimerization between these two receptors, which function to regulate striatal glutamatergic transmission, plasticity and learning.

Disclosures: J. Adler: None. S. Gong: None. C. Ford: None.

Poster

PSTR317: Pre- and Postsynaptic Modulation II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR317.16/B38

Topic: B.04. Synaptic Transmission

Support: 2025/MOB/01740

Title: Ketamine enhances glutamatergic drive onto mPFC-Projecting DRN Neurons

Authors: *C. HMAIED^{1,2}, S. OUBRAIM², S. KOULCHITSKY¹, I. GLADWYN-NG³, S. HAJ-DAHMANE², V. SEUTIN¹;

¹Univ. of Liège, Liege, Belgium; ²Dept. of Pharmacol. and Toxicology, Jacobs Sch. of Med. and Biomed. Sci., Univ. at Buffalo, Buffalo, NY; ³Taconic, Koeln, Germany

Abstract: Ketamine (KET), a non-competitive NMDA receptor antagonist, has recently gained attention for its rapid and sustained antidepressant effects following a single dose. However, the underlying mechanisms by which ketamine regulates the function of dorsal raphe nucleus (DRN) serotonin (5-HT) neurons, a key brain area involved in the regulation of depression, remain poorly understood. In this study, using ex-vivo whole cell recordings from rats and mice, we examined the effects of KET on synaptic transmission onto DRN 5-HT neurons. We found that KET (10 μ M) enhanced the frequency and amplitude of spontaneous excitatory α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated synaptic currents (sEPSCs) in a half of 5-HT neurons recorded, with the remaining neurons unaffected. Interestingly, KET had no effect on the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs), suggesting that the synaptic effect of KET depends on the neuronal network activity. To test this notion, we assessed the effects of KET on evoked AMPA-mediated EPSCs (eEPSCs) and found that KET induced a potentiation of their amplitude, along with a decrease on both paired-pulse ratio (PPR) and coefficient of variation (CV). Therefore, suggesting that KET potentiates DRN 5-HT neurons excitatory synapses by increasing presynaptic glutamate release. To identify the specific DRN 5-HT neurons responsive to KET, we used viral retrograde mapping to target DRN 5-HT projecting to the mPFC (DRN-mPFC) and found that glutamate synapses impinging onto these neurons are invariably potentiated by KET. Further experiments on 5-HT neurons projecting to other areas are underway. Taken together, these results indicate that KET exerts a cell-specific presynaptic potentiation of glutamate synapses in the DRN.

Disclosures: C. Hmaied: None. S. Oubraim: None. S. Koulchitsky: None. I. Gladwyn-Ng: None. S. Haj-Dahmane: None. V. Seutin: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.01/B39

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NINDS Center on Compulsive Behaviors Fellowship Award

Title: Identification and interrogation of novel long-range projecting hippocampal somatostatin interneurons across species

Authors: *A. VLACHOS^{1,2}, N. MCLEAN³, A. CACCAVANO⁵, G. A. VARGISH⁴, L. T. HEWITT⁶, X. YUAN⁷, S. HUNT¹, E. FURLANIS¹⁰, Y. WANG¹⁰, M. DAI¹², J. WU¹³, D. DWIVEDI¹⁵, J. DIMIDSCHSTEIN¹¹, M. A. ELDRIDGE¹⁶, B. B. AVERBECK¹⁷, G. J. FISHELL¹⁴, K. A. PELKEY⁸, C. J. MCBAIN⁹;

¹NICHD/NIH, Bethesda, MD; ²Brown Univ., Providence, RI; ³NICHD, 20892-3715, MD; ⁴Section on Cell. and Synaptic Physiol., NICHD, Bethesda, MD; ⁵Section on Cell. and Synaptic Physiol., NIH/NICHD, Washington, DC; ⁶Section on Cell. and Synaptic Physiol., NIH, NICHD, Bethesda, DC; ⁷NIH, Bethesda, MD; ⁸NICHD/LCSN, NIH, Silver Spring, MD; ⁹Lab. Cell/Molec Neurosci, NIH, Bethesda, MD; ¹⁰Broad Inst., Boston, MA; ¹¹Broad Inst., Cambridge, MA; ¹²Broad Inst. of MIT and Harvard, Cambridge, MA; ¹³Neurobio., Harvard Med. Sch., Boston, MA; ¹⁴Harvard Med. Sch., Jamaica Plain, MA; ¹⁵Harvard Med. Sch. Grad. Program In Neurosci., Boston, MA, ; ¹⁶Lab. of Neuropsychology, Newcastle Univ., Ponteland, United Kingdom; ¹⁷NIMH/NIH, Kensington, MD

Abstract: Though numerically sparse, a remarkably heterogeneous population of GABAergic inhibitory interneurons critically regulates information flow and processing amongst projection neurons of forebrain circuits. Uncovering inhibitory interneuron subtype-specific characteristics is imperative in understanding cellular components of cognitive processes underlying behavior. Furthermore, while interneuron diversity is best characterized in common experimental model organisms, such as rodents, the degree of conservation/divergence of discrete interneuron subpopulations in higher species is understudied but required to understand human brain function in health and disease. Using a novel enhancer-based cell-type specific labelling adeno-associated virus (BiSSTe10) injected into the hippocampus of mice and macaques, we discovered a subpopulation of previously undescribed hippocampal somatostatin interneurons (SST-INs) with unique molecular, anatomical, and electrical properties. BiSSTe10 labelled SST-INs co-stain for SST but not parvalbumin. Acute slice patch clamp recordings of BiSSTe10 cells intrinsic properties show a bimodal distribution of maximum sustained firing frequencies that span from regular-spiking (<100 Hz) to fast-spiking (>200 Hz) firing patterns. Additionally, cells exhibit a voltage sag at the onset of a hyperpolarizing step, indicative of HCN-channel current. This suggests there are two subpopulations of hippocampal stratum oriens BiSSTe10 interneurons in both mouse and macaque. Whole brain tissue clearing in mice reveals labelled SST-INs form extrahippocampal projections to the medial septum (MS) and demonstrate GABAergic output to MS cells. Ongoing experiments are monitoring calcium transients via enhancer AAV delivered GCaMP6f in hippocampal BiSSTe10 cells using two-photon imaging *in vivo* to determine the activity of MS-projecting SST-INs during behavior. Identification and investigation of this unique cell type provides insight to the hippocampal-septal circuit, and the diverse functions of SST-IN subtypes. This characterization sets the foundation to perturb this species-conserved discrete SST-IN population and discover its potential role in higher order neural circuits and human disease.

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Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.02/B40

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH NICHD Intramural Grant

Title: Cross-species electrophysiological comparison of fast-spiking inhibitory neurons across multiple brain regions

Authors: *L. T. HEWITT¹, R. CHITTAJALLU², A. CACCAVANO³, G. A. VARGISH⁴, G. PIZZIRUSSO⁵, A. VLACHOS⁷, N. MCLEAN⁶, X. YUAN⁸, S. HUNT¹¹, B. B. AVERBECK¹², K. A. ZAGHLOUL¹³, K. A. PELKEY⁹, C. J. MCBAIN¹⁰;

¹NIH, NICHD, Bethesda, MD; ²Lab. of Cell. and Synaptic Neurophysiol., NICHD, NIH, Bethesda, MD; ³Section on Cell. and Synaptic Physiol., NIH/NICHD, Washington, DC; ⁴Section on Cell. and Synaptic Physiol., ⁵NICHD, Bethesda, MD; ⁶NICHD, 20892-3715, MD;

⁷NICHD/NIH, Bethesda, MD; ⁸NIH, Bethesda, MD; ⁹NICHD/LCSN, NIH, Silver Spring, MD;

¹⁰Lab. Cell/Molec Neurosci, NIH, Bethesda, MD; ¹¹Natl. Inst. of Hlth., Bethesda, MD;

¹²NIMH/NIH, Kensington, MD; ¹³NINDS, Bethesda, MD

Abstract: Mouse models have been historically used in neuroscience research to determine potential therapeutic targets for human neurophysiological disease. However, there is a surprising lack of literature describing the overlap in the underlying function of mouse neurons compared to other species, including non-human primates and humans. It has become increasingly valuable to characterize similarities and innovations in non-human primate and human brain tissue in comparison to the rodent to determine the translational relevance of mouse physiology. Inhibitory neurons are vastly diverse and exhibit numerous sub-types, each providing exquisite spatio-temporal control over network dynamics. The sparse representation of GABAergic neuron subtypes has made them inherently difficult to study without targeted labeling through genetic/molecular approaches. Immense amounts of research indicate these inhibitory neurons are altered in disease states in the mouse brain; however, whether these neurons exhibit similar aberrant and baseline properties in humans and non-human primates is poorly understood. We investigated intrinsic properties using regional specific injections of the virus S5E2, which selectively labels fast-spiking (FS) parvalbumin neurons, in tandem with ex-vivo whole-cell patch clamp electrophysiology recordings. We recorded FS neurons in hippocampus and cortex across three species: mouse, macaque, and humans, in addition to recordings of striatal FS neurons in the mouse and macaque. Intrinsic electrophysiological properties from these experiments were placed in a non-biased k-means clustering algorithm to determine if regional and species dependent differences result in separation of FS neurons into discrete clusters. Recordings from humans, macaque, and mouse in the hippocampus and cortex divide into reliable populations of species-specific clusters. Data from mouse and macaque recordings in the striatum reveal a separation of mouse and macaque FS neurons. We also tested the intrinsic integration properties of FS neurons across species and regions using a simulated inhibitory input 'pause' protocol. Mouse FS neurons reliably exhibit a pause in high frequency firing in response to the pause protocol in the hippocampus, cortex, and striatum. Interestingly, macaque FS neurons do not exhibit the same pause response in the hippocampus and cortex. However, striatal FS neurons in the macaque do show a pause of high frequency firing. Our data

indicate the convergence and divergence of physiological properties exist in region and species dependent manners.

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Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.03/B41

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH grant R01MH131721

Title: Investigating the Circuit-Level Impact of Cell-Type Specific mGlu5 Ablation in Parvalbumin Interneurons

Authors: *L. PEETERS¹, B. A. GRUETER²;

¹Vanderbilt Univ. Med. Ctr., Nashville, TN; ²Anesthesiol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Cell-type specific ablation of mGlu5 receptors from parvalbumin interneurons (PVs) results in behavioral and physiological phenotypes that are consistent with neurodevelopmental disorders such as schizophrenia. Studies of PV-mGlu5 deletion have noted a brain region- and sex-specific reduction in PV number, and alterations in the formation and regulation of the perineuronal nets that surround these cells. PV-mGlu5 ablation has additionally been found to abrogate well-known sex differences in PV excitability in the medial prefrontal cortex. A reduction in the frequency of inhibitory post synaptic currents (IPSCs) in pyramidal neurons of the CA1 region of the hippocampus has additionally been reported. However, the impact of PV-mGlu5 ablation on the physiological properties of PVs and principal cells in other brain regions has been limitedly explored. The current study therefore sought to characterize the effects of PV-mGlu5 deletion by interrogating the excitability of PVs, as well as the balance of excitation and inhibition (E/I) within the principal cells they modulate in different brain regions. The effect of a heterozygous deletion of mGlu5, a more clinically translational manipulation, will additionally be interrogated. Similar to recent findings in the medial prefrontal cortex (mPFC), it is expected that PVs in the nucleus accumbens (NAc) with reduced mGlu5 expression will display altered excitability in a sex-dependent manner. As a result, it is expected that pyramidal neurons of the mPFC and medium spiny neurons in the NAc will display an E/I imbalance. This investigation will elucidate a critical role for mGlu5 receptors in the maturation of circuits implicated in various neuropsychiatric disorders, including schizophrenia.

Disclosures: L. Peeters: None. B.A. Grueter: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.04/B42

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NS10533
FNS119282A

Title: Two-pore potassium (K_{2P}) channels mediate plasticity of intrinsic excitability in L2/3 somatostatin interneurons in sensory cortex

Authors: *B. BOHANNON¹, D. FELDMAN²;

¹Neurosci., Berkeley, Oakland, CA; ²Neurosci., UC Berkeley, Berkeley, CA

Abstract: Experience drives robust plasticity of inhibitory circuits in sensory cortex, but the mechanisms for inhibitory circuit plasticity remain poorly understood. Somatostatin interneurons (SST INs) are a heterogeneous class of interneurons that preferentially target pyramidal cell (PYR) dendrites and may gate PYR plasticity. The extent and mechanisms of plasticity within sensory cortical SST INs themselves, however, have not been thoroughly studied. In hippocampus, SST INs exhibit plasticity of intrinsic excitability (IE) that is mediated by altered expression or functional regulation of voltage-gated ion channels including $K_{V7.2/3}$ and HCN channels (Incontro et al., 2021; Sammari et al., 2022). Whether sensory cortical SST INs also exhibit IE plasticity, and whether this occurs through similar or unique mechanisms, is unknown. In whole-cell current-clamp experiments, we found that 3 days of D-row whisker deprivation decreased IE of L2/3 SST INs in somatosensory cortex, depressing F-I curves by ~40%, accompanied by reduced input resistance, elevated rheobase, and reduced action potential bursting. We sought to understand the biophysical mechanisms that give rise to this reduction in SST IE. Deprivation did not change neuronal M-current (mediated by $K_{V7.2/3}$ channels) nor voltage sag (mediated by HCN channels). This suggests that SST INs in L2/3 of sensory cortex have different biophysical mechanisms for plasticity than in hippocampus. We used immunohistochemistry (IHC) to test for changes in $K_{V1.1}$ protein expression in SST INs, which is a known mechanism for deprivation-induced plasticity in parvalbumin (PV) INs in somatosensory cortex. We found no significant changes in $K_{V1.1}$ expression in SST INs, consistent with the stability of spike threshold after deprivation. We next examined leak potassium conductance (K_{leak}), which has been implicated in activity-dependent plasticity of IE in cultured cortical SST neurons. In whole-cell voltage-clamp, we quantified K_{leak} , which is predominantly mediated by two-pore potassium (K_{2P}) channels. We found that 3 days of D-row whisker deprivation significantly increased the K_{2P} current, measured as the Ba^{2+} -insensitive component of K_{leak} . Using IHC, we directly observed a significant increase in expression of the K_{2P} channel TRESK in SST INs after deprivation. We are currently testing whether increased TRESK expression is specific to Martinotti- vs. non-Martinotti SST cell subtypes. Thus, sensory deprivation reduces SST IE in sensory cortex by elevating K_{2P} current, adding to the diversity of

region- and cell-type specific biophysical plasticity mechanisms in different classes of interneurons.

Disclosures: B. Bohannon: None. D. Feldman: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.05/B43

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: KAKENHI 24H02336, 25K09842

Title: Simulation test for use-dependent changes in energy demand of axonal spikes on hippocampal mossy fiber

Authors: *H. KAMIYA;

Neurobio., Hokkaido Univ. Grad. Sch. of Med., Sapporo, Japan

Abstract: Axonal spikes are a highly energy-efficient neuronal code that propagates toward the presynaptic terminals for a long distance without attenuation. Pioneering work in squid giant axon displaying substantial temporal overlap of Na^+ inflow and the delayed K^+ outflow during action potentials and leading to the expenditure of extra energy to re-establish the electrochemical gradient through ionic pumps, thereby causing waste of energy consumption. In contrast, it was demonstrated that axonal action potentials in hippocampal mossy fibers were generated with a small overlap of Na^+ inflow and the delayed K^+ outflow, thereby saving energy demand of axonal spikes. In this study, it was attempted to investigate activity-dependent dynamic changes in the energy demand of axonal spike signaling by quantifying the temporal overlap of Na^+ influx and K^+ efflux during repetitive axonal spikes with computer simulation using the model of hippocampal mossy fiber. Axonal action potentials and the underlying Na^+ and K^+ currents were calculated and compared the time integral of the overlaps, which were expected to waste energy consumption. The overlaps of the currents decreased progressively with repetitive stimulation by delaying activation of K^+ currents during the train, possibly due to accumulated inactivation of K^+ channels. In support of this notion, when the model of the voltage-dependent K^+ channel was exchanged with a model of a non-inactivating type potassium channel, the use-dependent reduction of the overlap was not observed. These results suggested that the energy demand of axonal spikes is modulated by neuronal activity in an activity-dependent manner possibly due to use-dependent accumulated inactivation of K^+ channels.

Disclosures: H. Kamiya: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.06/B44

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: Ministry of Education (MoE), India

Title: Dorsoventral gradients in intrinsic neuronal excitability of rat hippocampal granule cells and mossy cells

Authors: *S. KUMARI, R. NARAYANAN;
Mol. Biophysics Unit, Indian Inst. of Sci., Bangalore, India

Abstract: The dorsal, intermediate, and ventral regions of the hippocampus are well characterized in terms of their behavioural roles, anatomical features, circuit connectivity, and gene expression profiles. However, there is notable paucity of studies investigating neuronal physiological properties along the dorsoventral axis of the dentate gyrus (DG), which serves as the primary gateway to the hippocampus proper. In this study, we systematically examined the electrophysiological properties of the two excitatory neuron types in the DG—granule cells (GCs) and mossy cells (MCs)—across the dorsal, intermediate, and ventral hippocampus of Sprague Dawley rats (4-8 weeks). Behaviorally, GCs have been implicated in spatial navigation, pattern separation, and engram formation, while MCs manifest multiple place fields and play key roles in novelty detection. We observed significant dorsoventral gradients in intrinsic excitability for both GCs and MCs, with dorsal neurons (GC: $n = 49$; MC: $n = 10$) exhibiting reduced excitability—inferred from significantly lower input resistance, impedance amplitude, and firing rates—compared to their intermediate (GC: $n = 43$; MC: $n = 14$) and ventral (GC: $n = 42$; MC: $n = 16$) counterparts. Strikingly, ventral GCs in the infrapyramidal blade manifested higher firing rates compared to their counterparts in the suprapyramidal blade. These blade-specific differences were limited to the ventral GCs, with negligible distinctions between GCs in the two blades of either dorsal or intermediate hippocampus. GCs functioned as integrators, exhibiting no sag and low-pass impedance profiles. MCs, on the other hand, manifested sag and exhibited robust delta-frequency (0.5-4 Hz) resonance. MCs also showed late firing of action potentials following suprathreshold current injections, with latencies ranging from 100-300 ms for a 250-pA current. Based on these observations, we hypothesized strong expression of HCN and *D*-type potassium channels in MCs. To test this, we applied ZD7288, a selective HCN channel blocker, which effectively abolished sag and delta-frequency resonance ($n = 10$). Furthermore, application of α -dendrotoxin, a peptide blocker of *D*-type potassium channels, significantly reduced firing latency to tens of milliseconds ($n = 7$) and increased firing rates by >5 fold. Importantly, both sub- and supra-threshold excitability were higher in ventral MCs compared to their dorsal counterparts. Collectively, our findings unveil marked inter-regional heterogeneity in the intrinsic electrophysiological properties of DG excitatory neurons and underscore the functional complexity of the hippocampus along its dorsoventral axis.

Disclosures: S. Kumari: None. R. Narayanan: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.07/B45

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH Grant R01MH134958

Title: Resting-state calcium dynamics reveal cell-type and hemispheric asymmetries in dorsal and ventral CA1 neurons

Authors: *C. KIM, J. KIM;

Neurosci. & Regenerative Med., Augusta Univ., Augusta, GA

Abstract: The dorsal and ventral hippocampal CA1 regions serve distinct but complementary functions: the dorsal CA1 (dCA1) primarily supports spatial memory and navigation, whereas the ventral CA1 (vCA1) is more involved in regulating emotional and social behaviors. Although *in vitro* studies show vCA1 neurons have higher intrinsic excitability and synaptic responsiveness than dCA1 neurons, how these differences manifest *in vivo* remains unclear. Additionally, little is known about hemispheric asymmetries or how promoter selection (e.g., CaMKII α vs. Synapsin I) influences calcium dynamics in these subregions. We first performed whole-cell current-clamp recordings in hippocampal slices from male control mice to compare membrane properties and evoked EPSPs at Schaffer collateral (SC)-CA1 synapses between dCA1 and vCA1. As expected, vCA1 neurons exhibited more depolarized resting potentials, higher input resistance, increased action potential firing, and larger EPSPs than dCA1 neurons. To assess whether these intrinsic differences are reflected *in vivo*, we focused on the home cage condition (resting state). Using three-color fiber photometry, we injected AAV-CaMKII α -jGCaMP8s and AAV-SynI-jRCaMP1b contralaterally into dCA1 and vCA1. During home cage recordings, vCA1 neurons in CaMKII α -injected mice exhibited higher calcium event counts and frequencies than dCA1 neurons, a difference not observed with SynI. This suggests promoter-dependent, cell-type-specific modulation of regional activity. Hemispheric asymmetry was also observed: left dCA1 (CaMKII α) showed greater calcium activity than right dCA1, with no such lateralization in SynI or vCA1. Lead-lag analysis revealed reduced solo calcium events between right dCA1 and left vCA1 compared to the opposite hemisphere pairing (left dCA1 $\leftrightarrow$ right vCA1), suggesting stronger interhemispheric synchronization along this axis in CaMKII α -expressing excitatory neurons. These findings reveal region-, hemisphere-, and promoter-specific organization of resting-state hippocampal CA1 activity and link *in vitro* excitability with *in vivo* dynamics.

Disclosures: C. Kim: None. J. Kim: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.08/B46

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: DOD Grant TS210063

Title: Altered intrinsic excitability of CA1 pyramidal neurons in a model of tuberous sclerosis

Authors: *D. H. BRAGER;

Sch. of Life Sci., Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Tuberous sclerosis is a monogenic disorder that is caused by a loss-of-function mutation in the *Tsc1* or *Tsc2* genes. *Tsc1/2* are negative regulators of the protein kinase mTOR which controls cellular processes across multiple organ systems including the brain. Neurological manifestations of tuberous sclerosis include neurodevelopmental delay, epilepsy, and TSC-associated neuropsychiatric disorders such as cognitive disability and autism spectrum disorder. Many studies focused on synaptic deficits as the contributing cause for hyperexcitability and cognitive impairments in tuberous sclerosis. Synaptic mechanisms, however, represent only part of the processes that regulate neuronal excitability. The complement of ion channels plays a critical role in controlling synaptic integration, propagation, and action potential output. We made whole-cell current clamp recordings from CA1 pyramidal neurons from *Tsc1*^{+/+} (control) and *Tsc1*^{+/-} mice. Although there was no significant difference in input resistance between control and *Tsc1*^{+/-} CA1 neurons, *Tsc1*^{+/-} CA1 neurons displayed significantly larger rebound and a higher resonant frequency, suggestive of elevated HCN channel function. Additionally, CA1 neurons from *Tsc1*^{+/-} mice fired significantly more action potentials compared to control CA1 neurons. An analysis of individual action potentials revealed that the threshold for action potential generation is hyperpolarized in *Tsc1*^{+/-} CA1 neurons, suggesting a difference in sodium and/or potassium channel function. These results suggest that heterozygous loss of *Tsc1* affects both subthreshold and suprathreshold voltage-gated ion channels and may contribute to altered intrinsic excitability of CA1 neurons in tuberous sclerosis.

Disclosures: D.H. Brager: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.09/B47

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH Grant R01NS135147

Title: Pneumonia-elicited neuronal hyperexcitability in human hippocampal organoids

Authors: *E. R. BUTLIG¹, M. T. LIN³, R. BALCZON⁴, T. STEVENS⁴, C. VENTETUOLO⁵, A. R. NELSON²;

¹Univ. of South Florida, Tampa, CA; ²Mol. Pharmacol. & Physiol., Univ. of South Florida, Tampa, FL; ³Physiol. & Cell Biol., ⁴Univ. of South Alabama, Mobile, AL; ⁵Brown Univ., Providence, RI

Abstract: BACKGROUND: Survivors of lower respiratory tract infections encounter cognitive impairment and an elevated risk for dementia, leading to a diminished quality of life threatened by both healthcare and financial burdens. Individuals with pneumonia develop cytotoxic tau variants in their bronchoalveolar lavage fluid. In experimental models, these tau variants interfere with the hippocampal information processing and trigger neural hyperexcitability, resulting in compromised learning and memory. However, these findings have yet to be translated to a human brain model. Hippocampal organoids have been proven to offer both 3D recapitulation of the complexity of various cell types as well as the functionality and relevance of neural circuitry compared to the hippocampus *in vivo*. The main aim of this study was to test the hypothesis that pneumonia-elicited peripheral tau will induce hyperexcitability in human hippocampal organoids. **METHODS:** Human hippocampal organoids were generated from induced pluripotent stem cells using previously described methods. Peripheral tau oligomers were isolated from the extracorporeal membrane oxygenator effluent of pneumonia patients via T22 immunoprecipitation, followed by elution and dialysis. Human hippocampal organoids were treated with peripheral tau oligomers for 90 minutes. Inward-rectifying calcium transients were visualized via confocal time-lapse imaging of GCaMP7f. Time-lapse recordings were processed in FIJI and processed in MATLAB. **RESULTS:** Hippocampal organoids exhibited staining for known hippocampal markers. Hippocampal organoids also contained granule cells of the dentate gyrus region and pyramidal neurons of the CA3 region of the hippocampus. Hippocampal organoids also possessed astrocytes, oligodendrocyte precursor cells, and inhibitory neurons. Hippocampal organoids treated with peripheral tau exhibited hypersynchronous neuronal activity. Neurons in T22-immunopurified oligomeric tau treated organoids exhibited increased frequency (1.672 vs 0.4675 spikes/min) and amplitude (4.771 vs 3.171) compared to those treated with vehicle. **CONCLUSION:** In this preliminary study, human hippocampal organoids were used to model the cellular diversity and functional circuitry of the human hippocampus. We found that oligomeric tau can be isolated from the plasma of pneumonia patients. Peripheral tau oligomers isolated from pneumonia patient plasma caused the neurons in hippocampal organoids to become hyperexcitable (e.g., increased frequency and amplitude of neuronal firing). Peripheral tau oligomers from pneumonia patient plasma also initiated a hypersynchronous firing pattern.

Disclosures: E.R. Butlig: None. M.T. Lin: None. R. Balczon: None. T. Stevens: None. C. Ventetuolo: None. A.R. Nelson: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.10/B48

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: Ray Gaskin's institutional grant

Title: Impaired Inhibitory Neuron Excitability is Reversible by a Selective KCNT1 Inhibitor in a KCNT1 R428Q Mouse Model of Severe Epilepsy

Authors: *M. SAFARI, G. JANG, A. SHORE;
Virginia Tech., Roanoke, VA

Abstract: Variants in the *KCNT1* gene, which encodes a Na⁺-activated-K⁺ channel, are known to cause severe epileptic conditions, including Epilepsy of Infancy with Migrating Focal Seizures (EIMFS) and Sleep-Related Hypermotor Epilepsy (SHE). These forms of epilepsy are characterized by early onset, and resistance to conventional medications. *KCNT1* mutations result in a gain-of-function (GOF) effect, leading to increased K⁺ efflux, neuronal network impairment, and subsequent seizure activity. A previous study on the SHE-linked variant Y796H showed selective impairment of GABAergic neurons, which become hypoexcitable, while glutamatergic neurons remain unaffected, whereas a study on the EIMFS-linked R455H variant demonstrated that both GABAergic and glutamatergic neuron dysfunction. The KCNT1 R428Q variant is a recurrent mutation that causes EIMFS. We generated a mouse model with Cre-dependent expression of the orthologous variant (R409Q). Germline expression via Sox2-Cre led to tonic and generalized tonic-clonic seizures, confirmed by EEG. Restricting expression to MGE-derived PV and SST interneurons using Nkx2.1-Cre produced similar seizure types, indicating that KCNT1 GOF in inhibitory neurons is sufficient to cause seizures. To investigate the cellular basis, we examined neuronal excitability in the motor cortex at P21-26, a stage and region showing hyperactivity in vivo by mesoscale Ca²⁺ imaging. We labeled PV and SST interneurons by crossing RQ mice with SST-Cre and PV-Cre lines and injecting AAV-hSyn-DIO-mCherry at P0-P2. Excitatory neurons were labeled using AAV8-CaMKII-GFP at the same age. We performed whole-cell patch-clamp recordings to assess intrinsic excitability across cell types and tested a selective, nanomolar-potent KCNT1 inhibitor by comparing firing properties with drug versus vehicle. Our results reveal that excitatory neuron excitability is not affected in *Kcnt1* R428Q mice. In contrast, SST interneurons' action potential firing becomes markedly hypoexcitable, exhibiting significant alterations in their firing properties. PV interneurons showed a significant increase in synaptic event frequency; however, their intrinsic excitability remains largely unchanged. This selective dysfunction likely contributes to overall circuit hyperexcitability, and the severe seizure phenotype observed in these mice. Treatment with a selective KCNT1 inhibitor at the cellular level, increased SST and PV neuron excitability. These findings provide important insights into the pathophysiology of KCNT1 related epilepsies and support the development of targeted therapies aimed at restoring inhibitory tone to control seizures.

Disclosures: M. Safari: None. G. Jang: None. A. Shore: None.

Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.11/B49

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIA-1P01AG071746 (PPG)

Title: Estrogen Modulates Electrophysiological Properties of Somatosensory Cortex Pyramidal Neurons in Middle-Aged Ovariectomized Female Mice on a High-Fat Diet

Authors: *F. FARAHANI^{1,2}, J. CALVO IGLESIAS^{3,2}, Z. PLUMLEY^{3,2}, I. FERNANDEZ UGIDOS^{3,2}, R. MOSTANY^{3,2};

¹Pharmacol., Tulane university, New Orleans, LA; ²Tulane Brain Inst., New Orleans, LA;

³Pharmacol. Dept., Tulane Univ., New Orleans, LA

Abstract: Growing evidence suggests that some cognitive impairments experienced by women during midlife are directly linked to the menopausal transition. Estrogen therapy remains the most effective treatment for menopause-associated symptoms. However, clinical trials have reported mixed results, with estrogen therapy showing variable efficacy across different populations. The overarching hypothesis of the Project Research Grant underpinning this study is that a woman's health status at the time of estrogen administration critically influences its effectiveness. In this context, we aim to investigate whether estrogen therapy can modulate the electrophysiological properties of pyramidal neurons in middle-aged, ovariectomized female mice that have been previously subjected to long-term high-fat diet feeding. Middle-aged (11-12 months old) female mice were used in this study. Starting at approximately 10 weeks of age, animals were randomly assigned to one of two dietary groups: control diet (CD) or high-fat diet (HFD). After 11 weeks on their respective diets, mice underwent bilateral ovariectomy (OVX) as a model of menopause. At the moment of this surgical procedure, mice were implanted with a subcutaneous capsule designed to deliver either vehicle or 17 β estradiol (E2) for the duration of the experiment. Three weeks post-OVX, mice were sacrificed, and acute brain slices were prepared for whole-cell patch-clamp recordings from layer 5 (L5) pyramidal neurons in the barrel field of the primary somatosensory cortex (S1BF). We found that E2 treatment differentially modulated excitability features in CD vs HFD. Specifically, E2 decreased the input resistance and evoked firing rate of L5 pyramidal neurons in HFD- but not in CD-fed mice. Consistently, E2 increased the first spike latency and rheobase current in HFD. Together, these results suggest that prior health conditions, such as diet-induced metabolic status, influence the effects of E2 on the excitability of pyramidal neurons in the cerebral cortex. Funding statement: Supported by NIA-1P01AG071746 (PPG).

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Poster

PSTR318: Intrinsic Properties and Modulation of Neuronal Firing II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR318.12/B50

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH RO1NS120099

Title: Investigating the effects of mild traumatic brain injury on cholecystokinin basket cells in the hippocampus

Authors: *A. HERNANDEZ¹, A. FARRUGIA², B. N. JOHNSON³, A. S. COHEN⁴;

¹Univ. of Pennsylvania, Philadelphia, PA; ²Children's Hosp. of Philadelphia, Allentown, PA;

³Children's Hosp. of Philadelphia, Wallingford, PA; ⁴Anesthesiol. and Critical Care Med., Children's Hosp Philadelphia, UPENN, Bala Cynwyd, PA

Abstract: Mild traumatic brain injury (mTBI) leads to impairments in learning and memory, and these cognitive deficits can be partly attributed to dysfunctions in hippocampal circuitry. The hippocampus, a brain region involved in learning and memory, is susceptible to damage from TBI. Augmented inhibition in hippocampal area CA1 was previously demonstrated by decreased stimulus-evoked action potential (AP) firing from pyramidal neurons (PNs). Local GABAergic interneurons, like cholecystokinin basket cells (CCK BCs), modulate the firing rate of CA1 PNs through perisomatic synapses. Therefore, this study will investigate the effect of mTBI on CCK BC physiology using whole-cell patch clamp recording. Mice will be injured using the well-validated lateral fluid percussion injury model of TBI. Animals will be assigned into two cohorts: LFPI injured animals who will receive surgery and injury, and sham animals who will receive surgery but do not undergo injury. For visualization of CCK BCs, CCK-Cre mice (Jackson Labs # 012706) will be injected with a Cre-dependent viral construct containing the mCherry fluorophore. I predict that CCK BCs will have increased excitatory synaptic input, a lower threshold of AP firing, increased AP frequency and larger input resistance after injury compared to sham animals, indicating increased CCK BC activity and excitability after mTBI. This work will explore the contribution of CCK BCs in hippocampal circuit dysfunction after mTBI and further our understanding of the precise mechanisms by which injury drives learning and memory deficits to aid in the development of therapies for TBI survivors

Disclosures: A. Hernandez: None. A. Farrugia: None. B.N. Johnson: None. A.S. Cohen: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR319.01/B51

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: Shanahan Foundation Fellowship

Title: Bounds on the computational complexity of neurons due to dendritic morphology

Authors: *A. AGRAWAL, M. A. BUICE;
Allen Inst., Seattle, WA

Abstract: The simple linear threshold units used in many artificial neural networks have a limited computational capacity. Famously, a single unit cannot handle non-linearly separable problems like XOR. In contrast, real neurons exhibit complex morphologies as well as active dendritic integration, suggesting that their computational capacities outperform those of simple linear units. Considering specific families of Boolean functions, we empirically examine the computational limits of single units that incorporate more complex dendritic structures. For random Boolean functions, we show that there is a phase transition in learnability as a function of the input dimension, with most random functions below a certain critical dimension being learnable and those above not. This critical dimension is best predicted by the overall size of the dendritic arbor. This demonstrates that real neurons have a far higher computational complexity than is usually considered in neural models, whether in machine learning or computational neuroscience. Furthermore, using architectures that are, respectively, more "apical" or "basal" we show that there are non-trivially disjoint sets of learnable functions by each type of neuron. Importantly, these two types of architectures differ in the robustness and generality of the computations they can perform. The basal-like architecture shows a higher probability of function realization, while the apical-like architecture shows an advantage with fast retraining for different functions. Given the cell-type specificity of morphological characteristics, these results suggest both that different components of the dendritic arbor as well as distinct cell types may have distinct computational roles. Our analysis offers new directions for neuron-level inductive biases in NeuroAI models using scalable models for neuronal cell-type specific computation.

Disclosures: A. Agrawal: None. M.A. Buice: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR319.02/B52

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: R01 NS130917

Title: Identifying Manifold Degeneracy and Estimating Confidence for Parameters of Compartmental Neuron Models with Hodgkin-Huxley Type Conductances

Authors: *S. NAMAZIFARD¹, A. KHADDAJ², M. HEINKENSCHLOSS², F. GABBIANI¹;
¹Baylor Col. of Med., Houston, TX; ²Computat. Applied Mathematics and Operations Res., Rice Univ., Houston, TX

Abstract: Much work has been devoted to fitting the biophysical properties of neurons in compartmental models with Hodgkin-Huxley type conductances. Yet, little is known on how reliable model parameters are, and their possible degeneracy. For example, when characterizing a membrane conductance through voltage-clamp (VC) experiments, one would like to know if the data will constrain the parameters and how reliable their estimates are. Similarly, when studying the responses of a neuron with multiple conductances in current clamp (CC), how robust is the model to changes in peak conductances. Such degeneracy is linked to biological robustness and is key in understanding the constraints posed by conductance distributions on dendritic computation. To address these issues, a compartmental model with Hodgkin-Huxley (HH) type conductances was used. We studied synthetic and experimental VC data of the H-type conductance (gH) that is widely expressed in neuronal dendrites. We also studied the original HH model in VC and CC. Finally, we considered a stomatogastric ganglion neuron model in CC. The ordinary differential equation solutions, parameters, and their sensitivities were simultaneously estimated using collocation methods and automatic differentiation. This allowed us to solve the non-linear least squares (NLLS) problem associated with each model. Parameter degeneracy manifold iterative tracing was performed based on the singular value decomposition of the NLLS residual Jacobian. We have also introduced a new objective function that allows us to identify parameter degeneracy manifold for membrane potential trajectories that are periodic at steady state and only differ in relative spike timings. This objective function is based on membrane potential characteristics, including the shape of the repeating spiking pattern, dominant frequencies and mean value, allowing to effectively eliminate the effect of phase shifts.

Disclosures: S. Namazifard: None. A. Khaddaj: None. M. Heinkenschloss: None. F. Gabbiani: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

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Program #/Poster #: PSTR319.03/B53

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Title: Towards a unified theory of dendritic inhibition

Authors: *Y. HE¹, K. DU², J. LU²;
¹Computer Sci., ²Peking Univ., Beijing, China

Abstract: We present a general theoretical framework for dendritic inhibition in morphologically and biophysically realistic neurons. Traditional models, such as shunting inhibition, rely on simplified neuron structures and steady-state assumptions, limiting their

applicability to neurons exhibiting complex dendritic nonlinearities. By reformulating NEURON's cable equation solver into a matrix representation, we derive analytical expressions for the partial derivatives of membrane voltage with respect to arbitrary model parameters. This enables precise quantification of how inhibitory inputs interact with dendritic nonlinearities to modulate somatic excitability. We define the Optimal Dendritic Inhibition (ODI) problem: identifying the minimal set of spatiotemporally distributed inhibitory inputs required to suppress somatic spiking in response to given excitatory stimuli. Employing a gradient-based greedy algorithm, we find that optimal inhibitory input locations—termed "inhibition hot-zones"—are highly dependent on the spatial and temporal characteristics of excitatory inputs. Further analysis decomposes the somatic voltage response into components attributable to dendritic intrinsic properties and synaptic input characteristics, introducing the concept of the neuron's "intrinsic input-output kernel." By examining how various ion channels influence this kernel, we establish a direct link between subcellular mechanisms and the neuron's computational functions, including dendritic inhibition, synaptic integration, and overall excitability. This framework bridges the gap between subcellular biophysics and neuronal computation, providing a versatile toolset for analyzing dendritic inhibition in complex neuronal models.

Disclosures: Y. He: None. K. Du: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR319.04/B54

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIMH R00MH126157

Title: Dendritic inhibition terminates plateau potentials in CA1 pyramidal neurons

Authors: *L. O. VAASJO¹, S. KOTERMANSKI², T. PATEL², J. SHI², S. CHAMBERLAND²;
²Dept. of Neurosci., ¹Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In CA1 pyramidal neurons (CA1-PYRs), plateau potentials control synaptic plasticity and the emergence of place cell identity. Here, we show that dendritic inhibition terminates plateaus in an all-or-none manner. Plateaus were initially resistant to inhibition but became increasingly susceptible to termination as they progressed. Between two distinct subtypes of dendrite-targeting interneurons, OLMNdnf generated slower postsynaptic currents that terminated plateaus more effectively than OLM α 2. Voltage-gated Ca²⁺ channels (VGCCs) were necessary for plateaus, which were prolonged by blocking small-conductance Ca²⁺-activated K⁺ channels (SK). A single-compartment model with these two conductances recapitulated core experimental findings and provided a mechanistic explanation for terminations. Plateaus arose from VGCCs maintained in the active state by sustained Ca²⁺ influx, a positive feedback loop that was quasi-balanced by ISK. Inhibition terminated plateaus by driving the membrane

potential below a dynamic threshold to deactivate VGCCs and end the positive feedback loop. Lastly, two-photon Ca²⁺ imaging showed that plateaus evoke large dendritic Ca²⁺ transients that were graded by terminations. Overall, our results demonstrate how the feedback inhibitory circuit interacts with intrinsic cellular mechanisms to regulate plateau potentials and shape dendritic Ca²⁺ signals in CA1-PYRs.

Disclosures: L.O. Vaasjo: None. S. Kotermanski: None. T. Patel: None. J. Shi: None. S. Chamberland: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

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Program #/Poster #: PSTR319.05/B55

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH Grant 5R01NS123396

Title: Functional properties of synaptic clustering throughout the dendritic tree under in vivo-like conditions

Authors: *J. SHI¹, D. B. HEADLEY²;

¹Ctr. for Mol. and Behavioral Neurosci., Newark, NJ; ²Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Neocortical pyramidal neurons receive thousands of synapses throughout their extensively branched dendritic trees. Recently it has been found that synapses with similar activities tend to cluster together on dendritic branches, and that learning can promote the formation of these clusters. Such clustering may promote the input efficacy through dendritic nonlinearities, such as NMDA spikes. Factors that may influence their elicitation of neuronal responses are their distance from the soma, presence on apical or basal branches, number of participating synapses, and ability to elicit calcium spikes. How these factors interact to affect neuronal integration has been partially investigated by both in vitro experimental and modeling work. However, these studies often lack a faithful replication of the in vivo conditions, where neurons are bombarded by large numbers of background synaptic activity throughout the dendritic tree. Moreover, the NMDA to AMPA ratio is often overestimated, potentially enhancing the impact of NMDA spikes. These discrepancies may distort our understanding of how synaptic clustering affects dendritic computation. To address this, we adapted a biophysically detailed model of a layer 5b pyramidal neuron and refined its passive and active properties to align with empirically grounded physiological conditions. Simulations with naturalistic presynaptic drive successfully reproduced realistic neuronal activities. Responses evoked by clusters were compared with randomly distributed synapses at matched distances from the soma. Activating synaptic clusters replicated the sigmoidal response function indicative of NMDA spikes. Relative to distributed synapses, synaptic clusters evoked comparable responses

when proximal to the soma, which transitioned to modestly enhanced responses at intermediate distances, and then weaker responses at the distal tips. This was true for both apical and basal branches. Any enhanced responses by clusters were NMDA-dependent. When NMDARs were eliminated from the model, clusters produced smaller responses than distributed synapses, especially as cluster size increased. Indeed, NMDARs seemed to promote equalization of responses across distance from the soma. These results suggest that local amplification by NMDA spikes enables synaptic clusters to achieve dendritic democracy and somatic linear summation on par with dispersed synapses. In the absence of NMDA spikes, clustered synapses would summate sublinearly. Thus, if learning promotes the formation of functionally important synaptic clusters, NMDA spikes may be necessary to keep those inputs from effectively weakening.

Disclosures: J. Shi: None. D.B. Headley: None.

Poster

PSTR319: Somatic and Dendritic Integration

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Topic: B.06. Intrinsic Membrane Properties and Signal integration

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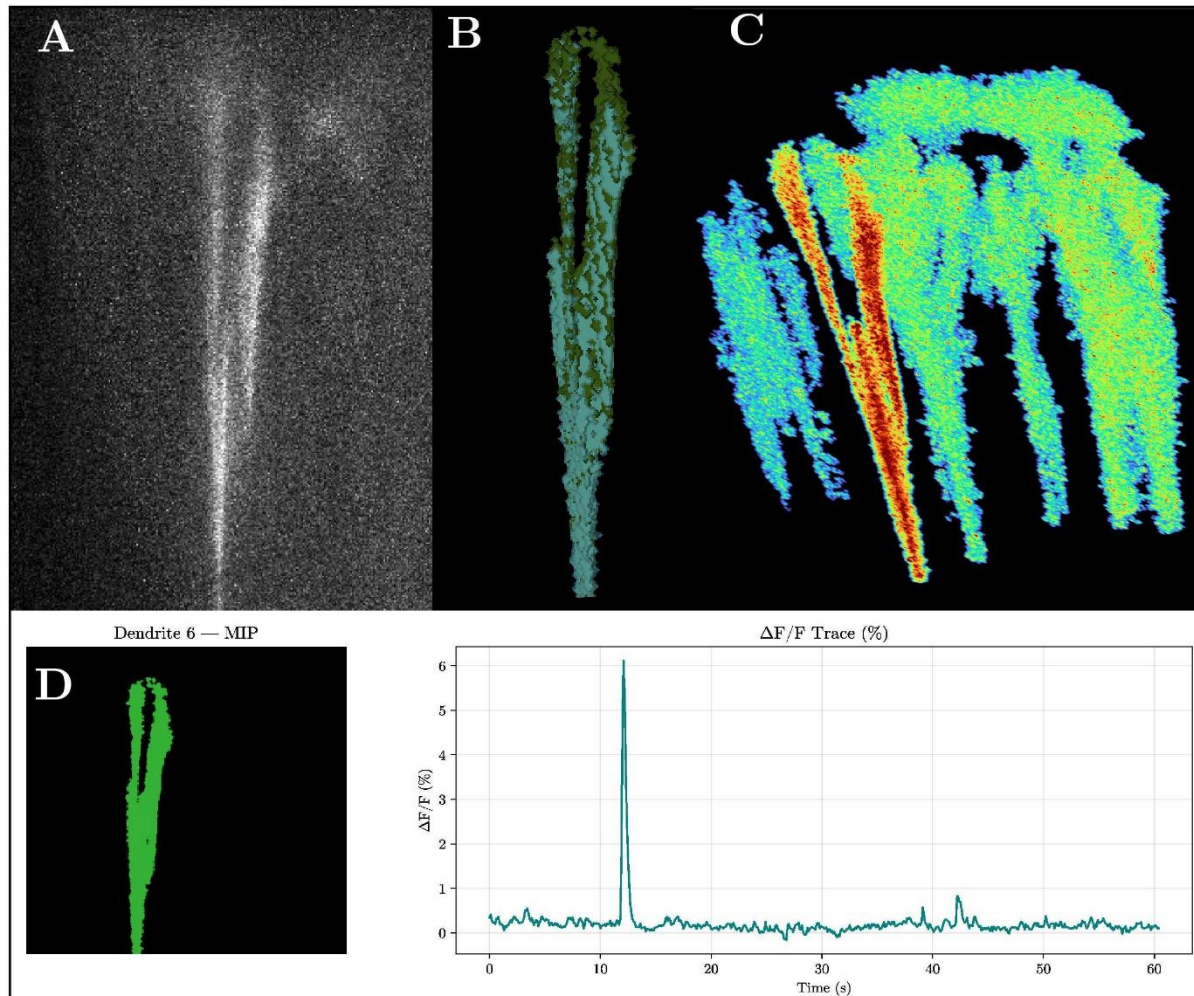
Title: Volumetric Imaging of Resting State Calcium Dynamics in Apical Dendrites

Authors: *D. BOGATOVA¹, D. BALOG², B. RAUSCHER², K. HERREMA², E. MARTIN², N. FOMIN-THUNEMANN³, K. KILIÇ⁴, Z. YAQOUB⁵, J. GIBLIN², A. CHEN², D. A. BOAS⁶, M. LONDON⁷, M. THUNEMANN⁶, A. DEVOR⁶;

¹Biol., ²Boston Univ., Boston, MA; ³Biomed. Engin., Boston Univ., BOSTON, MA; ⁴BME, ⁵Dept. of Biomed. Engin., ⁶Biomed. Engin., Boston Univ., Boston, MA; ⁷Edmond and Lily Safra Ctr. for Brain Sci. and Life Sci. Inst., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Apical dendrites of layer 5 pyramidal neurons are critical integrative hubs in cortical circuits. The repertoire of their activity patterns, including Ca and NMDA spikes, varies across brain states. However, the relationship between the dynamics of neurotransmitter-specific neuromodulatory activity and statistical properties of the dendritic Ca and NMDA spikes remains unclear. As a step towards this goal, we have implemented SCAPE (Swept, Confocally Aligned Planar Excitation) (Benezra et al., 2024) microscopy to image Ca activity in the top ~150 μm of layer 5 dendrites in 3D. We used awake, head-fixed Rbp4-Cre/Ai162 mice expressing GCaMP6s in layer 5 pyramidal neurons. We captured spontaneous Ca transients within a $270 \times 200 \times 150 \mu\text{m}^3$ volume in S1 cortex at a volumetric rate of 10 Hz. Panel A. shows XY maximum intensity projection (MIP) snapshot where individual firing dendrite is visible. To extract dendritic Ca transients, we developed a custom Python toolbox that allows segmentation of individual

dendrites from 3D data. We detect high-activity frames, make 3D masks (Panel B), and reconstruct volumetric $\Delta F/F$ activity maps (Panel C). We obtain the eye pupil diameter, whisking, and movement concurrently with GCaMP6s imaging to track alertness. For each active dendritic tree, we have extracted calcium transients. An example trace from a segmented dendrite (Panel D) shows a distinct activity at 12.1 seconds. Our next step is dual-color imaging of dendritic Ca and neuromodulatory neurotransmission (e.g., ACh) to linking dendritic Ca events to internally driven brain states in the awake animal.



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Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR319.07/B57

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH R01 MH135141

Title: Electrophysiological characterization of extracellular somatofugal and somatopetal signals along cortical dendrites in awake mice

Authors: *Z. ZHANG^{1,2}, M. HALGREN^{1,2}, V. D. TANG^{1,2}, M. T. HARNETT^{1,2};

¹McGovern Inst. for Brain Res., ²Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: The sensory cortex receives both bottom-up inputs from lower-order brain regions and top-down signals from higher-order areas. These pathways converge within the same cortical columns, where they exhibit distinct laminar termination patterns. Top-down and bottom-up interactions are key principles underlying cortical computation and cognition. Understanding how bottom-up and top-down pathways interact at the cellular and circuit level is therefore critical for uncovering the mechanisms by which the cortex processes sensory information and supports complex behaviors. Cortical pyramidal neurons, whose dendrites span across layers, are uniquely positioned to combine these different input streams. Yet how this happens remains unclear. The biophysical properties of pyramidal neurons support both linear and nonlinear synaptic integration, making the input-output transformation they perform on their many inputs in vivo difficult to determine. Although recent studies have begun to investigate this signal integration and transformation along dendrites in vivo using imaging approaches, capturing the millisecond-resolution layer-specific dynamics of bottom-up and top-down signaling during behavior remains a significant experimental challenge. In this study, we utilize the densely spaced recording sites of Neuropixels probes to capture fine-scale ‘footprints’ of dendritic activity across cortical layers. Probes were implanted perpendicular to the cortical surface in primary sensory areas of awake mice, allowing us to record single-unit activity with both subcellular spatial resolution and high temporal precision. We developed custom algorithms to analyze signal propagation patterns along the somato-dendritic axis. Consistent with previous findings, we observed frequent and robust somatopetal activity, in which action potentials backpropagated from the soma outwards along the apical dendrite. We further characterized the relationship between somatopetal propagation distance and neuronal firing pattern. We were also able to identify potential signatures of somatofugal events, in which activity appears to propagate from the apical dendrite toward the soma. These findings provide new insights into the bidirectional signaling dynamics along dendrites and offer a novel approach to dissecting bottom-up and top-down information flow in the awake brain.

Disclosures: Z. Zhang: None. M. Halgren: None. V.D. Tang: None. M.T. Harnett: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

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Program #/Poster #: PSTR319.08/B58

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH New Innovator Award, 1DP2NS136990
Chan Zuckerberg Initiative
Allen Institute for Neural Dynamics

Title: Measuring input-output transformations of cortical neurons using synaptic imaging

Authors: *M. SEYEDOLMOHADESIN¹, L. LI², M. XIE^{1,3}, M. HE¹, J. PARK¹, M. D. GOLUB², K. PODGORSKI¹;

¹Allen Inst. for Neural Dynamics, Seattle, WA; ²Paul G. Allen Sch. of Computer Sci. & Engin., Univ. of Washington, Seattle, WA; ³Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Understanding how individual neurons compute by transforming their thousands of synaptic inputs into spiking outputs remains a major challenge for neuroscience. To address this, we developed methods for high-speed, high-resolution, simultaneous in vivo imaging of individual neurons' synaptic inputs and somatic output. Using a dual-view Random Access Projection Microscope (SLAP2), we recorded simultaneous activity from 200-400 excitatory synapses per neuron at >100 Hz across multiple dendritic compartments (e.g., apical and basal dendrites) using the green glutamate indicator iGluSnFR4, and somatic spiking using a red calcium indicator.

Recording from layer 2/3 pyramidal neurons in the primary visual cortex while presenting mice with visual stimuli, we observed diverse tuning of converging presynaptic inputs. On a trial-averaged basis, the sum of these inputs poorly predicted somatic output, indicating that postsynaptic integration is more important than synaptic connectivity alone in determining neuronal tuning to the probed stimuli. However, on a single-trial basis, synaptic inputs predicted somatic activity more accurately than could be accounted for by their trial-averaged tuning. Coincident activation of many inputs occurred more frequently than chance. We identified oscillatory modes of synchronously active synapses that did not effectively drive calcium spikes, while other coincident input patterns predicted calcium spikes. Finally, morphological analysis revealed functional correlations between synaptic inputs that varied with pairwise distance, with distinct patterns of coactivity occurring at different length scales.

This study provides insight into the spatiotemporal organization of synaptic inputs to individual cortical neurons and how different input patterns relate to spiking output.

Disclosures: M. Seyedolmohadesin: None. L. Li: None. M. Xie: None. M. He: None. J. Park: None. M.D. Golub: None. K. Podgorski: None.

Poster

PSTR319: Somatic and Dendritic Integration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR319.09/B59

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: USA National Institute of Neurology and Stroke award (NS138991-01) to SDA
Connecticut IBACS Grant to KDM
Grant #4242 “NIMOCHIP” Science Fund Serbia to P. Andjus

Title: Recording voltage and calcium transients in striatal medium spiny neurons: an ex vivo study

Authors: *S. D. ANTIC^{1,2}, W. W. LYTTON³, K. MILICEVIC^{1,2};
¹Neurosci. Dept., Univ. of Connecticut Hlth., Farmington, CT; ²Inst. for the Brain and Cognitive Sci., Farmington, CT; ³DHSU, Brooklyn, NY.

Abstract: Striatal medium spiny neurons (SPNs), specifically those of the direct (SPNd) and indirect (SPNi) pathways, are the principal input units of the basal ganglia. Their intrinsic electrical properties and the way they integrate and propagate signals are fundamental to striatal organization and function. Computational models of striatal activity often simplify SPNs as point neurons, but such approaches overlook critical aspects of dendritic processing. Due to technical challenges (particularly the difficulty of recording from thin dendritic branches with glass microelectrodes), experimental data on dendritic voltage dynamics in SPNs remain scarce. While computational modeling of SPNd and SPNi provides valuable predictions about dendritic responses to synaptic inputs and action potential (AP) backpropagation, these models require validation against experimental data. In this project, we aim to expand the experimental dataset of dendritic electrical signals in SPNs. We achieve this by preparing acute brain slices and labeling SPNs with voltage-sensitive dyes (VSDs) of the styryl class. Because VSDs diffuse slowly into distal dendrites, obtaining reliable optical signals requires significant experimental optimization and time. To date, we have developed and refined several techniques to image voltage dynamics in thin dendritic branches during both synaptic input and AP backpropagation. Additionally, we used calcium-sensitive dyes to probe activity at dendritic tips, the terminal segments of SPN dendrites. Our calcium imaging experiments revealed robust calcium transients in a subset of dendritic tips during AP backpropagation, suggesting effective activation of voltage-gated calcium channels in these compartments. In summary, both voltage and calcium imaging experiments support the presence of active electrical signaling in a subset of distal dendritic branches of SPNs during axonal firing. These data provide essential empirical support for understanding dendritic integration in SPNs and will serve to constrain and validate future computational models.

Disclosures: S.D. Antic: None. W.W. Lytton: None. K. Milicevic: None.

Poster

PSTR319: Somatic and Dendritic Integration

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Program #/Poster #: PSTR319.10/B60

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: BBRF 30481
R01NS128079 to ITS

Title: Dendritic calcium hotspots enhance local inputs of mouse L2/3 pyramidal neurons

Authors: *L. M. ADSIT¹, I. T. SMITH²;

¹Mol. Cell. & Dev. Biol., ²Neurosci. Res. Inst., UC Santa Barbara, Santa Barbara, CA

Abstract: Dendrites have been characterized structurally, looking into branching points, dendrite radius, and channel concentrations, but the functional role of dendrites in dendritic integration is not well understood. Our lab previously employed whole cell patch-clamp recordings on layer 2/3 pyramidal neuron's distal dendrites and found independent electrical events originating at the dendrites. Other researchers employed optogenetic stimulation on presynaptic neurons, and were able to induce dendritic spikes that were visible with calcium indicators in the distal dendrites of pyramidal neurons. Knowing that activity is originating from the dendrites and that the spikes can be driven by individual strong inputs, we aim to understand specifically how dendritic spikes are connected to neuronal activity. We sparsely expressed a genetically encoded calcium indicator, GCaMP8m, in layer 2/3 pyramidal neurons of the visual cortex. Using two-photon calcium imaging of dendrites we could extract the backpropagating action potentials of the soma, as well as independent activity of individual synapses and segments of the dendrite. These neurons exhibit reliable orientation tuning. Consistent with previous work, we show that individual synapses, visible as dendritic spines, on a single neuron show orientation preferences for the full range of angles, with the largest fraction sharing the preference of the soma. The linear sum of spine signals can predict orientation preference, but not tuning selectivity of the soma. We developed a method of identifying dendritic spikes and extracting their orientation tuning. We found that dendritic spikes are found on dendritic areas more than 40 microns from the soma. The calcium events occur across a 15 to 20 micron segment of the dendrite. Longitudinal study of the dendrites for three months revealed that dendritic spikes were recurrently found in the same location within the dendritic arbor for up to one month, and new locations would emerge. The tuning of dendritic spikes is significantly more selective than the backpropagating action potentials and spines, representing a local signal enhancement. Most dendritic spikes share the tuning of the soma, but those that do not have significantly worse tuning selectivity. The activity profile of dendritic spikes is most highly correlated with spines located at the hotspot, especially in spines sharing the same orientation preference. We also found that from the spines that are not located on the site of the dendritic spikes, proximal spines are less likely to share activity than more distal spines, meaning their activity is less incorporated in causing these spiking events.

Disclosures: L.M. Adsit: None. I.T. Smith: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.01/C1

Topic: B.08. Epilepsy

Support: VIEP-BUAP to CA en Neuroendocrinología (BUAP-CA-288) 2025
Fellowship SECIHTI No. 799022

Title: Chronic hyperprolactinemia drives progressive prolongation of spike-wave discharges in a genetic model of H-ABC leukodystrophy

Authors: *E. HERNANDEZ ALVARADO¹, A. UGARTE¹, C. CORTES², J. R. EGUIBAR, Sr.³;

¹Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; ²Inst. of Physiol., B. Univ. Autonoma de Puebla, Puebla, Mexico; ³Behavioral Neurophysiol., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico

Abstract: The *taiep* rat has a point mutation in the *Tubb4a* gene, which encodes the protein tubulin beta 4A. This animal model presents electrophysiological and imaging characteristics similar to those of patients with human leukodystrophy named hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). *Taiep* rats also showed spike-wave discharges (SWDs) on chronic EEG recordings, that resemble those observed in patients with absence seizures. Prolactin is a hormone that neuromodulates different areas of the brain, including the thalamo-cortical circuit, associated with the generation of SWDs. The aim of this study was to evaluate the effect of chronic hyperprolactinemia in SWDs in adult female *taiep* rats. One-hundred-fifty-day-old female *taiep* rats were implanted with three stainless steel screw electrodes in the cerebral cortex and a braided bipolar electrode in the CA1 region of the hippocampus. Seven days later, a 24-hour EEG recording was performed. Two anterior pituitary glands from female *taiep* rats were then grafted into the renal capsule to induce a chronic hyperprolactinemia state. Twenty-eight days after grafting, one EEG recording was performed, followed by two subsequent EEG recordings, with seven days interval. The frequency and duration of SWDs in the hyperprolactinemia condition were quantified and compared with control conditions. Our results showed that hyperprolactinemia significantly increased the total frequency of SWDs compared to control recordings thirty-five days after grafting, SWDs increased 65%, from 805.5 ± 24.2 to 1323.5 ± 140.9 SWDs/24h (RM-ANOVA, $F_{(5,17)}=7.27$; $P<0.01$; followed by Dunnett's test $q=3.21$, $P<0.05$). Chronic hyperprolactinemia produced a progressive increase in the mean duration of SWDs, obtaining the longest duration forty-two days after grafting with respect to control conditions, going from 3.48 ± 0.2 s to 5.26 ± 0.5 s (RM-ANOVA, $F_{(5,17)}=4.63$; $P<0.05$; followed by Dunnett's test $q=3.1$, $P<0.05$). In conclusion, this neuropeptide modulates the thalamocortical circuit responsible for absence seizures, increasing both their frequency and duration. This is the first demonstration that hyperprolactinemia is capable to affect absence seizures in genetic neurodevelopmental diseases such as leukodystrophies.

Disclosures: E. Hernandez Alvarado: None. A. Ugarte: None. C. Cortes: None. J.R. Eguibar: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.02/C2

Topic: B.08. Epilepsy

Support: VIEP-BUAP 2025 for the CA in Neuroendocrinología (BUAP-CA-288)
SECIHTI fellow No. CVU: 108308.

Title: Differential effects of two testosterone esters on spike-wave discharges in *taiep* rats

Authors: *R. CASTILLO^{1,2}, C. CORTES², A. UGARTE³, J. R. EGUIBAR, Sr.⁴;
¹Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; ²Inst. of Physiol., ³B. Univ. Autonoma de Puebla, Puebla, Mexico; ⁴Behavioral Neurophysiol., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico

Abstract: The *taiep* rats were obtained at the Institute of Physiology in the Benemérita Universidad Autónoma de Puebla. They represent the first animal model of the human leukodystrophy named hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). The *taiep* rat had a tubulinopathy due to a point mutation in the *Tubb4a* gene. In magnetic resonance imaging (MRI) studies showed ventriculomegaly, hypomyelination and atrophy of putamen and cerebellum. Additionally, *taiep* rats display spike-wave discharges (SWDs) in the electroencephalogram (EEG), like those reported in humans with absence seizures. The SWDs are sexually dimorphic, being higher in frequency and duration in males with respect to females. In male rats, gonadectomized on postnatal day (PND) 2 significantly decreased the incidence of SWDs, and the opposite happen in adult orchiectomy which significantly increased the frequency of SWDs. The aim of this study was to evaluate the effect of testosterone propionate (TP) and testosterone enanthate (TE) on SWDs in orchiectomized *taiep* male rats. *Taiep* rats were orchiectomized at 165 ± 2 PND. One week later, they were implanted for electroencephalographic recordings (EEG). The rats were divided into two conditions: in group 1, two 24-hour EEG recordings per rat were made: one control after subcutaneous (s.c.) administration of olive oil (vehicle, 1 mL/Kg), and another after the administration of 2 mg/kg of TP s.c. In group 2, two 24-hour EEG recordings as previously described and another after the administration of 25 mg/kg of TE s.c. We analyze the frequency of the SWDs. Our results showed that after testosterone propionate administration decreased the total frequency of SWDs in orchiectomized male *taiep* rats with respect to control group ($t = 2.70$, $P < 0.05$). However, the administration of testosterone enanthate significantly increased the total frequency of SWDs ($t = 2.70$, $P < 0.05$) with respect to control group. In conclusion, testosterone propionate administration decreased SWDs, whereas testosterone enanthate administration increased them, supporting the notion that long-lasting testosterone compounds acts differently in the thalamo-cortical circuit that sustained that characterized absence seizures.

Disclosures: R. Castillo: None. C. Cortes: None. A. Ugarte: None. J.R. Eguibar: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.03/C3

Topic: B.08. Epilepsy

Support: NIH Grant 5p20GM103642
NIH 5R25GM061151-19
NSF Grant 1736026
NIH Grant 2R25Ms080687
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Title: Establishing a fast-track screening method for rufinamide derivatives using *Drosophila melanogaster* to identify potential antiepileptic drugs in genetically at-risk populations

Authors: *S. ITRIAGO FREITES¹, N. C. JIMÉNEZ REVELLES², A. CRUZ-CALDERON³, A. GHEZZI²;

¹Biol., Univ. de Puerto Rico, Rio Piedras, Rio Grande, PR; ²Dept. of Biol., ³Dept. of Chem., Univ. of Puerto Rico, Rio Piedras, San Juan, PR

Abstract: Epilepsy is a chronic neurological disorder characterized by recurrent seizures, often caused by mutations in voltage-gated sodium channels (VGSCs), particularly in the α -subunit Nav1.1. The condition affects nearly 2% of Puerto Rico's population, with children experiencing a higher prevalence that impacts cognitive development and contributes to a threefold increased risk of suicide. Drug resistance and genetic predispositions, including the Boricua Founder Variant in the *FRRS1L* gene, underscore the need for more effective, accessible, and personalized treatments. Rufinamide, an FDA-approved antiepileptic drug (AED), prolongs VGSCs inactivation, but its precise mechanism remains incompletely understood. This study introduces a scalable, cost-effective screening platform using *Drosophila melanogaster* to fast-track the identification of AED candidates, focusing on rufinamide and its structurally enhanced derivatives. Molecular docking simulations targeting Nav1.1 guided the design of rufinamide derivatives with improved binding affinities (up to -8.0 kcal/mol), surpassing the parent compound. To evaluate safety profiles, a serial dilution toxicity assay (0.005, 0.05, 0.5, and 5 mg/mL) in DMSO-yeast-sucrose media was performed in *Drosophila* to determine lethal doses (LD₅₀), revealing sex-specific tolerance patterns and minimal compound-related lethality. DMSO-related toxicity was observed, prompting testing of alternative solvents such as n-propanol, isopropanol, and butanol. Building on this, future phases will assess anticonvulsant efficacy using mechanical stimulation (vortex-induced seizures) and optogenetic seizure induction in genetically susceptible *para*-mutant fly models. This integrative approach combines computational modeling with rapid in vivo validation, establishing a streamlined pipeline for early-stage AED discovery. The platform holds particular promise for addressing treatment gaps in underserved populations with distinct genetic backgrounds, such as those in Puerto Rico. By democratizing drug development tools and emphasizing genetic relevance, this work lays the foundation for targeted, next-generation epilepsy therapeutics.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.04/C4

Topic: B.08. Epilepsy

Support: RS-2025-00520396

Title: Therapeutic Effects of C1q/TNF-Related Protein9 (CTRP9) Administration on Neuronal Damage and Impaired Angiogenesis Following Epilepsy

Authors: *H. YANG¹, M. PARK², H. JUNG⁵, M. LEE⁶, D.-S. SON³, S. SUH⁷, H. CHOI⁸, B. CHOI⁴;

¹Dept. of Physiology, Hallym University, Col. of Med., Chuncheon, Korea, Republic of; ²Dept. of Physiol., Hallym Univ., Chuncheon, Korea, Republic of; ³Data Sci., ⁴Dept. of Physical Educ., Hallym Univ., Chuncheon, Korea, Republic of; ⁵Dept. of Physiology, Col. of Medicine, Hallym university, Korea, Chuncheon, Korea, Republic of; ⁶Neurol., Hallym Univ. Sacred Heart Hosp., Anyang, Korea, Korea, Republic of; ⁷Dept. of Physiology, Hallym Univ., Chuncheon, Kang Won Do, Korea, Republic of; ⁸Neurol., Hallym Univ. Chuncheon Sacred Heart Hosp., Chuncheon, Korea, Korea, Republic of

Abstract: Epilepsy is one of the most common neurological disorders, often leading to significant neuronal death and cognitive impairments. Despite its widespread impact, effective treatments remain limited. Clinically, epilepsy is characterized by two or more unexplained seizures within a 24-hour period or chronic seizures. These seizures and related neurological conditions are often caused by abnormal cellular energy metabolism and inadequate energy supply through blood vessels. C1q/TNF-Related Protein 9 (CTRP9) is a protein similar to adiponectin, known for its anti-inflammatory and pro-angiogenic effects, primarily mediated through the activation of the AMP-activated protein kinase (AMPK) pathway. Although CTRP9 has been extensively studied in cardiovascular diseases, its role in neurological disorders, beyond global cerebral ischemia (GCI), remains largely unexplored. This study aimed to investigate the effects of CTRP9 in an epilepsy model. Neuronal excitotoxicity was induced using pilocarpine and L-glutamic acid, followed by treatment with 2 µg/ml CTRP9. Additionally, in a pilocarpine-induced epilepsy model in vivo, CTRP9 was administered intravenously at a dose of 1 mg/kg. Brain tissue was collected at various time points for histological and biochemical analyses. Our findings showed that CTRP9 treatment reduced neuronal death by decreasing glial cell activation and lowering the expression of pro-inflammatory factors. Furthermore, post-seizure CTRP9 treatment enhanced angiogenesis and improved pericyte function, indicating its potential as a therapeutic agent for epilepsy. These results suggest that CTRP9 may offer neuroprotective

effects in epilepsy through its anti-inflammatory and angiogenic properties, as well as by supporting energy metabolism and promoting endothelial repair, particularly through pericyte-mediated angiogenesis. Further basic and clinical research is needed to explore CTRP9's potential as a novel treatment strategy for epilepsy.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

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Topic: B.08. Epilepsy

Support: MEG resource facility, funded by the Department of Biotechnology, Ministry of Science & Technology, Govt. of India
[BT/MED/122/SP24580/2018]
IOE, University of Delhi

Title: Differential role of Casein Kinase 2 Catalytic Subunits α and α' in Nmdar Modulation Associated with Hyperexcitability in Temporal Lobe Epilepsy

Authors: *P. PRIYA¹, N. YADAV^{3,1}, R. MITTAL², S. SWEETY¹, A. B. DIXIT⁴, J. BANERJEE⁵;

¹Dr BR Ambedkar Ctr. for Biomed. Res., Delhi, India; ²Dr BR Ambedkar Ctr. for Biomed. Res., delhi, India; ³Bhaskaracharya Col. Of Applied Sci., Delhi, India; ⁴Dr B.R Ambedkar Ctr. for Biomed. Res. Univ. of Delhi, Delhi, India; ⁵Biophysics, All India Inst. of Med. Sci. (AIIMS), New Delhi, India, New Delhi Delhi, India

Abstract: Introduction: Mesial Temporal Lobe Epilepsy (MTLE) is associated with dysregulated excitatory-inhibitory balance in the brain. Numerous enzymes, protein kinases, that are modulated through phosphorylation have been linked with key processes involved in pathogenesis of epilepsy. Casein Kinase 2 (CK2) is a serine/threonine kinase constituting two catalytic subunits- CK2 α and CK2 α' , and two regulatory β subunits. CK2 phosphorylates the NR2B subunit of NMDARs at serine 1480 altering receptor localization. Although CK2 α and CK2 α' share high sequence similarity, they are encoded by distinct genes and have unique substrate specificities and cellular roles. However, the specific contributions of CK2 α and CK2 α' to NMDAR modulation and seizure activity in MTLE remain unclear. **Methods:** To investigate the roles of CK2 α and α' subunits in TLE, stereotactic surgery was performed to bilaterally inject siRNA targeting CK2 α or α' subunits into the hippocampus. An acute TLE model was developed using lithium-pilocarpine to induce status epilepticus. Seizure frequency and severity post siRNA was assessed using EEG. siRNA knockdown was confirmed using RT-PCR. NR2B expression and phosphorylation status was evaluated using western blot. H&E staining was performed to

confirm the pathophysiological changes. **Results:** Our results revealed a differential impact of CK2 catalytic subunit knockdown on seizure activity and NMDAR modulation. EEG analysis, displayed significant reduction in seizure frequency and severity post CK2 α subunit knockdown, whereas increased seizure susceptibility post CK2 α' knockdown. Reduced neurodegeneration and better-preserved neurons, were observed after CK2 α silencing, whereas, no significant changes were observed in CK2 α' knockdown group. Effective knockdown of CK2 α and α' was confirmed using RT-PCR. Elevated levels of NR2B phosphorylation were observed in CK2 α' knockdown group, using western blot, whereas no change was observed in CK2 α knockdown group. **Conclusion:** Current study demonstrates distinct role of CK2 catalytic subunits α and α' in NMDAR regulation and seizure activity in TLE. CK2 α induces NR2B phosphorylation, therefore leading to enhanced hyperexcitability, while CK2 α' exerted a protective effect by restricting NR2B phosphorylation. These results highlight the significance of subunit specific regulation of CK2 in MTLE pathophysiology and suggest possible therapeutic targets for intervention.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.06/C6

Topic: B.08. Epilepsy

Title: Mapping Lafora bodies and identifying ultrastructural changes in a Lafora disease mouse model

Authors: *V. CHIAPPINI¹, L. BARTESAGHI¹, R. PAROLISI¹, R. ALKHATER², F. DI CUNTO¹, P. J. MAGISTRETTI³, C. CALI¹;

¹Univ. of Turin, Turin, Italy; ²Pediatrics, Johns Hopkins Aramco Healthcare-KAUST, Dhahran, Saudi Arabia; ³BESE, King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia

Abstract: Lafora Disease (LD) is a rare, fatal genetic disorder characterized by the accumulation of glycogen aggregates, named Lafora bodies (LBs). LD belongs to the group of progressive myoclonus epilepsies and it is considered both a neurodegenerative disease and a glycogen metabolism disorder. LD is caused by mutations in *EPM2A* or *EPM2B* genes, encoding respectively for laforin and malin proteins. The laforin-malin complex is thought to prevent LB formation by regulating glycogen-associated proteins, likely through ubiquitination and their subsequent degradation via proteasomal or autophagic pathways. In the absence of this functional complex, aberrant glycogen granules accumulate as LBs, driving the cerebral degenerative disease progression. Recent studies have shown that LBs develop in both neurons and astrocytes. Neuronal LBs are larger and they are located in the somatic region, whereas astrocytic LBs are smaller, clustered, and resemble corpora amylacea observed in aging and in

neurodegenerative diseases. The role of LBs in LD-associated neurodegeneration remains unclear and their nanoscale morphology, subcellular localization, and colocalization with organelles, such as mitochondria, require further investigation. While mitochondrial dysfunction, including decreased ATP production, has already been described, a detailed examination of mitochondrial morphology, closely linked to their function, that may provide critical insights into the pathogenesis is still lacking. To address this, hippocampal samples from laforin knock-in mice (LD model) at 3 different disease stage (3 animal per time point), as well as from control mice, were prepared using the NCMIR protocol for Serial Block-Face Scanning Electron Microscopy (Volutome, ZEISS). Subsequently, 70x70x35 μm^3 volumes per sample were imaged at a voxel resolution of 5x5x50 nm³ (x, y, z). Preliminary qualitative observations of astrocytic processes revealed abundant LBs presence at various stages of maturation and the appearance of defective mitochondria containing vesicles-like materials deriving from the inner mitochondrial membrane. Deep learning models and state of the art software platforms were employed for 3D reconstructions of astrocytic LBs and the mitochondrial network. Quantitative data from reconstructions at 5 months revealed that LBs appear to be accumulated more frequently near blood vessels (61 % within 6 μm , 67 out of 110 LBs). Further analysis will provide deeper insights into the ultrastructural alterations in astrocyte morphology and the mitochondrial network during LD pathogenesis, shedding light on the role of LBs in neurodegeneration.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.07/C7

Topic: B.08. Epilepsy

Support: National Institute of Health R01 (NS133139)

Title: Inferior Colliculus and PAG Hyperexcitability as Mechanisms of Seizure-Induced Respiratory Arrest

Authors: *S. MOELLER RIVERA, C. DEZHA-BOLTEADA, I. C. WENKER;
Anesthesiol., UVA Sch. of Med., Charlottesville, VA

Abstract: Sudden Unexpected Death in Epilepsy (SUDEP) is defined as a sudden, unexpected, non-traumatic death in individuals with epilepsy, where no alternative cause of death is identified post-mortem. SUDEP accounts for between 8 and 17% of all epilepsy-related deaths, rising to 50% in patients for which current therapies are ineffective. SUDEP is the second most significant cause of lost life years among neurological disorders, only behind stroke, according to the Centers for Disease Control and Prevention. The most compelling clinical data comes from the MORTality in Epilepsy Monitoring Units (MORTEMUS), which analyzed 16 cases of

SUDEP from patients in epilepsy monitoring units (EMUs) worldwide. The study revealed that SUDEP resulted from apnea (i.e., respiratory arrest) following generalized tonic-clonic seizures, suggesting that impaired respiratory function triggers the sudden death. Recent studies using mouse models produce similar findings, where apnea, secondary to a seizure, is responsible for sudden death. Our group has found that in mouse models of SUDEP, fatal apneas are initiated during the tonic phase of seizures. Furthermore, silencing forebrain neurons increased seizure thresholds but did not prevent apneas during the tonic phase, suggesting that brainstem circuits, rather than forebrain activity, are primarily responsible for producing this fatal apnea. Current preliminary data from our lab indicates neurons in the periaqueductal grey (PAG) become highly activated proximal to seizures with concurrent apnea, compared to seizures without apnea. We hypothesized that hyperactivity of the PAG is sufficient to produce apnea during a seizure. To test this, we injected an adeno-associated virus to express Cre recombinase (AAV-Cre) selectively in PAG and inferior colliculus (IC), the latter of which we have shown can induce audiogenic seizures that do not present with apnea. Our preliminary data has indicated that this selective midbrain expression is sufficient to produce apnea during audiogenic seizures. Future studies will explore whether targeted inhibition of PAG neuronal activity in mouse models of SUDEP reduces fatal outcomes, directly testing the therapeutic potential of brainstem modulation. Clarifying the role of PAG could significantly enhance our understanding of the pathophysiological mechanisms of SUDEP and ultimately inform targeted therapeutic strategies for epilepsy patients, particularly those unresponsive to current treatments, thereby reducing the incidence and mortality associated with SUDEP.

Disclosures: S. Moeller Rivera: None. C. Dezha-Bolteada: None. I.C. Wenker: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.08/C8

Topic: B.08. Epilepsy

Support: R01NS133139
3R01NS133139-01S1

Title: Activation of periaqueductal gray neurons in mouse model of seizure induced apnea

Authors: *S. N. GARCIA DUBAR¹, I. C. WENKER²;

¹Anesthesiol., ²Dept. of Anesthesiol., Univ. of Virginia, Charlottesville, VA

Abstract: Sudden, unexpected, and unexplained death of a person with epilepsy, referred to clinically as Sudden Unexpected Death in Epilepsy (SUDEP), accounts for up to 17% of all epilepsy-related deaths, up to 50% if the person does not respond to standard treatments. While the mechanisms underlying SUDEP are not fully understood, there is increasing evidence that seizure-induced apnea (SIA) is the primary cause. We have previously shown in preclinical

models of epilepsy that SIA occurs minutes before terminal asystole and is not impacted by forebrain inhibition; thus, we propose that overactive brainstem neural circuitry produces SIA. We used a rapid kindling protocol on C57Bl/6J (WT) mice, which produces seizures on our modified Racine scale that display, wild running/jumping (stage 6), wild running/jumping and SIA (stage 6a), tonic extension and SIA (stage 7), and death from SIA (stage 8). Apnea duration, seizure threshold and duration, EEG power amplitude, heart rate, and breathing rate were assessed for each condition. In WT mice that were kindled for 3 days, 39% of mice died from SUDEP, with 100% of deaths occurring after a tonic seizure with apnea. We also observed apnea in a subset of stage 6 seizures that did not correspond with a tonic phase. Out of 83 seizures scored as 6 based on behavior, 44.6% had a pause in breathing > 1.5 seconds, which we categorized as 6a with an average apnea of 3.37 ± 0.35 seconds. 18.9% of 6a seizures had an apnea > 5 seconds. Using the same rapid kindling protocol, TRAP2 (Fos-iCre/tdTomato) mice were kindled until reaching their first seizure behavior stage 6 or 7, and then 30 minutes later injected with 4-hydroxytamoxifen to observe the activated neuronal populations in mice experiencing seizures. The most notable area where activated neurons were observed was the periaqueductal gray (PAG), a brainstem structure that has an important role in breathing integration. We found more activated neurons in the PAG of mice that had seizures with apnea versus seizures without apnea. In a subset of these experiments, we performed in situ hybridization to assess the subtypes of activated neurons and observed the majority to be glutamatergic. Our model reliably produces SIA and SUDEP within three days of kindling and can be used to further investigate differences between seizures with and without apnea. Additionally, our histology results suggest that excitatory neurons in the PAG are activated during seizures to produce the apnea that leads to SUDEP. The PAG represents a potential target for intervention to prevent SIA and SUDEP, and future studies examining the mechanisms by which the PAG produces SIA will be enlightening.

Disclosures: S.N. Garcia DuBar: None. I.C. Wenker: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.09/C9

Topic: B.08. Epilepsy

Support: NINDS R01 NS133139

Title: Induced seizures result in decrease in hypercapnic ventilatory response in a mouse model of scn8a epilepsy

Authors: *M. L. SCULIMBRENE¹, I. C. WENKER²;

¹Dept. of Anesthesiol., Univ. of Virginia, Charlottesville, VA; ²Dept. of Anesthesiol., Univ. of Virginia, Charlottesville, VA

Abstract: Sudden unexpected death in epilepsy (SUDEP) is a leading cause of death in patients with epilepsy. There is increasing evidence that apnea during a seizure is the primary cause of death following a seizure in SUDEP. However, the physiological mechanisms that drive breathing failure are completely unknown. The most critical drive to breathe is referred to as the central CO₂ chemoreflex: wherein the brainstem relies on the detection of tissue CO₂ levels to appropriately regulate breathing. There is evidence in epilepsy patients that seizures caused a prolonged decrease in the hypercapnic ventilatory response (HCVR), a measurement of CO₂ chemoreception. This has been replicated in *Scn8a*^{N1768D/+} (D/+) mice, a model of SUDEP that mimics the seizure activity of patients with *SCN8A* epileptic encephalopathy. The most well-documented risk factor for SUDEP is repeated generalized tonic-clonic seizures. Thus, we hypothesize that repeated seizures further suppress CO₂ chemoreception, increasing the likelihood of SUDEP. In this study, we explore the preictal and postictal HCVR before and after different numbers of induced seizures D/+ mice. 8-12-week-old D/+ and wild type littermate control (WT) mice were implanted with telemetric probes to record body temperature and, after recovery from surgery, whole-body plethysmography was used to record breathing parameters. Once mice were habituated to the experimental chamber (respiratory rate < 200 breaths per minute), the HCVR was tested by measuring the respiratory parameters at 0%, 3%, and 6% inspired CO₂ (21% O₂, balanced N₂). Next, one to four audiogenic seizures were evoked (15 KHz pure tone at 80 dB), each five minutes apart, and then the HCVR was tested 5 and 20 minutes postictal. Chamber temperature and humidity were measured throughout the experiment and, along with body temperature, were used to determine tidal volume. Minute ventilation was measured as a product of respiratory frequency and tidal volume, and HCVR was determined by the slope of minute ventilation and inspired CO₂. Baseline ventilation and response to 3% and 6% inspired CO₂ levels of WT and D/+ mice were not different (2-way ANOVA, *p* = 0.9725). There is a significant difference between preictal and postictal HCVR for D/+ mice exposed to two (2-way ANOVA, *p* = 0.009) and four (2-way ANOVA, *p* = 0.0002) audiogenic seizures. The data suggests that multiple consecutive seizures can lead to a blunted HCVR; however, it does not appear that more seizures further blunt the HCVR. The data can be used to support future experiments examining mechanisms of impaired HCVR and ultimately develop treatments to prevent SUDEP and improve the quality of life for persons with epilepsy.

Disclosures: M.L. Sculimbrenne: None. I.C. Wenker: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.10/C10

Topic: B.08. Epilepsy

Support: Clare Boothe Luce Fellowship

Title: Investigating the role of NaV channel dysfunction in seizure-induced neuronal damage & the therapeutic potential of XPC compounds

Authors: *M. LOKHANDWALA¹, C. ROCK¹, S. SIDHU², L. A. COCAS²;

¹Neurosci., ²Biol., Santa Clara Univ., Santa Clara, CA

Abstract: Status epilepticus (SE) causes lasting damage that initiates a cascade of degeneration increasing risk of future seizures. Current treatments for first seizure are primarily palliative rather than prophylactic. The SCN8A mutations implicated in epilepsy code for hyperactive Nav1.2 and Nav1.6 channels. XPC compounds were developed to selectively inhibit these channels and resulted in higher seizure thresholds in Scn8aN1768D/+ mice (Johnson et al., 2023). Existing studies do not address whether NaV1.6 continues to drive damage progression after a seizure occurs. We aim to explore whether acute post-seizure XPC treatment can preserve axon initial segment (AIS) structure, reduce glial reactivity, and protect myelin; and define a therapeutic window for post-seizure intervention.

We hypothesize that pilocarpine-induced SE causes early neuronal and glial injury, including AIS disruption, increased apoptosis, myelin degradation, and reactive gliosis, compared to healthy controls. We further hypothesize that acute post-seizure administration of XPC will reduce these effects and restore structure/function closer to control levels.

Our previous work demonstrated that acute seizure activity leads to significant pathological changes in adolescent mice, including widespread demyelination and increased cell death in the cortex, corpus callosum, and hippocampus (Foutch, Tilton, et al., 2025). These findings establish a strong precedent for studying early post-seizure injury and suggest that NaV1.6 inhibition may mitigate this damage.

Mice are assigned to control, seizure-only, or seizure + XPC rescue groups. Seizures are induced using the pilocarpine model, which causes widespread cortical excitation and overactivation of sodium channels, creating a hyperexcitable state closely mimicking the downstream effects of NaV1.6 dysregulation in epileptic conditions. XPC is administered immediately after observing SE. Immunohistochemistry is utilized to assess myelination, injury, glial activation, and synaptic integrity to evaluate damage and rescue.

We expect findings from the seizure-only group will support the theory that hyperexcitability driven by Nav1.6 amplifies damage even post-SE. In the XPC-treated group, we predict that NaV1.6 inhibition will demonstrate therapeutic potential with partial or full recovery when administered after seizure onset. Identifying these effects could reveal key targets for intervention during the window before injury becomes permanent. Our data may help specify the time frame in which NaV1.6 inhibition is most effective, providing insight into optimal timing for intervention after acute seizures

Disclosures: M. Lokhandwala: None. C. Rock: None. S. Sidhu: None. L.A. Cocas: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR320.12/C12

Topic: B.08. Epilepsy

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Title: Divergent Brain Network Reorganizations in Post-Traumatic and Kainate- Induced Epileptogenesis

Authors: *N. JAFARI¹, H. YEH², U. DEVARAJ², N. G. HARRIS^{3,4,5}, J. STERN², J. ENGEL, Jr.^{2,3,6}, A. BRAGIN^{2,3}, L. LI^{1,2};

¹Biomed. Engin., Univ. of North Texas, Denton, TX; ²Neurol., ³Brain Res. Inst., ⁴UCLA Brain Injury Res. Center, Dept. of Neurosurg., ⁵Semel Inst. for Neurosci. and Human Behavior,

⁶Neurobio. and Psychiatry, Univ. of California, Los Angeles, CA

Abstract: Epilepsy is increasingly recognized as a network disorder; however, the temporal evolution and pathological features of epileptogenic networks differ across etiologies. Limited knowledge exists on how functional connectivity (FC) reorganizes during the latent period of epileptogenesis. This study aimed to characterize early alterations in resting-state functional connectivity (rsFC) and their relationship with structural pathology in two preclinical models: posttraumatic epilepsy (PTE) induced by lateral fluid percussion injury (LFPI), and mesial temporal lobe epilepsy (mTLE) induced by intrahippocampal kainic acid (KA) injection. Resting-state fMRI and structural MRI were acquired at baseline, 1 week, and 8 weeks post-insult. Animals that developed spontaneous recurrent seizures were included in the analysis (mTLE: n=21; PTE: n=15). FC was evaluated across 33 predefined brain regions, with a focus on 12 key seed regions, and lesion volumes were quantified from structural MRI data. To examine the association between structural damage and functional network reorganization, correlations between lesion volume and FC strength across key brain regions were analyzed. Group comparisons assessed time-dependent changes in rsFC and lesion burden. In the mTLE model, hippocampal connectivity was significantly elevated at Week 1 ($p=0.024$), followed by global rsFC reduction at Week 8. Lesion expansion occurred in the prefrontal and sensorimotor cortices at both time points. Significant negative correlations were found between lesion volume and FC strength in the left hippocampus at Week 1 ($p=0.036$), and in the right hippocampus ($p=0.02$) and right sensorimotor cortex ($p=0.041$) at Week 8. In contrast, the PTE group showed progressive rsFC reductions in prefrontal and sensorimotor regions by Week 8. Lesion volume increased in the prefrontal cortex over time, but no correlation was found between lesion size and FC changes. Group comparison revealed higher FC at Week 8 in the mTLE model in the left ($p=0.025$) and right ($p=0.047$) prefrontal cortices, while the PTE model showed greater FC in the right hippocampus ($p=0.019$). Lesion volume was higher in mTLE at Week 1 in the right hippocampus ($p=0.023$) and left sensorimotor cortex ($p=0.046$) but became greater in PTE in the right hippocampus by Week 8 ($p=0.037$), with sensorimotor lesions stabilizing in both models. These findings reveal distinct temporal trajectories of functional and structural reorganization between TBI- and KA-induced epilepsy models. The divergent patterns of network plasticity underscore the importance of etiology-specific strategies for early detection and intervention in epilepsy.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

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Program #/Poster #: PSTR320.13/C13

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Title: Regional and circadian disruption of astrocytic K⁺ buffering in epileptic mice

Authors: *N. KIANI¹, K. ABUBAKAR¹, J. OUATAH¹, T. PETANJEK^{2,3}, M. ESCLAPEZ^{4,5}, A. I. IVANOV¹, C. BERNARD¹;

¹Aix Marseille Univ, INSERM, INS, Inst. Neurosci Syst, Marseille, France; ²Physiol. and Neurosci., Univ. of Zagreb, Zagreb, Croatia; ³Croatian Inst. for Brain Res., Zagreb, Croatia; ⁴Aix Marseille Univ, INSERM, INS, Inst. Neurosci Sys, Marseille Cedex 05, ; ⁵Aix Marseille Univ, Inserm, INMED, Inst. de Neurobiologie de la Méditerranée, Marseille, France

Abstract: Epilepsy is a chronic neurological disorder characterized by recurrent, unprovoked seizures, with medial temporal lobe epilepsy (mTLE) representing its most common and drug-resistant form. The hippocampal formation is central to seizure initiation, and both clinical and animal studies highlight regional and circadian influences on seizure susceptibility—specifically, the ventral hippocampus (VH) in rodents and the anterior hippocampus in humans show heightened excitability and damage vulnerability. In addition, almost 90% of drug-resistant epilepsy patients display circadian patterns in seizure occurrence, a phenomenon mirrored in rodent models. Seizure onset is often marked by a pronounced DC shift in baseline extracellular voltage, reflecting elevated extracellular potassium (K⁺) and suggesting disrupted K⁺ homeostasis. Astrocytes, via inward rectifying potassium (Kir4.1) channels and gap junctions, are key regulators of extracellular K⁺ through spatial buffering. Despite recent advances in elucidating the astrocytic role in the pathophysiology of epilepsy, it remains unclear to what extent astrocytic K⁺ buffering exhibits regional and circadian variation, and how these dynamics are altered in the epileptic brain. In this study, we investigated astrocytic K⁺ buffering via Kir4.1 channels and astrocytic networks in the dorsal (DH) and ventral (VH) hippocampi of healthy and chronically epileptic mice at different circadian time points. Our results revealed significant regional and circadian differences in K⁺ clearance efficiency, which were markedly altered in the epileptic state. Electrophysiological and pharmacological analyses showed that DH astrocytes in control animals exhibited larger Kir4.1 currents than those in VH. This regional variation was lost in epileptic mice, with overall loss of Kir4.1 current primarily in DH. K⁺-sensing electrode measurements indicated that control VH accumulates higher levels of extracellular K⁺ but clears it more rapidly than DH. In epileptic mice, however, VH K⁺

clearance is generally impaired and significantly slowed at times of peak seizure probability. Together, these findings underscore the critical role of VH excitability and astrocytic K⁺ buffering in seizure genesis, providing new insights into the spatial and circadian regulation of K⁺ homeostasis in epilepsy. This work advances our understanding of seizure onset mechanisms and may inform the development of targeted therapies for drug-resistant epilepsy.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.14/C14

Topic: B.08. Epilepsy

Title: Reduced Epileptogenic Threshold in Alzheimer's Disease: Experimental Validation and Neurochemical Interplay

Authors: *A. KAUR¹, R. K. GOEL²;

¹Dept. of Pharmaceut. Sci. and Drug Res., Punjabi Univ., Patiala, India; ²Pharmaceut. Sci., Professor,, Patiala, India

Abstract: Alzheimer's disease (AD) is increasingly associated with a reduced seizure threshold, suggesting a bidirectional relationship with epilepsy. Emerging evidence highlights shared neurochemical alterations, particularly disruptions in the balance between excitatory and inhibitory neurotransmission, as key contributors to neuronal hyperexcitability. The present study investigates seizure susceptibility in a streptozotocin (STZ)-induced AD model, using pentylenetetrazol (PTZ)-induced chemoconvulsions to assess seizure threshold. The overarching goal is to identify shared neurochemical targets relevant to the management of AD. Swiss albino mice received intracerebroventricular (ICV) injections of STZ, followed by PTZ kindling. Seizure susceptibility was evaluated by administering a PTZ (35 mg/kg) in STZ-treated mice. behavioral assessments, including the elevated plus maze, novel object recognition test and radial arm maze, were conducted across STZ, PTZ, and STZ + PTZ groups. Post-experiment, brain samples were collected for biochemical analysis (GSH, TBARS, ROS, AChE) and neurochemical profiling via HPLC-ECD. Results demonstrated a reduced seizure onset and increased seizure severity in STZ-treated mice, with convulsive episodes more prominent than in PTZ-treated controls. Behavioral tests revealed significant cognitive deficits in STZ, PTZ, and especially STZ + PTZ groups compared to saline-treated controls. Biochemically, decreased GSH and elevated TBARS, ROS, and AChE levels indicated heightened oxidative stress and cholinergic dysfunction. Neurochemically, increased glutamate and glutamine levels, along with reduced glycine and GABA levels, reflected excitatory-inhibitory imbalance. Additionally, diminished norepinephrine, dopamine, and serotonin levels were consistent with impaired cognitive function, with the most profound changes observed in the STZ + PTZ group. In

conclusion, PTZ administration lowered the seizure threshold in STZ-induced AD mice, correlating with increased oxidative stress, cholinergic disruption, excitatory-inhibitory neurotransmitter imbalance, and reduced monoaminergic transmission. These findings support a potential bidirectional link between epilepsy and AD, underscoring shared neurochemical targets.

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Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

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Program #/Poster #: PSTR320.15/C15

Topic: B.08. Epilepsy

Support: NIH R01 GM112715

Title: Latent Cortical Hyperexcitability Unmasked by GABAergic Disruption Following Neonatal Anesthesia Exposure

Authors: *D. P. AKSENOV^{1,2}, N. SERDYUKOVA², E. DOUBOVIKOV², M. MINHAIJ², S. GREENBERG²;

¹NorthShore Univ. HealthSystem, Evanston, IL; ²Endeavor Hlth., Evanston, IL

Abstract: General anesthesia is administered to millions of pediatric patients annually, yet its long-term impact on cortical development remains incompletely understood. Early-life anesthetic exposure might induce subtle neurological alterations that remain hidden under normal conditions but become evident following specific neurochemical challenges in adulthood. Here, we examined whether neonatal exposure to clinically relevant concentrations of sevoflurane anesthesia alters cortical excitability in rabbits. Neonatal rabbits underwent three sessions (2 hours each) of sevoflurane exposure at postnatal days 8, 11, and 14, without administration of any other drugs affecting brain activity. At adulthood, anesthesia-exposed animals and age-matched controls (mixed-sex, identical ages) were implanted with cortical electrodes and oxygen sensors for simultaneous recordings of electrophysiological activity and cortical oxygenation (PO₂) dynamics. Under baseline awake conditions, no differences in cortical activity or PO₂ fluctuations were observed between groups. However, localized GABA-A receptor antagonism with picrotoxin revealed significant latent hyperexcitability exclusively in anesthesia-exposed animals. These rabbits exhibited epileptiform-like discharges lasting 1-2 hours, coinciding with pronounced disturbances in cortical PO₂, indicative of impaired neurovascular coupling. These effects remained stable and reproducible over several months. Our findings suggest neonatal anesthesia exposure induces a latent vulnerability within cortical inhibitory networks, unmasked only upon disruption of GABAergic signaling. Understanding this latent susceptibility could explain variability in pediatric neurodevelopmental outcomes and highlights the need to investigate strategies to mitigate long-term risks of early anesthesia exposure.

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Poster

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1RF1NS129520

Title: Voltage imaging of excitatory and inhibitory neurons during epileptic biomarkers in a mouse model of cortical stroke

Authors: *D. C. SHAW¹, M. MOHAMMADU RIZAN², M. ABUMUAILEQ², C. RAVASIO², W. SHI⁶, L. DANG³, U. EDEN⁴, M. A. KRAMER⁵, C. J. CHU⁷, X. HAN²;

¹Grad. Program for Neurosci., ²Biomed. Engin., ³Undergraduate Program for Neurosci.,

⁴Mathematics and Statistics, ⁵Dept. of Mathematics and Statistics, Boston Univ., Boston, MA;

⁶Neurol., Massachusetts Gen. Hospital; Harvard Med. Sch., Boston, MA; ⁷Massachusetts Gen. Hosp. / Harvard Med. S, Cambridge, MA

Abstract: Interictal spikes, high-frequency ripples (80 - 250 Hz), and their co-occurrence (spike ripples, SRs) in human scalp or intracranial local field potential (LFP) recordings are established epileptic biomarkers found across etiologies. Though clinically significant, their physiological mechanisms remain unclear. To enable mechanistic investigation of the cellular and network mechanisms of these epileptic biomarkers, we first demonstrate that cortical photothrombosis in mice reliably produces spike, ripple, and SRs in peri-stroke cortex with morphology resembling those in human epilepsy. SRs, detected using automated procedures adapted from human intracranial data, best classified the pathological hemisphere. We then performed cellular voltage imaging of excitatory and inhibitory neurons using Voltron2 in mice with photothrombotic strokes. For imaging excitatory pyramidal cells, AAV1-syn-FLEX-Voltron2 and AAV9-CamKII-Cre were infused into superficial cortex near the stroke site in wild-type C57BL/6 mice, and then placed a 3 mm diameter glass window and a pair of LFP electrodes. Parvalbumin+ (PV) interneurons were targeted using AAV1-syn-FLEX-Voltron2 in PV-Cre mice. As Voltron2 requires chemical cofactors, JF-552 HaloTag ligand was administered 24 hours before imaging. Neuronal membrane voltage (Vm) and LFP were simultaneously collected, while mice were awake and head-fixed on a spherical treadmill. Aligning Vm to the corresponding epileptic

biomarkers revealed prominent Vm changes in both excitatory and inhibitory neurons around these LFP events. Many pyramidal cells exhibited robust Vm hyperpolarization preceding SRs, which transitioned to depolarization during SRs. However, changes in inhibitory neuron activity were less organized around these LFP events. These results demonstrate a robust platform to study the physiological mechanisms of epileptic biomarkers.

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Poster

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Title: Metabolic Underpinnings of SLC13A5 Epilepsy

Authors: *A. LIN¹, L.-J. CHEW², A. ZENG³, E. NOLAN⁴, K. WHITLEY⁴, I. S. JIN⁴, H. HIGASHIMORI⁵, J. S. LIU⁶;

¹Brown Univ. Neurosci. Grad. Program, Providence, RI; ²Dept Mol Biol, Cell Biol and Biochem, ³Dept. of Mol. Biology, Cell Biology, and Biochem., ⁵Sch. of Med., ⁶Neurol., ⁴Brown Univ., Providence, RI

Abstract: SLC13A5 Epilepsy (DEE25) is a rare genetic disorder caused by mutations in SLC13A5, a sodium-citrate transporter. Individuals born with SLC13A5 mutations are affected by severe multi-focal seizures soon after birth and subsequently develop both cognitive and motor impairments. SLC13A5 regulates citrate import across the plasma membrane; in humans, SLC13A5 is highly expressed in the brain and liver but the relationship between SLC13A5 expression sites, citrate levels, and the observed epileptiform activity in patients is unclear. To investigate the role of endogenous SLC13A5 on neuronal activity at various expression sites, we generated tissue-specific conditional SLC13A5 knockout mice: full-body SLC13A5 knockout, liver-specific SLC13A5 knockout, and brain-specific SLC13A5 knockout. We recorded video-electroencephalography (EEG) data from these mice and analyzed for epileptiform activity. Tissue-specific knockout of SLC13A5 was confirmed with quantitative PCR experiments. Preliminary analysis of EEGs has found seizures in liver-specific SLC13A5 knockout despite normal levels of citrate in the CSF, suggesting that liver-specific expression of SLC13A5 may play a role in epileptogenesis unrelated to citrate levels. To investigate the effect of citrate on

neuronal activity, we performed whole-cell patch clamp experiments to examine potential changes in intrinsic firing properties and network excitability secondary to citrate administration. Our results demonstrate no change in intrinsic firing properties of regular-spiking (RS) cells following citrate administration. Together, these results suggest that epileptiform activity may be correlated with a downstream effect of SLC13A5 deficiency rather than citrate levels themselves. Future directions include untargeted metabolomics of plasma samples collected from mice with varying SLC13A5 knockout to identify potential metabolites leading to neural hyperactivity.

Disclosures: A. Lin: None. L. Chew: None. A. Zeng: None. E. Nolan: None. K. Whitley: None. I.S. Jin: None. H. Higashimori: None. J.S. Liu: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.18/C18

Topic: B.08. Epilepsy

Title: The Expression of c-Src in the Limbic System and the Wnt/ β -Catenin Pathway in a Kindling Model of Epilepsy in Caloric-Restricted Rats
The Expression of c-Src in the Limbic System and the Wnt/ β -Catenin Pathway in a Kindling Model of Epilepsy in Caloric-Restricted Rats

Authors: *N. ESTEBAN-ROMÁN;

Neurophysiol., Inst. Nacional de Neurología y Neurocirugía, Ciudad de México, CDMX, Mexico

Abstract: Objective This study aims to evaluate the relationship between caloric restriction and the expression of the c-Src protein in the Wnt/ β -Catenin pathway. Specifically, we will examine, using immunohistochemistry and Western blot, how c-Src changes in neurons of the amygdala and hippocampus of Wistar epileptic rats (kindling model) that have been subjected to a restricted diet. Background Epilepsy is a neurological disorder characterized by recurrent seizures caused by abnormal neuronal activity. Caloric restriction (CR) exerts neuroprotective effects and reduces seizures, with possible implications in the modulation of the Wnt/ β -catenin pathway. Within this pathway, the c-Src protein is key: its inhibition decreases β -catenin activity and the expression of genes linked to neuronal hyperexcitability. In parallel, KATP channels regulate neuronal excitability by hyperpolarizing the membrane under low ATP levels. It's postulated that c-Src could be a regulatory convergence point between the Wnt/ β -catenin pathway and KATP channels, thereby controlling excitability. Studying c-Src is crucial for discovering new mechanisms in epilepsy and developing novel therapeutic strategies. Methods We used 24 male Wistar rats (250-300 g), equally divided into four groups: Control (no intervention), Kindling (limbic stimulation to induce epilepsy), Kindling + CR (stimulation + 80% caloric restriction), and CR (80% caloric restriction). Epilepsy was induced by repeated electrical stimulation of the basolateral amygdala until five generalized seizures were achieved.

Subsequently, hippocampus and amygdala samples were obtained to analyze c-Src expression using immunohistochemistry and Western blot. Results In the hippocampus, c-Src expression levels were: Control (1.00), CR (1.22), Kindling (1.40), and Kindling + CR (1.29). We observed that epileptic seizures increased c-Src activation, and CR showed an intermediate modulation of this activation. Conclusion Our findings indicate that CR modulates the expression of the c-Src protein in the context of epilepsy. This effect could be due to CR's influence on the Wnt/ β -Catenin pathway. We suggest that c-Src acts as a key integrator between cellular metabolism and neuronal excitability, possibly by interacting with KATP channels. The results show that CR exerts a neuroprotective effect by reducing c-Src activation, which could help decrease seizure frequency. These findings open the door to new non-pharmacological therapeutic strategies for refractory epilepsy. Keywords: c-Src, Wnt/ β -Catenin, epilepsy

Disclosures: N. Esteban-Román: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.19/C19

Topic: B.08. Epilepsy

Support: Welcome Trust, UK
Epilepsy Research, UK

Title: Cognitive impairment and ictal activity in a GABA_AR antibody-mediated encephalitis rodent model is prevented by Zolpidem treatment

Authors: *M. A. UPADHYA¹, J. KREYE², J. HALL¹, Z. ALOBAIDI¹, R. BILL¹, R. BADHAN¹, G. WOODHALL¹, H. PRUESS³, S. WRIGHT¹;
¹Aston Univ., Birmingham, United Kingdom; ²DZNE Berlin, Berlin, Germany; ³Charite Berlin, Berlin, Germany

Abstract: Background: Autoantibodies targeting GABA_A receptors (GABA_AR-Ab) in autoimmune encephalitis (AE) cause seizures and neuropsychiatric manifestations. Our previous study demonstrated that intra-cerebroventricular (ICV) infusion of patient-derived GABA_AR monoclonal antibodies (mAbs) caused severe epilepsy in rodents. Here, we evaluate zolpidem, a positive allosteric GABA_AR modulator, as a treatment in vivo. Method: Juvenile male Wistar rats (n=13) received ICV infusion of patient-derived GABA_AR mAbs for 7 days. On days 2-7, rats were treated daily with subcutaneous saline (n=7, GABA-saline) or zolpidem (1 mg/kg; n=6, GABA-zolpidem). Wireless EEG transmitters recorded from hippocampal CA3 for 9 days. EEG was analyzed by automated ictal event detection (Neuroarchiver) and a custom-made macro for power-band analysis. On days 2, 6 and 9, post-seizure behavioral battery (PSBB) and novel objection recognition (NOR) tests assessed hyperexcitability and cognition. Ex vivo, kainate-induced gamma oscillations (K γ O) in CA3 region were used to assess hippocampal network

integrity via local field potential (LFP) recordings. In silico studies were conducted to compare GABA_AR binding by mAbs ± zolpidem. Results: In silico results indicated zolpidem binds $\alpha 1$ and $\gamma 2$ subunits of GABA_AR at the same interface targeted by mAbs. Total ictal counts were significantly lower in GABA-zolpidem group (27 ± 24 vs. 246 ± 95 ; $p=0.01$) compared to GABA-saline. EEG delta waveband power was significantly increased in the GABA-saline group compared to the zolpidem-treated (7512 ± 522 vs. $3437 \pm 104 \mu V^2/Hz$; $p<0.0001$). The GABA-zolpidem group did not show hyperexcitability in behavioral testing compared to the GABA-saline group (4.8 ± 2.1 vs. 13.7 ± 2.2 ; $p<0.05$). The GABA-saline group showed significantly impaired cognitive function on day 6 (0.54 ± 0.03 vs. 0.83 ± 0.06 ; $p<0.001$) and day 9 (0.56 ± 0.04 vs. 0.81 ± 0.02 ; $p<0.01$) compared to the GABA-zolpidem group. In zolpidem-treated rats ($n=6$; $n=20$ CA3 slices) 100 nM kainate elicited a significant increase in K γ O power indicating normal hippocampal neuronal network function, in comparison to saline injected rats where the network failed to respond ($n=5$; $n=14$ CA3 slices; $p<0.05$). Discussion and Conclusion: Overall, this preliminary study demonstrates that zolpidem reduces ictal activity and prevents cognitive dysfunction in vivo in a GABA_AR-AE rodent model and restores aberrant network activity in vitro. Docking data confirm zolpidem's binding overlaps pathogenic mAb sites. These results highlight the potential of repurposing receptor specific treatments for the treatment of antibody-mediated epileptic encephalopathy.

Disclosures: M.A. Upadhy: None. J. Kreye: None. J. Hall: None. Z. Alobaidi: None. R. Bill: None. R. Badhan: None. G. Woodhall: None. H. Pruess: None. S. Wright: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.20/C20

Topic: B.08. Epilepsy

Support: TSC Alliance
Drs. Bonnie and Jonathan Rothberg & Family
Gerry and Bill Cowlin Foundation

Title: Dose-response of mTOR inhibitors in a nonclinical model of tuberous sclerosis complex (TSC) associated epilepsy - Conducted by the TSC Preclinical Consortium

Authors: *Z. FUCHS¹, M. CATRON², G. C. TOMBAUGH², R. RAKHIT³, R. Y. KIM³, N. KHAN³, N. T. HERTZ³, S. MORAIRTY², D. J. AGUIAR¹;

¹TSC Alliance, Silver Spring, MD; ²PsychoGenics, Inc., Paramus, NJ; ³Montara Therapeut., San Francisco, CA

Abstract: Introduction: TSC is a rare genetic disorder caused by mutations in either the *TSC1* or *TSC2* gene, leading to loss of function and increased activity of mechanistic target of rapamycin (mTOR). Approximately 85% of those affected with TSC exhibit epilepsy. To

address this, mouse models of TSC-specific epilepsy have been developed to further translational drug development. In the *Tsc1^{flox/flox}; GFAP-Cre⁺* conditional knockout (Tsc1 CKO), *Tsc1* is knocked out in glial fibrillary acidic protein (GFAP)-expressing cells, including astrocytes. The TSC Alliance Preclinical Consortium has tested more than 70 distinct compounds in this model. Historically, 3 mg/kg (mpk) rapamycin has been used as a positive control. In this study, we sought to define a dose response of mTOR inhibitors, rapamycin and everolimus, on seizure reduction and improve the differentiation of novel therapeutics. **Methods:** Tsc1 CKO mice derived from Michael Wong, MD, PhD (Washington University, St. Louis) (N=112) were balanced across six groups (0.03, 0.3, and 1 mpk) of rapamycin or everolimus and vehicle. Daily IP dosing began at P21 and continuous EEG was scored P45-54 for generalized tonic-clonic seizures. Whole brains were collected (P57) from a subset of animals (N=3-4 per group) to determine biomarker levels, including p70S6K and S6. Drug levels were determined by LC/MS. **Results:** A dose-dependent decrease in seizure frequency was observed across the treatment groups, with an approximate average EC₅₀ of 0.24 mpk. End of study survival was extended in a dose-dependent manner (Mantel-Cox test, all groups: p=0.0012. Brain and plasma drug concentrations showed proportionate dose-dependence across compartments. Brain concentration of mTOR inhibitors >20ng/g led to >50% reduction of mTOR biomarkers. Both rapamycin and everolimus reduce seizure frequency and improve survival in a dose-dependent manner in Tsc1 CKO mice. A similar relationship was observed in seizure reduction, where brain concentrations of mTOR inhibitors as low as 10.7ng/g (everolimus 0.3 mpk) and 14.8ng/g (rapamycin 0.3 mpk) led to a ≥50% reduction in seizures compared to vehicle average. **Conclusion:** This study provide a baseline dose, PKPD, and efficacy correlations for mTOR inhibitor treatment in a widely used model for seizures in TSC. This clinically relevant translational model of TSC can be used to inform and de-risk drug testing of new therapies for TSC.

Disclosures: **Z. Fuchs:** None. **M. Catron:** None. **G.C. Tombaugh:** None. **R. Rakhit:** A. Employment/Salary (full or part-time): Montara Therapeutics. **R.Y. Kim:** A. Employment/Salary (full or part-time): Montara Therapeutics. **N. Khan:** A. Employment/Salary (full or part-time): Montara Therapeutics. **N.T. Hertz:** A. Employment/Salary (full or part-time): Montara Therapeutics. **S. Morairty:** None. **D.J. Aguiar:** None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.21/C21

Topic: B.08. Epilepsy

Title: Functional ultrasound assessment of seizure threshold modulation during slow kainic acid infusion in naive and epileptic mice

Authors: R. IMMONEN¹, T. BOLKVADZE¹, T. BRAGGE¹, ***J. RYTKÖNEN**²;

¹Charles River Discovery Res. Services, Kuopio, Finland; ²Charles River Discovery, Kuopio, Finland

Abstract: Chronic epileptic animals have increased susceptibility to seizures, i.e. they are prone to seizures upon application of external stressor, such as a chemoconvulsant challenge.

Antiepileptic and anticonvulsant treatments aim to modulate the excitability and the seizure susceptibility. In this study, we used functional ultrasound (fUS), a non-invasive, neuroimaging method, to record the brain hemodynamic response to a sub-convulsive dose of kainic acid (KA) - an epileptogenic chemoconvulsant.

Three parallel cohorts of adult C57BL/6J male mice were studied. In the first cohort, a model of chronic epileptic activity was established by induction of status epilepticus with fractionated kainic acid (KA) injections (10 mg/kg + 10 mg/kg + 5 mg/kg + 5 mg/kg, 30-min interval, i.p., total dose 30 mg/kg). The development of epileptic activity was confirmed by continuous 3-week EEG recording (cortical electrodes over the hippocampus). In the second cohort, fUS was used to characterize the activation pattern induced by slow KA infusion in naïve mice under ketamine-medetomidine anesthesia (KA i.p. 10 mg/kg, 10 mL/kg over 30 min infusion). The 55-min fUS scan consisted of a 5-min baseline, and a 50-min follow-up of the response in relative cerebral blood volume (rCBV) during and after the 30-min infusion. In the third cohort, epilepsy was induced by fractionated KA injections as established in the first cohort and fUS imaging utilizing the KA infusion challenge developed in the second cohort was performed in the chronic epileptic animals 4 weeks after the KA challenge.

Induction of chronic epileptic activity with fractionated KA injections in mice was successful with 0% acute mortality, and spontaneous epileptiform activity, spiking and seizures manifesting in all animals. A slow infusion of KA in naïve mice resulted in a 30% increase in the rCBV in hippocampus while no activation was detected in thalamus or cortex. The onset of the hippocampal response was observed 22 to 32 min after the KA infusion start with a response maximum at 45 min, corresponding to a cumulative dose of 30 mg/kg. Pre-treatment with diazepam 5 mg/kg suppressed the increase in rCBV response. In chronic epileptic animals, a fUS challenge consisting of slow infusion of KA was expected to induce a faster onset and higher magnitude in the hippocampal response, spreading to the cortex and thalamus indicating epileptiform activation and seizure generalization. The fUS platform with sub-seizure KA challenge allows testing of novel seizure threshold modulating treatments.

Disclosures: **R. Immonen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **T. Bolkvadze:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **T. Bragge:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **J. Rytkönen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.22/C22

Topic: B.08. Epilepsy

Support: Special Research Fund - BOF

Title: Investigating the effect of photopharmacological modulation on epileptic neural circuits with semi-transparent, soft and conformable neural microelectrode arrays

Authors: *D. KC¹, L. SOHAIL¹, S. DRAKOPOULOU¹, J. SPANOGHE², R. RAEDT², G. SPYROPOULOS¹;

¹Dept. Information Technol., Ghent Univ., Zwijnaarde, Belgium; ²Dept. of Head and Skin, Ghent Univ., Ghent, Belgium

Abstract: Drug-resistant epilepsy remains a significant clinical challenge, necessitating pathway-specific interventions that modulate excitatory and inhibitory imbalances. Among emerging approaches, activation of adenosine A1 receptors (A1Rs) has shown promise by inhibiting presynaptic glutamate release and promoting postsynaptic hyperpolarization. However, conventional pharmacological delivery lacks spatial and temporal specificity, leading to widespread drug diffusion and continuous receptor activation. This broad activity not only hampers the ability to control focal seizures but also increases the risk of systemic side effects, including sedation and cardiovascular complications. Photopharmacological techniques offer a compelling alternative by enabling light-driven, spatially precise receptor activation. Yet, the network-level dynamics of such interventions remain largely unexplored on a multiregional scale. In this study, we present a soft, semi-transparent, wide-coverage neural probe engineered with a honeycomb architecture, low-impedance conductive polymer electrodes, and large perforations to maintain cortical access and flexibility. This platform enables real-time, high-resolution monitoring of cortical seizure dynamics during photopharmacological modulation. We investigated the effect of a photocaged A1R agonist (cCPA; Craey et al., 2024) in ten-week-old Sprague-Dawley rats across two acute chemoconvulsant seizure models: 4-Aminopyridine (4AP) and picrotoxin (PTX), which disrupt potassium and chloride-mediated inhibition, respectively. cCPA was microinjected at four neocortical sites surrounding a central seizure focus (central site of chemoconvulsant injection), and 405 nm light pulses were delivered via optical fibers or LEDs under various stimulation protocols and in various locations of neocortex. This setup allowed for systematic assessment of seizure propagation and confinement patterns in response to localized A1R activation. Our ongoing work will further explore cCPA's modulatory efficacy in electrically induced seizure models to compare suppression dynamics across chemical and non-chemical paradigms.

Disclosures: D. Kc: None. L. Sohail: None. S. Drakopoulou: None. J. Spanoghe: None. R. Raedt: None. G. Spyropoulos: None.

Poster

PSTR320: Mechanistic Studies of Epilepsy in Animal Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR320.23/Web Only

Topic: B.08. Epilepsy

Support: Department of Biotechnology, Government of India
Institution of Eminence (IOE), University of Delhi

Title: Isoform-specific regulation of Wnt/ β -catenin signaling by Casein Kinase 2 in Temporal Lobe Epilepsy

Authors: *R. MITTAL¹, P. PRIYA², N. YADAV³, J. BANERJEE⁴, A. B. DIXIT⁵;

¹Dr B R Ambedkar Ctr. for Biomed. Res., Univ. of Delhi, Delhi, India; ²ACBR, Univ. of Delhi, Delhi, India, Bazpur, India; ³Univ. of Delhi, North Campus, New Delhi, India; ⁴Biophysics, All India Inst. of Med. Sci. (AIIMS), New Delhi, India, New Delhi Delhi, India; ⁵ACBR, ACBR, Univ. of Delhi, Delhi, India

Abstract: INTRODUCTION: Mesial temporal lobe epilepsy is the most common form of drug-resistant epilepsy and is hypothesised to be a cause of imbalance between the excitation and inhibition activity in the brain. The canonical Wnt signaling has been associated with aberrant neurogenesis, neuroinflammation, and hyperexcitability. Casein Kinase 2 (CK2), a serine/threonine kinase, is known to phosphorylate multiple components of Wnt/ β -Catenin pathway, making them key substrates in the regulation of the signaling cascade. The two catalytic subunits- CK2 α and CK2 α' , though share high homology, differ in subcellular localization and substrate specificity, implicating the differential regulation of Wnt/ β -Catenin signaling by the two isoforms. Dysregulation of the CK2 isoform-specific modulation of Wnt signaling may play a crucial role in the pathophysiology of MTLE. **METHODOLOGY:** siRNA targeting CK2 α and CK2 α' was injected bilaterally into the hippocampus of male Sprague-Dawley rats through stereotaxic surgery. Post knockdown, the acute lithium-pilocarpine model of TLE was generated. Seizure activity was video-monitored and the hippocampal and anterior temporal lobe (ATL) tissues were collected. H & E staining confirmed histopathological changes. Post CK2 isoform-specific inhibition, the mRNA and protein expression levels of key components of Wnt signaling cascade were analysed using quantitative real-time PCR and Western Blotting. **RESULTS:** CK2 α knockdown showed significant reduction in the seizures, unlike CK2 α' knockdown, where the seizure grades were comparable to untreated epileptic controls. Histological analysis further revealed distinct outcomes in neuronal integrity between the two isoforms. Molecular studies demonstrated significant alterations in the expression of β -Catenin, Dishevelled, and Lef-1 at both mRNA and protein levels in the hippocampus and ATL, highlighting region-specific modulation of Wnt/ β -Catenin signaling by CK2 isoforms in TLE. **CONCLUSION:** These findings reveal the differential regulatory roles of CK2 catalytic isoforms in modulating Wnt/ β -Catenin signaling pathway in TLE. The region-specific alterations in the expression of Wnt signaling molecules driven by CK2 isoform specific modulation, not only highlights the functional specificity of the two catalytic subunits but also determines their distinct influence on signaling cascade spatially, indicating unique contributions of each isoform to TLE pathophysiology.

Disclosures: R. Mittal: None. P. Priya: None. N. Yadav: None. J. Banerjee: None. A.B. Dixit: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.01/C23

Topic: B.09. Glial Mechanisms

Support: NIH R01 DC007695
NIH R01 DC015901

Title: Three-dimensional reconstructions illustrate morphogenesis of developing astrocytes during neural circuit formation in the auditory brainstem

Authors: *E. M. AMICK, A. GABBARD, V. MADEM, J. M. AHMED, A. NISHAD, D. T. HELLER, G. A. SPIROU;
Med. Engin., Univ. of South Florida, Tampa, FL

Abstract: Astrocytes perform critical functions in the mature central nervous system, including maintaining the blood-brain barrier via perivascular endfeet and modulating synaptic activity through perisynaptic processes. Electron microscopy (EM) is required to resolve these fine structures, but the complexity and size of EM datasets present analytical challenges. Light microscopy lacks the resolution to capture the full complexity of astrocyte nanoarchitecture, necessitating the use of large-volume EM approaches. While mature astrocyte ultrastructure is increasingly well-characterized, little is known about how these features emerge during early development. To address this, we examined astrocyte morphology across postnatal development in the medial nucleus of the trapezoid body (MNTB), an auditory brainstem region that undergoes rapid, stereotyped circuit maturation and is emerging as a valuable model for glial development. We utilized our unique developmental series (postnatal day (P)2, 4, and 6) of serial block-face scanning EM (SBEM) image volumes to observe the change in the astrocyte morphology. All astrocytes within each volume were skeletonized to assess branching patterns across development (P2: N = 39; P4: N = 12; P6: N = 27), revealing a consistent presence of 3-4 primary processes per cell at each age. Across all developmental stages, astrocyte nuclei were consistently eccentric in position and had a lobular shape. Furthermore, we generated the first complete reconstructions of astrocytes at P2, P4, and P6, enabling detailed analysis of their morphological features across early development. Qualitative comparison of the reconstructions revealed the presence of perivascular endfeet and contacts with nerve terminals as early as P2. Quantitative analysis showed that astrocytes formed 3, 3, and 2 endfeet at P2, P4, and P6, respectively. We made the novel observation that large portions of MNTB principal neuron (PN) somata were covered by vellus, or sheet-like, astrocytic processes at all developmental stages. Each astrocyte extended vellus processes onto multiple PNs, contacting 5, 6, and 7 cells at P2, P4, and P6, respectively. At P4 and P6, large-caliber axons were observed to be partially ensheathed by astrocytic processes. Although myelination in the MNTB begins by P9, astrocytes at this stage continued to extend processes that wrapped around myelinated axons. These findings provide new insights into the early morphological development of astrocytes and suggest that key structural features associated with mature function are established during critical periods of synaptic and vascular maturation.

Disclosures: E.M. Amick: None. A. Gabbard: None. V. madem: None. J.M. Ahmed: None. A. Nishad: None. D.T. Heller: None. G.A. Spirou: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); syGlass.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.02/C24

Topic: B.09. Glial Mechanisms

Support: CIHR Grant
NSERC

Title: Effects of noradrenaline-induced astrocyte purinergic signalling on PVN CRH neuron activity

Authors: *P. GROVUE¹, G. PERINGOD¹, T. CHOMIAK^{1,2}, M. ROJAS-CARVAJAL¹, J. S. BAINS^{1,3,4}, G. R. GORDON¹;

¹Hotchkiss Brain Institute, Dept. of Physiol. and Pharmacol., Univ. of Calgary, Calgary, AB, Canada; ²Cumming Sch. of Med. Optogenetics Core Facility, Calgary, AB, Canada; ³Krembil Res. Inst., Toronto, ON, Canada; ⁴Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The paraventricular nucleus of the hypothalamus (PVN) is vital to the regulation of the neuroendocrine response to stress. Corticotropin-releasing hormone (CRH) neurons within the PVN initiate this response by releasing CRH, which ultimately drives activation of the hypothalamic-pituitary-adrenal (HPA) axis. During stress, noradrenaline (NA) released into the PVN acts upon both CRH neurons and neighbouring astrocytes via G-protein-coupled adrenoceptors, of which the $\alpha 1$ subtype is most heavily engaged in Gq signalling pathways. Astrocytes are known modulators of neuronal activity across the brain and play an integral role in the ability of NA to modulate synapses. This positions PVN astrocytes to play a role in modulating CRH neuron activity, potentially altering HPA axis outputs. However, it is not fully known how astrocytes may modulate CRH neuron activity, nor have possible mechanisms been fully investigated in the PVN. Using 2-photon imaging in acute mouse brain slices, we first sought to elucidate how astrocytes respond to NA. We found that PVN astrocyte responses to bath application of the $\alpha 1$ -agonist phenylephrine (PE) were nearly abolished by $\alpha 1A$ -adrenoceptor antagonism, while the CRH neuron response was only partially blocked. Given that Gq signalling in astrocytes drives calcium-dependent gliotransmission, we pursued a well-known astrocyte gliotransmission pathway: the release of ATP, which is readily degraded into adenosine in the extracellular space. Biosensors localized to astrocytic membranes revealed increases in both ATP and adenosine following PE application. To assess the impact of ATP/adenosine release on CRH neurons, we showed that bath application of adenosine caused an overall inhibition of CRH neuron calcium activity, and blocking adenosine A1 receptors triggered an

increase in CRH neuron calcium activity. To directly test astrocytic influence, we expressed CalEx—a calcium-extruding plasma membrane pump—in astrocytes to clamp their calcium activity. This manipulation led to increased spontaneous calcium activity in CRH neurons. Ultimately, these findings outline a PVN astrocyte signalling pathway that could serve to modulate CRH neuron activity, suggesting a role for astrocytes in the regulation of the stress response.

Disclosures: P. Grovue: None. G. Peringod: None. T. Chomiak: None. M. Rojas-Carvajal: None. J.S. Bains: None. G.R. Gordon: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.03/C25

Topic: B.09. Glial Mechanisms

Support: NIA AG085666

Title: Functional Gap Junction Coupling of Atypical Astrocytes in Normal Aging

Authors: *E. SOJKA¹, P. TIRJA², V. RAO³, K. LEFTON³, M. GOOD⁴, M. ARMBRUSTER³, C. G. DULLA³;

¹Tufts Univ., Boston, MA; ²Tufts Univ. Grad. Sch. of Biomed. Sci., Boston, MA; ³Dept. of Neurosci., Tufts Univ. Sch. of Med., Boston, MA; ⁴Mol. Cardiol. Res. Ctr., Tufts Med. Ctr., Boston, MA

Abstract: Astrocytes play many roles in the brain, including controlling neurotransmission, maintaining blood-brain-barrier function and integrity, and shaping inflammation. Astrocytes form large intercellular networks via gap junction coupling, which allows for communication, trafficking, and redistribution of molecules. While astrocyte protein expression undergoes characteristic, spatially broad changes during normal aging, preliminary data also establishes an increasing proportion of astrocytes that sporadically lose expression of key functional proteins, impairing their essential functions. Referred to as atypical astrocytes (AtAs), these cells acquire this novel, distinct phenotype on a cell-by-cell basis and are concentrated in the prefrontal cortex (PFC) and retrosplenial cortex (RSC). AtAs have also been reported following repeated mild traumatic brain injury where they show similar phenotypes to those found in normal aging. Proteins lost in AtAs include excitatory amino acid transporters, Kir4.1, and aquaporin-4, which clear glutamate from synapses, mediates potassium buffering, and removes amyloid beta, respectively. Additionally, preliminary data indicates crucial astrocytic gap junction proteins—connexin 30 (Cx30) and connexin 43 (Cx43)—are decreased in aging-induced AtAs as compared to their typical astrocyte counterparts. Likewise, RNA sequencing data from fluorescence-activated cell sorting (FACS)-isolated AtAs reveals GJB6 and GJA1 mRNA levels—encoding Cx30 and Cx43 proteins, correspondingly—are also downregulated.

In this study, we use immunohistochemistry and electrophysiology in juvenile and aged mice to investigate the functional coupling of AtAs. To characterize gap junction protein expression profiles of AtAs, we performed immunohistochemistry for Cx30 and Cx43 in 2-month-old and 1-year-old C57BL/6 mice. We found AtAs have significantly decreased Cx30 and Cx43 immunoreactivity compared to typical astrocytes. To evaluate whether AtAs are functionally uncoupled due to their lack of Cx30 and Cx43, we employed a dye diffusion assay in acute cortical slices. We filled individual astrocytes in the PFC or RSC with biocytin, a small molecule permeable to gap junctions. Biocytin diffusion to neighboring astrocytes will be assessed to quantify the functional coupling of the astrocytic network. We hypothesize biocytin will not spread into or out of AtAs, which will be validated with subsequent immunolabeling for Cx30 or Cx43. These findings will provide insights into whether astrocytic networks become progressively uncoupled in the aging brain and will inform a mechanism by which they may do so.

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Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.04/C26

Topic: B.09. Glial Mechanisms

Support: NIH/NIA R01AG059848
NIH/NIA P30 AG066515

Title: Divergent astrocyte responses in cortical gray and white matter in Alzheimer disease

Authors: *J. PAN¹, A. SANKARAESWARAN², J. SANZ-ROS⁷, X. JIANG⁸, J. FORES MARTOS², D. PARK³, S. IVEZICH⁴, J. JOHNSON⁵, B. LU⁶, J. J. PALOP⁹, I. COBOS¹⁰;
¹Stanford Univ., Palo alto, CA; ²Stanford Univ., Palo Alto, CA; ³Dept. of Pathology, Stanford Univ., Stanford, CA; ⁴Stanford Univ., Menlo park, CA; ⁵Biol., Stanford Univ., Fort Lauderdale, FL; ⁶Stanford Univ., palo alto, CA; ⁷Dept. of Pathology, Stanford Univ. Sch. of Med., Mountain View, CA; ⁸Gladstone Institutes, UCSF, San Francisco, CA; ⁹Gladstone Inst. & UCSF, South San Francisco, CA; ¹⁰STANFORD UNIVERSITY, Stanford, CA

Abstract: Astrocytes undergo molecular and functional changes during the progression of Alzheimer disease (AD), yet the heterogeneity of their responses in gray matter (GM) and white matter (WM) remains poorly understood. Here, we combined single-nucleus and spatial transcriptomics in prefrontal GM and WM to define region-specific molecular subtypes of astrocytes and their correspondence with morphologically defined subtypes across AD progression in the human brain. We found that the pan-activator astrocyte marker CD44 was increased in both WM and GM in AD, but it exerted divergent functions in these regions. In

WM, increased CD44 in fibrous astrocytes, through interactions with its ligand hyaluronic acid, led to an increase in oligodendrocyte precursor cells (OPCs), decreased oligodendrocytes and myelin, and a disrupted remyelination environment, a phenotype exacerbated in *APOEε4* compared to *APOEε3* donors. In contrast, increased CD44 in protoplasmic astrocytes resulted in enhanced migration and clustering around amyloid-beta (Aβ) plaques, mediated by interactions with its ligand osteopontin (*SPPI*), secreted by microglia. Our findings highlight cellular and region-specific astrocyte states in AD and underscore the importance of spatially resolved omics in uncovering the complexities of AD pathogenesis.

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Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.05/C27

Topic: B.09. Glial Mechanisms

Title: Mapping Astrocyte Heterogeneity in the Mouse Brain Using an RNA-Protein Multomics Assay

Authors: *D. WAKHLOO¹, C. ZHOU², Y. WANG², L.-C. WANG², A. DIKSHIT²;
¹Spatial Biol. - ACD, ²Spatial Biol. Div., Bio-Techne, Newark, CA

Abstract: Astrocytes are critical regulators of central nervous system (CNS) function, contributing to synaptic homeostasis, metabolic support, and neuroinflammation. Their phenotypic and functional heterogeneity underlies region-specific roles in brain development and disease. To explore astrocytic heterogeneity and neuron-glia interactions in the mouse brain, we implemented a spatial multiomics workflow integrating RNA in situ hybridization and immunofluorescence. Here, we introduce a protease-free workflow for the RNAscope™ Multiplex Fluorescent v2 assay, combining RNA in situ hybridization (ISH) with protein immunofluorescence to detect both RNA and protein markers simultaneously on the same frozen mouse brain tissue sections. This manual multiplex protease-free workflow enables the visualization of RNA and protein targets without enzymatic digestion, preserving tissue morphology and antigenicity. Using this workflow, we profiled canonical astrocyte markers—Slc1a3 (encoding GLAST), Aldh1l1, and Glul (encoding glutamine synthetase)—and co-localized them with glial fibrillary acidic protein (GFAP), a pan astrocytic marker. To examine spatial associations between astrocytes and neurons, we co-detected Gja1 mRNA (encoding the gap junction protein connexin-43 in astrocytes) alongside MAP2 protein, a neuronal dendritic marker. This spatial multiomics approach enables detection of variation in astrocyte gene expression and subtype distribution, highlighting diverse functional states within the astrocyte lineage. Importantly, our protease-free method preserved delicate cellular features, enabling

accurate co-detection of molecular signals that inform astrocyte identity and their interactions with neurons. RNAscope™ manual multiplex assay, provides a robust tool for dissecting cell-type-specific molecular landscapes, facilitating deeper insights into glial biology across development, aging, and neurodegeneration.

Disclosures: **D. Wakhloo:** A. Employment/Salary (full or part-time): Bio-Techne. **C. Zhou:** A. Employment/Salary (full or part-time): Bio-Techne. **Y. Wang:** A. Employment/Salary (full or part-time): Bio-Techne. **L. wang:** A. Employment/Salary (full or part-time): Bio-Techne. **A. Dikshit:** A. Employment/Salary (full or part-time): Bio-Techne.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.06/C28

Topic: B.09. Glial Mechanisms

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Title: Astrocyte-norepinephrine signaling tunes neural encoding for behavioral adaptation

Authors: ***M. CELOTTO**¹, G. DRUMMOND², A. NATESAN¹, J. PARK², Y. OSAKO¹, S. SOOTER⁴, G. FERNANDES³, S. PANZERI⁵, M. SUR⁶;

²Brain and Cognitive Sci., ³Neurobio., ¹MIT, Cambridge, MA; ⁴Univ. of Arkansas Fayetteville, Fayetteville, AR, ; ⁵Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; ⁶Dept. of Brain and Cognitive Sci., MIT, Dept. of Brain and Cognitive Sci., Cambridge, MA

Abstract: Decision-making in adaptive behavior relies on internal models of the world refined by reinforcement learning (RL). Biological organisms outperform artificial RL in generalization and rapid adaptation to novel contexts, suggesting undiscovered brain mechanisms. Emerging evidence implicates astrocytes - glia once relegated to metabolic support - in regulating neural circuits over long timescales. Here, we combined computational analyses and modeling with experimental data to show how astrocytes transform transient neuromodulatory signals into sustained, context-dependent cortical modulation, enabling real-time RL. We focused on norepinephrine (NE)-driven behavioral adaptation following unexpected outcomes. Using a go/no-go task in mice, we showed that surprising outcomes with different valences triggered distinct updates in behavioral policy. Phasic locus coeruleus (LC)-NE release transiently (<100 ms) encodes reward prediction errors (RPEs), and cortical astrocytes extend NE-RPE signals to

modulate neuronal population activity over ~6-second inter-trial intervals. Crucially, disrupting LC activity, astrocytes dynamics or astrocyte-neuronal signaling abolished post-surprise behavioral adaptation, establishing causal links between astrocyte-NE signaling and behavior. Notably, astrocytes modulated neuronal responses in a context-specific way. After an unexpected punishment, NE-astrocyte signaling enhanced stimulus information in prefrontal cortex (PFC), increasing behavioral sensitivity on subsequent trials. Conversely, unexpected rewards caused astrocytes to suppress action-related neuronal signals, driving adaptive reductions in action execution. To formalize these mechanisms, we are developing computational models that integrate astrocytes into recurrent neural networks (RNNs) trained to solve different RL tasks. These astrocyte-RNNs incorporate astrocytes' temporal buffering of RPE and modulation of neuronal responses. We hypothesize that such models could improve policy updates in real-time RL tasks by retaining contextual information across delays - a known weakness in conventional RNNs. Our work reveals a unified principle for astrocytes in flexibly converting transient neuromodulatory cues into sustained, task-relevant tuning of cortical neuronal encoding, providing a biological solution to real-time RL. On the one hand, we show that astrocytes play a direct role in mediating a fundamental component of learning. On the other hand, we bridge these biological discoveries with machine learning, advancing a new paradigm for adaptive decision-making with long behavioral timescales.

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Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.07/C29

Topic: B.09. Glial Mechanisms

Title: Glycogen dynamics during memory consolidation: Integrated biochemical and 3D ultrastructural analyses

Authors: *M. F. VELOZ CASTILLO^{1,4,5}, R. SICAT², C. GENOUD⁶, H. FIUMELLI³, P. J. MAGISTRETTI¹, C. CALI^{4,5};

¹Biol. and Envrn. Sci. and Engin., ²Visualization Core Lab., ³Biol. and Envrn. Sci. & Engin., King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia; ⁴Neurosci., Univ. degli Studi di Torino, Turin, Italy; ⁵Neurosci. Inst. Cavalieri Ottolenghi, Orbassano, Italy; ⁶Univ. of Lausanne, Lausanne, Switzerland

Abstract: Glycogen is the principal energy reservoir in the brain and is exclusively stored in astrocytes under physiological conditions. It can be rapidly mobilized to support neuronal metabolism during heightened activity, notably through its conversion to lactate, which is shuttled to neurons via monocarboxylate transporters (MCT 1 and 4) to fuel their tricarboxylic acid cycle as part of the astrocyte-neuron lactate shuttle (ANLS) (Pellerin & Magistretti, 1994).

Glycogen metabolism has been implicated in memory formation and consolidation, learning-dependent synaptic stabilization, and maintenance of long-term potentiation (Suzuki, et al. 2011; Vezzoli, et al. 200; Scavuzzo, et al. 2021). However, accurate quantification of brain glycogen during memory consolidation remains challenging due to post-mortem enzymatic degradation. To overcome this, we used Focused-Beam Microwave (FBMW) brain fixation to instantaneously heat-inactivate metabolic enzymes while preserving the brain ultrastructure. Unlike conventional chemical fixation, FBMW rapidly halts enzymatic activity, minimizing post-mortem glycogen degradation and preserving a more accurate *in vivo* metabolic profile. Male mice underwent the novel object recognition test, and glycogen levels were measured in the dorsal hippocampus and prefrontal cortex at 1, 4, and 9 hours after the test. Biochemical quantifications revealed dynamic time-dependent changes in glycogen content, consistent with its proposed role as a metabolic buffer during memory consolidation. Complementary ultrastructural analyses using volume electron microscopy showed preserved cytoarchitecture in FBMW fixed tissue. Under these conditions, glycogen granules and mitochondria were segmented from 3D datasets using manual annotation and a convolutional neural-network pipeline, enabling the correlation of energy reserves with organelle distribution at the subcellular level. Together, these integrated analyses provide a validated platform to explore and understand glycogen metabolism in relation to memory processing.

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Poster

PSTR321: Astrocytes: Biology I

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Program #/Poster #: PSTR321.08/C30

Topic: B.09. Glial Mechanisms

Support: NINDS NS127819
NINDS NS113499

Title: Assaying the contributions of Kir4.1 to focal astrocyte depolarizations via astrocyte genetically encoded voltage imaging

Authors: J. GARCIA¹, *M. ARMBRUSTER¹, D. K. MULKEY², C. G. DULLA³;
¹Tufts Univ., Boston, MA; ²Dept. Physiol. and Neurobio., Univ. of Connecticut Physiol. & Neurobio., Storrs Manfld, CT; ³Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Astrocytes have traditionally been viewed as electrically passive cells in the CNS, with a hyperpolarized membrane potential that does not deviate much from their resting potential in response to physiological activity. Astrocytes are electrically coupled through gap junctions, have a very low membrane potential due to high K⁺ conductance, which results in a very small membrane space constant. This space constant highlights that somatic patch clamp recordings

may not be sensitive to changes in the fine distal astrocytic processes and potentially enable focal depolarizations. Astrocytic genetically encoded voltage indicators, GEVIs, have shown that neuronal activity can induce large, fast, and focal astrocyte depolarizations, challenging this view. Many of the components that shape and contribute to these focal depolarizations are not well understood. Here, we hypothesize that the astrocytic inward rectifying K⁺ channel, Kir4.1, and the astrocyte specific $\alpha 2$ Na⁺/K⁺/ATPase, a2NKA, distinctly shape the focal astrocyte depolarizations in the peripheral processes of cortical astrocytes. Utilizing acute slice imaging of somatosensory cortices using an astrocyte targeted ASAP3 GEVI we show that: Cortical astrocyte depolarizations are driven primarily by presynaptic K⁺ release via inhibition of the presynaptic Kv1.1 channel; the repolarization time-constant scales with neuronal stimuli number; focal loss of Kir4.1 via focal adult astrocytic conditional knockout of Kir4.1 increases the peak depolarization and speeds up the repolarization time constant; and inhibition of a2NKA slows the repolarization time-constant. These results and new hotspot analysis suggest that Kir4.1 can act both acutely at the hotspot to dampen the depolarizations and inducing a later extra-synaptic depolarization, showing complex extracellular K⁺ dynamics.

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Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.09/C31

Topic: B.09. Glial Mechanisms

Support: NIH R01 NS129888

Title: Magnetogenetic activation of astrocytic calcium response regulates neuronal activity

Authors: *M. HERNANDEZ-MORALES¹, L. TIAN², N. TIAHJONO³, C. LIU⁴;
¹Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; ²Biochem. and Mol. Med., Max Planck Florida Inst. for Neurosci., Jupiter, FL; ³Univ. of California, Davis, Davis, CA; ⁴Univ. of California, Berkeley, Berkeley, CA

Abstract: Astrocytes play essential roles in brain function, but their contribution to behavior remains poorly understood. Astrocytic calcium activity is thought to modulate neuronal circuits involved in behavior. We used the FeRIC technique (Ferritin-iron Redistribution to Ion Channels) to manipulate astrocytic calcium signaling by activating ion channels coupled to ferritin using radiofrequency (RF) magnetic fields. FeRIC utilizes the ferritin binding domain 5 of kininogen to link endogenous ferritin with ion channels, including TRPV1, TRPV4, and TRPA1. RF stimulation of astrocytes expressing TRPV4^{FeRIC} induces calcium responses that are dependent on both extracellular calcium influx and calcium release from the endoplasmic reticulum. In cocultures of astrocytes expressing FeRIC channels and neurons expressing GCaMP6f, RF stimulation activates astrocytes and induces two effects on neurons: increased

spiking activity and slow calcium transients. In the presence of tetrodotoxin, which blocks synaptic transmission, RF activates astrocytes but induces only slow neuronal calcium transients, with no increased spiking activity. Our results demonstrate that FeRIC enables the remote, noninvasive activation of astrocytic calcium activity, which in turn modulates neuronal activity. This method advances magnetogenetics for controlling astrocytic calcium signaling, providing a novel tool to investigate astrocyte-mediated regulation of neuronal circuits and behavior.

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Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.10/C32

Topic: B.09. Glial Mechanisms

Support: R01 NS129888

Title: Controlling astrocytic calcium activity with the ferritin-based magnetogenetic technique FeRIC

Authors: *T. TRAN¹, M. HERNANDEZ-MORALES², C. LIU³;

¹Neurosci. PhD Program, Univ. Of California, Berkeley, Berkeley, CA; ²Dept. of Electrical Engin. and Computer Sciences, & Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; ³Dept. of Electrical Engin. and Computer Sciences, & Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA

Abstract: Astrocytes play crucial functions in the brain, and their calcium signaling is postulated to modulate many of these functions, including synaptic transmission, neuronal circuit activity, and behavior. Calcium signaling in astrocytes exhibits high complexity, with differences in amplitude, frequency, and propagation between the soma and processes. These differences may play specific roles in astrocytic functions. Elucidating astrocytic contributions to behavior requires controlling their calcium signaling at cellular and subcellular levels. Here, we show that magnetic field stimulation can induce calcium responses in astrocytes using the magnetogenetic technique FeRIC (Ferritin Iron Redistribution to Ion Channels), which relies on ferritin as the magnetic transducer. In FeRIC, ion channels, such as TRPV1, TRPV4, and TRPA1, are linked with endogenous ferritin using the ferritin binding domain 5 of kininogen. Astrocytes were genetically targeted by the expression of FeRIC constructs under the astrocyte-specific promoter gfaABC1D, and their calcium signaling was examined using GCaMP6f. Stimulation with radiofrequency (RF) magnetic fields increases the calcium signaling in astrocytes. We performed a comprehensive cell-based statistical analysis to characterize the RF-induced astrocytic calcium responses in two *in vitro* models. Our results indicate that FeRIC increases the astrocytic calcium activity at both the soma and processes upon RF stimulation. These findings are promising for

the application of FeRIC to control astrocytic calcium signaling safely, noninvasively, and remotely in freely moving animal models.

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Poster

PSTR321: Astrocytes: Biology I

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Program #/Poster #: PSTR321.11/C33

Topic: B.09. Glial Mechanisms

Support: Astrocyte grant: R01 NS129888

Title: Magnetogenetic activation of astrocytes increases synaptic transmission and neuronal firing

Authors: *J. MORALES-ZÚÑIGA¹, M. HERNANDEZ-MORALES², T. TRAN³, C. LIU⁴;
¹EECS & HWNI, Univ. of California, Berkeley, Berkeley, CA; ²Dept. of Electrical Engin. and Computer Sciences, & Helen Wills Neuros, Univ. of California Berkeley, Berkeley, CA; ³HWNI & Dept. of Neurosci., Univ. Of California, Berkeley, Berkeley, CA; ⁴EECS & HWNI, Univ. of California, Berkeley, Berkeley, CA

Abstract: Magnetogenetics enables the control of the activity of genetically targeted cells using magnetic fields. In this study, we used the magnetogenetic method called FeRIC (Ferritin-iron Redistribution to Ion Channels) to control astrocyte activity and assess its influence on synaptic transmission and neuronal firing. FeRIC uses radiofrequency (RF) magnetic fields to activate ion channels (TRPV1, TRPV4, and TRPA1) coupled with endogenous ferritin, using the ferritin-binding domain 5 of kininogen as a linker. In FeRIC, ferritin converts the RF energy into biochemical stimuli that activate the channels. We evaluated the effectiveness of FeRIC in controlling astrocytes using electrophysiological recordings of cultured hippocampal astrocytes and neurons, with astrocytes selectively expressing FeRIC constructs. In voltage-clamped astrocytes expressing FeRIC, RF stimulation (180 MHz at 10-20 micro Tesla) induced slow inward currents. Next, we evaluated the effects of the magnetic activation of astrocytes on synaptic transmission by patch-clamping neighboring neurons. Compared to basal firing, RF-induced astrocyte activation resulted in an increase in the total number of neuronal action potentials (APs) after the stimulation phase. This effect was partially inhibited by glutamate receptor antagonists. Moreover, in the presence of tetrodotoxin to prevent the generation of APs, magnetic RF-induced activation of astrocytes increased the frequency of miniature excitatory postsynaptic currents (mEPSCs), indicating an increased probability of release (Pr) at the presynaptic terminal. Our results suggest: 1) The FeRIC method allows remote activation of astrocytes, 2) magnetic activation of astrocytes increases neuronal activity by increasing AP firing and Pr, 3) astrocytic regulation of neuronal activity occurs independently of synaptic transmission, and 4) glutamate signaling is involved in the astrocytic control of neuronal activity.

In conclusion, FeRIC is a method that can be applied to remotely control genetically targeted astrocytes to study the astrocytic regulation of neuronal activity and synaptic transmission.

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Poster

PSTR321: Astrocytes: Biology I

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Program #/Poster #: PSTR321.12/C34

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01NS115809
American Parkinson Disease Association (APDA)

Title: A critical role for S100B secretion by substantia nigra pars compacta astrocytes in accelerating 6-hydroxydopamine-induced parkinsonism in mice

Authors: *C. RODRIGUEZ¹, S. NALLURI², N. HARRISON², E. BANCROFT², R. SRINIVASAN^{3,4};

²Dept. of Neurosci. & Exptl. Therapeut., ³Dept. of Neurosci. and Exptl. Therapeut., ¹Texas A&M Univ. Syst. Hlth. Sci. Ctr., Bryan, TX; ⁴Dept. of Texas A&M Inst. for Neurosci. (TAMIN), Texas A&M Univ., College Station, TX

Abstract: Parkinson's disease (PD) is a devastating neurodegenerative disorder with no known cure. Understanding pathogenic processes in early PD is therefore critical for developing neuroprotective treatments. Multiple reports point to a pathogenic role of secreted S100B in clinical PD, and we have previously shown that extracellularly applied recombinant human S100B peptide inhibits A-type voltage-gated potassium channels in primary cultured dopaminergic (DA) neurons thereby pathologically increasing L-type voltage-gated calcium channel-mediated calcium fluxes. As a next step, to assess if S100B secreted from astrocytes leads to similar effects on DA neurons, we develop and test a novel astrocyte-specific adeno-associated virus (AAV) that expresses a non-secreted form of S100B. We generated two AAV constructs expressing either a non-secreted (S100Bnon-sec) or a secreted form of S100B (S100Bsec). Both constructs contain the mCherry reporter followed by an internal ribosome entry sequence (IRES) at the N-terminus of S100B and are driven by the astrocyte-specific GfaABC1D promoter. Using primary DA neuron-astrocyte mouse co-cultures, we found that when compared to full-length S100B, non-secreted S100B decreased Sec13 labeled ER exit sites (ERES) in astrocytes. This decrease in Sec13-ERES corresponded with a significant increase of endogenous S100B protein, a decrease in S100B in culture media, changes in calcium signaling events in DA neurons and a decrease in the nuclear translocation of the phosphorylated cyclic-AMP response element binding (pCREB) in DA neurons. Further, when comparing the ability of S100Bnon-sec versus S100Bsec to accelerate DA loss in vivo, S100Bsec accelerated

neurodegeneration in our preclinical 6-hydroxydopamine (6-OHDA) PD mouse model. These results suggest that developing drugs to prevent interactions between extracellular S100B and SNc DA neurons can be a viable approach for disease modifying treatments against PD.

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Poster

PSTR321: Astrocytes: Biology I

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Title: Astrocyte networks connect specific brain regions through gap junctions

Authors: *M. L. COOPER¹, M. SELLES², A. S. SAAB³, S. A. LIDDELOW⁴, M. V. CHAO⁵;
¹NYU Med. Ctr., NEW YORK, NY; ²Skirball, NYU Grossman Sch. of Med., New York City, NY; ³MPI of Exp. Med., Goettingen, Germany; ⁴Neurosci. Inst., NYU Sch. of Med., New York, NY; ⁵Skirball Inst. of Biomolecular Med., New York Univ. Neurosci. & Physiol., New York, NY

Abstract: Canonically, brain regions are functionally connected to one another by neuronal axons. However, astrocytes throughout the brain also form extensive networks of cells connected by gap junctions. Astrocyte gap junctions are necessary for memory formation, synaptic plasticity, coordination of neuronal signaling, and closing the visual and motor critical periods. In disease, gap junction linked astrocytes redistribute metabolic resources across the central nervous system to protect degenerating neurons. Current methods cannot examine the structural complexity of *in vivo* astrocyte gap junctional networks and limit mechanistic work to slice electrophysiology, omitting connections between astrocytes in different planes and altering the network through injuries induced by the preparation itself. To address this knowledge gap, we developed a vector that marks functional astrocyte networks by biotinylating molecules fluxed by astrocyte gap junctions, enabling visualization of astrocyte networks through whole-brain tissue clearing. We show that astrocyte networks traverse the mouse brain, connecting and omitting specific regions rather than diffusing indiscriminately. These networks vary in size and organization, with some local networks limited to a single brain region and other long-range

networks robustly interconnecting many regions across hemispheres. Astrocyte networks directly link brain regions not connected by neurons, indicating previously unassociated brain regions communicate with one another through networks of gap junction-linked astrocytes.

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Poster

PSTR321: Astrocytes: Biology I

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Title: Modulation of stress-related physiological and behavioral responses by lateral septal astrocytes

Authors: *S. WON¹, K. SEO¹, H.-Y. LEE², S.-Y. CHO², H. LEE¹;

¹Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu, Korea, Republic of; ²Seoul Natl. Univ. Sch. of Dent., Seoul, Korea, Republic of

Abstract: The lateral septum (LS) is an inhibitory hub in the basal forebrain that regulates mood, anxiety, fear, and stress responses. While previous studies have emphasized the role of LS in stress-related behaviors, the function of astrocytes within LS remains largely unexplored. In this study, we used fiber photometry to monitor astrocytic Ca²⁺ dynamics in the LS of adult male and female C57BL/6J mice. We found that aversive sensory and social stimuli, but not rewarding or non-threatening stimuli, elevated intracellular Ca²⁺ levels in astrocytes within both dorsal (LSd) and intermediate (LSi) subregions of the LS. To examine the effect of astrocytic activity on neuronal properties, we performed whole-cell patch-clamp recordings in a septal brain slice. Chemogenetic activation of hM3Dq-expressing astrocytes decreased excitatory synaptic transmission in both LSd and LSi neurons. Concurrently, inhibitory synaptic transmission was enhanced in LSi neurons. Previous research has shown that adenosine released from astrocytes can modulate synaptic dynamics in other brain regions, such as the striatum and hypothalamus. Therefore, we hypothesized that LS astrocytes mediate neuronal inhibition *via* adenosine as a gliotransmitter. Indeed, the adenosine A1 receptor (A₁R) antagonist reversed the suppression of sEPSC in LSd and LSi neurons, while the adenosine A2A receptor (A_{2A}R) antagonist blocked the enhancement of sIPSC in LSi neurons. In adult male mice, chemogenetic activation of LSd astrocytes increased social avoidance and anxiety under social stress conditions, but not in naïve mice. In contrast, activation of LSi astrocytes elevated heart rate and blood corticosterone levels

in unstressed mice. Together, these findings demonstrate the region-specific roles of astrocytes in the LS and their adenosine-mediated interactions with neurons in modulating diverse coping behaviors in response to stressful experiences.

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Poster

PSTR321: Astrocytes: Biology I

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Topic: B.09. Glial Mechanisms

Support: Narodowe Centrum Nauki 2020/39/D/NZ3/03070

Title: Astrocytic β -catenin signaling via TCF7L2 orchestrates brain osmoregulation and restricts neuronal excitability

Authors: M. POPEK¹, K. GORYCA², D. ADAMSKA², J. URBAN-CIECKO³, K. HRYNIEWIECKA², A. LAWICKA², M. LIPIEC⁴, *L. SZEWCZYK²;

¹Mossakowski Med. Res. Institute-Polish Acad. of Sci., Warsaw, Poland; ²Univ. of Warsaw, Warsaw, Poland; ³Dept. of Neurobio., Nencki Inst. of Exptl. Biol., Warszawa, Poland; ⁴Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Astrocytes undergo extensive differentiation and maturation during postnatal development. While some mechanisms guiding astrocyte development are beginning to be identified, the molecular mechanisms that link astrocytic maturation with neuronal function remain incompletely understood. We investigated the role of the Wnt/ β -catenin signaling and its effector, TCF7L2 transcription factor, in postnatal astrocytes using single-nucleus RNA sequencing, imaging, morphometric analysis, microdialysis, and electrophysiological approaches in a *Tcf7l2* conditional knockout (*Tcf7l2* cKO) mice. We discovered that loss of astrocytic *Tcf7l2* resulted in broad transcriptional dysregulation across astrocytes, particularly in genes governing amino acid, ion transport, and membrane potential. cKO mice exhibited disrupted amino acid homeostasis, astrocyte swelling, and altered extracellular potassium clearance in the somatosensory cortex. These biochemical and morphological changes in astrocytes were accompanied by dysregulated gene expression in cortical pyramidal neurons, impaired changes in their excitability, and hyperpolarized resting membrane potential. Our data suggest that postnatal maturation of astrocytes regulated by TCF7L2 is required to coordinate ion and amino acid transport in adulthood, contributing to extracellular homeostasis and neuronal functional integrity. These findings identify the novel role of Wnt/ β -catenin effector TCF7L2 as a critical regulator of astrocyte-mediated neurophysiological support and underscore the importance of astrocytic maturation during a postnatal developmental window.

Disclosures: M. Popek: None. K. Goryca: None. D. Adamska: None. J. Urban-Ciecko: None. K. Hryniewiecka: None. A. Lawicka: None. M. Lipiec: None. L. Szweczyk: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.16/C38

Topic: B.09. Glial Mechanisms

Support: Vassar College URSI Funding

Title: Aging and ketogenic diet have brain region specific effects on glutamine synthetase and glial fibrillary acidic protein in astrocytes

Authors: *T. DOYLE, E. BAGADE;
Psychological Sci., Vassar Col., Poughkeepsie, NY

Abstract: Insulin resistance increases with age, leading to increases in blood glucose, particularly after carbohydrate consumption. This dysregulation of blood glucose can lead to increases in reactive oxygen species and cognitive impairment. The ketogenic diet, which is nutritionally complete and has medium chain triglycerides (MCTs) for caloric content, can reduce the need for insulin and has the potential to preserve cognitive functioning across the lifespan. Astrocytes are important in controlling blood flow through neurovascular coupling, glucose processing, and the storage and breakdown of glycogen in learning and memory. It has been proposed that aging and neurodegenerative diseases can disrupt these astrocytic functions. Our study examines whether age-related changes in astrocytic functioning, and therefore cognitive decline, can be mitigated with a ketogenic diet. Young adult (6 months) and aged (24 months) male and female F344 x Brown Norway F1 hybrid rats were assigned a time-restricted ketogenic MCT-enriched diet (76% MCT oil), a time/calorie-matched control diet (65% carbohydrates), or ad libitum access to standard chow (65% carbohydrates) for 8 weeks and then were tested on the water maze task. We measured expression levels of glutamine synthetase (GS), an astrocyte-specific enzyme involved in glutamate and GABA recycling, and of glial fibrillary acidic protein (GFAP), an astrocyte-specific structural protein that increases with astrocytic activity and with neuroinflammation, in the agranular insular cortex (AIP), the caudate putamen (CPu), and the perirhinal cortex (PRh). The CPu had higher GFAP expression and lower GS expression than in the AIP and PRh. Moreover, there was a higher AIP:CPu ratio of GFAP staining density in rats receiving the ketogenic diet compared to the time/calorie-matched control diet rats and a lower PRh:CPu ratio of GFAP staining area in the ketogenic diet rats compared to time/calorie-matched control diet rats. Additionally an increase in GS and GFAP ratio of staining density in the AIP:CPu correlated with improved learning scores in young rats receiving the ketogenic diet. These results suggest a potential role of ketogenic diet in ameliorating age-related changes in astrocyte activity and emphasize the importance of investigating astrocytic functioning, glucose dysregulation, and aging.

Disclosures: T. Doyle: None. E. Bagade: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.17/C39

Topic: B.09. Glial Mechanisms

Support: 1R21DA061153-01

Title: Network-wide effects of endogenous astrocyte mu opioid receptor in dorsal hippocampus cal

Authors: *S. THOMPSON¹, S. HUSEYNOV³, O. PLETNIKOVA⁴, K. MURLANOVA⁵, M. PLETNIKOV²;

¹State Univ. of New York, Univ. at Buffalo, Buffalo, NY; ²State Univ. of New York, Univ. at Buffalo, ORCHARD PARK, NY; ³Physiol. and Biophysics, State Univ. of New York at Buffalo, Buffalo, NY; ⁴Pathology and Anatom. Sci., Jacobs Sch. of Med. and Biomed. Sci. Univ. at Buffalo, Buffalo, NY; ⁵Physiol. and Biophysics, SUNY, Univ. at Buffalo, Buffalo, NY

Abstract: In the hippocampus, mu opioid receptor (MOR) is expressed on GABA neurons and astrocytes. Activation of MOR on hippocampal GABAergic interneurons results in decreased GABA release and disinhibition of the activity of glutamatergic pyramidal neurons. However, the function of astrocyte MOR in vivo remains limited. Here we show network-wide effects of astrocyte targeted MOR KD in dorsal hippocampus CA1 cell-specific AAV-Cre mediated transduction in Oprm1fl/fl mice. Astrocyte MOR KD was associated with up-regulation of oxidative phosphorylation in astrocytes, astrocyte reactivity, elevated neuroinflammation and oxidative stress in the adjacent brain tissue, and altered activity of pyramidal neurons during social interaction. Our findings for the first time provide evidence for the role of astrocyte endogenous opioid signaling in regulating astrocyte bioenergetics and reactivity to affect neuronal functions and behaviors in mice.

Disclosures: S. Thompson: None. S. Huseynov: None. O. Pletnikova: None. K. Murlanova: None. M. Pletnikov: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.18/C40

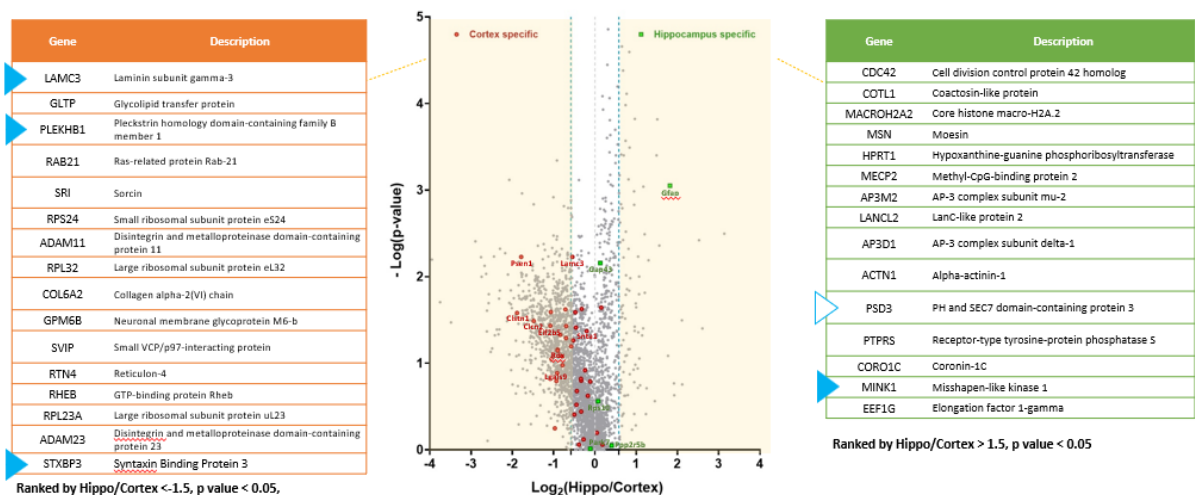
Topic: B.09. Glial Mechanisms

Title: Study of whole cell proteome- the proteomic difference of astrocyte between mouse brain cortical and hippocampal regions

Authors: *J.-C. LIAO¹, C.-C. HUANG⁵, T. CHONG², H. HUANG³, Y.-D. CHEN¹, E. CHUNG¹, H.-J. CHANG⁴;

²Applications Dept., ³Systems, ⁴Chem. Biol., ¹Syncell Inc., Taipei City, Taiwan; ⁵Technol. Application Res. Dept., Syncell, Inc., Taipei City, Taiwan

Abstract: Astrocytes, a subtype of glial cells in the Central Nervous System, play key roles in neurogenesis, synaptogenesis, blood-brain barrier regulation, and extracellular homeostasis. Notably, astrocytes in different brain regions exhibit distinct morphological and functional characteristics. This study aims to characterize proteomic differences in astrocytes across regions of the mouse brain. We employed the Microscoop® microscopy-based proteomics platform, which performs high-content, microscope-guided photo-biotinylation, to study astrocyte proteomes in the cortical and hippocampal regions. In cryo-sections of mouse brain, astrocytes were immunostained with glial fibrillary acidic protein (GFAP) and identified by Microscoop® as targets for two-photon laser illumination. The laser activates proprietary, photosensitive biotin probes in the sample, triggering localized protein biotinylation. Microscoop® automates this cycle across thousands of fields of view, generating sufficient biotinylated protein for streptavidin pull-down and LC-MS/MS proteome identification. This optoproteomics workflow enables subcellular proteomic discovery with high specificity, sensitivity, and resolution. We established distinct proteomes of cortical and hippocampal astrocytes, detecting well-known markers such as GFAP, LAMC3, and PARK7. Comparative analysis revealed novel region-enriched candidates. Among them, PLEKHB1 and SYBP3 colocalized with GFAP and were more abundant in cortical astrocytes. Conversely, MINK1 was specifically validated in hippocampal astrocytes. These findings may offer insight into region-specific astrocyte functions and generate testable hypotheses. This study highlights the potential of whole-proteome profiling—beyond transcriptomics—for defining cell types, subtypes, and states. Microscoop® enables unbiased spatial proteomics, advancing the discovery of novel astrocyte markers unique to specific brain regions.



Disclosures: **J. Liao:** A. Employment/Salary (full or part-time): Syncell Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc. **C. Huang:** A. Employment/Salary (full or part-time): Syncell Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc. **T. Chong:** A. Employment/Salary (full or part-time): Syncell Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc. **H. Huang:** A. Employment/Salary (full or part-time): Syncell Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc. **Y. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc.. F. Consulting Fees (e.g., advisory boards); Syncell Inc. **E. Chung:** A. Employment/Salary (full or part-time): Syncell Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc. **H. Chang:** A. Employment/Salary (full or part-time): Syncell Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Syncell Inc..

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.19/C41

Topic: B.09. Glial Mechanisms

Support: NIH Grant NS111583

Title: Consequences of astrocyte morphology loss in vivo

Authors: ***P. CHEN**¹, C. NEUPANE¹, L. WU¹, V. PANDEY², J. WOHLSCHLEGEL², J. ZUCHERO³, B. S. KHAKH¹;

¹Physiol., UCLA David Geffen Sch. of Med., Los Angeles, CA; ²Biol. Chem., UCLA, Los Angeles, CA; ³Neurosurg., Stanford, Stanford, CA

Abstract: Astrocytes exhibit complex and intricate morphology, forming connections with thousands of synapses per cell. A growing body of research seeks to understand the relationship between astrocyte morphology and neuronal function *in vivo*. Morphological alterations in astrocytes have been observed across a growing range of diseases and models, including Alzheimer's disease, obsessive-compulsive disorder, Huntington's disease, aging, and trauma. However, the consequences of astrocyte morphology loss for tissue or cellular homeostasis are poorly understood. Using disassembly-promoting, encodable actin tools (DeAct), we developed astrocyte-specific DeActs to directly target the actin cytoskeleton of striatal astrocytes *in vivo*. miRNA targeting sequences (4x6T) were incorporated to increase astrocyte specificity. This strategy efficiently disrupted the astrocyte actin cytoskeleton, leading to reduced territory and

increased soma size using both histology and live brain slice imaging. Using longitudinal *in vivo* two-photon imaging, we observed that DeAct positive astrocytes gradually thickened their major branches, reduced their territories, and increased their somata sizes. Astrocyte membrane conductance was decreased, while the resting membrane potential remained unchanged as expected for cells with predominantly open potassium channels. These physiological alterations align with a downward trend in Kir4.1 expression. To gain a holistic view of the molecular consequences of actin disruption, we performed astrocyte-specific RNA sequencing using a RiboTag-based viral strategy. Gene ontology (GO) analysis revealed downregulation of metabolism and synapse pathways and upregulation of reactive astrocyte pathways. We further investigated the impact on surrounding cell types. While neuronal density remained unaffected, neurons exhibited depolarized resting membrane potentials and increased intrinsic excitability. Additionally, the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) was reduced, likely attributable to a decreased density of synaptic puncta. Microglia activation related pathways were upregulated, consistent with increased microglial density and the appearance of amoeboid morphologies. Endothelial cell density remained unchanged as shown by CD31 and lectin staining, even though astrocytes lost their AQP4 positive end feet. In summary, our work introduces a genetically encoded tool for specifically disrupting the astrocyte actin cytoskeleton *in vivo*. Using this tool, we explored *in vivo* functions served by the morphological complexity of astrocytes within the brain parenchyma.

Disclosures: P. Chen: None. C. Neupane: None. L. Wu: None. V. Pandey: None. J. Wohlschlegel: None. J. Zuchero: None. B.S. Khakh: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

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Topic: B.09. Glial Mechanisms

Support: JSPS KAKENHI Grant 23H04689
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AMED JP22dm0207084
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JST CREST JPMJCR21E2

Title: Converging perisynaptic astrocytic processes from distinct astrocytes onto active dendritic segments following motor learning in the mouse primary motor cortex

Authors: *Y. YANAGAWA^{1,2}, M. YOUSSEF^{2,3}, J. SOHN⁴, S. UPRETI², N. L. KAMIJI², E. Z. TÓTH², R. SASAKI^{2,5}, K. SEMBA⁶, N. OHNO^{1,2}, Y. KUBOTA^{2,1};

¹Jichi Med. Univ., Shimotsuke, Tochigi, Japan; ²Natl. Inst. for Physiol Sci., Okazaki, Aichi, Japan; ³Vet. Med. Fac., South Valley Univ., Qena, Egypt; ⁴Osaka Univ., Suita, Osaka, Japan;

⁵Dept of Physiol Sci., SOKENDAI, Okazaki, Aichi, Japan; ⁶Med. Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Astrocytes play a critical role in regulating synaptic transmission through their participation in tripartite synapse formation. It is known that each astrocyte occupies a distinct domain with minimal overlap. However, the plasticity of these domains, particularly under conditions of learning-induced synaptic remodeling, remains unclear. Here, we report that following motor learning, perisynaptic astrocytic processes (PAPs) from multiple astrocytes converge onto a short dendritic segment (<40 μ m in length) of the apical tuft of a layer 5 pyramidal neuron in the mouse primary motor cortex (M1). Using two-photon imaging in Thy1-GFP mouse trained on a forelimb seed-reaching task for 8 days, we identified dendritic segments of layer 5 pyramidal neurons that exhibited significant spine turnover (Sohn et al., Science Advances, 2022). We revealed that these active dendritic segments were contacted by PAPs originating from 3-6 distinct astrocytes with correlated light and electron microscopy (CLEM) using volume electron microscopy (vEM) acquired via automated tape collecting ultramicrotome (ATUM) and scanning electron microscopy (SEM). These processes appeared to project directly and purposefully toward the active dendritic segment. Despite this convergence, individual dendritic spines were typically contacted by PAPs from only a single astrocyte, suggesting a high degree of spatial precision in astrocyte-synapse interactions. Our findings indicate that astrocytic processes dynamically reorganize in response to experience, potentially contributing to synapse-specific modulation during motor learning.

Disclosures: Y. Yanagawa: None. M. Youssef: None. J. Sohn: None. S. Upreti: None. N.L. Kamiji: None. E.Z. Tóth: None. R. Sasaki: None. K. Semba: None. N. Ohno: None. Y. Kubota: None.

Poster

PSTR321: Astrocytes: Biology I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR321.21/C43

Topic: B.09. Glial Mechanisms

Title: Fluoride exposure upregulates cystine/glutamate exchanger activity in glial cells

Authors: *L. C. HERNANDEZ¹, A. OCHARAN², E. CALDERON-ARANDA², A. ORTEGA²;

²Toxicología, ¹Cinvestav-IPN, Mexico City, Mexico

Abstract: Fluoride exposure in drinking water impairs cognitive functions in a period, dose and time-dependent manner. Although the molecular mechanisms are not clearly defined, evidence suggests that glial cells participate in both acute and chronic fluoride toxicity. Traditionally, fluoride toxicity involves dysregulation of the reactive oxygen species (ROS) and more recently, disruption of glutamatergic neurotransmission. In glial cells, excitatory cystine/glutamate

exchanger (xCT) regulates extra-synaptic glutamate levels and internalized cystine plays an important role in ROS control as a precursor of GSH. In this context, it is clear that xCT lies at the interface between excitotoxicity and the antioxidant response. Accordingly, we decided to evaluate the expression and regulation of the cystine/glutamate exchanger after fluoride exposure. We demonstrate the functional expression of the cystine/glutamate exchanger in U373 human glioblastoma cells, HMC3 human microglia cells and chicken cerebellar Bergmann glia cells. Using [³H]-L-Glutamate uptake assays, we demonstrate that fluoride increases the activity of the exchanger in a time and dose-dependent manner. This increase is mitigated by the antioxidant trolox. By determining the kinetic parameters of the exchanger and using a cytoskeletal transport inhibitor, cytochalasin B, we determined that the increases in activity at 30 minutes of exposure to fluoride are due to increases in the number of exchangers in the plasma membrane. To better understand the mechanisms of fluoride neurotoxicity, we evaluated its effect on a RNA-binding protein, HuR. We evidenced a significant increase in cytoplasmic HuR protein after 6 h of fluoride exposure and increases in the mRNA half-life of HuR and xCT. Our data suggest that HuR plays a critical role in the stability of xCT mRNA by binding to its 3'-UTR end promoting its stability and thus its translation. Taken together, our data provides a new perspective on the effect of fluoride on a novel target of regulatory proteins and supports the idea of a fundamental role of glial cells in fluoride neurotoxicity. Keywords: Fluoride, glutamate/cystine exchanger, HuR, glia.

Disclosures: L.C. Hernandez: None. A. Ocharan: None. E. Calderon-Aranda: None. A. Ortega: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.01/C44

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

Support: NIH Grant AG081037

Title: Progressive loss of astrocytic AIBP expression during Alzheimer's disease pathology

Authors: *S. DING, S. CHOI, N. NAZARENKOV, Y. MILLER;
UCSD, La Jolla, CA

Abstract: Astrocytes and microglia play crucial roles in mediating neuroinflammation during Alzheimer's disease (AD) progression. ApoA-I binding protein (APOA1BP, also known as AIBP or NAXE) attenuates neuroinflammation by protecting against amyloid β -induced TLR4 inflammatory formation and oxidative stress. In *Apoa1bp*-deficient APP/PS1 mice, microgliosis is exacerbated, leading to increased A β plaque burden, neuronal cell death, and reduced survival at 6 months of age. Although AIBP is both secreted and localized to the cytoplasm, and its mRNA is ubiquitously expressed in humans, its spatial and cell-type-specific distribution in the

brain remains unclear. To examine AIBP expression in the human brain, we performed immunohistochemistry on hippocampal sections from 14 postmortem human subjects (aged 75-96, both sexes). Using GFAP and IBA1 to label astrocytes and microglia, respectively, we found that AIBP protein was highly expressed in astrocytes, but not in microglia. When human subjects were grouped by Braak stages (I-II, III-IV, V-VI), astrocytic AIBP expression showed a progressive decline with advancing AD pathology. Notably, a number of AIBP-positive astrocytes were significantly reduced in Braak V-VI compared to Braak I-II, with an intermediate reduction at Braak III-IV. These findings demonstrate that AIBP is predominantly expressed in astrocytes and its expression declines with AD progression, suggesting a potential role for AIBP in astrocyte-mediated neuroprotection and AD pathogenesis.

Disclosures: S. Ding: None. S. Choi: None. N. Nazarenkov: None. Y. Miller: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.02/C45

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

Support: JSPS KAKENHI Grant Number JP19H0101
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Strategic Research Program for Brain Sciences from the Japan Agency for Medical Research and Development Grant Number 23dm0207073h0005
Moonshot R&D from Japan Science and Technology Agency Grant Number JPMJMS2024

Title: Investigation of the regulatory mechanism of expression of KLK7, an A β -degrading protease.

Authors: *R. NAKAYOSHI, C.-J. SUNG, Y. HORI, T. TOMITA;
Lab. of Neuropathology and Neuroscience, the Dept. of Pharmaceut. Sci. of the Grad, Tokyo, Japan

Abstract: Alzheimer disease (AD) is pathologically characterized by the aggregation and deposition of amyloid-beta peptide (A β) in the brain. We have previously identified Kallikrein-related peptidase 7 (KLK7) as an A β -degrading protease secreted by astrocytes (Kidana *et al.*, EMBO Mol Med, 2018). We have also demonstrated that A β deposition is exacerbated in the brains of *Klk7* knockout mice, and that the mRNA level of *KLK7* is significantly decreased in the brains of AD patients. These results indicate that KLK7 plays a crucial role in AD pathogenesis.

However, the regulatory mechanisms of KLK7 expression remain unclear. We examined the *Klk7* mRNA level in AD model mice (*App*^{NL-G-F/NL-G-F} mice) and in mouse primary astrocytes stimulated with A β . The *Klk7* mRNA level increased in 5-month-old mice; on the other hand, it decreased in 18-month-old mice. Additionally, 24 hours of acute A β stimulation increased the *Klk7* mRNA levels in mouse primary astrocytes. Conversely, continuous A β stimulation for 3 weeks as a chronic stimulation decreased *Klk7* mRNA levels. These results suggest that astrocytes have a characteristic mechanism for sensing A β and regulating KLK7 expression. To identify the receptors involved in this mechanism, we carried out the knockdown screening for known A β -binding receptors (Jarosz-Griffiths *et al.*, J. Biochem. 2016) using a neuroblastoma cell line, Neuro2a cells, which showed the upregulation of *Klk7* mRNA under acute A β stimulation. As a result, we have identified 18 candidate receptors whose knockdown suppressed the upregulation of *Klk7* mRNA levels. We will further validate these receptors and elucidate the downstream molecular mechanisms.

Disclosures: R. Nakayoshi: None. C. Sung: None. Y. Hori: None. T. Tomita: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.03/C46

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

Support: PNRR-MCNT2-2023-12377363

Title: Investigating astrocytes in Alzheimer's disease: targeting Calcineurin with FK506

Authors: *E. MURARI¹, G. FORLONI², C. BALDUCCI³;

¹Inst. di Ricerche Farmacologiche Mario Negri, Milano, Milano, Italy; ²Neurosci., Inst. Di Ricerche Farmacologiche Mario Negri, Milano, Italy; ³Neurosci., Inst. di Ricerche Farmacologiche Mario Negri, Milano, Italy

Abstract: Alzheimer's disease (AD), the leading cause of dementia, affects over 30 million people globally. *Post-mortem* diagnosis reveals extracellular β -amyloid (A β) plaques and intracellular hyperphosphorylated tau tangles that trigger chronic neuroinflammation and synaptic loss, leading to cognitive decline. Oligomeric A β species (A β O), the most toxic forms, impair synapses and memory *via* glial activation, which release pro-inflammatory cytokines, promoting A β production and worsening pathology. Neuron-glia crosstalk plays a central role in AD. We now know that calcineurin (CaN), a calcium/calmodulin-dependent phosphatase, is overactive in astrocytes from AD patients and mouse models. We showed that astrocyte-specific depletion of CaN's regulatory B subunit prevents A β O-induced memory deficits and microglial activation in wild-type mice and, in 3xTg-AD mice, halts cognitive decline, A β /tau accumulation, and neuroinflammation. Pharmacological CaN inhibition using FK506 (Tacrolimus) restores synaptic plasticity and memory in AD mice and reduces AD risk in

transplant patients. This study aimed to inhibit astrocytic CaN in AD human astrocytes, using FK506 encapsulated in astrocyte-targeting polymeric nanogels (FK506-NGs) and test the ability to restore a physiological phenotype. Human iPSC-derived astrocytes from a healthy donor and an isogenic AD line carrying the PSEN1 A246E mutation were used. Morphological differences were evaluated by immunofluorescence (IF) using α -tubulin as a marker for cellular shape, classifying astrocytes as arborized, fibroblastic, or polarized. Inflammatory markers (GFAP, aquaporin 4, S100 β , S100A10) were assessed *via* IF, qRT-PCR, and transcriptomics. AD astrocytes showed increased fibroblast-like morphology, compared to the arborized predominance in control cells, and higher expression of inflammatory markers, as well as enhanced CaN pathway activity and elevated NFATC1 and NFATC2 expression as evidenced by transcriptomics. FK506 alone, at 1-10 nM concentration, significantly lowered GFAP, S100 β , and S100A10 levels in AD astrocytes without affecting controls. FK506-NGs were preferentially taken up by AD astrocytes (50-80%) compared to controls (5-35%) and, at 0.5-2 nM, significantly downregulated inflammatory markers only in AD cells. In conclusion, iPSC-derived AD astrocytes exhibit a distinct inflammatory phenotype that is reversed by astrocyte-specific CaN inhibition. These results support targeted modulation of astrocytic neuroinflammation as a promising therapeutic strategy for AD, warranting further investigation in complex models.

Disclosures: E. Murari: None. G. Forloni: None. C. Balducci: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.04/C47

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

Title: Therapeutic effect of FK506 derivatives in Alzheimer's disease.

Authors: *Y. LEE, G. HA, D. LEE, E. CHEONG;
Biotech., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Pathological features of Alzheimer's disease (AD) include the accumulation of amyloid-beta (A β) plaques, formation of neurofibrillary tangles composed of hyperphosphorylated tau, and significant synaptic loss. Additionally, recent studies have highlighted the involvement of glial cells, particularly astrocytes and microglia, in the pathogenesis of AD. These glial cells play crucial roles in maintaining and modulating synaptic function. For instance, astrocytes are essential for preserving neurotransmitter homeostasis and ionic balance in the synaptic environment, while microglia actively monitor synaptic activity and contribute to synaptic pruning through phagocytosis, thereby influencing synaptic plasticity. FK506 (Tacrolimus) is an immunosuppressive agent widely used in organ transplantation. Beyond its established role in immunosuppression, recent clinical findings suggest that FK506 may exert beneficial effects in the context of neurodegenerative diseases, including dementia. However, CNS-specific downstream mechanisms of FK506—particularly in glial modulation—

remain poorly understood. To address this gap, we aim to investigate the effects of FK506 and its derivatives with reduced calcineurin-binding affinity, focusing on their actions in glial cells and their potential influence on synaptic plasticity. We performed age-dependent electrophysiological assessments in CA1 pyramidal neurons in the hippocampus from 3 to 12 months of age in naïve 5xFAD and wild type mice. Specifically, we found electrophysiological changes in CA1 pyramidal neurons to assess the effects of FK506 and FK506 derivatives on synaptic transmission. Furthermore, to elucidate the underlying mechanisms of FK506 action, we conducted immunohistochemistry and quantitative RT-PCR analyses, aiming to identify downstream signaling pathways.

Disclosures: Y. Lee: None. G. Ha: None. D. Lee: None. E. Cheong: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.05/C48

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

Support: National Natural Science Foundation of China (32371036)

Title: A subset of amyloid plaque-associated astrocytes with elevated expression of genes for glycolysis

Authors: *L. SHI MIN, P. LIAN, N. DING, J.-T. YU, P. YUAN;
Fudan Univ., Shanghai, China

Abstract: Defective glucose metabolism is a crucial feature of Alzheimer's disease (AD), and is closely associated with cognitive decline. In the brain, the primary source of energy in neurons is lactate secreted from astrocytes. Astrocytes take up glucose from the blood flow and convert it to lactate via glycolysis. Therefore, astrocytic glycolysis function is of critical importance for neuronal energy metabolism. Previous studies identified dysfunction of glycolysis process in astrocytes in transgenic 5xFAD mice, providing a potential mechanism for defective glucose metabolism observed among AD patients. To identify the spatial distribution of metabolic dysfunctional astrocytes in AD brains, we performed spatially resolved transcriptomic analysis in the brains of 5xFAD mice. Surprisingly, we discovered an astrocyte subpopulation in the vicinity of amyloid plaques exhibited elevated expression of genes involved in glycolysis. Functional analysis of the same subpopulation showed diminished expression in the glutamate signaling pathways, indicating reduced interaction with neurons. We characterized thrombospondin-4 (Thbs4) as a marker gene for this subpopulation. Immunostaining of Thbs4 and other enzymes further corroborated elevated expression of glycolytic genes in these cells. These results suggest that there is a subpopulation of plaque-associated astrocytes in AD with elevated expression of glycolytic enzymes. Our findings indicate that insufficient glycolysis enzymes in astrocytes is

unlikely the underlying mechanism for glucose hypometabolism in AD, and have implications in the development of interventions targeting brain metabolic dysfunction.

Disclosures: L. Shi min: None. P. Lian: None. N. Ding: None. J. Yu: None. P. Yuan: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

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

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Title: Neocortical region-specific changes to astrocytes and microglia near amyloid-beta plaques and vasculature in Alzheimer's disease

Authors: A. K. MCKENDELL¹, A. PELLAGRINI², S. MAGALHAES², A. M. BADINA³, E. MCDONOUGH⁴, L. LOWERY⁴, B. B. TOURNIER³, J. I. LUEBKE⁵, D. E. MEYER⁴, P. R. HOF¹, *M. T. VARGHESE⁶;

¹Nash Family Dept. of Neuroscience, Friedman Brain Institute, Ronald M. Loeb Ctr. for AD,

²Nash Family Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ³Dept. of Psychiatry, Univ. Hosp. of Geneva and Univ. of Geneva, Geneva, Switzerland; ⁴GE HealthCare Technol. & Innovation Ctr., Niskayuna, NY; ⁵Anat. & Neurobio., Boston Univ., Boston, MA;

⁶George & Anne Ryan Inst. for Neuroscience, Dept. of Biomed. and Pharmaceut. Sci., Univ. of Rhode Island, Warwick, RI

Abstract: Alzheimer's disease (AD) shows a regional pattern of pathology and neurodegeneration across the brain that affects cognition including memory. However, the understanding of what contributes to this regional vulnerability is limited. To address this, we performed highly multiplexed immunofluorescence staining (MxIF) on postmortem human samples for 26 markers relating to cell types, cell features, cell states, tissue architecture, and AD neuropathology. We compared an AD-susceptible brain area, the dorsolateral prefrontal cortex (DLPFC) to an AD-resistant brain area, the primary visual cortex (V1), and related our findings in AD to age-matched, cognitively healthy women and men. Subjects were grouped as follows: for clinical dementia rating (CDR) scores, AD (n = 3, CDR 3), mild cognitive impairment (n = 4, CDR 0.5) and healthy controls (n = 5, CDR 0); for Thal stage, AD (n = 2, stage 4), pre-AD (n = 8, stages 1-3), and control (n = 2, stage 0); for Braak stage, AD (n = 5, stages III-VI), control (n

= 7, stages I-II). Images from the 20x field-of-view for each marker were corrected for illumination field uniformity, stitched into composite panels, registered across rounds using cell nuclei as reference, and the autofluorescence background was subtracted. Using the image analysis program *QuPath*, the images were segmented for aldehyde dehydrogenase 1 family member 1 (all astrocytes) and glial fibrillary protein (reactive astrocytes), ionized calcium-binding adapter molecule 1 (all microglia) colocalizing with CD68, major histocompatibility complex class II or translocator protein (reactive microglia), collagen IV (vasculature), and amyloid β protein (A β). Astrocyte reactivity increased specifically near A β plaques with worsening CDR only in the V1 ($p = 0.022$). Similarly, microglial coverage and reactivity increased near A β plaques with worsening CDR in the V1 ($p \leq 0.05$). Interestingly, we found a decrease in juxtavascular reactive astrocytes in layer 1 of the DLPFC and an increase in the white matter of the V1 ($p \leq 0.04$). Ongoing work assessing vascular A β will help determine if juxtavascular astrocytic changes are a response to cerebral amyloid angiopathy or a ubiquitous change with worsening Thal stage. Our preliminary results suggest a localized glial response to pathological A β deposits in V1 may confer neuroprotection, whereas changes in astrocytic coverage of blood vessels may influence changes in blood-brain barrier integrity. We are continuing investigation of markers for glial morphology and cell state to assess changes across the neocortex with worsening cognition and ultimately to discern glial contribution to selective neuronal vulnerability in AD.

Disclosures: **A.K. McKendell:** None. **A. Pellagrini:** None. **S. Magalhaes:** None. **A.M. Badina:** None. **E. McDonough:** A. Employment/Salary (full or part-time): GE HealthCare Technology & Innovation Center. **L. Lowery:** A. Employment/Salary (full or part-time): GE HealthCare Technology & Innovation Center. **B.B. Tournier:** None. **J.I. Luebke:** None. **D.E. Meyer:** A. Employment/Salary (full or part-time): GE HealthCare Technology & Innovation Center. **P.R. Hof:** None. **M.T. Varghese:** None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.07/C50

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

Support: CureAlzheimer's Fund
Paul F. Glenn Center for Biology of Aging Research at the Salk Institute
Fellowship

Title: The role of astrocyte-secreted Igfbp2 in the progression of Alzheimer's disease

Authors: ***T. TAO**¹, J. SHOU², N. J. ALLEN¹;

¹Salk Inst. for Biol. Studies, La Jolla, CA; ²Univ. of California San Diego, La Jolla, CA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder, yet despite significant research efforts, effective therapies to prevent or slow its progression remain limited, highlighting the urgent need for new therapeutic targets. Clinical studies have shown a strong association between AD pathology and elevated levels of insulin-like growth factor binding protein 2 (IGFBP2) in both the circulation and cerebrospinal fluid (CSF) of patients, implicating IGFBP2 in disease progression. In the brain, IGFBP2 is predominantly expressed by astrocytes. By binding insulin-like growth factors (IGFs), IGFBP2 can either sequester them, preventing receptor activation, or stabilize them in the extracellular space, modulating their bioavailability. This dual role, capable of both inhibiting and promoting IGF signaling, underscores the importance of investigating astrocyte-derived IGFBP2 in AD. To answer the question whether IGFBP2 play a beneficial or harmful role on neurons, iPSC-derived neurons were treated with recombinant IGFBP2 for 24 hours and we found that IGFBP2 significantly reduced average neurite length, compared with vehicle-treated controls. To determine whether IGFBP2 inhibits synaptic function, prolonged exposure to IGFBP2 during a three-week neuronal maturation period further reduced expression of key synaptic proteins, including PSD-95, Synapsin I, and Synaptophysin. To complement these *in vitro* findings, immunohistochemistry using an IGFBP2-specific antibody revealed significantly elevated IGFBP2 expression in the dentate gyrus of 4-month-old female TauP301S mice. Furthermore, overexpression of IGFBP2 in astrocytes, driven by a minimal *Gfap* promoter and delivered via AAV.PHP.eB in 4-month-old C57BL/6 mice, resulted in a trend toward reduced PSD-95 levels in the hippocampus and a significant reduction in Synapsin I. In the cortex, IGFBP2 overexpression significantly decreased both PSD-95 and Synapsin I. We are currently knocking out IGFBP2 in TauP301S model by CRISPR to see whether reducing IGFBP2 level will improve the synaptic function and behavior performance in TauP301S model. Together, our work suggests that elevated astrocyte-derived IGFBP2 may contribute to synaptic dysfunction in AD by negatively impacting neuronal morphology and synaptic function, highlighting its potential as a novel therapeutic target.

Disclosures: T. Tao: None. J. Shou: None. N.J. Allen: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.08/C51

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

Support: NRF-2020M3E5D9079742
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HU23C0217
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NIH (R01NS109537)

Title: Astrocytic Autophagic Plasticity Facilitates A β Clearance and Cognitive Function in Alzheimer's Disease

Authors: *S. KIM¹, Y. KIM¹, U. PARK¹, J. CHU¹, S. KIM¹, H. RYU²;

²Brain Disorders, ¹Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Reactive astrocytes in Alzheimer's disease (AD) exhibit dynamic plasticity in response to amyloid beta (A β), yet their role in autophagy-mediated A β clearance remains unclear. We investigated how A β -induced autophagic responses in astrocytes influence A β detoxification and neuropathology. We employed A β -treated human and mouse astrocytes, AAV-CRE-ON-mediated astrocyte-specific knockdown or overexpression of autophagy genes (LC3B and SQSTM1), and AD transgenic mouse models (APP/PS1, 5XFAD). Autophagic flux, mitochondrial function, and urea cycle metabolites were assessed via confocal imaging, Western blotting, RNA-seq, LC/MS, and behavior tests (NOR, NOPR). A β oligomers transiently upregulated LC3B and SQSTM1 in astrocytes, inducing autophagic flux that mitigated mitochondrial oxidative stress and supported cellular viability. Pharmacological or genetic inhibition of astrocytic autophagy exacerbated A β plaque accumulation, increased GFAP-positive astrocytes, and reduced NeuN signals in APP/PS1 mice. Moreover, autophagy inhibition suppressed urea cycle metabolite production and heightened ROS levels, indicating disrupted A β catabolism. In contrast, astrocyte-specific overexpression of LC3B reduced A β burden and improved cognitive performance. Notably, increased LC3B and SQSTM1 levels were observed in hippocampal astrocytes of human AD patients. Our findings demonstrate that astrocytic autophagic plasticity is essential for A β clearance and maintenance of cognitive function in AD. Enhancing astrocyte-specific autophagy represents a promising therapeutic strategy to attenuate A β pathology and preserve neuronal health.

Disclosures: S. Kim: None. Y. Kim: None. U. Park: None. J. Chu: None. S. Kim: None. H. Ryu: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.09/C52

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

Support: NIH Grant. 5 P30AG066468
NIH Grant. 5 R01 AG069912
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PA Dept. of State 4100087331

Title: DNA damage impacts mature oligodendrocytes less than oligodendrocyte precursor cells - survival and APP localization

Authors: F. STAUFFER¹, F. MA², R. KINGSTON¹, M. BAGNELL¹, N. PRAVEEN¹, G. GUTTA¹, E. BRENNER¹, *K. HERRUP^{3,1};
¹Neurobio., ²Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; ³Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Alzheimer's Disease (AD) is primarily a disease of aging, yet relatively little is known about how age-related processes such as chronic DNA damage, a major driver of aging, affect brain cell health and viability. It has previously been shown that the accumulation of DNA damage reduces the myelination of axons by oligodendrocytes (OLs). Further, single cell RNAseq studies have shown that OLs are the primary producers of amyloid precursor protein (APP) in the central nervous systems of both mouse and human. Because its proteolytic cleavage generates amyloid-beta, APP is commonly regarded as a deleterious protein. Some products of APP processing, however, have been shown to have neurotrophic effects. Others, such as the AICD (APP-IntraCellular-Domain) translocate to the nucleus and alter gene transcription and are used in DNA repair. To explore the links between DNA damage and APP expression, we established primary cultures of OLs from P2-P5 mouse cortices. We subjected the cultures to a modest DNA damage using sub-micromolar concentrations of the topoisomerase-II inhibitor, etoposide. We used immunocytochemistry of neural/glial antigen 2 (NG2) and myelin basic protein (MBP) to follow oligodendrocyte precursor cells (OPCs) and OLs respectively. Higher etoposide concentrations led to significant losses of both cell types. As we lowered the levels of etoposide, however, we found that mature OLs (MBP⁺ cell count) survived in greater numbers than OPCs (NG2⁺ cell count) growing in the same culture, indicating that they were more resilient to DNA damage. Analysis of MBP⁺ processes revealed that OL structure was also dramatically affected by even low levels of etoposide. At lower doses of etoposide OL branching density appeared to increase while we observed an apparent retraction of processes at higher doses. APP localization also changed with increasing DNA damage. Using antibodies against the C terminus of APP, we found that with increasing DNA damage, APP localization qualitatively shifted from a concentrated, perinuclear location and spread more diffusely throughout the cytoplasm, with a more prominent signal appearing in the OL processes. This altered subcellular location is consistent with data from mouse cortical neurons in vivo where increased DNA damage led to a similar shift in the pattern of APP intracellular location. These data further support a role for APP in the DNA damage response of brain cells and help to link APP and the genetics of familial AD to the process of aging in both neurons and oligodendrocytes.

Disclosures: **F. Stauffer:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine. **F. Ma:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine. **R. Kingston:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine. **M. Bagnell:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine. **N. Praveen:** None. **G. Gutta:** None. **E. Brenner:** None. **K. Herrup:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.10/C53

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

Support: UNMC startup funds to S Sil.

Title: Chronic-binge alcohol consumption leads to Alzheimer's-like pathology, via modulating epitranscriptomic pathways

Authors: A. SAHA¹, M. KUMAR², S. RAY⁴, S. VISWANATHAN¹, *S. SIL³;

²Pharmacol. and Exptl. Neurosci., ³Dept. of Pharmacol. & Exptl. Neurosci., ¹Univ. of Nebraska Med. Ctr., Omaha, NE; ⁴Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. center, Omaha, NE

Abstract: Alcohol consumption is a critical global public health issue, contributing to nearly 2.6 million worldwide deaths and associated chronic conditions, including Alcohol Use Disorder (AUD) and Alcohol-Related Brain Damage (ARBD). Alcohol intake & Alzheimer's Disease (AD) have a nuanced relationship, & the former has been posited as a potential risk factor for AD, leading to a wide range of memory impairment(s). Chronic-alcohol exposure has been linked to accelerated brain aging and cognitive decline. This study investigates how chronic-binge alcohol consumption induces Alzheimer's-like pathology leading to neurodegeneration, and its association with epitranscriptomic changes, to identify potential therapeutic targets. C57/BL6 mice were subjected to chronic-binge alcohol exposure, and brain regions were analyzed for AD markers, neuronal injury & glial activation. Results demonstrated a significant increase in Alzheimer's-related markers, including amyloid-precursor-protein, A β 42, and phosphorylated-tau, in the hippocampus and cortex. This was accompanied by elevated levels of β -secretase and Presenilin-2. Increased expression of synaptic proteins i.e., Synaptophysin, Gephyrin, GAD65, and a decrease in Synapsin, indicated alterations in synaptic function, which could affect neurotransmission. Concomitantly, chronic-alcohol exposure led to significant astrocyte and microglial activation, as evidenced by increased GFAP expression, enhanced astrocytic branching complexity, and microglial morphological changes indicative of a pro-inflammatory state, reinforcing the role of ethanol-induced neuroinflammation in exacerbating neurodegeneration. To further elucidate the molecular mechanisms of alcohol in neurodegeneration, hippocampal epitranscriptomic analysis was performed, and significant alteration in methylation status was observed in mRNAs related to cognitive functions, glial activation, and neurogenesis. These findings highlight the profound impact of chronic-binge alcohol consumption on neurodegeneration, synaptic dysfunction, and glial activation, leading to AD pathology. Understanding these mechanisms will aid in developing therapeutic strategies to counteract alcohol-induced cognitive decline and neurotoxicity, in AUD and ARBD.

Disclosures: A. Saha: None. M. Kumar: None. S. Ray: None. S. Viswanathan: None. S. Sil: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.11/C54

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

Support: NIH 3RF1AG076124-01A1S1

Title: Senescence accelerated in APOE4 astrocytes by cholesterol transporter ABCA1

Authors: *C. HUGO¹, S. WANG², H. N. YASSINE³;

¹Neurol., ²Keck Sch. of Med., ³USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease comprising 60-70% of dementia cases. The strongest risk factors for late-onset AD are aging and *APOE* ε4 genotype. Aging and *APOE4* are associated with increased cellular senescence, a cellular state defined by loss of proliferative ability and a secretory phenotype. Senescence in Alzheimer's disease has not been well-studied, particularly with regards to lipid metabolic dysfunction. Lipids, which accumulate more in AD and *APOE4*, have been described as important players in senescence. The pathways and mechanisms that may drive a senescent phenotype through lipid modulation is a large gap in our understanding of AD senescence. Past research in our lab has identified ATP-binding cassette transporter (ABCA1), a cholesterol transporter, as a potential driver of senescence through its effects on lipid accumulation, causing a buildup of lipids, particularly, oxidized cholesterol, in lysosomes. In AD and *APOE4*, ABCA1 appears to be trapped at lysosomes, which increases intracellular cholesterol accumulation and promotes inflammation and other key pathways involved in senescence. This work proposes that in *APOE4*, senescence is accelerated via lysosomal ABCA1 and cholesterol accumulation. Considering the difficulties of obtaining and maintaining primary human brain cells, induced pluripotent stem cells (iPSCs) allow for investigative studies on brain cells. In human iPSC-derived astrocytes (iAs) of *APOE3* and *APOE4* genotypes, I have first established a senescent phenotype via DNA damage. Delving further into a potential mechanism, I will assess whether a senescent phenotype can be reduced via pharmacological and genetic inhibition of ABCA1. I will also examine the potential crosstalk between senescent astrocytes and neurons, assessing neuronal senescence and dysfunction. The goal of this work is to examine the role of ABCA1 in cellular senescence with a focus on *APOE4*, furthering our understanding of how *APOE* genotype and cholesterol-related pathways may influence a senescent phenotype. Outcomes of this work may advance our understanding of how senescence is accelerated in AD astrocytes, as well as identify therapeutic targets to alleviate this process.

Disclosures: C. Hugo: None. S. Wang: None. H.N. Yassine: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: PSTR322.12/C55

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

Support: SFNS grant 310030_212322

Title: Spatial transcriptomics analysis of glial activation dynamics throughout the progression of Alzheimer's disease

Authors: *A. BADINA¹, K. CEYZÉRIAT², L. ABJEAN¹, S. TSARTSALIS³, P. MILLET³, B. B. TOURNIER⁴;

¹Dept. of Psychiatry, Fac. of Medicine, Univ. of Geneva, Switzerland, Geneva, Switzerland;

²CIBM Ctr. for Biomed. Imaging, Geneva, Switzerland; ³Dept. of Psychiatry, Univ. Hosp. of Geneva, Geneva, Switzerland, Geneva, Switzerland; ⁴Univ. of Geneva / Univ. Hosp. of Geneva, Geneva, Switzerland

Abstract: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder, currently affecting 50 million people worldwide and projected to rise to 130 million by 2050. It is driven by the accumulation of amyloid- β (A β) and Tau, which form extracellular plaques and intracellular tangles. Astrocytes and microglia detect these deposits and adopt activated phenotypes. Doing so, they release pro-inflammatory mediators in order to recruit and activate further glial cells, causing a vicious circle of neuroinflammation eventually contributing to damaging neurons. However, it remains unclear how much of this glial activation is directly caused by proximity to A β and Tau pathology and how much is amplified by later glia-glia signaling. We used spatial transcriptomics (10x Visium) to characterize the cellular microenvironment in amyloid-driven neurodegeneration on human hippocampal slices. 16 subjects were distributed in three age-matched groups: controls, early stages AD and late stages AD based on their Braak stages. We then distributed spots in regions of interest depending on their proximity to A β plaques, according to A β immunostaining. Cell type deconvolution (DWLS method) was used to identify cell type proportion in each spot, conducted on two levels. The first level revealed neuronal subtypes and glial types, and the second level was focused on glial subtypes (3 astrocyte subtypes, 2 microglial subtypes and 2 oligodendrocyte subtypes). We quantified cellular communication through ligand-receptor expression (CellChat package), and computed cell-type abundance and activation marker expression as a function of proximity to A β plaques using custom R scripts. Differential expression revealed a plaque-proximal upregulation of microglial inflammatory modules and astrocytic reactive genes that declined with distance to plaques. Within the same distance to plaques, glial cells showed increased activation with the disease's progression. Our data demonstrate spatially graded glial activation and maladaptive intercellular signaling in the amyloid microenvironment. These findings underscore the importance of microenvironmental context in Alzheimer's pathogenesis and nominate plaque-proximal cell-cell communication pathways through ligand-receptor pairs in order to identify potential therapeutic targets.

Disclosures: A. Badina: None. K. Ceyzériat: None. L. Abjean: None. S. Tsartsalis: None. P. Millet: None. B.B. Tournier: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.13/C56

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

Title: Germination of astrocytes-rich cerebral organoids

Authors: *Y. LIUQING;
Inst. of biophysics, Beijing, China

Abstract: Alzheimer's disease (AD) is one of the most common dementias in the elderly. Currently, AD is considered to be an irreversible neurodegenerative disease caused by multiple factors; its characteristic pathological changes are manifested as atrophy of the cerebral cortex, senile plaques formed by the deposition of β -amyloid protein in the brain, neurofibrillary tangles formed by abnormal phosphorylation of Tau protein, damage to a large number of memory-related neurons, and loss of synapses. Astrocytes and microglia, the cells involved in the inflammatory response in the brain, play a key role in the occurrence and development of AD. Because of the characteristics of AD degenerative diseases, most of the current brain organoids that simulate AD diseases require more than 90 days of culture to produce the corresponding phenotype of β -amyloid protein deposition, and they are usually brain organoids that only contain neurons. The emergence of stem cell technology allows us to easily obtain cells from AD patients and reprogram them into corresponding iPSCs. By inducing iPSCs from AD patients, we can generate corresponding astrocytes and microglia. Compared with normal stem cell-induced cell types, astrocytes generated by AD-iPSCs exhibit inflammatory properties and have stress morphology, and microglia induced by AD-iPSCs show weaker phagocytic ability to detect the environment. We also co-cultured AD-iPSC-generated astrocytes with AD-iPSCs to generate astrocytes-rich brain organoids, and these organoids can simulate the corresponding AD phenotypes. Our multi-cell brain organoid can be a useful tool to reveal the mechanism of AD.

Disclosures: Y. liuqing: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.14/C57

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

Title: Mapping Mitochondrial Dysregulation in Glial Networks of Alzheimer's Disease Brains

Authors: C. STRINGER¹, E. HEAD², W. NGUYEN¹, *A. ZAHEDI¹;
²Pathology & Lab. Med., ¹Univ. of California Irvine, Irvine, CA

Abstract: Alzheimer's disease (AD) is characterized by progressive cognitive decline, neuroinflammation, oxidative stress and mitochondrial dysfunction. In AD transgenic mice, inactivation of one SOD2 allele accelerates the onset of behavioral defects and neuronal deterioration. This partial SOD2 loss led to a decrease in parenchymal amyloid-beta (A β) plaques burden but an increase in cerebrovascular amyloid deposition, highlighting its complex role in AD pathology and limited information about spatial distribution and cellular associations. In this study, we spatially mapped SOD2 expression in postmortem human brain tissues from AD patients and age-matched controls, sourced from UC Irvine's Alzheimer's Disease Research Center (ADRC). Immunohistochemical analyses identified elevated SOD2 expression in GFAP⁺ astrocytes and plaque-associated Iba1⁺ microglia, in particular around dense, neuritic "cored" plaques. These findings suggest a glial-specific upregulation of SOD2 associated with plaque morphology, indicating a dysregulated mitochondrial response in glial networks contributing to plaque development and neurotoxicity. Additionally, Bax, a pro-apoptotic mitochondrial membrane-associated protein was found to be upregulated in cerebral amyloid angiopathy (CAA)-affected regions, indicating a link between vascular amyloid deposition, mitochondrial dysfunction, and vascular fragility. Building on our previous work identifying mitochondrial-targeted therapeutics to alleviate oxidative stress and inflammation, we evaluated the effects of repurposed drugs on human induced pluripotent stem cell (iPSC)-derived microglia (iMGs). Treatment with a mitochondrial biogenesis drug, Bezafibrate, enhanced A β 42 phagocytosis in iMGs over a 24-hour period. Ongoing investigations will explore the impact of the APOE4 genotype on microglial phagocytic dysfunction and assess the therapeutic potential of lipid metabolism modulators in 5xFAD transgenic mouse model. These findings underscore the pivotal role of mitochondrial dysfunction and glial activation in AD pathology and support the therapeutic potential of targeting mitochondrial pathways to mitigate disease progression.

Disclosures: C. Stringer: None. E. Head: None. W. Nguyen: None. A. Zahedi: None.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

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Program #/Poster #: PSTR322.15/C58

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

Support: ABC-DS U19AG068054
Brightfocus BFF17_0008
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UCI MIND ADRC P30AG066519
UCI UROP

Title: Exploring the Role of Astrocytic Aquaporin-4 in Response to Beta-Amyloid Deposition and Neuroinflammation in Down Syndrome with Alzheimer Disease Pathology

Authors: *K. A. GHADIA, C. STRINGER, E. HEAD;
Dept. of Pathology & Lab. Med., Univ. of California Irvine, Irvine, CA

Abstract: This study investigates astrocyte-mediated neuroinflammation in Alzheimer's disease (AD) pathology in Down Syndrome (DS) through analysis of the markers S100 β (a pro-inflammatory factor), Complement Component 3 (C3; associated with reactive astrogliosis), and Glial Fibrillary Acidic Protein (GFAP; a marker of astrocyte reactivity). Marker expression and inter-marker correlations were examined across four diagnostic groups for gray matter: Aged Controls, Late-Onset Alzheimer's Disease (LOAD), DS, and DS with AD (DSAD). Although no statistically significant differences in expression levels were observed between diagnostic groups, the correlations among neuroinflammatory markers were weaker in DS and DSAD. This may suggest altered or late-stage astrocytic responses in these groups, potentially reflecting a chronic inflammatory state in which typical signaling pathways are no longer tightly coordinated. These findings highlight the complexity of astrocyte involvement in DS-related AD pathology and suggest that expanded sample sizes may further clarify these neuroinflammatory patterns.

Disclosures: K.A. Ghadia: None. C. Stringer: A. Employment/Salary (full or part-time): University of California Irvine. E. Head: A. Employment/Salary (full or part-time): University of California Irvine.

Poster

PSTR322: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR322.16/C59

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

Support: Arc Institute

Title: Development of Complementary 3D Human Neural Model Systems Integrating Multiple Cell Types for Alzheimer's Disease Research

Authors: A. RAJAN¹, *A. ATAMIAN¹, S. RAO¹, D. AL-AZZEH¹, L. SAAVEDRA¹, E. HERGENREDER²;

¹Arc Inst., Palo Alto, CA; ²Arc Inst., Palo Alto, CA.

Abstract: Research on human neurodegenerative disorders have mainly been limited by the lack of complex human cell-based systems that recapitulate human disease pathogenesis and phenotype. To address this gap, we developed two complementary 3D human neural model systems: a neural progenitor cell (NPC)-derived organoid model and an induced neuron with induced astrocyte (iN/iA)-based spheroid model. These models demonstrate the ability to integrate multiple exogenous human cell types that can be used in parallel as a translationally relevant human stem-cell derived 3D neural model systems to study the cell-cell interactions and disease-relevant cellular dynamics.

As a proof of concept, we demonstrate the successful integration of human pluripotent stem cell (hPSC)-derived hematopoietic progenitor cells (HPCs) with the goal of deriving microglia. Integrated HPCs maintained viability, differentiated into microglia inside the models, displayed characteristic morphologies and function, and could be retrieved for downstream molecular analyses including flow cytometry, single-cell RNA sequencing, and functional assays. The integration of these human stem cell derived 3D neural models with CRISPR tools, omics technologies, and computational approaches will advance our understanding of neurodegenerative disease mechanisms and lead to potential therapeutic discovery.

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Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.01/C60

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

Title: Building up colonies of non-human primate neurodegenerative disease models for translational research

Authors: *L. YANG, G. WANG, Y. XU, S. ZHA, J. GABRIEL, R. PEREZ, J. KOPRICH; Kunming Biomed Intl., Kunming, China

Abstract: The development of non-human primate (NHP) models of central nervous system (CNS) diseases, such as Alzheimer's (AD) and Parkinson's (PD), is needed due to their close phylogenetic relationship to humans, similar neuroanatomy, comparable genetics, and higher-order cognitive function, making them better models for translational research. To identify a cohort of animals for AD research projects we are screening animals from a population of 100 aged (18-27 yrs) and 20 young (4-6 yrs) cynomolgus macaques using AV45-PET [18F-florbetapir] to assess brain levels of beta-amyloid 42 (A β 42). To this end, 5mCi 18F-AV45 is injected, i.v., to each animal 60 minutes before the scanning is performed for image collection and reconstruction. The A β 42 deposition is then quantified by standardized uptake values (SUVs) derived using SUV (measured radioactivity concentration in ROIs/(injection dose/body weight)), then calibrated by using muscle as the internal control to achieve the SUV ratio (SUVR) of the frontal cortex and hippocampus determined by the following formula: $SUVR = (SUV_{ROIs} - SUV_{muscle}) / SUV_{muscle}$. Currently, approximately 60% of the aged animals screened to date show various extents of beta-amyloid deposition in brain, which were not closely correlated with advancing age within the aged cohort, although increased compared to the young cohort. CSF levels of A β 42, measured by ELISA, showed a similar pattern to AV45-PET results. In a small subset of animals, A β 42 deposition findings by AV45-PET was confirmed using immunohistochemistry and revealed that only in the aged cohort was deposition shown in the hippocampus and frontal cortex compared to young animals. Furthermore, aged

animals showed immunoreactivity for phosphorylated Tau. To develop a PD cohort of NHPs, we generated 20 cynomolgus macaques with alpha-synucleinopathy. Using MRI-guidance, animals were stereotactically administered with AAV9-hA53T-aSyn to a single hemisphere of the substantia nigra. After 18 months, degeneration of dopaminergic (DA) terminals of the injected side was observed by VMAT2-PET using the radioactive tracer AV133 [FP-(+)-DTBZ] which detects DA terminals projecting to the putamen and caudate. Alpha-synuclein induced degeneration was supported by both human A53T a-synuclein and tyrosine hydroxylase immunohistochemistry in the midbrain. Biochemical analyses of dopaminergic function and behavioural performance will be reported. In conclusion, we have developed and continue to characterize large colonies of both AD and PD NHP models that support the preclinical evaluation of candidate therapeutics for neurodegenerative disease.

Disclosures: L. Yang: None. G. Wang: None. Y. Xu: None. S. Zha: None. J. Gabriel: None. R. Perez: None. J. Koprach: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.02/C61

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

Title: Associations of white matter integrity with asymmetry and temporal coordination of grasping movements in healthy older adults and mild cognitive impairment patients: An exploratory study.

Authors: *O. VASYLENKO¹, T. R. VANGBERG², K. WATERLOO³, C. RODRÍGUEZ-ARANDA⁴;

¹UiT Arctic Univ. of Tromsø, Tromsø, Norway; ²Univ. Hosp. of North Norway, Tromsø, Norway; ³Univ. Hosp. of North Norway, Tromsø, Norway; ⁴Dept. of Psychology, Univ. of Tromsø, Tromsø, Norway

Abstract: Grasping skills are important for daily activities and are affected by aging and mild cognitive impairment (MCI). Both unimanual and bimanual grasping decline. For instance, recent research has demonstrated reduction of asymmetry and disrupted timing in grasping movements. However, it is unclear how these changes relate to brain structure. Decline in white matter integrity (WMI) is documented in aging and MCI, and previous studies have identified cerebrospinal tract (CST) and corpus callosum (CC) as correlates of dexterity performance. Given that many cognitive abilities are involved in the control of grasping, it is useful to expand on these findings. **Aim:** to explore how WMI of all major tracts is associated with 1) asymmetry of right- and left hand movements during unimanual grasping, and 2) temporal coordination during synchronous bimanual grasping in healthy older adults and MCI patients. **Methods:** Forty-five healthy older adults and 37 MCI patients, all right-handed, were tested with the Purdue Pegboard Test and recorded with 2D Vicon Motus motion capture. Asymmetry was

assessed by laterality indices of linear and angular velocities and path lengths for each hand. Bimanual coordination was assessed by time lags between movement onset for each hand. MR images were acquired on a 3T scanner and FreeSurfer TRACULA was used to extract 40 white matter tracts. WMI was assessed by fractional anisotropy and mean diffusivity. **Preliminary results:** Grasping performance was lower in the MCI group. WMI was lower in the MCI group in CST, prefrontal body of CC, middle cerebellar peduncle (MCP), optic radiation (OR), inferior longitudinal fasciculus (ILF), and fornix (FX). Significant associations between grasping asymmetry and WMI did not differ by group. Less asymmetry in path length was related to lower WMI in prefrontal and parietal body of CC, OR, right ILF, and left FX. For bimanual coordination, some associations did not differ by group. Longer time lags were related to lower WMI in CST, rostrum and central body of CC, right OR, extreme capsule, right ILF, superior longitudinal fasciculus (SLF) II, and frontal aslant tract. Additionally, significant group interactions were found for MCP, left middle longitudinal fasciculus, right uncinate fasciculus, arcuate fasciculus, and SLF III. The associations between time lags and WMI of these tracts were stronger in the MCI group. **Conclusion:** The present data suggest that the decline in dexterity in MCI relies on accelerated degeneration of WM and not on qualitative differences between groups. Longitudinal studies are needed to better understand the role of WMI decline in hand function along the aging continuum.

Disclosures: O. Vasylenko: None. T.R. Vangberg: None. K. Waterloo: None. C. Rodríguez-Aranda: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.03/C62

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

Support: NIH Grant R01AG057602

Title: Diffusion MRI as a biomarker for early treatment with a neurotrophic peptidergic compound in the 3xTg-AD mouse model of Alzheimer's disease (AD)

Authors: M. FALANGOLA¹, J. VOLTIN², M. COLE², P. NIETERT³, F. LIU⁴, K. IQBAL⁵, *J. H. JENSEN²;

¹Neurosci., Med. Univ. of South Carolina - Radiology, Charleston, SC; ²Neurosci., ³Publ. Hlth. Sci., Med. Univ. of South Carolina, Charleston, SC; ⁴New York State IBR, Staten Island, NY;

⁵Dept Neurochem, NYS Inst/Basic Res., Staten Island, NY

Abstract: The 3xTg-AD transgenic (TG) mouse has been extensively used as an Alzheimer's disease (AD) model. Treatment with neurotrophic factor peptide mimetic P021 reduces abnormal hyperphosphorylation of tau, attenuates soluble A β , increases BDNF expression, and rescues deficits in dendritic and synaptic plasticity in TG mice. Diffusion MRI (dMRI) is widely used to

noninvasively detect changes in brain microstructure. We previously showed that dMRI is able to capture brain structural changes related to AD pathology progression in TG mice. Our aim here is to demonstrate the potential of dMRI to quantify brain changes due to P021 treatment in this model. Female 3xTg-AD [MMRRC Stock No: 34830-JAX] and age/gender-matched controls (NC) [101045 B6129SF2/J] were purchased from *The Jackson Laboratory*. Treatment (P021) was administered from 2.5 to 8 months of age. TG-P mice (n=30) were put on mouse chow (AIN-76, Research Diets, New Brunswick, NJ) containing 60 nmoles P021/g. TG-V mice (n=26) and NC-V mice (n=23) were treated with the same diet (vehicle) but without the peptide. *In vivo* dMRI was performed at 8 months on a 7T Bruker system using a diffusional kurtosis imaging acquisition generating four diffusion tensor metrics [mean diffusivity (MD), axial diffusivity (D_{ax}), radial diffusivity (D_{rad}), fractional anisotropy (FA)] and four diffusional kurtosis metrics [mean kurtosis (MK), axial kurtosis (K_{ax}), radial kurtosis (K_{rad}), kurtosis fractional anisotropy (KFA)]. Regions of interest (ROI) were defined using ImageJ in the corpus callosum (CC), external capsule (EC), and cortex [cingulate (Ctx-Cg) and retrosplenial cortex (Ctx-Rsp)]. Regional dMRI values were obtained by averaging all voxel-based values within each ROI. One-way ANOVA was performed with FDR-corrected p -values to assess group differences. P -values ≤ 0.05 were considered statistically significant. In the TG-V, FA was significantly lower in the CC, EC and Ctx-Cg, D_{ax} was lower in the CC, and K_{ax} was higher in the Ctx-Rsp compared to NC-V. These dMRI changes in the TG-V reflect white matter defects (e.g., less density/coherence of fibers, less myelin and dystrophic neurites). After a short-term treatment (5½ months), TG-P dMRI values shifted towards NC-V values resulting in no statistical differences between NC-V and TG-P for KFA in the CC and K_{ax} in the Ctx-Rsp. Also, TG-P and TG-V were significantly different for FA in the CC, EC, Ctx-Cg; for KFA in the EC; for D_{ax} in the CC. These TG-P dMRI changes may be due to P021-mediated increase in BDNF expression, promoting myelination and decreasing GSK3 β activity and neuritic damage. Our results support dMRI as a potential biomarker to monitor P021 therapeutic effects.

Disclosures: M. Falangola: None. J. Voltin: None. M. Cole: None. P. Nietert: None. F. Liu: None. K. Iqbal: A. Employment/Salary (full or part-time): Phanes Biotech, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); the inventor and patent holder of P021. J.H. Jensen: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.04/Web Only

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

Support: R01 AG060610
U01 AG068057

Title: Generating diffusion MRI scalar maps from T1-weighted images using Reversible GANs

Authors: *T. CHATTOPADHYAY¹, G. MEHENDALE¹, S. I. THOMOPOULOS¹, P. M. THOMPSON²;

¹USC, Los Angeles, CA; ²Neurol., USC, Palos Verdes, CA

Abstract: Diffusion tensor imaging (DTI) offers crucial insights into brain tissue microstructure but acquiring high-quality data is time-intensive and not always feasible. Synthetic DTI generated from more accessible structural MRI (T1w) can address data scarcity, reduce acquisition cost, and enable broader research and clinical application.

We present a novel framework using 3D Reversible Generative Adversarial Network (RevGAN) to directly translate standard T1w MRI scans to create synthetic mean diffusivity (MD) maps, evaluating fidelity and utility of synthetic images for downstream neuroimaging tasks. MD maps are particularly sensitive to amyloid pathology and dementia.

The datasets comprised 930 subjects (497 controls, 317 MCI, and 116 with dementia) from Alzheimer's Disease Neuroimaging Initiative (ADNI), with an independent test cohort of 226 Indian subjects from NIMHANS. T1w images were preprocessed (N4 bias correction, skull stripping, nonlinear registration, min-max scaling) and aligned to ENIGMA template. 3D RevGAN architecture incorporated invertible neural network for bidirectional mapping between T1w and MD, ensuring information preservation and cycle consistency without additional networks. Training used adversarial loss with two discriminators and was performed for 600 epochs. We evaluated synthetic maps using quantitative metrics (PSNR, MS-SSIM, Mutual Information, FID, MMD) across clinical subgroups (Cognitively Normal, Mild Cognitive Impairment, Dementia). A 3D CNN was trained for sex and dementia classification on real and synthetic MD maps; performance was tested on ADNI and NIMHANS cohorts.

Synthetic maps closely resembled real images, with average PSNR of 17.54 and MS-SSIM of 0.81 across all subjects. Mutual information was highest in dementia subjects (1.01), indicating preserved structural relationship. Distributional similarity metrics (FID: 41.20, MMD: 0.02) confirmed high-quality synthesis. Models trained on synthetic MD achieved balanced accuracy, and AUC-ROC comparable to those trained on real MD for both sex (0.82/0.89 vs. 0.79/0.86) and dementia classification (0.82/0.86 vs. 0.82/0.85). Evaluation on NIMHANS data showed only minor performance drop, demonstrating strong cross-population generalizability. RevGAN enables accurate, single-step synthesis of DTI-MD maps from T1ws, producing images suitable for downstream applications. Synthetic maps supported robust classification performance, even in external datasets. This approach can alleviate DTI data scarcity and enhance neuroimaging workflows. Future work will target finer anatomical fidelity and multi-channel DTI synthesis.

Disclosures: T. Chattopadhyay: None. G. Mehendale: None. S.I. Thomopoulos: None. P.M. Thompson: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.05/C63

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

Support: AARG-23-1149996
R01AG058854
RF1AG057892
R01AG087513
U01AG068057
U19AG024904
RF1NS136995

Title: Microstructural correlates of Alzheimer's Disease: comparing DTI and NODDI biomarkers across white matter tracts

Authors: *K. LIOU¹, S. I. THOMOPOULOS¹, H. YOO¹, C. R. JACK, JR.², M. W. WEINER³, P. M. THOMPSON¹, T. M. NIR¹;

¹Imaging Genet. Center, Mark and Mary Stevens Neuroimaging and Informatics Inst., USC, Marina del Rey, CA; ²Dept. of Radiology, Mayo Clin., Rochester, MN; ³Dept. of Radiology, Sch. of Med., Univ. of Calif San Francisco, San Francisco, CA

Abstract: Diffusion MRI (dMRI) is a powerful tool for assessing white matter (WM) microstructural abnormalities in Alzheimer's disease (AD). In its fourth phase, the AD Neuroimaging Initiative (ADNI) has expanded from a single to multiple multishell dMRI protocols across GE, Philips and Siemens scanners (**Fig1A**), enabling both traditional and advanced dMRI model analyses. Single shell data can yield diffusion tensor imaging (DTI) metrics, while multishell dMRI supports advanced models like neurite orientation dispersion and density imaging (NODDI), capturing non-Gaussian diffusion components linked to specific tissue compartments. We compared DTI and NODDI WM metrics' sensitivity to key clinical AD indicators, including cognitive impairment (CI) and amyloid (A β) pathology. We analyzed multishell dMRI from 282 ADNI3/4 subjects (**Fig1B**). We extracted mean DTI (FA, MD, RD, AxD) and NODDI metrics (ICVF, ISOVF, ODI) from 31 JHU atlas WM regions. Mixed effects linear models assessed associations between regional dMRI and clinical metrics: CDR-sob, MCI diagnosis vs control, and A β PET positivity (A β +). Fixed effect covariates included age, sex, age by sex interaction, education, and race/ethnicity. dMRI protocol was modeled as a random effect to account for acquisition differences. We found widespread associations between dMRI measures and CI (i.e. CDR-sob, MCI) across nearly all dMRI indices ($P_{FDR}<0.05$; **Fig1C,D**); fewer associations were found with ODI. The strongest effects localized to limbic tracts, such as the cingulum hippocampal bundle and fornix. A β + was linked to higher RD, AxD and ISOVF, with largest effects in the posterior corona radiata (**Fig1E**). Overall, the strength and distribution of DTI and NODDI associations with CI were comparable, but NODDI ISOVF was more sensitive to A β . NODDI may also offer added biological specificity. Lower DTI FA and greater diffusivity suggest WM deterioration, but are nonspecific. Lower ICVF and ODI, and higher ISOVF suggest these effects may reflect factors such as neuronal loss, reduced tissue complexity, and inflammation/edema, respectively.

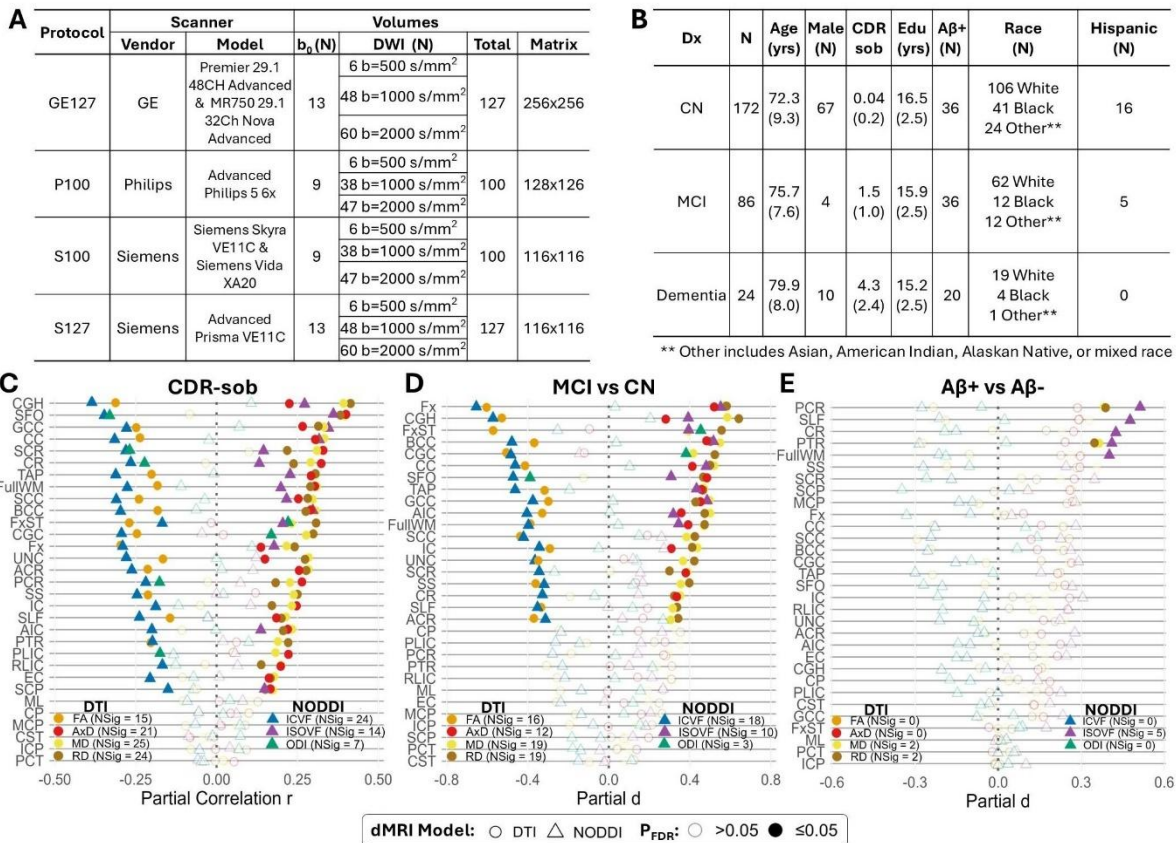


Figure 1. (A) Available ADNI3 and ADNI4 multishell protocols. DTI was fit to the subset of b_0 and $b=1000$ s/mm² DWI volumes; NODDI was fit across all DWI. **(B)** Clinical and demographic details for ADNI multishell subjects. **(C)** Effect sizes for associations between CDR-sob and DTI and NODDI metrics. **(D)** Effect sizes for group differences in DTI and NODDI metrics between CN and MCI subjects. **(E)** Effect sizes for group differences in dMRI metrics between A β + and A β - subjects. We note that we additionally covaried for diagnosis in A β analyses. **KEY:** DWI: Diffusion Weighted Imaging; CDR-sob: Clinical Dementia Rating scale sum-of-boxes; CN: Cognitively Normal; MCI: Mild Cognitive Impairment; A β : Amyloid Beta; FA: Fractional Anisotropy; MD: Mean Diffusivity; RD: Radial Diffusivity; ICVF: Intracellular Volume Fraction; ISOVF: Isotropic Volume Fraction; ODI: Orientation Dispersion Index.

Disclosures: K. Liou: None. S.I. Thomopoulos: None. H. Yoo: None. C.R. Jack, Jr.: None. M.W. Weiner: None. P.M. Thompson: None. T.M. Nir: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.06/C64

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

Title: Identifying early neuroanatomical biomarkers of cognitive decline using T1-weighted MRI and brain-specific machine learning approach in subjective cognitive decline and mild cognitive impairment

Authors: *Y.-C. CHEN¹, J.-D. ZHU^{1,2};

¹Dept. of Occup. Therapy, Chung Shan Med. Univ., Taichung City, Taiwan; ²Occup. Therapy Room, Chung Shan Med. Univ. Hosp., Taichung City, Taiwan

Abstract: Subjective cognitive decline (SCD) and mild cognitive impairment (MCI) are widely recognized as potential early stages of Alzheimer's disease (AD), with some individuals finally progressing to AD. Therefore, early identification of individuals at high risk is crucial, particularly by identifying them from cognitively normal controls (CNs). This study used T1-weighted MRI and brain-specific machine learning approach to classify individuals with SCD and MCI from CNs and to identify key brain regions that may serve as early indicators of AD progression. The data were sourced from the Alzheimer's Disease Neuroimaging Initiative 2 database, including 69 individuals with SCD, 93 with MCI, and sex- and age-matched CNs based on the first two clinical groups. Each group was randomly divided into training and test datasets at a ratio of 8:2. After image preprocessing, we constructed 116 brain-specific classification models using an automated anatomical labelling atlas in the training dataset. Feature selection was performed using elastic net regression, followed by five-fold cross-validation with multiple classification algorithms. The trained models were applied to the independent test dataset. All procedures were repeated ten times using MATLAB 2024b. We classified the SCD and CN groups separately, as well as the MCI and CN groups. Shared brain regions were identified in the two groups by intersecting the top 20 brain regions ranked by mean test accuracy. We then performed a correlation coefficient analysis between the gray matter volume of these brain regions and Mini-Mental State Examination (MMSE) scores to evaluate their association with cognitive function and potential as early biomarkers of disease progression. Our results showed that the logistic regression algorithm achieved the highest classification performance, with mean test accuracies of top 20 brain regions ranging from 88.7% to 98.26% for the SCD group and 80.69% to 93.1% for the MCI group across 116 brain-specific models. Among the seven shared brain regions, five exhibited significantly positive correlations with MMSE scores after false discovery rate correction ($r = 0.23-0.34$, adjusted $p < 0.05$), located in the bilateral postcentral gyri, right angular gyrus, left middle frontal gyrus, and left middle temporal gyrus. Our findings identified seven key brain regions associated with early cognitive decline using MRI-based machine learning classification. Five shared brain regions between SCD and MCI groups showed significant correlations with MMSE scores, highlighting their potential as early neuroanatomical biomarkers for cognitive decline and possible progression toward AD.

Disclosures: Y. Chen: None. J. Zhu: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.07/D1

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

Support: The Football Association
Rugby Football Union
Premiership Rugby

Title: Default mode network hyperconnectivity and plasma pTau217 in retired elite rugby players

Authors: *J. HAIN¹, E.-J. MALLAS¹, T. PARKER¹, K. A. ZIMMERMAN¹, E. J. ROONEY¹, Y. LEE², M. DEL GIOVANE¹, M. C. B. DAVID¹, L. M. LI³, N. S. GRAHAM¹, D. FRIEDLAND¹, R. SYLVESTER⁴, D. J. SHARP¹;

¹Imperial Col. London, London, United Kingdom; ²Univ. of Reading, Reading, United Kingdom; ³Division of Brain Sciences, Imperial Col. London, Computational, Cognitive and Clin. Neuroimaging, London, United Kingdom; ⁴Dept. of Neurodegenerative Dis., UCL Inst. of Neurol., London, United Kingdom

Abstract: Contact sport athletes with exposure to repetitive head impacts face increased risk of neurodegenerative disease and cognitive decline. Using functional MRI, increased functional connectivity (FC) in the default mode network (DMN) has been observed following traumatic brain injury (TBI) and in early Alzheimer's disease (AD), whereas reduced FC has been observed in later stages of AD. This suggests a non-linear trajectory, from hyper- to hypo-connectivity, as a response to neurological disruption (fig.1A). We investigated FC across key large-scale brain networks and its association with blood-based biomarkers of AD pathology and cognitive performance across three cohorts (fig.1B).

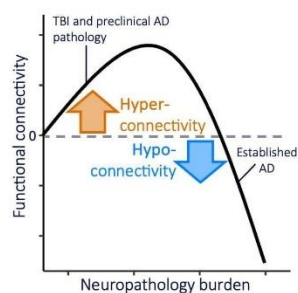
Retired rugby players (N=173), moderate/severe TBI (N=50), AD (N=64) and age-matched controls (N=100) underwent resting-state fMRI, neurodegenerative blood biomarker sampling (pTau217, GFAP, NfL, A β 42/A β 40), and cognitive assessment (Trail Making, WMS-IV, ACE). FC was analysed using seed-based dual regression.

Rugby players showed hyperconnectivity in the DMN and sensorimotor network (SMN) relative to controls (fig.1C). Rugby players had elevated pTau217 compared to HC (fig.1D). In rugby players both within-DMN and DMN-to-SMN connectivity was associated with increased pTau217 (fig.1E). In TBI, within-SMN hyperconnectivity was observed acutely, while at 6- and 12-months post-injury, hyperconnectivity extended to within-DMN and DMN-to-SMN connections (fig.1F). In contrast, AD patients exhibited hypoconnectivity across within-DMN, within-SMN, and DMN-to-SMN compared to HC (fig.1G).

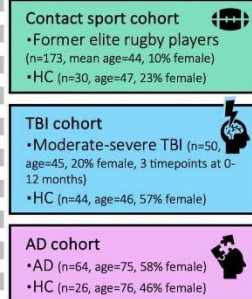
Our findings support a model where FC changes reflect stage-specific responses to neurodegeneration. Hyperconnectivity developing early in TBI reflects an initial network response, while hypoconnectivity marks established disease. Hyperconnectivity in mid-life rugby players may signal early vulnerability to neurodegeneration. Results suggest that resting-state fMRI could be a useful biomarker of proximity to cognitive decline alongside pathology-specific biomarkers.

Figure 1

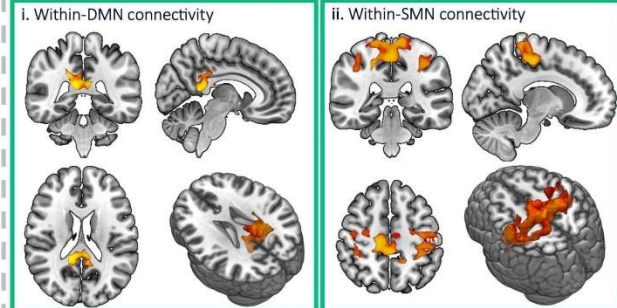
A Hypothesis



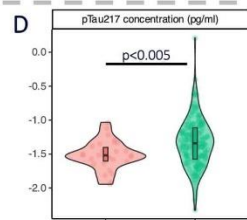
B Study cohorts



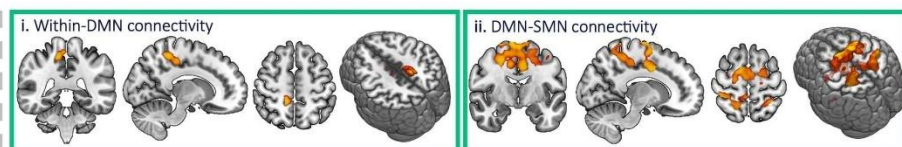
C Rugby > HC



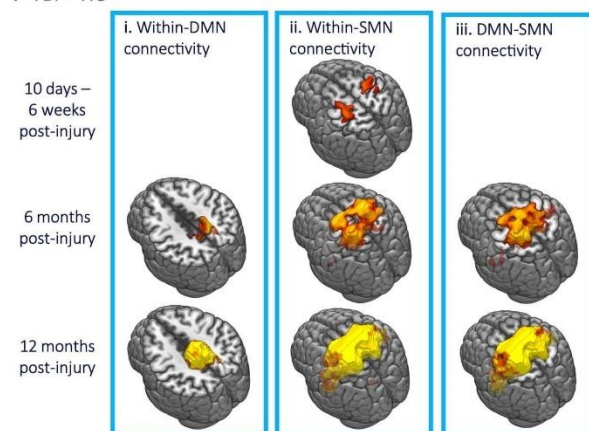
D



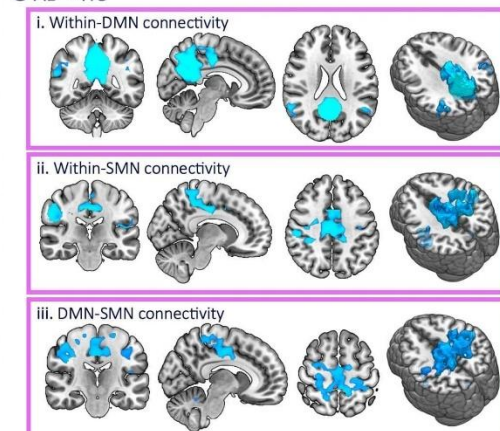
E Effect of pTau217 in rugby



F TBI > HC



G AD < HC



Disclosures: **J. Hain:** A. Employment/Salary (full or part-time): The Football Association, Rugby Football Union, Premiership Rugby. **E. Mallas:** A. Employment/Salary (full or part-time): UKDRI CR&T. **T. Parker:** A. Employment/Salary (full or part-time): NIHR clinical lectureship. **K.A. Zimmerman:** None. **E.J. Rooney:** A. Employment/Salary (full or part-time): The Football Association, Rugby Football Union, Premiership Rugby. **Y. Lee:** None. **M. Del Giovane:** A. Employment/Salary (full or part-time): The Football Association, Rugby Football Union, Premiership Rugby. **M.C.B. David:** None. **L.M. Li:** None. **N.S. Graham:** A. Employment/Salary (full or part-time): NIHR clinical lectureship. **D. Friedland:** F. Consulting Fees (e.g., advisory boards); undertakes clinical private practice, including in the Advanced Brain Health Clinic which receives funding support from the RFU, FA, and PFA. **R. Sylvester:** F. Consulting Fees (e.g., advisory boards); on the concussion advisory board of the English Rugby Football Union, independent concussion consultant for the World Rugby Union, on the head injury advisory board of the English Football Association, undertakes clinical private practice, including in the Advanced Brain Health Clinic which receives funding support from the RFU, FA, and PFA. **D.J. Sharp:** A. Employment/Salary (full or part-time): funded by the UK Dementia Research Institute, of which the MRC is the principal funder. F. Consulting Fees (e.g.,

advisory boards); on the concussion advisory board of the English Rugby Football Union, undertakes clinical private practice, including in the Advanced Brain Health Clinic which receives funding support from the RFU, FA, and PFA.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.08/D2

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

Title: Clinical and preclinical comparison of neurovascular and metabolic dysregulation in alzheimer's disease

Authors: *J. K. CHONG CHIE¹, S. A. PERSOHN¹, O. R. SIMCOX³, A. COLLINS¹, P. SALAMA⁴, P. R. TERRITO²;

¹Stark Neurosci. Res. Inst., ²Med., Indiana Univ., Indianapolis, IN; ³Wood Col. of Osteo. Med., Marian Univ., Indianapolis, IN; ⁴Purdue Univ., Indianapolis, IN

Abstract: Clinical imaging diagnostics in AD focuses on detecting amyloid and tau; however, several studies suggest that neurometabolic and vascular (NMV) dysregulation (MVD) begins at an early age, potentially preceding amyloid β accumulation, leading to AD onset. Metabolic dysfunction is linked to energy deficits, contributing to neuronal damage. At the same time, vascular dysregulation is increasingly recognized as a critical component, with chronic hypoperfusion observed in both AD patients and mouse models. These MVD changes persist in preclinical and clinical cases across the disease spectrum; therefore, comparing these changes could provide valuable insight into disease onset and determine disease progression. MVD was evaluated using ¹⁸F-FDG, a surrogate for metabolism (MET), and cerebral blood flow (CBF), using ASL or 64Cu-PTSM, in clinical and preclinical subjects. These regional values were z-scored, and projected into Cartesian space to determine the MVD phenotype. In addition, we developed a region set enrichment analysis (RSEA) to evaluate brain functional category (BFC) changes based on metabolic variations. MVD showed changes across the disease spectrum in clinical cases, and across time in mouse models follow similar trajectories. In both cases, early stages show Type 1 ($\downarrow$ MET $\uparrow$ CBF) and Type 2 ($\uparrow$ MET $\downarrow$ CBF) uncoupling, and late stages show prodromal ($\uparrow$ MET $\uparrow$ CBF) and NMV failure ($\downarrow$ MET $\downarrow$ CBF). Although there is no direct one-to-one relationship between BFC in mice and humans, RSEA results showed significant changes across comparable categories: motor, learning, and perception in mice; while motor, memory, cognition, and language changed in humans. Our findings suggest that disease progression follows a disease stage-dependent dysregulation pattern that can identify at risk brain regions in both populations. In addition, our study shows that MVD and RSEA changes vary by sex and disease stage in both populations, making it a sensitive tool for early AD diagnosis that can be used preclinically and clinically, which may improve disease stratification and therapeutic testing.

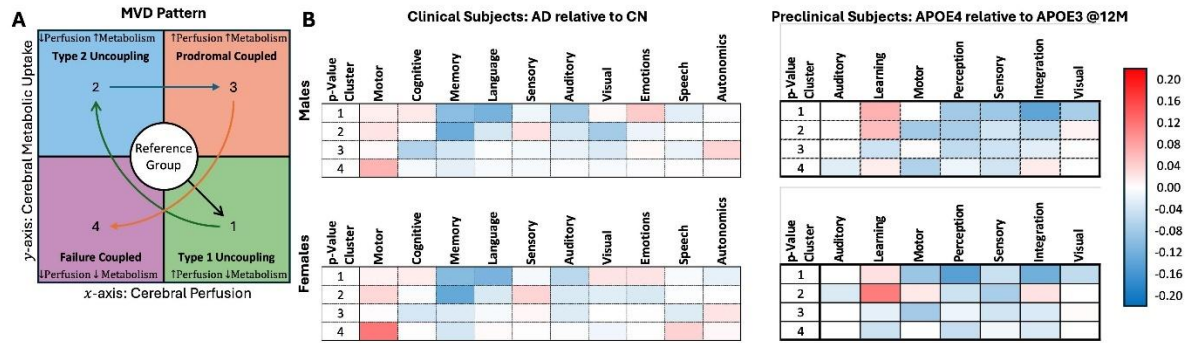


Figure 1 – (A) MVD Pattern and (B) RSEA results for AD relative to cognitive normal (in humans) and APOE4 relative to APOE3 (in mouse models).

Disclosures: J.K. Chong Chie: None. S.A. Persohn: None. O.R. Simcox: None. A. Collins: None. P. Salama: None. P.R. Territo: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.09/D3

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

Support: NIA/NIH: R01AG056519
NIA/NIH: P30AG072972

Title: The association of GFAP with regional white matter hyperintensities, amyloid, and memory in the oldest-old: The Life After 90 Study

Authors: *B. RIZVI¹, C. B. YOUNG¹, A. M. BRICKMAN², P. GILSANZ³, P. MAILLARD⁴, M. M. CORRADA⁵, R. A. WHITMER¹;

¹Univ. of California, Davis, Davis, CA; ²Columbia Univ., New York, NY; ³KPNC Div. of Res., Pleasanton, CA; ⁴Neurol., Univ. of California Davis, Davis, CA; ⁵Univ. of California, Irvine, Irvine, CA

Abstract: Glial fibrillary acidic protein (GFAP) is a marker of reactive astrocytic activity that is elevated in Alzheimer's disease (AD). Previous work showed an association of elevated plasma GFAP levels with greater amyloid burden and age- and AD-related cognitive decline. Past work also linked GFAP to white matter hyperintensities (WMH), a marker of small vessel cerebrovascular disease, in amyloid-positive individuals. Moreover, WMH in posterior regions have shown to be more strongly associated with memory impairment. Here, we assessed whether plasma GFAP is related to regional WMH and amyloid-beta burden. We further tested whether amyloid-beta moderates the association of regional WMH with memory performance in the oldest-old. We included participants from *LifeAfter90*, a racially and ethnically diverse cohort aged 90 years and older. Levels of GFAP were measured in the plasma. A 3T MRI with T1 and FLAIR images was acquired to derive lobar and total WMH volumes. Regional and global

amyloid-beta were measured with amyloid-PET, using AV45 and a standard SUVR analysis with cerebellar gray matter as the reference region. We assessed verbal episodic memory with the Spanish and English Neuropsychological Assessment Scales. There were 387 participants included (mean age of 93.09 ± 2.22 , 59.4 % women, 26.1% Asian, 22.5% Black, 20.4% Latinx, 25.1% White, mean education of 14.5 years) who had imaging or plasma collected. Elevated plasma GFAP was only associated with greater occipital WMH ($b=45.09$, 95% CI [17.22, 72.96], $p=0.002$), but not regional or global amyloid-beta burden (all $p>0.05$). Plasma GFAP was not associated with verbal episodic memory ($b=0.0001$, 95% CI [-0.001, 0.001], $p=0.855$). Amyloid-beta moderated the association between occipital WMH and memory performance, such that when there was lower amyloid-beta burden, greater occipital WMH was associated with lower memory performance (interaction $b=0.807$, 95% CI [0.211, 1.403], $p=0.008$). These findings suggest that early markers of AD, including GFAP, may promote regionally selective cerebrovascular disease, which affects memory in the oldest-old.

Disclosures: B. Rizvi: None. C.B. Young: None. A.M. Brickman: None. P. Gilsanz: None. P. Maillard: None. M.M. Corrada: None. R.A. Whitmer: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.10/D4

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

Support: Health and Aging Brain Study - Health Disparities U19AG078109

Title: Hemoglobin in relation to structural and fluid markers of neurodegeneration across a multi-ethnic cohort

Authors: *S. GANESAN¹, V. R. TENNANT¹, N. L. LEE¹, M. W. HAND¹, M. L. DAVISON¹, Z. E. TSOKOLAS¹, S. E. O'BRYANT², A. W. TOGA¹, K. YAFFE³, M. N. BRASKIE¹;

¹USC Stevens Neuroimaging and Informatics Inst., USC, Los Angeles, CA; ²Inst. for Translational Res., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; ³Dept. of Psychiatry, Neurology, and Epidemiology and Biostatistics, Univ. of California San Francisco, San Francisco, CA

Abstract: Background: Low hemoglobin (Hb), clinically manifesting as anemia, has been associated with cortical atrophy, white matter injury, and elevated risk of Alzheimer's disease. However, the mechanisms linking Hb to early neurodegeneration remain unclear. To address this, we investigated the relationship between Hb and established neurodegeneration markers—meta-ROI cortical thickness and plasma neurofilament light (pNfL) levels—in a large, multi-ethnoracial cohort of cognitively unimpaired adults, controlling for factors that may be linked to anemia and neurodegeneration. Methods: We analyzed data from 830 cognitively unimpaired individuals from the Health and Aging Brain Study - Health Disparities cohort (64.8 ± 8 years;

583 F; 326 Hispanic, 384 non-Hispanic White (NHW), 120 non-Hispanic Black (NHB)). Participants underwent a 3T T1-weighted MRI for meta-ROI cortical thickness estimation (FreeSurfer v5.3), pNfL quantification (Quanterix), and blood Hb assessment. We ran separate multiple linear regressions to assess Hb's relationship with meta-ROI thickness and pNfL. All continuous variables were standardized. Models were adjusted for age, sex, education, self-described ethnorracial group, scanner type, APOE4 status, body mass index, diabetes, hypertension, dyslipidemia, estimated glomerular filtration rate (measure of renal function), and amyloid positivity. Interactions between Hb and age, sex, APOE4 status, amyloid positivity, and ethnorracial group were assessed. Results: Lower continuous levels of Hb were correlated with thinner cortex in our meta-ROI ($B = 0.11$, $p = 0.004$) and higher pNfL ($B = -0.07$, $p < 0.001$). The Hb by ethnorracial group interaction on pNfL was significant ($B = -0.09$, $p = 0.038$), with the strongest association observed in NHB ($B = -0.13$, $p < 0.001$), followed by Hispanic ($B = -0.07$, $p = 0.001$) and NHW ($B = -0.04$, $p = 0.081$) groups. No significant interactions were detected with age, sex, APOE4, or amyloid status. Conclusion: In cognitively unimpaired individuals, low Hb levels were associated with markers of axonal injury (pNfL) and cortical atrophy (meta-ROI), independent of amyloid status and vascular-metabolic comorbidities. Stronger associations in NHB individuals may reflect the influence of various socioeconomic, environmental, or genetic factors associated with ethnorracial identity.

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Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.11/D5

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

Title: Rsi vs. nodd: a head-to-head comparison of multicompartiment diffusion mri models

Authors: *V. D. HERNANDEZ^{1,2}, S. K. SOLDERS³, J. A. ELMAN⁴, E. T. REAS³;
¹Cognitive Sci., ²Psychology, ³Neurosciences, ⁴Psychiatry, UCSD, La Jolla, CA

Abstract: Diffusion MRI (dMRI) has been shown to be sensitive to microstructural brain changes during aging and with associated conditions such as Alzheimer's disease and Mild Cognitive Impairment (MCI). Restriction Spectrum Imaging (RSI) and Neurite Orientation Dispersion and Density Imaging (NODDI) are dMRI models that have shown use in diagnostic and research settings. Even so, RSI and NODDI have not been directly compared, leaving uncertainty regarding their comparability and which technique is more sensitive to associations of brain microstructure with aging or disease. We hypothesized that RSI and NODDI metrics are not directly comparable, and may be differentially sensitive to associations with aging or cognitive impairment. RSI and NODDI models were applied to dMRI data from 225 older adult

participants (84% cognitively normal (CN), 15% MCI) from the Rancho Bernardo Study of Healthy Aging (RBS) and UC San Diego Alzheimer's Disease Research Center (ADRC). RSI and NODDI metrics were measured across global white and gray matter. Partial correlation matrices assessed associations between RSI and NODDI metrics, and linear regressions examined associations between diffusion metrics and age (adjusted for sex), as well as cognitive composite scores (processing speed, verbal fluency, executive function, episodic memory, and visuospatial memory; adjusted for age, sex, education). RSI and NODDI metrics were moderately correlated in expected patterns, with stronger correlations in white matter than in gray matter. Unexpectedly, in white matter, NODDI's neurite density correlated most weakly with RSI's neurite density (partial $r = 0.40$) and most strongly with RSI's restricted isotropic diffusion (partial $r = 0.71$); in gray matter, NODDI's neurite density showed no correlation with RSI's neurite density. In white matter, age correlated most strongly with NODDI's free water (partial $r = 0.53$) and with RSI's total restricted diffusion (partial $r = -0.51$). In gray matter, only NODDI's free water, but most RSI metrics significantly correlated with age. Most RSI metrics were associated with cognitive composites in white matter and gray matter. All NODDI metrics were associated with cognitive composites in white matter, but showed minimal associations in gray matter. Overall, our results suggest that RSI and NODDI are not directly interchangeable, but may capture distinct microstructural tissue properties. Differences in associations with age and cognition across tissue type hold important implications for model selection depending on research or clinical application.

Disclosures: V.D. Hernandez: None. S.K. Solders: None. J.A. Elman: None. E.T. Reas: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.12/D6

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

Support: Health and Aging Brain Study - Health Disparities U19AG078109

Title: The relationship between anxiety and brain amyloid levels in cognitively unimpaired older adults

Authors: *S. T. NIMMO¹, D. LUU¹, M. W. HAND¹, N. N. LEE¹, A. W. TOGA¹, S. O'BRYANT², K. YAFFE³, M. N. BRASKIE¹;

¹USC, Los Angeles, CA; ²Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; ³Univ. of California San Francisco, San Francisco, CA

Abstract: Background: Neuropsychiatric symptoms—particularly anxiety—have emerged as important risk factors in the progression of Alzheimer's Disease (AD). Notably, 40% of individuals with mild to severe cognitive impairment develop new anxiety symptoms. Anxiety is

a potential predictor of cognitive decline; elevated anxiety combined with amyloid-beta ($A\beta$) positivity may accelerate neurodegenerative progression. Few studies have explored distinct features of anxiety related to underlying AD pathology. This study investigates whether the relationship between anxiety and brain amyloid levels differs by $A\beta$ status and examines the potential of anxiety as a preclinical behavioral marker for AD.

Methods: We observed 1857 cognitively unimpaired (CU) participants (mean age 64.8; 595 males, 1262 females) from the Health and Aging Brain Study-Health Disparities. Global amyloid standardized uptake value ratio (SUVR) was derived from Florbetaben positron emission tomography (PET) scans acquired and analyzed using ADNI3 protocols with a whole cerebellum reference region. $A\beta+$ was defined as global SUVR >1.08 (318 $A\beta+$ and 1539 $A\beta-$ participants). Anxiety was defined by a past medical diagnosis (331 with anxiety, 1526 without anxiety). Linear regression models included age, self-reported sex and ethnoracial group, years of education, PET scanner, depression diagnosis, and subjective memory complaints (SMC) as covariates.

Results: While anxiety was not associated with global amyloid burden in the main model ($\beta = -0.09$, $p = 0.19$), a significant interaction was observed between anxiety and $A\beta$ status ($\beta = 0.34$, $p < 0.01$), with anxiety associated with lower global amyloid in $A\beta+$ individuals ($\beta = -0.32$, $p = 0.08$) and higher global amyloid in $A\beta-$ individuals ($\beta = 0.05$, $p = 0.08$). In a subsample of 1652 participants with available *APOE* genotype, the interaction between anxiety and $A\beta+$ on global amyloid level remained significant ($\beta = 0.24$, $p = 0.02$) after controlling for *APOE4* carrier status. In all participants, SMC mediated the relationship between anxiety and $A\beta$ levels with a significant indirect effect ($\beta = 0.02$, $p = 0.02$), but no significant direct effect of anxiety on amyloid ($\beta = -0.09$, $p = 0.15$).

Conclusion: These findings suggest that in $A\beta-$ CU individuals, greater amyloid is associated with more anxiety, as expected. However, in $A\beta+$ CU individuals, greater amyloid is associated with less anxiety, which may reflect diminished insight or neurodegenerative changes impacting emotional regulation and self-awareness. Anxiety may serve as an early behavioral marker for individuals at risk of AD, but its utility may diminish as pathology advances and cognitive awareness declines.

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Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.13/D7

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

Support: NIH Grant R01 NS119593
NIH P51OD010425

Title: $A\beta$, Tau, and FDG-PET synthesis from multi-contrast MRI using deep learning

Authors: ***R. IRITANI**¹, H. JAHANIAN², A. YAZDAN-SHAHMORAD³;

¹Bioengineering, ²Radiology, ³Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA

Abstract: With the FDA's recent approval of the anti-amyloid antibodies lecanemab and aducanumab, the prospect of disease-modifying therapies for Alzheimer's disease has become a clinical reality. However, these treatments are only effective in the early stages of the disease before significant neurodegeneration has occurred. Positron Emission Tomography (PET) has the ability to detect the spread and density of key biomarkers associated with AD including Amyloid- β , Tau, and reduced glucose metabolism, which can be detected up to 20 years prior to clinical symptoms occurring. However, PET is largely unavailable in many countries and even in the U.S. is limited and costly. PET also requires the injection of a radiotracer, exposing the patient to radiation and potential dangerous side effects. For these reasons, a more accessible and equally accurate early detection method is needed. Here, we present a deep learning framework for synthesizing A β , Tau, and Fluorodeoxyglucose (FDG)-PET from multi-contrast MR images, which can reduce reliance on standard PET imaging by reconstructing the same pathology from learned representations in the MR inputs. To accomplish this, we developed and tested a U-Net framework on clinical data from the Alzheimer's Disease Neuroimaging Initiative (ADNI). This network features an encoder and a decoder, each containing pooling layers and multiple convolutions per layer, with skip connections that forward the output of each encoder layer to the corresponding decoder layer's input. Different loss functions were evaluated with this framework. Both perceptual loss and L1 norm loss yield results superior to L2 loss, with perceptual loss generating PET images that retain pathological features optimally. Additionally, the impact of the learning rate and depth of the network on model performance was measured. Across the various PET modalities (A β , Tau, and FDG), parameter changes affected performance differently, with optimal hyperparameters varying by type. We find that our UNet is able to synthesize A β , Tau, and FDG-PET with high structural similarity (SSIM > 0.93) and can depict regions of pathology, with the best performance on FDG-PET synthesis (SSIM > 0.97). These results highlight the possibility of synthesizing clinically relevant PET scans from multi-contrast MRI for the diagnosis and monitoring of Alzheimer's disease progression, potentially bypassing the need for traditional PET imaging. This will make early screening of Alzheimer's more accessible, and improve the efficacy of disease-modifying treatments which require early intervention.

Disclosures: **R. Iritani:** None. **H. Jahanian:** None. **A. Yazdan-Shahmorad:** None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.14/D8

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

Title: Mini Nutritional Assessment is correlated with thinning of the retinal ganglion cell layers, with global cognitive impairment, and body mass index in women with sporadic Alzheimer's disease

Authors: *M. GUERRERO VILLALPANDO^{1,2}, C. MARIN ARANDA³, N. HERNÁNDEZ SEBASTIÁN⁵, M. SOLIS-ORTIZ⁴, M. CARDONA ALVARADO²;

¹Dept. of Med. Sci., Univ. of Guanajuato, Silao, Mexico; ²Dept. of Med. Sci., ³Dept. de Ciencias Médicas, Univ. of Guanajuato, León, Mexico; ⁴Dept. de Ciencias Médicas, Univ. of Guanajuato, Leon, Mexico; ⁵Optics Res. Ctr., León, Mexico

Abstract: Alzheimer's disease AD is a neurodegenerative disease that leads to severe cognitive impairment. Retinal ganglion cells are an excellent biomarker for detecting AD, given their connection to the brain and obesity has been linked to cognitive decline. Although multifactorial interactions are evident in AD, nutrition plays an essential role in the pathogenesis and evolution of this disease, even in early. However, the impact nutritional status on cognitive decline, retinal ganglion cell layer thickness and anthropometric measurements in AD is not well known. The objective of the present study was to investigate the association of Mini-Nutritional Assessment scores with retinal ganglion cell layer thickness, global cognitive performance, and body mass index in women with sporadic AD. Seventy seven elderly women between 60 and 80 years of age participated in the study. The elderly women were 28 clinically diagnosed with mild cognitive impairment, 32 with moderate cognitive impairment and 17 with severe cognitive impairment in the progression of AD, in accordance with The Montreal Mental Cognitive Assessment test scores. This test was also used to assess global cognitive performance. Mini Nutritional Assessment test was used to assess nutritional status. The body mass index was also obtained. An Ocular Coherence Tomography was performed on the participants to measure the minimum and average thickness of the retinal ganglion cell layers. Elderly women with severe cognitive impairment showed thinning of the ganglion cell layers in both eyes, lower scores on the MoCa test and MNA and overweight. This lower nutrition status was positively correlated with thinning retinal ganglion cell thinning and global cognitive impairment and was negatively correlated with body mass index. These findings suggest that nutrition status may influence retinal ganglion cell thinning and impaired cognitive function. Furthermore, the inflammation caused by excess weight may be damaging retinal ganglion cells in women with sporadic AD.

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Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.15/D9

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

Support: NIH/NIA RF1AG085638
NI/NIA P30AG086401

Title: The role of functional network health in tau resilience

Authors: *D. S. JIN¹, J. S. PERUCHO¹, Y.-H. FANG¹, K. M. VISSCHER²;
¹Univ. of Alabama at Birmingham, Birmingham, AL; ²Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: The importance of tau protein in Alzheimer disease (AD) and mild cognitive impairment (MCI) has long been noted. However, many individuals with large tau burdens do not exhibit the severe attentional and memory impairment seen in Alzheimer's disease, showing resilience to tau deposition. We hypothesize that strong underlying functional network architecture of key networks can provide protection from tau's neurocognitive effects. One measure of healthy functional network architecture is network segregation, the degree to which brain connections within a network are stronger than connections between networks. Our hypothesis predicts that among all individuals with high tau burden, those with healthier segregation should be more resilient than those with weaker segregation; thus, in those participants with high tau, segregation should be stronger in MCI and healthy cognition participants than in participants with AD symptoms. We used resting-state functional connectivity (rs-fMRI) from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset to assess the functional network health of 329 patients (N = 207 Healthy, 94 MCI, and 28 AD). We calculated network segregation in patients separated into high-tau (>1.26 cerebellar SUVR whole brain average) and low-tau groups. We found that, within the cingulo-opercular network, the high-tau MCI participants showed significantly stronger segregation than the high-tau AD participants, whereas this pattern was not found for the group with low tau. Furthermore, consistent with our hypothesis that individuals with better functional architecture are resilient to the severest of symptoms, the average cingulo-opercular segregation of high-tau MCI patients (0.95) exceeded that of low-tau MCI patients (0.90), though this difference was not significant. We also found the segregation of the default mode and dorsal attention networks to be compromised in AD, regardless of tau levels, suggesting that their functional network architecture may not confer resilience to tau. Our findings are consistent with the hypothesis that strong network architecture in the cingulo-opercular network confers a degree of resilience to the deleterious effects of tau.

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Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.16/D10

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

Support: T32 NS082168

Title: Automated Imaging Differentiation for Dementia: Comparing Deep Residual Network, Support Vector Machine, and Random Forest

Authors: ***R. CHEN**¹, S. CHIU⁵, J. C. DESIMONE², W.-E. WANG², A. BARMPOUTIS³, C. MCMILLAN⁷, D. IRWIN⁷, L. R. CLARK⁷, K. KANTARCI⁶, D. E. VAILLANCOURT⁴;

¹J. Crayton Pruitt Family Dept. of Biomed. Engin., ²Dept. of Applied Physiol. and Kinesiology, ³Digital Worlds Inst., ⁴Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL; ⁵Neurol., Mayo Clin., Scottsdale, AZ; ⁶Mayo Clin., Rochester, MN; ⁷Univ. of Pennsylvania, Philadelphia, PA

Abstract: Background: Freewater imaging is a non-invasive diffusion imaging metric that is associated with neurodegeneration and neuroinflammation. Neurodegeneration and neuroinflammation are hallmarks of dementia disorders such as Alzheimer's disease (AD) and dementia with Lewy bodies (DLB). We developed Automated Imaging Differentiation for Dementia (AIDD), a support vector machine (SVM) model trained on free-water imaging, which can discriminate between AD, DLB, and normal aging. Aim: The main aim of the study is to compare different machine learning algorithms, namely deep residual network (ResNet) and random forest (RF), to the established support vector machine model. Methods: Diffusion MRI from 387 retrospective subjects (129 AD, 129 DLB, and 129 control) were rendered and processed into free-water maps. Free-water maps were randomly assigned to training (n=312) and testing (n=75) cohorts for the support vector machine. The RF and ResNet models used the same test-train split to match the SVM. SVMs and RFs were trained on free-water values within 120 regions of interest, while ResNets were trained on three-dimensional free-water maps. All three models used a 5-fold cross-validation algorithm. The primary outcomes are the accuracies and areas under the curve (AUCs) across four comparisons: AD versus DLB, AD versus control, DLB versus control, and control versus AD/DLB. Results: For AD versus DLB, all three models (SVM, RF, ResNet) had AUCs greater than 0.870 in the testing set, showing good robustness in differentiation. The SVM model showed a greater AUC (0.995, 95% CI 0.984-1.000) compared to RF (0.955, 95% CI 0.898-1.000) and ResNet (0.888, 95% CI 0.799-0.977). Conclusion: This study suggests that the support vector machine has the best performance using free-water metrics compared to random forest and residual network models.

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Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.17/D11

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

Support: Alzheimer's Drug Discovery Foundation (ADDF)
NIH Grant AG067916
Cure Alzheimer's Fund

Title: Longitudinal PET Imaging of RIPK1-Mediated Neuroinflammation in Alzheimer's Disease Using the FDA-Authorized Probe

Authors: *E. S. TOKLUCU^{1,2}, P. BAI³, C. WANG³, C. ZHANG²;

¹Acibadem Mehmet Ali Aydinlar Univ., Istanbul, Turkey; ²Genet. and Aging Res. Unit, McCance Ctr. for Brain Health, Dept. of Neurology, Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA; ³Athinoula A. Martinos Ctr. for Biomed. Imaging, Dept. of Radiology, Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA

Abstract: Receptor-interacting protein kinase 1 (RIPK1) is a key regulator of necroptosis and inflammation, two critical biological processes increasingly linked to the onset and progression of Alzheimer's disease (AD). Despite growing evidence implicating RIPK1 in AD neuropathology, its dynamic involvement across disease stages has remained poorly characterized in vivo. To address this gap, we employed [¹⁸F]CNY07, a novel fluorine-18-labeled PET radiotracer derived from the potent and selective RIPK1 inhibitor Nec-1s. Blocking experiments with unlabeled CNY07 reduced tracer signal by ~30%, confirming binding specificity. [¹⁸F]CNY07 was recently authorized for investigational use by the FDA in 2024 and exhibits high target affinity, excellent blood-brain barrier permeability, and favorable pharmacokinetics. In this study, we performed a longitudinal in vivo imaging analysis using [¹⁸F]CNY07 in transgenic AD mouse models, spanning early (3 months), mid (6 months), late (12 months) and aged (18 months) stages of pathology (n=4 per group, male or female). Dynamic PET/CT scans were acquired post-intravenous radiotracer administration. Brain images were coregistered to a murine atlas, and quantitative analysis of regions of interest (ROIs) was conducted using PMOD software. We observed progressive, region-specific increases in radiotracer uptake, particularly in hippocampus, amygdala, and cortex, reflecting the evolving neuroinflammatory state. Data were analyzed using two-way ANOVA (p<0.05), with values reported as mean ± SEM. These findings reveal a robust and temporally resolved pattern of RIPK1 activation in AD, consistent with hallmark neurodegenerative changes. Our study validates [¹⁸F]CNY07 as a translational molecular imaging tool for investigating RIPK1-mediated neuroinflammation in AD and highlights its potential utility in monitoring therapeutic response to RIPK1-targeted interventions.

Disclosures: E.S. Toklucu: None. P. Bai: None. C. Wang: None. C. Zhang: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.18/D12

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

Support: 1R01MH132746

Title: Fiber-specific alterations in white-matter architecture in adults with autism across the lifespan

Authors: *C. BAYOUTH¹, Y. ZHANG², B. BRADEN³, E. OFORI⁴;

²Col. of Hlth. and Solutions, ¹Arizona State Univ., Tempe, AZ; ³Dept. of Speech and Hearing Sci., Arizona State Univ., Phoenix, AZ; ⁴Arizona State Univ., Pheonix, AZ

Abstract: Abstract: Fixel-based analysis (FBA) overcomes the limitations of diffusion tensor imaging (DTI) in resolving crossing fibers and provides fiber-specific metrics—fiber density (FD), fiber cross-section (FC), and their combined measure, fiber density and cross-section (FDC) that quantify both microstructural and macrostructural properties of white matter. While current FBA studies in autism spectrum disorder (ASD) have mainly focused on children and adolescents, far less attention has been given to fiber-specific white matter changes in autistic adults. To address this gap, this study employed FBA to characterize micro- and macrostructural white matter alterations in autistic adults across the lifespan compared to matched neurotypical (NT) controls.

Methods: Participants included 111 ASD (mean age = 41, 36% F, ~15y education, WAIS = ~52) and 97 NT control (mean age = 43, 39% F, ~16y education, WAIS = ~51), all right-handed, aged 18-71. Diffusion data ($b = 2,500$ s/mm², 32 directions) was curated using a customized open-source MRtrix3 pipeline. Following single-shell 3-tissue constrained spherical deconvolution, subject FODs were warped to a study-specific template. Between group comparisons for whole-brain FD, FC, and combined FDC were performed using connectivity-based fixel enhancement with non-parametric permutation (5,000 iterations).

Results: Compared to NT adults, autistic adults exhibited higher FD and FDC in bilateral anterior corona radiata, genu of the corpus callosum, and frontal segments of the superior longitudinal and uncinate fasciculi (peak $t = 4.3$, p 's < 0.05). Additionally, ASD adults showed marked FD reductions in the posterior thalamic radiations, inferior cerebellar peduncles, and the posterior body of the corpus callosum. These findings highlight widespread fiber-specific white matter abnormalities in ASD adults, particularly in association and commissural tracts implicated in executive function and interhemispheric integration.

Conclusion: These findings indicate a fronto-posterior altered integrity in autistic adults across the lifespan. By leveraging FBA in a rigorously harmonized sample, our preliminary results provide the most comprehensive map to date of pathway-level white-matter vulnerabilities in ASD. The pattern reinforces models of impaired cerebro-cerebellar integration in ASD and identifies fiber-specific targets for longitudinal monitoring and intervention. The results underscore the importance of investigating aging-related neurobiological changes in autism, with potential implications for understanding cognitive decline and dementia risk in this population.

Disclosures: C. Bayouth: None. Y. Zhang: None. B. Braden: None. E. Ofori: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: PSTR323.19/D13

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

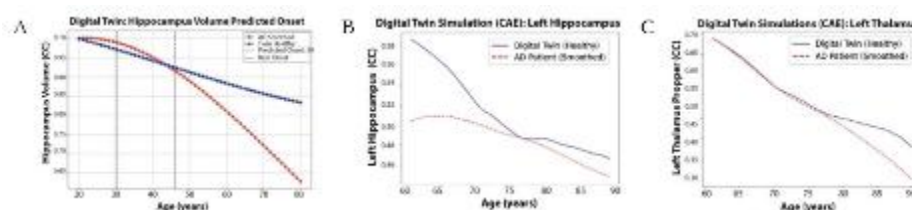
Support: NIA U19 AG076581-01A1

Title: Digital Twin Prediction of long-COVID trajectories of cognitive decline in an Amerindian population.

Authors: ***J. M. TORANZO**¹, J. MARTINEZ¹, D. PEREZ², M. J. USCAMAYTA AYVAR³, I. ZWIR⁴, G. A. DE ERAUSQUIN⁵;

¹Lab. of Brain Develop. , Modulation and Repair., ²Lab. of Brain Develop. , Modulation and Repair, Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX; ³The Glenn Biggs Inst. for Alzheimer's and Neurodegenerative Dis., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; ⁴Inst. of Neurosci., Univ. of Texas Rio Grande Valley, Harlingen, TX; ⁵Lab. of Brain Develop. , Modulation and Repair, Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX

Abstract: We analyzed data from the ISAVRAD cohort of older adults exposed to SARS-CoV-2 infection during the acute phase of the pandemic. To generate digital twins from cross-sectional data including volumetry, cognitive performance, and SARS-CoV-2 infection severity, we implemented a Conditional Autoencoder (CAE) trained on statistically relevant neuroimaging biomarkers and clinical risk factors. The model was designed to learn a deterministic latent representation of brain structures, conditioned on age and a composite risk score. Preprocessing included normalization and feature selection for robust training. The CAE architecture comprises an encoder that maps input features into a latent space and a decoder that reconstructs them using conditioning variables. Optimal latent dimensionality was determined via grid search with K-fold cross-validation. Once trained, the decoder simulated brain aging trajectories, interpolating across age values while controlling for disease risk. This approach provides a personalized model of cognitive decline, offering insights into post-COVID-19 cognitive and structural brain changes. Comparisons between healthy controls and at-risk individuals reveal early risk anticipation and age-related patterns. Figure. A Digital Twin (DT) framework estimating the onset of disease-specific brain atrophy (MRI) in (A) synthetic healthy vs. AD patients. (B-C) DT using CAE and applied to real-world data from the ISAVRAD study reveals similar deviation points but in a focused adult cohort.



Disclosures: J.M. Toranzo: None. J. Martinez: None. D. Perez: None. M.J. Uscamayta Ayvar: None. I. Zwir: None. G.A. de Erausquin: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.20/D14

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

Support: National Institute on Aging (R21AG085605)

Title: Mri analysis of perivascular space morphology in alzheimer's disease

Authors: *M. ABBASPOUR¹, P. WANG¹, B. PRICE¹, T. A. MCGAUGHEY², M. HAUT¹, R. MEHTA¹;

¹West Virginia Univ., Morgantown, WV; ²WVU-RNI, Morgantown, WV

Abstract: Perivascular spaces (PVS) are fluid-filled channels surrounding penetrating cerebral vessels and are involved in intracerebral fluid circulation and brain waste removal. Alterations in PVS morphology, including PVS enlargement, have been implicated in various neurological conditions, including Alzheimer's disease (AD) and may serve as an early biomarker of AD. While PVS analysis in humans has relied primarily on visual rating scales on T2-weighted MRI, few studies have implemented a quantitative approach to examine PVS in AD. Those that have used a quantitative approach of PVS analysis have mostly studied PVS structure using T1-weighted images, despite that T2-weighted imaging provides higher sensitivity for PVS detection. In this study, we analyzed in vivo structural T2-weighted MRI data from 146 individuals evaluated in our memory clinic (median age: 69, 45-83 years, 57.5% female), including persons with early-onset AD (EOAD), late-onset AD (LOAD), non-AD pathology, and normal controls. All participants were imaged on the same Siemens Magnetom Prisma 3-Tesla MRI scanner using standardized protocol and sequence parameters. The Frangi filter was applied to estimate vesselness features on high resolution 0.5 mm³ isotropic 3D T2-SPACE MRI data, and thus to segment cerebral PVS using an automated approach. Using FreeSurfer (v7.4.1) and Lesion Segmentation Tool (LST-AI), PVS masks were then generated in the basal ganglia and normal appearing white matter. Using connected component analysis, PVS morphology was further characterized by extracting count, length, width, and volume features from PVS masks in these regions. These PVS measurements were correlated with clinical data, including AD diagnosis and cognitive test scores. Subgroup analyses were performed to assess differences between groups. We found statistically significant relationships between the number of PVS, i.e. PVS count, in both the cerebral white matter and basal ganglia regions in the EOAD group, with EOAD having a lower PVS count than the control group ($p < 0.05$). The LOAD and non-AD groups showed negative effect sizes and would be considered significant at the 0.1 level. Note all analyses are adjusted for age, sex, education, and APOE. While these trends show promise, the statistical significance is impacted by underpowered samples and shows the necessity for further study of PVS in larger AD patient samples.

Disclosures: M. Abbaspour: None. P. Wang: None. B. Price: None. T.A. McGaughey: None. M. Haut: None. R. Mehta: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.21/D15

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

Support: 5R01AG07934502

Title: Resting state alpha changes during monoclonal anti-amyloid treatment

Authors: *J. GLUECK¹, M. HE², N. SAMUDRA³, S. SHA³, P. L. PURDON⁴;

¹Anesthesiology, Perioperative and Pain Med., Stanford Univ., Stanford, CA; ²Dept. of Anesthesiology, Perioperative and Pain Medicine; Dept. of Psychology, ³Dept. of Neurol. and Neurolog. Sci., ⁴Dept. of Anesthesiology, Perioperative and Pain Med., Stanford Univ., Palo Alto, CA

Abstract: The U.S. Food and Drug Administration recently approved two monoclonal antibody therapies (mAbs), lecanemab and donanemab, for the treatment of early Alzheimer's disease (AD). The approval of these medications relied on clinical dementia rating scales to measure cognitive and behavioral changes, as well as neuroimaging including MRI and PET to characterize brain volumetric changes and amyloid and tau burden. Previous studies using electroencephalography (EEG) have identified disrupted brain oscillations in older adults with AD and mild cognitive impairment (MCI), and diminished alpha activity has been shown to predict future cognitive decline. No study has yet characterized changes in neural activity as a result of mAbs, representing a gap in our understanding of how brain oscillations change as a result of treatment, and whether EEG can provide additional insight into the efficacy of mAbs. In the current study, we employed a longitudinal, within-subjects study design using scalp EEG recordings to characterize changes in brain oscillations in patients receiving mAbs for AD and MCI. We hypothesize that (1) alpha power within subjects will increase after treatment compared to baseline, and (2) the alpha spectral peak will become more tuned due to improved neural coherence. In collaboration with providers at the Stanford Center for Memory Disorders, patients prescribed intravenous mAbs for AD and MCI were recruited. Study participants underwent saline cap EEG recordings and cognitive tasks at Baseline before any mAb treatment and after 3 months of infusions. The cognitive tasks included an eyes-closed/eyes-open resting state task, an object-location working memory task, and an auditory oddball task. Additionally, participants underwent resting state EEG recordings immediately after infusions 1 and 2 to measure for possible acute post-infusion oscillation changes. All subjects provided written informed consent before any study procedures were performed. All procedures were approved by the Stanford University Institutional Review Board. Baseline and Month 3 resting state recordings were available for two participants. One participant received lecanemab and one participant received donanemab. Multitaper spectral analysis was performed to compare Baseline and Month 3 data within each subject. Preliminary analyses revealed that both participants showed a general trend of decreased occipital alpha at Month 3 compared to Baseline. Longitudinal data collection is ongoing. Data from future timepoints at Months 6 and 12, as well

as data from additional participants, will inform a clearer pattern in occipital alpha changes over the course of treatment.

Disclosures: **J. Glueck:** None. **M. He:** None. **N. Samudra:** Other; Dr. Samudra has given a CME talk supported by a stipend from Lilly, which manufactures donanemab. **S. Sha:** A. Employment/Salary (full or part-time): Dr. Sha receives salary support from Aribio, Biogen, Cognition Therapeutics, Eisai, Eli Lilly, and Janssen for clinical trials.. 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.; Dr. Sha also receives salary support from the NIH for her role in the Stanford ADRC and other clinical trials.. F. Consulting Fees (e.g., advisory boards); Dr. Sha receives consulting support from Guidepoint Global and Expert Connect. **P.L. Purdon:** A. Employment/Salary (full or part-time): PLP is a co-founder of PASCALL Systems, Inc., a start-up company developing closed-loop physiological control systems for anesthesiology..

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.22/D16

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

Support: DOD CDMRP awarded to Dr. Adamson
SPIRE VA Award granted to Dr. Adamson
support from WOMEN CoE center at VA Palo Alto

Title: Sex differences among predictors of brain age gap in traumatic brain injury patients and healthy controls

Authors: ***M. M. ADAMSON**¹, J. P. COETZEE², E. L. DENNIS³;
¹Neurosurg., Stanford University/VA Palo Alto Healthcare Syst., Palo Alto, CA; ²Stanford Univ., Palo Alto, CA; ³Imaging Genet. Center, SNII, Keck Som USC, Marina Del Rey, CA

Abstract: Motivation:

Traumatic brain injury (TBI) is associated with increased dementia risk. This may be driven by processes of accelerated brain aging underlying biological changes resulting from the injury. Machine learning algorithms have been developed that can use structural MRIs to give a predicted brain estimate age (pBA). When the estimated age is greater than the chronological age (CA), this is called the brain age gap (BAg). We analyzed this outcome in men and women with and without TBI.

Methods:

In an exploratory, hypothesis-generating analysis, we analyzed data from 85 TBI patients and 22 healthy controls (HCs). High-resolution T1W images were processed using FreeSurfer 7.0. pBAs

were calculated from T1s. Differences between the two groups were tested using the Mann-Whitney U. Associations between the BAG and other factors were tested using partial Pearson's r within groups, controlling for CA, followed by construction of regression models.

Results:

Pairwise comparisons using Mann-Whitney U found that compared to TBI participants, HCs had greater cortical thickness (CT) ($p=.002$). TBI participants had higher scores on PCL-5 ($p=.011$). A partial Pearson's r, controlling for (CA), identified correlates of BAG within each group. Correlates that survived an FDR were used in regression models.

Four linear regression models were constructed, one for women and men with TBIs, and for women and men HCs. All models controlled for CA. For women with TBIs, higher hippocampal volume (HV) predicted lower BAG ($\beta = -0.566$, $p = .006$). For men with TBIs, higher CT predicted lower BAG ($\beta = -0.442$, $p=.003$), as did higher CA ($\beta = -0.295$, $p=.046$). For women and men HCs, higher CA predicted lower BAG, with ($\beta=-0.675$, $p=.023$) and ($\beta=-0.701$, $p=.016$) respectively.

Conclusion:

These results offer tentative support to the view the factors associated with BAG among individuals with TBI differ from factors associated with BAG among HCs, and between men and women. Specifically, BAG among individuals with TBI is predicted by neuroanatomical changes factors, while among HCs it is predicted only by CA. These findings require replication using a larger dataset, and may reflect features of the algorithm, an underlying biological process, or both.

Disclosures: M.M. Adamson: None. J.P. Coetzee: None. E.L. Dennis: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.23/D17

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

Support: NIH Grant R21AG083589

Title: Prediction of Mild Cognitive Impairment to Alzheimer's Dementia Using Excitation-Inhibition Imbalance

Authors: *G. LI¹, V. HU², P.-T. YAP³;

¹Univ. of North Carolina Chapel Hill, Chapel Hill, NC; ²Cary Acad., Cary, NC; ³Univ. of North Carolina, Chapel Hill, NC.

Abstract: Alzheimer's disease (AD) is a serious neurodegenerative disorder characterized by progressive cognitive decline and irreversible memory loss. Mild cognitive impairment (MCI) is a prodromal stage of AD that provides a critical window for early intervention. To help prevent further cognitive decline, it is of crucial importance to identify progressive MCI (pMCI) from

stable MCI (sMCI). Recent studies suggest that excitation-inhibition (E-I) imbalance plays an important role in AD pathophysiology, raising the possibility of E-I imbalance as a potential early AD biomarker. To evaluate the utility of E-I balance in early AD identification, we apply a Large-scale nEural Model Inversion (LEMI) framework to a resting-state functional MRI (rs-fMRI) dataset collected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database to estimate E-I balance. We include 53 pMCI (25/28 males/females, age: 73.9 ± 9.5 years) who converted to AD in follow-up visits and 53 sMCI (26/27 males/females, age: 73.4 ± 5.8 years) who remained stable for at least 36 months. Regional averaged BOLD time series are extracted from 46 regions covering the default mode, salience, executive control and limbic networks using the Desikan-Killiany atlas. LEMI estimates both local and inter-regional E-I connectivity (EIC) for each subject, from which local, inter-regional and total E-I balance are calculated for each region. We use a combined filter-wrapper approach to identify the best discriminative features and employ a support vector machine (SVM) classifier with a Gaussian kernel to classify pMCI from sMCI. The evaluation is performed for ten repetitions of 5-fold SVM classification with randomly partitioned training and testing sets. The classification results are evaluated by accuracy (ACC), sensitivity (SEN) and specificity (SPE) and compared between EI features and functional connectivity (FC) features. We found that SVM achieves the highest accuracy using local E-I balance as features (ACC: $67.16 \pm 2.27\%$; SEN: $78.31 \pm 3.05\%$; SPE: $56.05 \pm 3.41\%$) compared to inter-regional E-I balance (ACC: $60.94 \pm 2.74\%$; SEN: $57.82 \pm 5.84\%$; SPE: $64.24 \pm 4.06\%$) and total E-I balance (ACC: $59.96 \pm 2.83\%$; SEN: $50.96 \pm 4.47\%$; SPE: $69.05 \pm 2.22\%$). Also, the accuracy using EIC features (ACC: $63.73 \pm 2.62\%$; SEN: $79.55 \pm 2.97\%$; SPE: $48.05 \pm 3.48\%$) is higher than FC features (ACC: $61.33 \pm 2.95\%$; SEN: $69.25 \pm 5.67\%$; SPE: $53.67 \pm 5.69\%$). Overall, we show that E-I balance could be a useful biomarker for early AD diagnosis and local E-I balance contains the most discriminative features for MCI to AD prediction. In addition, we demonstrate that EI features can better predict AD progression than conventional FC features.

Disclosures: G. Li: None. V. Hu: None. P. Yap: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.24/D18

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

Support: JST KSAC2025-24
JSPS KAKENHI JP23H03722

Title: Fluorine-19 MRI for detecting A β oligomers using a keto form of fluorinated curcumin derivative

Authors: *D. YANAGISAWA^{1,2}, T. KATO³, S. TAKAHATA³, S. ISHIGAKI², I. TOOYAMA³;

²Mol. Neurosci. Res. Ctr., ³Med. Innovation Res. Ctr., ¹Shiga Univ. of Med. Sci., Otsu, Japan

Abstract: Alzheimer's disease (AD) is the most common form of dementia. In the AD brain, amyloid β ($A\beta$) aggregates into undesirable, long, insoluble fibrils that accumulate as extracellular deposits known as senile plaques. In contrast, recent studies have revealed that $A\beta$ also forms soluble, low-molecular-weight oligomers that exhibit high neurotoxicity. The $A\beta$ oligomers are now considered key contributors to the progression of AD; however, the distribution of $A\beta$ oligomers in the brain is not fully understood. In the present study, we synthesized a novel fluorinated curcumin derivative with a fixed keto form, named as Shiga-Y51, which exhibits high selectivity for $A\beta$ oligomers. We investigated whether Shiga-Y51 could be used to detect $A\beta$ oligomers in a mouse model of AD using ¹⁹F MRI. To assess the binding activity, we performed quartz crystal microbalance (QCM) analysis. The QCM frequency significantly decreased upon the addition of Shiga-Y51 to the electrode coated with $A\beta$ oligomer, whereas no significant change was observed when applied to the electrode coated with $A\beta$ fibrils. These results suggest that Shiga-Y51 selectively binds to $A\beta$ oligomers. We then conducted ¹⁹F MRI using a 7T MR scanner to detect Shiga-Y51 in the brains of APP/PS1 transgenic mice. A significantly strong ¹⁹F signal was detected in the brains of APP/PS1 mice, compared with wild-type controls, indicating successful in vivo detection of $A\beta$ oligomers. Based on these findings, Shiga-Y51 enables the non-invasive detection of $A\beta$ oligomers in the brain using ¹⁹F MRI. Furthermore, fluorinated curcumin derivatives with a fixed keto form may serve as promising seed compounds for the development of imaging probes specifically targeting $A\beta$ oligomers.

Disclosures: D. Yanagisawa: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Japanese patent application (JP2020-033405) on Shiga-Y51. T. Kato: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Japanese patent application (JP2020-033405) on Shiga-Y51. S. Takahata: None. S. Ishigaki: None. I. Tooyama: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Japanese patent application (JP2020-033405) on Shiga-Y51.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.25/D19

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

Support: NIH Grant P20GM10902
NIH Grant P20AG068053

Cleveland Clinic Neurological Institute Emergent Clinician-Scientist
Career Award

Title: Free water mapping predicts elevated amyloid- β and cognition in older adults without dementia

Authors: *A. R. BENDER, P. PATTIAM GIRIPRAKASH, Z. YANG;
Lou Ruvo Ctr. for Brain Hlth., Cleveland Clin., Las Vegas, NV

Abstract: Background: Alzheimer's disease (AD) is characterized by neuropathological features, including amyloid- β (A β) depositions. The effects of A β on tissue microstructure can lead to increased extracellular cerebral spinal fluid (CSF), or 'free water.' Common diffusion MRI (dMRI) methods to quantify free water such as free water elimination (FWE) and neurite orientation dispersion diffusion imaging (NODDI) are sensitive to neuropathology and cognitive function. In addition, multi-shell multi-tissue (MSMT) constrained spherical deconvolution (CSD) can model CSF but has yet to be validated with other measures. We tested the sensitivity of three dMRI free water mapping methods to A β levels and cognition in a clinical aging cohort. Methods: Study participants (N=122) were clinically characterized as cognitively unimpaired (n=58) or with amnesic mild cognitive impairment (n=64); all had complete data from cognitive testing, and from A β PET and dMRI imaging. MRI acquisition: Siemens 3T, b-values = 0, 500, 1000, 2500 s/mm², 237 directions. PET processing: ADNI methods to calculate A β SUVR, rescaled to centiloids. dMRI processing followed MRtrix3 MSMT-CSD pipeline to fit fiber orientation distributions (FOD), build a sample FOD template, and transform CSF images to the template. We also fit NODDI and FWE models to estimate isotropic volume fraction (FISO), and free water fraction (FWF), transformed to the template. Voxel-based analyses modeled total centiloid level as predictor of whole-brain differences in FWF, FISO, and MSMT-CSF, covarying for age, sex, handedness, and head size; results are corrected for multiple tests. Results: Higher centiloid predicted greater FWF, FISO, and MSMT-CSF (for all, $r \geq 0.51$, $p < .001$) with overlapping effects in bilateral entorhinal cortices, hippocampi, and amygdalae, as well as left fornix crus and inferior temporo-occipital regions; however, FWF and MSMT-CSF effects were more widespread than FISO. Higher A β also predicted higher FWF and MSMT-CSF in bilateral basal forebrain, fornix body, retrosplenium, posterior cingulum, splenium of corpus callosum, and precuneus. Only FWF was positively associated with A β in anterior thalamic radiation, and caudate nucleus. All three measures significantly correlated with performance on cognitive tests of visual reproduction ($r \leq -0.46$), working memory ($r \leq -0.21$), category fluency ($r \leq -0.36$), speeded processing ($r \geq 0.21$) and executive function ($r \geq 0.20$). Conclusion: In adults without dementia, elevated A β predicts higher extracellular CSF in brain regions associated with A β and pTau pathology. Free water mapping appears sensitive to AD neuropathology and cognitive function.

Disclosures: A.R. Bender: None. P. Pattiam Giriprakash: None. Z. Yang: None.

Poster

PSTR323: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR323.26/D20

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

Support: NIA Grant K01-073584

Title: 3D Residual network classification of Alzheimer's disease clinical staging using multimodal MRI

Authors: *Y. YANG¹, A. SATHE¹, A. DURANT¹, P. KANAKARAJ², M. KIM², C. GAO³, N. NEWLIN², K. RAMADASS^{3,2}, N. MOHD KHAIRI³, Z. LI³, T. YAO⁴, Y. HUO^{2,3}, S. L. RISACHER^{5,6}, D. TOSUN⁷, A. W. TOGA⁸, P. M. THOMPSON⁹, P. ZHANG^{10,14}, K. SCHILLING^{11,12}, B. A. LANDMAN^{3,2}, A. L. JEFFERSON^{15,13}, A. J. SAYKIN^{5,6}, M. L. CUCCARO¹⁶, T. J. HOHMAN^{15,13}, D. B. ARCHER^{15,13};

¹Vanderbilt Memory & Alzheimer's Ctr., Nashville, TN; ²Dept. of Computer Sci., ³Dept. of Electrical and Computer Engin., ⁴Dept. of Computer Science, Vanderbilt Univ., Nashville, TN; ⁵Dept. of Radiology and Imaging Sci., ⁶Indiana Alzheimer's Dis. Res. Ctr., Indiana Univ. Sch. of Med., Indianapolis, IN; ⁷Dept. of Radiology and Biomed. Imaging, Univ. of California San Francisco, San Francisco, CA; ⁸Mark and Mary Stevens Inst. for Neuroimaging and Informatics, USC, Los Angeles, CA; ⁹Mark and Mary Stevens Inst. for Neuroimaging and Informatics, USC, Marina del Rey, CA; ¹⁰Dept. of Biostatistics, ¹¹Dept. of Radiology & Radiological Sci., ¹²Vanderbilt Univ. Inst. of Imaging Sci., ¹³Dept. of Neurol., Vanderbilt Univ. Med. Ctr., Nashville, TN; ¹⁴Vanderbilt Memory and Alzheimer's Ctr., ¹⁵Vanderbilt Memory and Alzheimer's Ctr., Vanderbilt Univ. Sch. of Med., Nashville, TN; ¹⁶Human Genet., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Recent advancements in deep learning have enabled researchers to leverage large-scale neuroimaging data to predict disease-related outcomes such as Alzheimer's disease (AD) risk. However, prior studies that developed AD-prediction algorithms have largely used T1-weighted structural magnetic resonance imaging (MRI), thus only capturing gray matter atrophy patterns while overlooking white matter decline. Here, we leveraged T1 and free-water (FW) corrected diffusion MRI data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to classify clinical stages of cognitively unimpaired (CU), mild cognitive impairment (MCI), and dementia (AD) using 3D residual network (ResNet) models. Only baseline imaging sessions were included (n=990; CU=545, MCI=349, AD=96). The resulting dataset consisted of 53.5% female with average age of 72.9 (7.97) years. For each diffusion MRI session, we used a FW correction pipeline to create FW-corrected fractional anisotropy (FA_{FWcorr}) and FW maps to delineate between tissue and cerebrospinal fluid compartments, thereby improving signal specificity. We then trained the 3D ResNet models on T1, FA_{FWcorr}, and FW images over 5-fold cross-validation across 100 epochs, randomly splitting the dataset into 80% training and 20% validation in each fold; the distribution of CU, MCI and AD classes was balanced across folds. We explored several models of increasing depth and complexity: ResNet10 concatenated with a multi-layer perceptron (MLP) with a hidden layer, ResNet18 concatenated with an MLP without a hidden layer, and ResNet18 concatenated with an MLP with a hidden layer. All models included standard covariates: sex, race/ethnicity, age at imaging, and years of educational attainment. Among models that used T1, FA_{FWcorr}, and FW images, all were able to accurately classify CU, MCI, and AD stages in the training set, with ResNet10 [train acc=0.78(0.18)] and ResNet18+MLP without hidden layer [train acc=0.78(0.19)] achieving higher training accuracy

than ResNet18+MLP with hidden layer [train acc=0.73(0.17)]. However, in the held-out validation set, the ResNet18+MLP with hidden layer model [val acc=0.53(0.11)] outperformed both the ResNet10 [val acc=0.50(0.13)] and ResNet18+MLP without hidden layer [val acc=0.49(0.13)] models. Our results suggest that 3D ResNet models could classify clinical stages of CU, MCI, and AD from T1 and FW-corrected diffusion MRI images. The lower validation accuracy could be due to model overcorrection on the AD class. Future work will continue to optimize these models, with the goal of creating a sensitive brain signature which captures both gray and white matter contributions to disease.

Disclosures: Y. Yang: None. A. Sathe: None. A. Durant: None. P. Kanakaraj: None. M. Kim: None. C. Gao: None. N. Newlin: None. K. Ramadass: None. N. Mohd Khairi: None. Z. Li: None. T. Yao: None. Y. Huo: None. S.L. Risacher: None. D. Tosun: None. A.W. Toga: None. P.M. Thompson: None. P. Zhang: None. K. Schilling: None. B.A. Landman: None. A.L. Jefferson: None. A.J. Saykin: None. M.L. Cuccaro: None. T.J. Hohman: F. Consulting Fees (e.g., advisory boards); TJH has served on the scientific advisory board for Vivid Genomics.. Other; TJH serves as deputy editor and senior associate editor for Alzheimer's & Dementia: TRCI and Alzheimer's & Dementia respectively.. D.B. Archer: None.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.01/D21

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

Support: R01 AG053555

Title: Sex-specific associations between estradiol, inflammation, and white matter microstructure in older adults at risk for alzheimer's disease

Authors: *D. PARKER¹, E. YI³, L. MCMILLAN⁵, E. A. THOMAS⁴, J. N. ADAMS⁵, M. A. YASSA²;

²Neurobio. and Behavior, ¹Univ. of California Irvine, Irvine, CA; ⁴Epidemiology and Biostatistics, ³Univ. of California, Irvine, Irvine, CA; ⁵Neurobio. and Behavior, UC Irvine, Irvine, CA

Abstract: Alzheimer's disease (AD) is a major public health concern that continues to grow as the population ages. Women are at twice the risk of AD as men, yet the mechanisms leading to this dramatic difference in risk are still not understood. Converging evidence suggests that women have an accelerated rate of AD pathogenesis during the preclinical stage. A key mechanism may be associated with inflammatory changes in the brain that are linked to menopause-related declines in estrogen. The impact of such changes on white matter microstructure has not been previously examined. We hypothesized that inflammation would be associated with altered WM features in key medial temporal lobe (MTL) pathways linked to

memory function. Neurite orientation dispersion and density imaging (NODDI) analyses of diffusion MRI data in a cohort (n=84) of deeply phenotyped cognitively unimpaired postmenopausal women (n=54) and men (n=30) from the BEACoN study at UC Irvine. Salivary samples were used to assess estradiol (E2) and cytokine levels using the MSD platform. Interestingly, variability in E2 levels was associated with inflammatory cytokines in women, but not in men, despite a similar parametric range. In women, E2 was negatively correlated with TNF α and IL1 β and showed a trending negative correlation with IL-6 (Fig1A). Women had lower neurite density index (NDI) across MTL white matter tracts compared to men (hippocampal cingulum, fornix, uncinate fasciculus; p's<0.001; Fig1B), reflecting reduced integrity. Within women, lower E2 levels were associated with lower fornix NDI (Fig1C). These findings suggest that lower levels of estradiol may be associated with an inflammatory state that is unique to women and impacts white matter integrity in memory-related pathways.

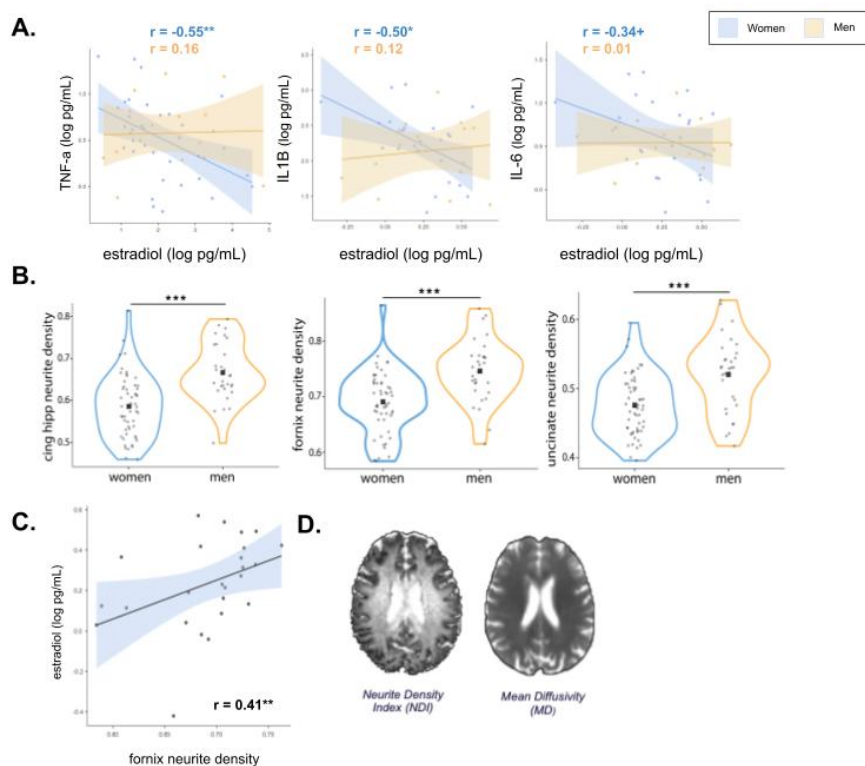


Figure 1. A, Higher inflammatory cytokines are associated with lower estradiol measured in saliva within women, but not men. B, NODDI measures demonstrate significantly lower neurite density in women compared to men across medial temporal lobe white matter. C, Lower estradiol is associated with lower fornix neurite density within women. D, Neurite Density Index (NDI) and Mean Diffusivity (MD) maps.

Disclosures: D. Parker: None. E. Yi: None. L. McMillan: None. E.A. Thomas: None. J.N. Adams: None. M.A. Yassa: None.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.02/D22

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

Support: NIH Grant R01 AG053555

Title: Sex differences and estradiol effects on regional amyloid- β and hippocampal-dependent memory in cognitively unimpaired older adults

Authors: *E. YI¹, D. M. PARKER⁴, L. TAYLOR², S. KIM⁴, N. TUTEJA¹, L. MCMILLAN², E. A. THOMAS³, J. N. ADAMS², M. A. YASSA⁴;

²Neurobio. and Behavior, ³Epidemiology and Biostatistics, ¹Univ. of California, Irvine, Irvine, CA; ⁴Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Evidence suggests menopause-driven estradiol withdrawal increases vulnerability to Alzheimer's disease (AD) pathogenesis in women. While prior studies indicate that women and men accumulate global beta amyloid (A β) at similar rates, the regional specificity of sex- and estradiol-related A β has not been studied. Notably, regions exhibiting a high density of estrogen receptors, such as the entorhinal cortex (EC) and posterior cingulate cortex (PCC), are also among the earliest affected in AD. We examined estradiol's relation to sex-specific regional A β deposition and hippocampal-dependent memory in older cognitively unimpaired (CU) postmenopausal women and men. We analyzed data from 129 participants (64% female; 60-87 years) from the BEACoN study. Salivary estradiol was measured using a saliva-optimized immunoassay in a subset of 87 participants (72% female). We measured A β with 18F-florbetapir-PET, and quantified global A β and regional A β in the hippocampus, EC, amygdala, fusiform, PCC, and retrosplenial cortex (RSC). Hippocampal-dependent memory was measured using mnemonic discrimination tasks (MDT) probing object, spatial, and temporal pattern separation. Mann-Whitney U tests were used to assess sex differences and Spearman's rank correlations were used to test relationships between continuous variables. There were no sex differences in global A β or salivary estradiol levels, nor was there a relationship between estradiol and global A β in either men or women. However, regional analyses revealed significant sex differences in A β deposition in the left EC (U = 1434, p = .020) and bilaterally in the fusiform (left: U = 1481, p = .036; right: U = 1432, p = .019), with women exhibiting higher A β levels than men. In women, lower estradiol levels were significantly associated with higher A β in the left EC (rs(51) = -.310, p = .025), but were not associated with A β levels in the fusiform gyrus. Further, higher EC A β in women was significantly associated with poorer object (EC-R: rs(71) = -.242, p = .037) and temporal (EC-L: rs(67) = -.331, p = .005) MDT performance, and higher A β in the left fusiform gyrus was significantly associated with poorer temporal MDT performance (rs(67) = -.302, p = .010). These findings support regionally-specific patterns of A β deposition that are related to decreased estradiol levels in women, which may confer deficits in

hippocampal-dependent memory. These biomarkers may reflect AD-related cognitive deficits unique to women, with potential to help narrow the window for intervention.

Disclosures: E. Yi: None. D.M. Parker: None. L. Taylor: None. S. Kim: None. N. Tuteja: None. L. McMillan: None. E.A. Thomas: None. J.N. Adams: None. M.A. Yassa: None.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.03/D23

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

Title: Evaluating Plasma Ptau-217 Sensitivity in Detecting Individual Differences in Early-stage Tau Aggregation in Cognitively Unimpaired Older Adults

Authors: *L. VARGHESE¹, L. TAYLOR², E. A. THOMAS³, J. N. ADAMS⁴, M. A. YASSA⁵; ²Dept. of Neurobio. and Behavior and Ctr. for the Neurobio. of Learning and Memory, ³Epidemiology and Biostatistics, ¹Univ. of California, Irvine, Irvine, CA; ⁴Neurobio. and Behavior, UC Irvine, Irvine, CA; ⁵Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Plasma p-tau217 is a promising biomarker for detecting Alzheimer's pathology. However, previous studies have shown weak or no correlations with tau-PET in cognitively unimpaired (CU) individuals. Early tau aggregates in focal clusters, likely due to local seeding or cell-specific vulnerability (e.g., stellate cells in the entorhinal cortex). Traditional mean SUVR-based ROI analysis of tau may be insufficiently sensitive to these focal changes in early tau aggregation, possibly leading to weak correlations with p-tau217. Here, we use 3 new approaches to enhance the sensitivity of PET analysis to early tau's cluster-like aggregation and re-assess its relationship with p-tau217. We analyzed 142 CU older adults (95 A β -/47 A β +) from ADNI with plasma p-tau217 (Janssen assay) and 18F-Flortaucipir PET. SUVR maps were thresholded at 1.3, and clusters were identified using MATLAB's bwconncomp. ROIs included: entorhinal cortex (EC), parahippocampal gyrus (PHC), amygdala (AMY), and fusiform gyrus (FUS). In addition to mean SUVR, we computed (1) % suprathreshold voxels (≥ 1.3 SUVR), (2) cluster volume (total volume of all clusters / the number of clusters and ROI volume), and (3) cluster-weighted mean SUVR (mean SUVR across clusters weighted by their respective volumes). Spearman's rank correlation and commonality analyses were utilized. In A β - participants, cluster volume in EC showed the strongest correlation with plasma p-tau217 ($\rho = 0.32$, $p = 0.01$), while mean SUVR was not significant. In A β + participants, cluster volume showed the strongest correlation with plasma p-tau217 in later-stage regions like PHC and FUS ($\rho = 0.33$, $p = 0.02$; $\rho = 0.31$, $p = 0.03$), whereas mean SUVR was not significant. Commonality analysis in A β - participants revealed that in EC, cluster volume explained 24% of p-tau217 variance. In AMY and PHC, cluster-weighted mean SUVR accounted for 40% and 23% of variance, respectively. Cluster metrics (volume and weighted mean) of tau-PET were stronger

than traditional measures in detecting early tau aggregation in regions not yet saturated by pathology. In the EC for A β - participants, cluster volume was the strongest plasma p-tau₂₁₇ predictor, consistent with the sparse, cluster-like distribution of early tau. Once tau spreads outside the EC, it becomes more widespread and diffuse, which could be why cluster-weighted mean SUVR outperforms cluster volume in late-stage MTL regions (AMY/PHC). These results suggest that plasma p-tau₂₁₇ correlations with tau aggregation may have been previously underestimated. Using cluster-based metrics reveals these associations and suggests that plasma p-tau can serve as a useful biomarker for early tau aggregation.

Disclosures: L. Varghese: None. L. Taylor: None. E.A. Thomas: None. J.N. Adams: None. M.A. Yassa: None.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.04/D24

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

Support: R01AG053555

Title: Linking distinct neuropsychological profiles to structural markers of neurodegeneration in cognitively unimpaired older adults

Authors: *E. CHANG¹, B. D. HITT¹, Z. MELIKYAN¹, L. TAYLOR², S. SIMMONS¹, N. MEZA¹, B. AVILA³, N. TUTEJA¹, L. MCMILLAN³, E. A. THOMAS⁴, M. A. YASSA⁵, J. N. ADAMS³;

²Dept. of Neurobio. and Behavior and Ctr. for the Neurobio. of Learning and Memory,

³Neurobio. and Behavior, ⁴Epidemiology and Biostatistics, ¹Univ. of California, Irvine, Irvine, CA; ⁵Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Prior studies have identified cognitive subtypes of older adults with Alzheimer's disease (AD) with the goal of identifying risk prodromes for cognitive decline. However, few have examined cognitive subtypes during the presymptomatic phase. The current study aimed to determine whether certain neuropsychological profiles are associated with imaging biomarkers of AD and could be used for early detection of AD or other dementias during the presymptomatic phase. 167 cognitively unimpaired older adults (60-89 years old, 66% female) underwent NACC v3 and PACC batteries as part of the BEACoN study at UC Irvine. Raw scores were converted to z-scores controlling for age, sex, and education. Latent profile analysis (LPA) was performed to classify participants into distinct profiles, and a 5-class solution was found to be most optimal based on fit indices. The five distinct neuropsychological profiles identified included: a High Cognition group (n=36) with strong performance across all cognitive domains, a Low Cognition group (n=20) with lower scores overall, a Standard Ager group (n=54) with average scores overall, a Dysexecutive group (n=26) with low executive but high

memory performance, and a Discordant Memory group (n=31) with average performance on the Rey Auditory Verbal Learning Test (RAVLT) but lowest scores on the Craft Story 21 and the Benson Complex Figure Task. Identified profiles were then examined in association with structural MRI Freesurfer segmentations (volume or cortical thickness) of brain regions implicated in AD. The Discordant Memory group showed significantly greater hippocampal volume relative to the High Cognition group (p=0.022) and greater amygdala volume relative to the High Cognition (p=0.007), Low Cognition (p=0.040), and Dysexecutive (p=0.018) groups. The Dysexecutive group had significantly lower entorhinal cortical thickness compared to the High Cognition (p=0.012) and Discordant Memory (p=0.027) groups, but greater anterior cingulate cortical thickness compared to Standard Aged (p=0.006). No significant difference in inferior parietal cortical thickness was found between groups. These findings indicate that distinct neuropsychological profiles may be associated with regional patterns of neurodegeneration that may signal different mechanisms. Unexpectedly, the Discordant Memory group showed greater hippocampal and amygdala volumes compared to the High Cognition group despite lower memory performance. Future analyses will examine differences in amyloid, apolipoprotein E4 genotype, and white matter hyperintensities to further determine profile-based risk for AD and related dementias.

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Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.05/D25

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

Support: R01AG053555
R21AG075464
F32AG074621

Title: Locus coeruleus integrity is associated with regionally-specific medial temporal lobe neurodegeneration in cognitively unimpaired older adults

Authors: *S. CHAND¹, S. SIMMONS⁴, L. FISCHER⁵, L. TAYLOR², E. CHANG¹, B. AVILA³, N. MEZA¹, N. TUTEJA¹, L. MCMILLAN⁶, M. A. YASSA⁸, J. N. ADAMS⁷;
²Dept. of Neurobio. and Behavior and Ctr. for the Neurobio. of Learning and Memory,
³Neurobio. and Behavior, ¹Univ. of California, Irvine, Irvine, CA; ⁴UCI, Irvine, CA; ⁵German Ctr. for Neurodegenerative Dis., Magdeburg, Germany; ⁶Neurobio. and Behavior, UC Irvine, Long Beach, CA; ⁷Neurobio. and Behavior, UC Irvine, Irvine, CA; ⁸Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: The locus coeruleus (LC), a brainstem nucleus critical for cognition through noradrenergic regulation, has been implicated in Alzheimer's disease (AD). This region projects densely to memory-critical medial temporal lobe (MTL) subregions that are differentially vulnerable to AD. The transentorhinal cortex (BA35), entorhinal cortex (EC), and CA1 subfield of the hippocampus exhibit early tau pathology and atrophy in AD, while the dentate gyrus (DG) is spared of pathology until late stages. Given the dense noradrenergic innervation from the LC to these subregions, we tested the hypothesis that LC degeneration may compromise integrity and exacerbate pathological cascades in these regions. Cognitively unimpaired older adults (N = 29, mean age = 69.8, 83% female) from the BEACoN study at UC Irvine underwent MRI using an LC-specific T1 TSE sequence to measure LC integrity. These LC scans were normalized by the pontine tegmentum, and the voxel with the peak signal intensity from right and left LC masks was quantitatively identified and averaged as a measure of LC integrity. Hi-res T1 MPRAGE and T2 TSE scans were processed with Automated Segmentation of Hippocampal Subfields (ASHS) to extract volumes of BA35, EC, CA1, and DG. AD pathology was assessed with 18F-MK6240 and 18F-florbetapir to measure MTL tau (composite of entorhinal, hippocampus, amygdala) and amyloid- β (A β), respectively. Memory was assessed with the Rey Auditory Verbal Learning Test delayed recall score. LC signal intensity was significantly lower in A β + compared to A β - older adults ($t(26) = 2.36$, $p = 0.026$) and demonstrated a trending relationship with MTL tau pathology ($r = -0.36$, $p = 0.10$). Lower LC signal intensity was significantly associated with reduced CA1 volume ($r = 0.42$, $p = 0.035$), but was not associated with BA35 or EC volume. Further, lower LC integrity was associated with reduced DG volume ($r = 0.52$, $p = 0.007$). There was no significant relationship between LC integrity and memory ($r = -0.12$, $p = 0.56$). These preliminary findings suggest that LC integrity may serve as an early biomarker of subfield-specific MTL neurodegeneration in preclinical AD, beginning before evidence of memory deficits. While the association between LC integrity and CA1 atrophy aligns with known CA1 vulnerability in AD, the association with DG atrophy suggests that noradrenergic dysfunction may impair hippocampal integrity through alternative mechanisms beyond classical pathology propagation that may be tied to decreases in LC functional integrity. Future research with larger and more diverse samples will expand on these results and mechanisms linking LC and MTL subregional degeneration.

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Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.06/D26

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

Support: NIH/NIA 2R01 AG053555-06

Title: Cerebral Blood Flow and Metabolism Coupling is Associated with Alzheimer's Disease Pathology and Memory Performance

Authors: *Y. ". ZHAO¹, J. N. ADAMS², J. R. BOCK¹, S. KIM³, M. A. YASSA⁴;

¹Univ. of California, Irvine, Irvine, CA; ²Neurobio. and Behavior, UC Irvine, Irvine, CA;

⁴Neurobio. and Behavior, ³Univ. of California Irvine, Irvine, CA

Abstract: Reduced resting cerebral metabolic rate of glucose (CMRglu) and reduced cerebral blood flow (CBF) have been independently reported to be associated with Alzheimer's Disease (AD) progression. However, it is unclear how the coupling between CBF and metabolism affects AD pathology and cognition. In this work, we hypothesized that reduced coupling between CBF and CMRglu would limit the brain's ability to obtain sufficient oxygen and glucose, as well as clear metabolic waste through vasculature, leading to the accumulation of amyloid and tau and impairing cognition. We analyzed 86 ADNI participants (38F/48M, mean age=72.8±8.1) who completed FDG-PET and 3D-PASL scans within one year. The sample included 3 cognitively unimpaired, 21 individuals with mild cognitive impairment (MCI), and 62 individuals diagnosed with dementia. Voxel-wise CBF/CMRglu ratios were calculated to index CBF-CMRglu coupling. Group-level analysis revealed that individuals with dementia showed significantly lower coupling than those with MCI in precuneus. To examine whether CBF-CMRglu coupling is associated with AD pathology, we split participants based on their CBF and CMRglu profile into three groups: a high-risk group (Low-CBF/Low-CMRglu), a low-risk group (High-CBF/High-CMRglu) group, and a "mixed" group (Low-CMRglu/High-CBF or High-CMRglu/Low-CBF). Kruskal-Wallis tests showed that CBF-CMRglu coupling profiles were significantly associated with A β burden ($H(2) = 20.19, p < .001$), temporal lobe tau burden ($H(2) = 21.79, p < .001$), and memory performance ($H(2) = 23.15, p < .001$). Post-hoc Wilcoxon rank-sum tests showed that compared to low-risk group, high-risk group had significantly higher A β burden ($W = 91, p < .001, r = .64$), higher temporal lobe tau load ($W = 96, p < .001, r = .62$), and worse memory performance ($W = 640, p < .01, r = .45$). These findings underscore the critical role of vascular-metabolic coupling in AD pathophysiology and support its potential as integrated biomarkers for AD staging.

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Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

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

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NIA R01AG053555
NIA F31AG074703

NIA R21AG079552
NIA T32AG00096
NIA F31AG084308
AASM Foundation SRA-1818

Title: Sleep signatures of brain health: a data-driven approach to predicting cerebral small vessel disease burden in older adults

Authors: *D. E. BERISHA¹, B. RIZVI⁴, N. JANSSEN⁵, N. J. TUSTISON⁸, M. CHAPPEL-FARLEY⁹, A. DAVE⁶, R. M. BENCA², M. A. YASSA⁷, B. A. MANDER³;

¹Univ. of California, Irvine, IRVINE, CA; ²Psychiatry and Behavioral Med., Univ. of California, Irvine, Winston Salem, NC; ³Psychiatry and Human Behavior, Univ. of California, Irvine, Irvine, CA; ⁴Univ. of California, Davis, Davis, CA; ⁵Univ. of California Irvine, La Laguna, Spain; ⁶Dept. of Cognitive Sci., ⁷Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; ⁸Univ. of Virginia, Charlottesville, VA; ⁹Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Introduction: Cerebrovascular pathology, associated with sleep disturbance, increases Alzheimer's disease (AD) risk and exacerbates AD outcomes. Here, we use a data-driven approach to characterize which sleep measures predict distinct cerebral small vessel disease (CSVD) imaging markers. Materials and Methods: Forty older adults (72.3±5.8 years; 26 female, Apnea Hypopnea Index=15.7±19.4) underwent overnight polysomnography (PSG). Sleep architecture variables were derived, including measures of sleep fragmentation and intermittent hypoxia. T1-MPRAGE and T2-FLAIR scans were acquired at 3 Tesla. Total perivascular space (PVS) volume, periventricular white matter hyperintensity (WMH), deep WMH, and total WMH volume (all in cm³) were derived. Average time between MRI and PSG was 1.5±0.97 years. To identify the most informative sleep-derived predictors of distinct CSVD outcomes, we applied recursive feature elimination (RFE) with Ridge regression ($\alpha = 1.0$) as the base estimator across the four target variables where predictor variables were PSG features. The top three ranked variables for each were then evaluated with multiple linear regressions including age at PSG, sex, and concurrent treatment for hypertension and/or hyperlipidemia. Results: NREM minimum O₂ saturation was the top predictor of PVS volume (MSE = 2.11), followed by REM minimum O₂ saturation and N2 sleep percentage. For periventricular WMH (MSE = 1.32), the top three were REM minimum O₂ saturation, NREM minimum O₂ saturation, and N2 sleep percentage. Deep WMH was most strongly predicted by REM sleep percentage (MSE = 1.38), with N1 and N2 sleep percentages contributing next. For total WMH (MSE = 14.73), the top predictors were REM minimum O₂ saturation, REM sleep percentage, and N2 sleep percentage. Conclusions: These results suggest that hypoxic burden during sleep, particularly REM sleep, may play an important role in CSVD pathology, while alterations in sleep stage distribution (percent spent in REM sleep) may contribute additional cerebrovascular risk. The differential predictors across PVS and WMH outcomes may reflect distinct mechanisms of vulnerability in microvascular vs. white matter compartments to sleep-related physiological features. This work highlights the potential mechanistic targets for intervention in aging populations with cerebrovascular risk.

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that research relationship even if those funds come to an institution.; Eisai. F. Consulting Fees (e.g., advisory boards); Eisai, Genentech, Haleon, Idorsia, Sage. **M.A. Yassa:** None. **B.A. Mander:** F. Consulting Fees (e.g., advisory boards); Eisai.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.08/D28

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

Support: R01AG053555
R21AG075464
F32AG074621

Title: Measurement of implicit statistical learning in young adults and cognitively unimpaired older adults

Authors: ***J. R. BOCK**¹, L. FISCHER³, D. TAYLOR², E. CHANG², B. AVILA², N. MEZA², N. TUTEJA², L. MCMILLAN², M. A. YASSA², J. N. ADAMS²;
²Neurobio. and Behavior, ¹Univ. of California, Irvine, Irvine, CA; ³German Ctr. for Neurodegenerative Dis. (DZNE), Magdeburg, Germany

Abstract: Statistical learning (SL) is the ability to develop inferences about stimuli based on co-occurrences with other stimuli in an environment. In addition to explicit decision-making based on these inferences, SL is thought to be a largely implicit memory process, which can be measured, through response time (RT), across repeated co-occurrences. However, recent findings on the impact of healthy aging on SL have been mixed. To explore this, we measured implicit SL by quantifying RT for identifying shape stimuli in a task with two samples: young adults (n = 41; age M = 23.37 years, range = 18 - 33) and older adults (n = 17; age M = 73.41 years, range = 63 - 89), totaling 58 participants (64% female), recruited at the University of California, Irvine. The SL task, in three runs with separate sets of stimuli, presented 6 unpaired (U) shapes that never co-occurred with any other shape and 4 triplets of shapes that were presented in the same sequence each time (positions A, B, and C), each presented 12 times. We hypothesized that stimuli at positions B and C may become predictable as SL occurs, leading to faster RTs for these stimuli compared to unpredictable stimuli (U, A). To obtain a measure of RT, participants were asked to identify whether each shape contained rounded features or not, without being explicitly informed of the triplet structure. The RT for each stimulus was recorded, log-transformed, and used to derive *RT facilitation*, an index of implicit SL. This was calculated by subtracting log(RT) of a given stimulus from the sample mean log(RT) for all position U stimuli in a given run, in which higher values of facilitation reflect more SL (faster, lower RTs). A mixed-effects linear regression model was evaluated for each run with RT facilitation as the outcome. The second run demonstrated the greatest overall performance, after familiarization but before fatigue, where RT for stimuli positions B and C was facilitated 6.8% and 3.7% better than

U, respectively (position A n.s.). Facilitation increased by 0.5% per repeated presentation overall and by a further 0.4% for position A, but canceled out for position B (-0.4%). No statistically significant effect was observed across age, between age groups, across group-by-position interaction, nor across group-by-position-by-presentation interaction ($\alpha = .05$). These results suggest that implicit SL is occurring for the predictable stimuli B and C, especially B with rapid learning by a non-linear trajectory, in both age groups, corroborating recent findings that indicate preservation of implicit SL into old age. Further research is underway to characterize learning trajectories across age and cognitive decline due to Alzheimer's disease.

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Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.09/D29

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

Support: NIH Grant U19AG065169
NIH/NIA 2R01 AG053555
NIA R44 AG079718

Title: Mnemonic discrimination task performance across the lifespan: Analysis of web-based, large-scale data from MindCrowd

Authors: *S. KIM¹, J. BOCK¹, J. JANECEK¹, A. KENNEDY SCOTT², M. DE BOTH³, M. HUENTELMAN⁴, L. RYAN⁵, L. MCMILLAN⁶, M. A. YASSA^{1,7};

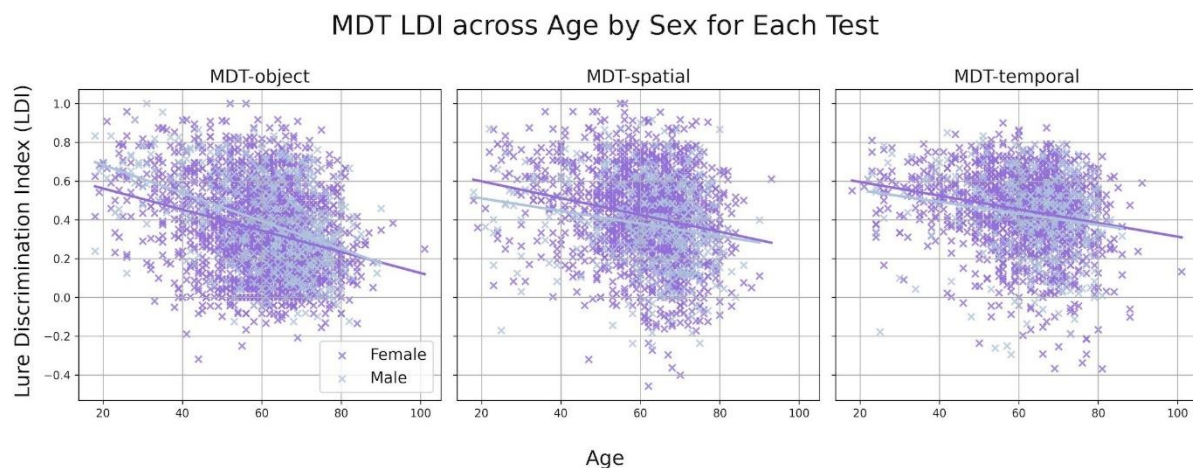
¹Univ. of California Irvine, Irvine, CA; ²Augnition Labs, New York, NY; ⁴Neurogenomics,

³TGen, Phoenix, AZ; ⁵Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ;

⁶Neurobio. and Behavior, UC Irvine, Long Beach, CA; ⁷Ctr. for the Neurobio. of Learning and Memory, Irvine, CA

Abstract: Episodic memory declines in normal aging and is significantly impacted in the earliest stages of Alzheimer's Disease. Mnemonic discrimination, the ability to differentiate similar events into distinct memories, is an integral facet of episodic memory and is known to rely on hippocampal pattern separation. Prior work has demonstrated the utility of this task as a potential digital cognitive biomarker for age-related memory loss and Alzheimer's disease. While several studies have previously demonstrated impaired mnemonic discrimination with older age and in patients with mild cognitive impairment, large-scale, web-based unsupervised testing has not been previously validated across the lifespan. In this study, we analyzed data from the Mnemonic Discrimination Task (MDT), implemented in MindCrowd (<https://mindcrowd.org/>), a web-based cognitive assessment platform administered on participants' choice of device (e.g., phone, tablet,

computer) without supervision. We used data from N = 3404 (mean age = 61.09, range = 18 - 101 yrs, 82% Female) who took at least one of the three versions (object, spatial, or temporal domain) of the MDT between June 2022 and November 2024. We analyzed performance with the lure discrimination index (LDI) as the outcome in one regression model for each MDT domain (LDI object mean = 0.35, SD = 0.23; spatial mean = 0.41, SD = 0.23; temporal mean = 0.44, SD = 0.20), predictors including age, sex, and an age-by-sex interaction. In all the three domains, an age effect was seen: per year of age, object LDI changed by -0.005 (95% CI = -0.006, -0.005); spatial LDI changed by -0.004 (95% CI = -0.005, -0.003); and temporal LDI changed by -0.004 (95% CI = -0.004, -0.003), all p 's < .001. Our findings support the feasibility of remote, unsupervised testing of MDTs in large samples across a wide range of ages. We also demonstrate that performance in mnemonic discrimination declines with age across object, spatial and temporal domains.



Disclosures: **S. Kim:** None. **J. Bock:** None. **J. Janeczek:** None. **A. Kennedy Scott:** A. Employment/Salary (full or part-time): Augnition Labs. **M. De Both:** None. **M. Huentelman:** None. **L. Ryan:** None. **L. McMillan:** None. **M.A. Yassa:** F. Consulting Fees (e.g., advisory boards); Augnition Labs.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.10/D30

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

Support: NIH, NIA Grants R43AG065039 and R44AG065039

Title: Liquid biopsy multi-omic TempO-Seq platform for diagnosis of Alzheimer's Disease, Parkinson's Disease, and other diseases from a fingerstick

Authors: B. SELIGMANN¹, M. HERNANDEZ¹, Z. CHEN¹, M. OPICHKA¹, S. CAMIOLO², J. YEAKLEY¹, *G. SAHAGIAN^{3,4};

¹BioSpyder, Carlsbad, CA; ²BioClavis, Glasgow, United Kingdom; ³The Neurol. Ctr. of Southern California, CARLSBAD, CA; ⁴Profound Res., Nashville, TN

Abstract: Background and Significance: There is a diagnostic need for neurological disease tests that use samples collectable in any clinic or through self-collection. While liquid biopsy assays are well described for plasma obtained from venipuncture, we used whole blood from a fingerstick, spotted onto 903 Protein Saver card filter paper, to profile whole transcriptome gene expression with the TempO-Seq® DBS assay. Self-collection makes this approach a potentially transformative diagnostic tool. We established a classifier for Alzheimer's Disease (AD) and Parkinson's Disease (PD), achieving 98% accuracy in classifying patients (Seligmann et. al., J. Alz. Dis. 101(2024): 813-822). Here we described expanding this fingerstick assay to measure DNA variants and protein biomarkers, increasing its diagnostic potential. We also tested archived frozen blood obtained from biobanks, spotted onto 903 Protein Saver cards. Methods and Results: Blood was spotted and dried on the filter cards. Areas from each spot were punched into wells to measure the whole transcriptome (mRNA), alternatively spliced isoforms, the whole miRNome (miRNA), select DNA variants (APOE2, 3, and 4), and select protein biomarkers, providing a multi-omic profile of each sample. Commercial TempO-Seq assays were used, with additional attenuators added to reduce signal from highly expressed blood genes. Data demonstrated that AD diagnostic criteria and immunotherapy suitability could all be assessed from the same fingerstick blood sample. Results will be presented. Conclusions: The results demonstrate the utility of the TempO-Seq DBS multi-omic liquid biopsy assay for profiling gene expression, DNA variants, and protein biomarkers from a fingerstick. We show potential to diagnose disease using AD and PD as exemplars. Since fingerstick samples can be self-collected, TempO-Seq DBS tests offer a) molecular-based diagnosis of Alzheimer's and Parkinson's Disease; b) tools to address health disparities; c) early at home diagnosis; and d) improved clinical trial recruitment.

Disclosures: **B. Seligmann:** A. Employment/Salary (full or part-time): BioSpyder. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioSpyder. **M. Hernandez:** A. Employment/Salary (full or part-time): BioSpyder. **Z. Chen:** A. Employment/Salary (full or part-time): BioSpyder. **M. Opichka:** A. Employment/Salary (full or part-time): BioSpyder. **S. Camiolo:** A. Employment/Salary (full or part-time): BioSpyder. **J. Yeakley:** A. Employment/Salary (full or part-time): BioSpyder. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioSpyder. **G. Sahagian:** A. Employment/Salary (full or part-time): The Neurology Center of Southern California, Profound Research. 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.; BioSpyder research funding, NIH Funding, Regeneron, argenx, Lilly, Roche, Immunovant, UCB Pharma, Abbvie, Cartesian. F. Consulting Fees (e.g., advisory boards); argenx, UCB Pharma.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.11/D31

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

Title: Simultaneous Detection of NF-L and Total Tau in CSF and Blood Using the SMC[®] FemtoQuest Platform and Millipore[®] Automated Assay Workstation (AAWTM)

Authors: *J. HWANG¹, D. DROLL², L. MARQUARDT¹, F. WIESE³;

¹R&D, ²Product Mgmt., Milliporesigma, Saint Louis, MO; ³R&D, Milliporesigma, St Louis, MO

Abstract: The quantification of neuronal protein biomarkers is essential for advancing our understanding of brain health, particularly in neurodegenerative disease research. Current methodologies primarily focus on biomarkers in cerebrospinal fluid (CSF), but the invasive nature of sample collection limits its practicality for routine analysis. The newly developed SMC[®] Neurofilament Light Chain (NF-L) and Total Tau 2-plex high sensitivity immunoassay kit addresses this gap by enabling the accurately quantification of these 2 critical biomarkers in human blood samples as well. Verified for use in CSF, serum, and plasma, this assay exhibits a high degree of specificity with < 5% cross-reactivity between analytes and precision of a coefficient of variation (CV) < 15%. Achieving LLoQs of 0.87 pg/mL and 2.34 pg/mL for NF-L and Total Tau, respectively, NF-L was 100% detectable in all sample types tested while Total Tau was detectable in all CSF and plasma samples with >80% detectability in serum samples. Statistically significant differences between presumed healthy vs Alzheimer's disease CSF samples were also observed. Integration of the Millipore[®] Automated Assay Workstation (AAWTM) into the 2-plex SMC[®] assay workflow enables enhanced reproducibility, minimizes hands-on time, and reduces the potential for human error. The automated assay workflow using the AAWTM resulted in lower LLoQ than manual assays with NF-L achieving 0.43 pg/mL and Tau achieving 0.58 pg/mL. An increased shift in sample detection was observed in comparison of healthy vs. Alzheimer's disease plasma samples. The NF-L and Total Tau sample ranges for healthy samples were 3.53-21.4 pg/mL and 0.639-10.2 pg/mL, respectively, and 6.03-42.7 pg/mL and 1.76-26.3 pg/mL for disease samples. Finally, performance on the AAWTM results in a 57% decrease in hands-on time compared to manual assay performance. In summary, this robust immunoassay enables the generation of accurate and reproducible data in multiple sample types and facilitates more efficient research of neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and Multiple Sclerosis.

Disclosures: J. Hwang: A. Employment/Salary (full or part-time): Milliporesigma. D. Droll: A. Employment/Salary (full or part-time): Milliporesigma. L. Marquardt: A. Employment/Salary (full or part-time): Milliporesigma. F. Wiese: A. Employment/Salary (full or part-time): Milliporesigma.

Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.12/D32

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

Support: R01AG086493
R21AG 065621

Title: Plasma biomarkers of neurodegeneration in middle-aged and older autistic adults

Authors: *D. CHRISTENSEN¹, S. RAYAPROLU², D. SHIRLEY³, H. GEMMELL¹, A.-M. ORLANDO⁴, J. C. DESIMONE⁵, D. LOEWENSTEIN⁷, D. E. VAILLANCOURT⁶, Z. WANG⁶; ²Neurol., ¹Univ. of Florida, Gainesville, FL; ³Univ. of Florida, Gainesville, FL, FL; ⁴Psychiatry, ⁵Dept. of Applied Physiol. and Kinesiology, ⁶Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL; ⁷Psychiatry and Behavioral Sci., Univ. of Miami, Miami, FL

Abstract: Despite the documented prevalence of increased neurodegenerative conditions (e.g., dementia) in middle-aged and older autistic adults, empirical research that quantifies neurodegenerative biomarkers and further reveals the underlying pathophysiological substrates in autistic adults is scarce. To address this critical gap, we examined established plasma-based amyloid/tau/neurodegeneration (A/T/N) biomarkers in cognitively normal autistic adults and neurotypical controls to identify potential biomarker deviations that may elucidate vulnerable neurobiological mechanisms potentially exacerbating the developmental course of dementia in autism spectrum disorder (ASD). We quantified amyloid beta ratio (A β 42/40), phosphorylated tau (pTau181 & pTau217), glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL) in 30 autistic (14F), 31 neurotypical (20F) of matched aged 30 to 68 years. To examine between-group differences while controlling for the covariates of age, sex, and full-scale IQ (FSIQ), we conducted statistical analysis in two parts. First, we employed a robust linear model (rlm) with the biomarker as the response and Age, Sex and FSIQ as the predictors and then extracted the residuals. We then plugged the residuals into a Wilcoxon model for both groups and subtracted NT from ASD to examine the mean residual difference. Our preliminary results demonstrate that autistic adults show significantly reduced plasma concentrations of A β 40 ($p = 0.03$), and similar values of A β 42. A β 42/40 ratio was significantly greater in autistic adults ($p = 0.02$). Further, NfL levels were significantly elevated in autistic adults compared to neurotypical controls ($p = 0.003$), while GFAP was similar between groups. For pTau, 9 ASD and 12 NT participants had their levels evaluated, where values for both pTau181 and pTau217 were similar between groups. Our findings highlight a unique profile of aging among middle and older aged autistic adults compared to controls, where neurodegeneration is indicated by elevations in NfL, while amyloidogenic influence is not evident as a pathological factor. This study lays the foundation for future pathophysiological research in autistic adults using plasma biomarkers.

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Poster

PSTR324: Alzheimer's Disease and Related Dementias: Biomarkers

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR324.13/Web Only

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Grants from the Malakoff Humanis company, malakoffhumanis.com
French federation for research on the brain (Fédération pour la Recherche sur le Cerveau) by fund FRC 2020 “Le cerveau protégé de/par son environnement”

Title: At-home digital phenotyping of apathy in behavioral variant of Frontotemporal Dementia and Alzheimer’s Disease and investigation of its impact on caregiver burden

Authors: I. SEZER^{1,2}, *J. BEHENSKA^{1,3}, S. AFLALO¹, C. BERNER¹, E. KARPINSKI¹, M. TOBA^{1,4}, K. N'DIAYE¹, M. IRISH³, A. FILIPCHUK², V. GODEFROY⁵, B. BATRANCOURT¹, R. LEVY¹;

¹Sorbonne Université, Inst. du Cerveau - Paris Brain Inst. – ICM, Inserm, CNRS AP-HP, Hôpital de la Pitié Salpêtrière, Paris, France; ²Healthy Mind, Paris, France; ³Brain and Mind Centre, Sch. of Psychology, The Univ. of Sydney, Sydney, Australia; ⁴Lab. of Functional Neurosciences (UR UPJV 4559), Univ. Hosp. of Amiens and Univ. of Picardie Jules Verne, Amiens, France; ⁵Univ. Claude Bernard Lyon 1, CNRS, INSERM, Ctr. de Recherche en Neurosciences de Lyon CRNL U1028 UMR5292, F-69500, Bron, France

Abstract: Neuropsychiatric symptoms (NPS) are core features of dementia and significantly affect both patients and their caregivers. One of these symptoms is apathy, which is present in up to 90% of people with dementia, including Alzheimer’s disease (AD) and the behavioral variant of frontotemporal dementia (bvFTD) (Kumfor et al, 2018), and is marked by reduced goal-directed behavior (Levy & Dubois 2006; Robert et al, 2018), undermining autonomy and quality of life. There is a critical need for objective, reliable methods to monitor these symptoms, as clinical scales are often biased by patient anosognosia and caregiver subjectivity. Digital health technologies, like sensors, offer continuous, objective monitoring. Actigraphy, using accelerometer data, is especially useful for assessing apathy by tracking movement and Rest-Activity Rhythm (RAR), providing metrics such as daytime activity and sleep quality, which are linked to apathy.

The ECOCAPTURE program at the ICM aims to define digital phenotypes of apathy with the current study (ECOCAPTURE@HOME, NCT04865172) focusing on in-home conditions with patient-caregiver dyads. Data was collected during 28 days for each patient-caregiver dyad (5 AD, 6 bvFTD, 6 Healthy Control), using Empatica wristbands (Empatica Inc., USA). Analyses focused on two actigraphy metrics: the mean amplitude deviation (MAD), which quantifies physical activity levels across periods, and Fragment, counting daytime rest episodes. Validated clinical scales and ecological momentary assessments (EMA) completed by the caregiver helped to contextualize the digital markers further.

General MAD analysis showed a significant difference between AD patients and caregivers ($p = 0.026$; $\chi^2 = 4.97$) and a trend in the FTD dyads ($p = 0.0502$; $\chi^2 = 3.83$). In FTD, a significant Group:Phase interaction ($p = 0.037$; $\chi^2 = 4.37$) indicated that MAD values differed between

patients and caregivers depending on the RAR phase (active vs. rest). When comparing MAD across patient groups, no significant main effect of Group was found, but a strong Group:Phase interaction emerged ($p = 1.6e-07$; $\chi^2 = 27.42$). Fragment analysis revealed no significant intradyadic differences, nor any significant Group effect when comparing Fragment values between AD and FTD patients. Correlations showed that actigraphy-based apathy measures are linked to caregiver burden as measured by clinical scales. These findings underline the promising nature of an objective, multimodal approach to assess apathy in dementia.

Disclosures: **I. Sezer:** A. Employment/Salary (full or part-time): Association nationale de la recherche et de la technologie (ANRT) (2/3), Healthy Mind PhD Fellowship (1/3). **J. Behenska:** A. Employment/Salary (full or part-time): PhD Fellowship from the IUIS: Institut Universitaire Economie de la Santé, Sorbonne Université. **S. Aflalo:** None. **C. Berner:** A. Employment/Salary (full or part-time): PhD Fellowship from the IUIS: Programme Instituts et Initiatives, Sorbonne Université. **E. Karpinski:** None. **M. Toba:** None. **K. N'diaye:** None. **M. Irish:** None. **A. Filipchuk:** A. Employment/Salary (full or part-time): Healthy Mind. **V. Godefroy:** None. **B. Batrancourt:** None. **R. Levy:** None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.01/D33

Topic: C.03. Parkinson's Disease

Support: NINDS (R01NS124735)
NIH (R01AG079898)

Title: Cell type-specific senescence-associated transcriptional regulators

Authors: ***T. RUSSO**, J. PLESSIS-BELAIR, R. SHER, M. RIESSLAND;
Dept. of Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Cellular senescence of brain cell types has become an increasingly important perspective for both aging and neurodegeneration, specifically in the context of Parkinson's Disease (PD). Recent advancements have been made to provide a 'minimum criteria' to define a senescent cell *in vivo* based on observed phenotypes, however senescent cells *in vivo* are very low in number and transient in their presence as they can be cleared within days by immune cells. In contrast, *in vitro* models of senescence are advantageous where an unlimited number of cells can be generated and molecular investigations into the nuanced differences in the expression of senescent phenotypes across different cell types can be made more accessible. Here, we describe a cell type-specific profile of senescence through the investigation of various canonical senescence markers in five human midbrain cell lines (astrocytes, endothelial cells, microglia, oligodendrocytes, and neurons) using chronic 5-Bromodeoxyuridine treatment as a model of DNA damage-induced senescence. Importantly, our findings highlight cell type-

specific profiles of key hallmarks displayed in cellular senescence. We used principal component analysis and subsequent regulatory transcriptional network inference to define both unique and common senescence profiles in the cell types investigated, as well as revealed senescence-associated transcriptional regulators (SATRs). Functional characterization of one of the identified regulators, transcription factor AP-4, further highlights the cell type-specificity of the expression of the various senescence hallmarks. Taken together, our data indicates that SATRs modulate cell type-specific profiles of induced senescence in key midbrain cell types that play an important role in the context of aging and PD. Our characterization of cell type-specific senescence can inform more precise, effective, and safe therapeutic interventions, particularly in complex diseases like PD where evidence suggests that multiple cell types contribute to pathology.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.02/D34

Topic: C.03. Parkinson's Disease

Support: Stony Brook Hartman Grant

Title: Loss of MeCP2 gives rise to a senescent phenotype in dopaminergic neurons.

Authors: ***J. F. MARTIN**, M. RIESSLAND;
Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Dopaminergic (DA) neurons of the midbrain make up a large majority of the total amount of DA neurons in the central nervous system and are involved in a wide range of crucial brain functions including the control of voluntary movement, emotion, and reward. Progressive loss of midbrain DA neurons in the substantia nigra pars compacta (SNpc) is a well known hallmark of Parkinson's Disease (PD), an age-related neurodegenerative disorder. However, it is unknown why DA neurons in the SNpc are particularly vulnerable to degeneration upon the onset of PD. Because of the strong correlation between PD and inflammaging (age-related inflammation), cellular senescence has recently been implicated as a potential key player in the cellular pathology of PD. Cellular senescence is a process that normally occurs in mitotic cells to prevent DNA-damaged cells from proliferating. However, new evidence of senescence occurring in post-mitotic DA neurons has been published. Methyl-CpG-binding protein 2 (MeCP2) was recently identified as a neuroprotective "vulnerability factor" of DA neurons that mediates their response to degeneration, along with SATB1, a risk factor for PD whose ablation in DA neurons results in senescence. Mutations in MeCP2 cause Rett Syndrome (RTT), a neurodevelopmental disorder that occurs mainly in females and is characterized by the dysregulation of MeCP2 in all cell types, including SNpc DA neurons. Consequently, RTT patients exhibit motor symptoms

that mirror PD, and patients who do not face early lethality as a result of RTT go on to develop early onset Parkinsonism. In a study looking into the cognitive pathology of RTT, loss of MeCP2 in interneurons was found to cause an upregulation in senescence-associated genes *in vitro*. However, senescence has not yet been implicated in the age-related motor pathology of RTT-Parkinsonism. Using stem cell-derived dopaminergic neurons and murine animal models, we show that there is an upregulation of DNA damage markers and a senescence phenotype in the absence of MeCP2 both *in vitro* and *in vivo*, as well as changes in the motor coordination of mice following MeCP2 knockdown in the murine SNpc. These experiments provide insight into the mechanistic pathology of RTT-related Parkinsonism and PD and can lead to potential preventative treatments to alleviate DA-associated symptoms in RTT and PD patients using FDA-approved senolytics.

Disclosures: J.F. Martin: None. M. Riessland: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.03/D35

Topic: C.03. Parkinson's Disease

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BaCaTec 9 [2021-2]
Bavarian Research Consortium "Interaction of Human Brain Cells"
(ForInter)

Title: Vimentin network dysregulation mediates SNCA duplication-induced neurite deficits in patient-iPSC-derived midbrain neurons

Authors: *A. KOLLER¹, Y. SCHNEIDER¹, A. SCHWEIGERT¹, M. ANDERT¹, L. HOFFMANN², S. O'BRIEN³, P. KIELKOWSKI², J. WINKLER¹, J. SCHLACHETZKI³, W. XIANG¹;

¹Mol. Neurol., Univ. Hosp. Erlangen, Erlangen, Germany; ²Dept. of Chem., Ludwig-Maximilians-Universität München, Munich, Germany; ³Cell. and Mol. Med., UCSD, La Jolla, CA

Abstract: SNCA gene duplication (*SNCA*^{Dupl}), elevating α -synuclein (aSyn) expression is a known genetic cause of Parkinson's disease (PD). While the association between aSyn accumulation and PD is well-established, the mechanisms by which neuronal dysfunction is driven by aSyn pathology remain unclear. Here, we investigate how increased aSyn levels due to *SNCA*^{Dupl} impacts midbrain neurons derived from human-induced pluripotent stem cells

(hiPSCs) originated from *SNCA*^{Dupl} patients. To dissect the specific effects of aSyn, we generated an isogenic control hiPSC line (*SNCA*^{Iso}) from a *SNCA*^{Dupl} PD patient via CRISPR/Cas9-mediated correction of *SNCA* gene dosage. We then characterized midbrain neurons generated from *SNCA*^{Dupl}, *SNCA*^{Iso} and healthy donor hiPSC lines. *SNCA*^{Dupl} neurons exhibited pronounced neurite deficits, including reduced neurite fiber density and dystrophy accompanied by decreased levels of β -tubulin III, a key cytoskeletal component in neurons. These deficits were restored by *SNCA* dosage correction. Multi-omics profiling of *SNCA*^{Dupl} neurons revealed significantly dysregulated pathways involved in neuronal differentiation and synaptic function, along with upregulation of the intermediate filament protein vimentin. Further biochemical and immunocytochemical analyses showed significantly increased vimentin levels and truncation, phenotypes that were also observed in the post-mortem putamen tissue from sporadic PD cases and in brain tissue from aSyn overexpressing mouse models. Moreover, we observed increased vimentin solubility and reduced distribution along neurites in *SNCA*^{Dupl} neurons. Importantly, pharmacological targeting of the vimentin network via okadaic acid and withaferin A reversed neuritic deficits and restored β -tubulin III levels in *SNCA*^{Dupl} neurons. Together, our findings identify vimentin dysregulation as a potential mediator of aSyn-induced cytoskeletal disorganization and neurite degeneration, highlighting the vimentin network as a potential therapeutic target to counteract aSyn-mediated neuritic dysfunction.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.04/D36

Topic: C.03. Parkinson's Disease

Support: NINDS Intramural Competitive Fellowship Award (NCFA) - F32 equivalent, to AML
NINDS Intramural Research Grant NS003134, to ZMK

Title: Axonal function is impaired early in a mouse model of progressive parkinsonism

Authors: *A. M. LIPKIN, N. L. O'PREY, A. BORONAT-GARCÍA, Z. M. KHALIQ;
Cell. Neurophysiol. Section, NIH/NINDS, Bethesda, MD

Abstract: Parkinson's disease is caused by the death of dopamine-producing neurons in the substantia nigra pars compacta (SNc). It is hypothesized that the death of the nigrostriatal axons of these neurons precedes and contributes to cell death in the SNc. In support of this hypothesis, loss of striatal dopaminergic fibers occurs before SNc cell death in a mouse model of progressive parkinsonism resulting from mitochondrial dysfunction, the MitoPark model. Striatal dopamine

release is also decreased before striatal fiber loss in this model, suggesting that impairments in axonal function may be present before axonal degeneration. Here, we explore if axonal function is impaired before the onset of axon loss in the MitoPark mouse model of Parkinson's disease. To determine if deficits in axonal function precede axon loss, we performed calcium imaging in the axons of MitoPark mice and age-matched controls at several pre-symptomatic timepoints preceding SNc cell death and striatal fiber loss (6 weeks, 9 weeks, and 12 weeks of age). We examined both the ability of striatal axons to follow evoked high-frequency activity (20 Hz and 100 Hz) following evoked pacemaker activity (2 Hz) as well as their ability to propagate signals down highly-branching axonal arbors. We measured calcium signals both using bulk slice photometry and two-photon microscopy to determine calcium signals in single dopaminergic axons. We found a reduction in stimulation-evoked calcium signals after 9 weeks of age in MitoPark but not control mice, suggestive of an impairment in nigrostriatal axonal function that begins before the overt loss of dopaminergic fibers. Understanding how axonal signaling is changed before axon die-back and neuronal death may illuminate new strategies to prolong the survival of axons and prevent the death of SNc dopamine neurons in the face of energetic challenges.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.05/D37

Topic: C.03. Parkinson's Disease

Title: Characterization of Ophthalmate as a New Neurotransmitter

Authors: *D. SHEVACHMAN¹, A. ALACHKAR²;

¹Univ. of California Irvine, Irvine, CA; ²Pharmaceut. Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Ophthalmic acid (OA) is a tripeptide composed of L- γ -Glutamyl-L-2-Aminobutyryl-Glycine (γ -Glu-2AB-Gly) that is structurally analogous to glutathione (GSH). We recently discovered a critical physiological role for OA in motor function and Parkinson's disease through the activation of calcium-sensing receptor (CaSR), a class C G protein-coupled receptor (GPCR). The presence of OA in the brain, its enzymatic synthesis, and its ability to activate CaSR in neurons suggest that OA may act as a neurotransmitter. Here, we investigated whether OA fulfills key neurotransmitter criteria, including receptor-mediated signaling, specific uptake, and activity-dependent release. First, G-protein activation by OA was assessed using [35S]GTP γ S binding assays in mouse brain tissue, with and without the CaSR antagonist NPS-2143. OA significantly enhanced GTP γ S binding, indicating GPCR activation; this effect was blocked by NPS-2143, confirming CaSR as the target receptor. Uptake studies were conducted in primary striatal neurons using radiolabeled OA to characterize transporter-mediated

accumulation. OA showed saturable uptake into neurons, mediated by a glutathione transporter. OA release was measured from acute striatal slices following high K⁺-induced depolarization in the presence or absence of extracellular calcium. We found that OA release was depolarization-induced, and calcium-dependent, supporting a vesicular exocytosis mechanism. These findings collectively support OA's classification as a neurotransmitter and point to its role in striatal neuromodulation via CaSR.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.06/D38

Topic: C.03. Parkinson's Disease

Support: Korea Research Institute of Bioscience and Biotechnology Research Initiative Program [KGM4562532]
National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-RS-2023-00229062)

Title: Mitochondrial fission through the Calpain-2/CDK5/p25/Drp1 signaling axis in MPTP-induced cynomolgus monkey models of Parkinson's disease

Authors: *J. PARK, J. SEO, J. WON, Y. LEE;
Natl. Primate Res. Ctr., Korea Res. Inst. of Biosci. and Biotech., Cheongju, Korea, Republic of

Abstract: Dopaminergic neuron integrity was evaluated via tyrosine hydroxylase (TH) expression in the substantia nigra (SN). Mitochondrial morphology and distribution were analyzed using TH/TOM20 immunofluorescence, and molecular pathways involved in mitochondrial dynamics were examined by assessing phosphorylation of Drp1(S616) and expression of Calpain-2, CDK5, and p25 via western blotting. TH-positive dopaminergic neurons in the SN were significantly reduced from 1W and continued to decline up to 48W. GFAP expression peaked at 1W and gradually decreased, while Iba1 expression remained elevated until 12W before decreasing at 48W. Mitochondria in dopaminergic neurons exhibited fragmentation and restricted perinuclear distribution from 1W, with average mitochondrial length significantly shortened and sustained through 48W. Drp1(S616) phosphorylation was highest at 1W and declined over time, indicating early activation of mitochondrial fission. Calpain-2 expression showed a biphasic pattern, peaking at 1W and 48W, while p25 expression increased progressively throughout the post-MPTP period, suggesting activation of the Calpain-2/CDK5/p25 signaling axis in the maintenance of mitochondrial dysfunction. The MPTP-induced PD monkey model replicates key features of human PD, including progressive dopaminergic loss, neuroinflammation, and mitochondrial abnormalities. These findings

highlight early mitochondrial fragmentation and sustained CDK5 pathway activation as potential contributors to long-term neurodegeneration in PD.

Disclosures: J. Park: None. J. Seo: None. J. Won: None. Y. Lee: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.07/D39

Topic: C.03. Parkinson's Disease

Title: A high-content, multiplex platform for investigating lysosomal dysfunction in patient-derived fibroblasts: applications in neurodegenerative drug discovery

Authors: D. LEITE¹, R. LENHAM², T. R. ROSENSTOCK¹, S. THOMSON¹, T. PHILLIPS¹, *N. R. MIRZA¹;

¹Sygnature Discovery, Nottingham, United Kingdom; ²Nottingham Univ., Nottingham, United Kingdom

Abstract: Lysosomes are acidic, membrane-enclosed organelles responsible for cellular waste degradation and recycling, which also contribute to other functions: membrane repair, metabolic regulation, and immune signalling. Evidence implicates lysosomal dysfunction in neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD). As lysosomal proteins gain prominence as therapeutic targets, robust disease-relevant human *in vitro* cellular assays are essential for functional assessment and pharmacological screening. We have compared human dermal fibroblasts from healthy donors and AD and PD patients to investigate lysosomal integrity and autophagy. A suite of multiplexed assays was validated to assess lysosomal biology: lysosome abundance (LAMP1 and LysoTracker™ staining), luminal pH (LysoSensor), enzymatic activity (cathepsin B), and autophagy initiation (p62 accumulation). Assays were miniaturised to a 384-well format, semi-automated, and analysed via high-content imaging with AI/ML-assisted quantification (MetaXpress™). Statistical analysis and graphics were performed in GraphPad Prism. Compared to healthy donors, fibroblasts from AD and PD patients exhibited reduced lysosomal content (40-80%) under 24 hours of nutrient-deprived conditions. In this scenario, total fluorescence was measured for LAMP1 (Healthy: 3150 ± 396.2 , AD: 2149 ± 348.6 , and PD: 1519 ± 177.5 ; mean $\pm$ SEM) and LysoTracker (Healthy: 18028 ± 1951 , AD: 8399 ± 1432 , and PD: 4042 ± 997.5) (mean $\pm$ SEM). Fibroblasts from AD and PD patients also showed impaired autophagic flux (52%) under 18 hours of nutrient-deprived conditions. Specifically, total p62 values were as follows: Healthy: 10860 ± 1360 , AD: 7964 ± 575.5 , and PD: 5516 ± 1036 (mean $\pm$ SEM). Lysosomal pH and cathepsin B activity remained comparable between groups. After chloroquine treatment for 24 hours, fibroblasts from healthy individuals and PD patients showed an increase in LAMP1 and LysoTracker™ signals, as well as cathepsin B activity. Notably, the increase in these markers was greater in healthy individuals than in PD patients (LAMP1: Healthy: 7338 ± 414.8 and PD: 4762 ± 272.4 ;

LysoTracker: Healthy: 3154 ± 38.16 and PD: 2750 ± 85.83 ; cathepsin B activity: Healthy: 50085 ± 2939 and PD: 38930 ± 2572) (mean $\pm$ SEM). These findings indicate disrupted lysosomal function in cells from PD patients. These findings show that patient-derived fibroblasts recapitulate key lysosomal and autophagic defects seen in neurodegeneration. Our multiplexed assay platform offers a robust, scalable system for pharmacological interrogation of lysosomal pathways supporting early-stage AD and PD drug discovery.

Disclosures: D. Leite: None. R. Lenham: None. T.R. Rosenstock: None. S. thomson: None. T. Phillips: None. N.R. Mirza: None.

Poster

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Program #/Poster #: PSTR325.08/D40

Topic: C.03. Parkinson's Disease

Support: PAPIIT-UNAM Grant IN211524
SECTEI Grant 146/2024

Title: Regulation of Cav1.3 channels by the leucine-rich repeat kinase 2 variant LRRK2^{G2019S}: possible pathogenic implications in Parkinson's Disease

Authors: *A. CORZO LOPEZ¹, A. SANDOVAL¹, P. DURAN², D. C. TOVAR-SOTO¹, B. VARGAS CABALLERO³, V. GALICIA SALDAÑA¹, R. GONZALEZ-RAMIREZ⁴, R. FELIX⁵;

¹Sch. of Med. FES Iztacala, Natl. Autonomous Univ. of Mexico UNAM, Tlalnepantla, Mexico;

²Mol. Pathobiology, New York Univ., New York, NY; ³Cell. Biol., Ctr. for Res. and Advanced Studies of the Natl. Polytechnic Inst., Ciudad Nezahualcoyotl, Mexico; ⁴Dept. of Mol. Biol. and Histocompatibility, "Dr. Manuel Gea González" Gen. Hosp., Mexico City, Mexico; ⁵Cinvestav-IPN, Mexico City, Mexico

Abstract: Voltage-gated calcium channels (Cav) are integral membrane protein complexes comprising a main pore-forming subunit (Cav α_1) and auxiliary subunits (Cav $\alpha_2\delta$ and Cav β). They are found in a variety of tissues, including the nervous system, where they mediate calcium influx in response to plasma membrane depolarizations. This increase in intracellular calcium regulates fundamental processes such as neuronal excitability and neurotransmitter release. LRRK2 is a serine-threonine kinase that has been shown to regulate the activity of presynaptic Cav2.1 channels by phosphorylating their Cav β auxiliary subunit. However, its potential actions on Cav1.3 channels, which are essential for the pacemaker activity of dopaminergic neurons in the substantia nigra and whose dysfunction is linked to Parkinson's disease (PD), have not been explored. Here, we identified possible phosphorylation sites for LRRK2 on the Cav β_3 subunit sequence. Using immunoprecipitation techniques and electrophysiological recordings in HEK-293 cells expressing recombinant Cav1.3 channels, with or without wild-type LRRK2 or its

G2019S mutant form (associated with familial PD), we show that the G2019S variant significantly increases the current density through Cav1.3 channels, an effect that depends on the presence of Cav β ₃. Furthermore, phosphorylation of the S152 residue in Cav β ₃ is necessary and sufficient to mediate this abnormal regulation. This mechanism may help explain the hyperexcitability of dopaminergic neurons observed in PD models. These findings provide evidence of how pathogenic LRRK2 mutations disrupt calcium homeostasis by post-translational modulation of Cav1.3 channels.

Disclosures: A. Corzo Lopez: None. A. Sandoval: None. P. Duran: None. D.C. Tovar-Soto: None. B. Vargas Caballero: None. V. Galicia Saldaña: None. R. Gonzalez-Ramirez: None. R. Felix: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.09/D41

Topic: C.03. Parkinson's Disease

Support: NIH Grant AA029811
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Title: Loss of auxilin in *C. elegans* causes motor deficits and degenerative changes in dopaminergic neurons

Authors: *H. KIM¹, M. PATINO-MARTINEZ²;
¹Chicago Med. School/Rosalind Franklin Univ., North Chicago, IL; ²Chicago Med. School, Rosalind Franklin Univ., North Chicago, IL

Abstract: Parkinson's disease (PD) is characterized by the progressive and selective degeneration of dopaminergic neurons. Many PD-linked genes are involved in endocytic trafficking and synaptic vesicle (SV) recycling, highlighting intracellular vesicle trafficking dysfunction as a central mechanism in PD pathogenesis. Auxilin (DNAJC6), one such PD-linked gene, encodes a HSP70 co-chaperone that facilitates clathrin uncoating. Loss of auxilin reduces the pool of free clathrin and decreases SV number, but does not fully eliminate endocytosis. The mechanism by which auxilin dysfunction leads to selective dopaminergic neurodegeneration, however, remains poorly understood. To further address the role of auxilin in PD pathogenesis, we used the nematode *C. elegans*, which has a highly conserved synaptic architecture and orthologs of most PD-associated genes, including auxilin (*dnj-25*). Using CRISPR/Cas9 genome editing, we generated a split GFP-tagged *dnj-25* strain. In vivo imaging revealed both diffuse cytosolic localization and distinct puncta in apical epithelial cells. Time-lapse imaging showed dynamic "flickering" of DNJ-25 puncta, consistent with its reversible recruitment to clathrin lattices and rapid turnover. We next generated a *dnj-25* null mutant, which arrested at the L3 larval stage, indicating that DNJ-25 is essential for development. To investigate its neuronal

function, we generated a neuron-specific deletion mutant (*dnj-25(-neuron)*), which was viable and fertile but displayed severe, age-dependent locomotor deficits. Notably, adult *dnj-25(-neuron)* animals exhibited near-complete loss of thrashing activity and dopaminergic neuron degeneration. Together, these findings suggest that *dnj-25* is required not only for synaptic vesicle recycling but also for other aspects of synaptic and neuronal maintenance. This work establishes *C. elegans* as a powerful model to dissect auxilin function in vivo and may provide insights into shared mechanisms underlying both familial and idiopathic forms of PD.

Disclosures: H. Kim: None. M. Patino-Martinez: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.10/D42

Topic: C.03. Parkinson's Disease

Title: Necroptosis is activated in nigral dopaminergic neurons of *Prkn*R275W mice

Authors: *C. MINI^{1,2,3}, M. REGONI^{2,3}, L. ZANETTI⁴, G. DI GIORGIO³, E. MONZANI³, D. CHIERICHETTI³, G. CONSALEZ^{2,3}, L. P. CROCI³, M. BACIGALUPPI⁵, F. VALTORTA^{2,3}, J. SASSONE^{2,3};

¹Dept. of Statistics, Computer Science, Applications (DiSIA), Univ. of Florence, Florence, Italy;

²Vita-Salute San Raffaele Univ., Milan, Italy; ³San Raffaele Scientific Inst., Milan, Italy;

⁴Columbia Univ., New York, NY; ⁵Univ. Zürich, Schlieren, Switzerland

Abstract: Inactivating mutations in the ubiquitin ligase Parkin are a leading cause of Autosomal Recessive Juvenile Parkinsonism (ARJP), a neurodegenerative disease characterized by early dysfunction and death of dopaminergic (DAergic) neurons in substantia nigra pars compacta (SNpc). A better understanding of the molecular mechanisms underlying degeneration of DAergic neurons could inform the design of drugs that promote neuron survival, an unmet medical need in the field. It has been recently showed that Parkin blocks necroptosis, a regulated form of cell death that is triggered by inflammatory signals and causes the release of immunogenic endogenous molecules. The latter can promote neuroinflammation and microglia activation, exacerbating neuronal death. The core necroptotic pathway comprises RIPK1 (receptor-interacting protein 1 kinase), RIPK3 (receptor-interacting protein 3 kinase) and MLKL (mixed lineage kinase domain-like pseudokinase), the executor of necroptosis that, once phosphorylated, oligomerizes and translocates to the plasma membrane where it induces the formation of pores, resulting in membrane permeabilization and rupture. Here we investigated the potential involvement of necroptosis in a preclinical model of ARJP, *Prkn*R275W mice, harboring the loss-of-function mutation R275W in mouse *Prkn* gene. This is the missense mutation with the highest allelic frequency in *PRKN*-mutated ARJP patients. *Prkn*R275W mice display neurodegeneration of SNpc DAergic neurons starting from 6 months of age, but these neurons present early defects already at 1 month of age. We analyzed biomarkers for necroptosis

and neuroinflammation in SNpc of *Prkn*R275W mice at 1 and 6 months of age. Immunofluorescence staining shows an increase of RIPK3 and phosphorylated MLKL levels in nigral DAergic neurons of *Prkn*R275W mice at 1 and 6 months of age. RIPK3 and MLKL mRNAs were also upregulated in *Prkn*R275W mice at the same age. Regarding neuroinflammation, we found increased microglial and astrocytic activation in *Prkn*R275W mice starting at 6 months of age. Moreover, a bulk RNA-sequencing analysis on SNpc of 6-month-old WT and *Prkn*R275W mice revealed an upregulation of inflammatory pathways in mutated mice. Overall, these results suggest that necroptosis is activated in nigral DAergic neuron of *Prkn*R275W mice, possibly playing a role in neurodegeneration in ARJP.

Disclosures: C. Mini: None. M. Regoni: None. L. Zanetti: None. G. di giorgio: None. E. Monzani: None. D. Chierichetti: None. G. Consalez: None. L.P. Croci: None. M. Bacigaluppi: None. F. Valtorta: None. J. sassone: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.11/D43

Topic: C.03. Parkinson's Disease

Support: IU School of Medicine Start Up
SNRI Start Up

Title: Degeneration of Dopamine Neurons Reduces Function of Striatal Glycine Receptors

Authors: *S. COOK¹, B. MUNOZ^{1,2};

¹Pharmacol. & Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN; ²Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: Parkinson's Disease (PD) is caused by the degeneration of dopamine neurons in the basal ganglia. The most effective treatment to alleviate motor symptoms is the use of Levodopa, however, its prolonged use leads to dyskinesia and episodic psychosis events. At this time, there is no treatment to delay or stop the progression of PD. Recent studies have shown that the development of dyskinesia and episodic psychosis events can be treated using glycine transporter type 1 (GlyT1) inhibitors such as bitopertin. Interestingly, using GlyT1 inhibitor in the striatum increases glycine concentration leading to the activation of striatal glycine receptors (GlyRs), increasing dopamine release and promoting neuroprotection. Using patch clamp electrophysiology, we previously demonstrated that GlyRs are highly potentiated by the activation of dopamine receptors. These results implicate a direct interaction between dopamine, dopamine receptors, and GlyRs. Here, using a PD mouse model of selective neurotoxic disruption (6-OHDA), patch clamp electrophysiology, and qPCR, we showed that the expression and function of GlyRs in medium spiny neurons (MSNs) are affected by the loss of the dopamine synapses. Specifically, we found that β GlyR subunit expression increased at 21 days following

6-OHDA treatment. Additionally, GlyR-evoked currents decreased at 7, 14, and 21 days following 6-OHDA treatment. These data suggest a specific role of striatal GlyRs in mediating synaptic plasticity and dopaminergic tone, which could be involved in the progression of PD.

Disclosures: S. Cook: None. B. Munoz: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.12/D44

Topic: C.03. Parkinson's Disease

Title: Vps26b deficiency enhances mpp⁺-induced neurotoxicity by impairing pink1 stabilization and mitophagy in dopaminergic sh-sy5y cells

Authors: *J. SEONG¹, Y. LEE²;

¹Korea Res. Inst. of Biosci. & Biotechnology/National Primate Res. Ctr., Cheongwon-gu, Cheongju-si, Korea, Republic of; ²Natl. Primate Res. Ctr., NPRC, KRIBB, Cheongju, Korea, Republic of

Abstract: Vacuolar protein sorting 26B (VPS26B), a component of the retromer complex, has been implicated in endosomal trafficking and mitochondrial quality control. However, its role in dopaminergic neuronal survival under conditions of 1-methyl-4-phenylpyridinium (MPP⁺) exposure remains poorly understood. In this study, we investigated the effects of VPS26B knockdown on mitochondrial integrity and mitophagy in SH-SY5Y cells exposed to MPP⁺, a neurotoxin commonly used to model Parkinson's disease. VPS26B-deficient SH-SY5Y cells exhibited enhanced mitochondrial swelling, accompanied by increased expression of PINK1, cathepsin D, and LC3-II, suggesting an accumulation of damaged mitochondria and dysregulated mitophagic flux. These alterations were associated with increased vulnerability to MPP⁺-induced neurotoxicity. Our findings suggest that VPS26B is critical for maintaining mitochondrial homeostasis through the stabilization of PINK1 and proper execution of mitophagy, and that its deficiency exacerbates neurotoxic responses under MPP⁺ exposure.

Disclosures: J. Seong: None. Y. Lee: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.13/D45

Topic: C.03. Parkinson's Disease

Support: NIH Grant ES034846
Parkinson's Foundation: PF-VSA-1476761

Title: Endolysosomal dysfunction as a central mechanism of environmental toxicant-induced Parkinson's neurodegeneration

Authors: *T. E. WALTERS¹, G. COHEN², A. ADAMSON², A. MANABAT⁴, K. L. ESKOW JAUNARAJ³, L. A. VOLPICELLI-DALEY⁴, A. I. BERNSTEIN⁵, B. R. DE MIRANDA²;
¹The Univ. of Alabama at Birmingham, Birmingham, AL; ²Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; ³Neurol., Univ. of Alabama at Birmingham, Springville, AL; ⁴Neurol., UAB, Birmingham, AL; ⁵Pharmacol. and Toxicology, Rutgers Univ., Piscataway, NJ

Abstract: Environmental exposures are linked to risk for neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (PD, AD). We and others have previously shown that exposure to industrial byproducts, like the organic solvent trichloroethylene (TCE), induces neurotoxicity within the dopaminergic system and recapitulates hallmark PD pathology such as alpha-synuclein (α Syn) aggregation, oxidative stress, damage to endocytic vesicles, and endolysosomal dysfunction in adult mice. Mitochondrial dysfunction has been postulated to be a central component of toxicant induced PD, however, the endolysosomal system may play a more integral role in modulating toxicant induced neurodegeneration and disease phenotype. We have previously published that dopaminergic neuron loss is induced in mice after 12 weeks of 100 ppm TCE inhalation exposure. To test whether endolysosomal damage occurs prior to neuron loss we exposed mice to 100 ppm TCE for 4 weeks and found that exposed animals displayed a significant reduction of cathepsin D within dopaminergic neurons ($p < 0.0001$) prior to degeneration. We also observed that TCE exposed mice displayed increased insoluble, aggregated α Syn ($p < 0.0001$) in brain lysates at this same 4-week timepoint. To investigate how TCE may cause specific endolysosomal pathway impairment, we exposed GBA1^{L444P +/-} (GBA HET) mice that express a heterozygous mutation in *GBA1* to 4 weeks of 100 ppm TCE inhalation. TCE exposed GBA HET mice showed a significant reduction in dopaminergic neuron cathepsin D ($p < 0.0001$), but not control GBA HET animals, suggesting that TCE, rather than GBA1 caused cathepsin D dysfunction. In contrast, GBA HET animals displayed reduced GCase activity in brain tissue, but no further GCase reduction was observed in TCE exposed animals. Interestingly, the combined TCE exposed GBA HET mice did display a loss of striatal terminal intensity ($p < 0.0012$) when compared to wildtype TCE and GBA HET controls. Thus, TCE exposure likely impacts the endolysosomal system in a parallel, but divergent mechanism from the PD mutation GBA and this toxicant-induced loss of endolysosomal function could be an initiating and key feature of PD pathogenesis from environmental exposures.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.14/Web Only

Topic: C.03. Parkinson's Disease

Support: UNAM-PAPIT: IA205025
IN216023
IN218924
UNAM-PAPIME: PE304525

Title: Rol of CB₁endocannabinoid and A_{2A}receptors in calcium currents and striatal activity in Parkinson Disease and Levodopa-induce dyskinesias

Authors: *E. A. RENDON-OCHOA^{1,3}, A. LUNA², V. CALDERON⁴, V. AVILÉS ROSAS^{5,6}, M. A. ARIAS⁷, M. PADILLA⁸, M. PALOMERO RIVERO⁹, A. GÓMEZ PAZ², E. GALARRAGA¹⁰;

¹Psychopharmacology, Univ. Nacional Autonoma de Mexico, Estado de México, Mexico; ²Univ. Nacional Autonoma de Mexico, Ciudad de México, Mexico; ³Facultad de Estudios Superiores Iztacala, Estado de México, Mexico; ⁴Inst. de Neurobiología, Juriquilla, Mexico; ⁵Facultad de Química, Ciudad de México, Mexico; ⁷Lab. de Neurociencia Traslacional, ⁶Facultad de Psicología, Ciudad de México, Mexico; ⁸UNAM, Ciudad De Mexico, Mexico; ⁹Inst. de Fisiología Celular, Ciudad de México, Mexico; ¹⁰Neurosciecias Cognitiva, Inst. Fisiologia Celular, Mexico DF 04510, Mexico

Abstract: Chronic treatment with L-DOPA for motor complications of Parkinson disease (PD) leads to levodopa-induced dyskinesias (LID). Other non-dopaminergic approaches emerged to alleviate symptomatology. In this regard, CB₁ agonist and A_{2a} antagonist are promising drugs. CB₁ agonist and A_{2a} antagonist have proven efficacy in reducing anormal-involuntary movements (AIMs) in animals and humans with advanced PD, however, to date, information about their molecular mechanisms is scare and whether changes in microcircuit striatal dynamics remain unclear. With the use of identified dissociated striatal projection neurons (SPN) and calcium imaging recordings this we asked 3 principal questions: whether CB₁ activation influences in calcium currents in both populations of SPN and this activation habilitates A_{2a} receptors; whether exist a change in the modulation of CB₁ in control, 6-OHDA or LID conditions; whether the CB₁ modulation changes the dyskinetic striatal microcircuit dynamics, facilitating or decreasing SPN populations or produces changes in striatal ensembles sequence. Current recordings in dissociated neurons showed that CB₁ agonist, WIN, could effectively decrease calcium currents in all conditions, reducing the activity. In addition, WIN habilitated A_{2a} receptors in iSPN (indirect spiny projection neurons), restoring calcium currents to control values in all experimental conditions. In agreement with dissociated recordings, calcium imaging showed that WIN decreased striatal microcircuit activity and produced changes in ensembles sequence. We confirmed that exist neurons that facilitated, decreased or were unaffected by WIN. We concluded that CB₁ agonist reduced general SNP activity. This reduction in activity led to changes in dyskinetic striatal activity, that could be, in part, responsible for therapeutics outcomes with the use of CB₁ agonists, possibly reducing activity in dSPN (direct spiny projection neurons).

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.15/D46

Topic: C.03. Parkinson's Disease

Support: NIH R01-NS122226-01

Title: Dorsal raphe nucleus vesicular glutamate transporter 3 knockdown on levodopa-induced behaviors and striatal neurotransmission in hemiparkinsonian rats

Authors: *C. BUDROW¹, I. M. SANDOVAL², F. P. MANFREDSSON², C. R. BISHOP³; ¹SUNY - Binghamton Univ. Behavioral Neurosci., Binghamton, NY; ²Translational Neurosci., Barrow Neurolog. Inst., Phoenix, AZ; ³Psychology, Binghamton Univ., Binghamton, NY

Abstract: Parkinson's disease (PD) is a movement disorder marked by progressive loss of substantia nigra (SNc) originating dopamine (DA) neurons. While levodopa (L-DOPA) remains the gold-standard treatment, chronic use can result in L-DOPA-induced dyskinesia (LID), typified by abnormal involuntary movements (AIMs) and dystonia. Maladaptive neuroplasticity of the raphe-striatal serotonin (5-HT) system is a key driver of LID, with emerging evidence revealing a capacity for dorsal raphe nucleus (DRN) 5-HT neurons to co-release glutamate (GLUT) via vesicular GLUT transporter 3 (VGLUT3). This suggests a novel and complex neurotransmitter convergence. This study examined the role of DRN-5-HT dependent VGLUT3 co-expression in LID. TPH2-Cre+ Long Evans rats (N=17; M=9, F=8) received unilateral 6-hydroxydopamine lesions, followed three weeks later by DRN-targeted VGLUT3 knockdown (KD; AAV9-FlexUTR2-rVGLUT3-miRNA999; n=9) or control (AAV-SCR; n=8) viral infusion with concurrent striatal cannula implantation ipsilateral to lesion. After assessing baseline motor performance, rats were treated with L-DOPA (6 mg/kg, s.c.) for 14 days, with AIMs scored on days 1, 7, and 14. On day 15, *in vivo* microdialysis was performed prior to and during L-DOPA alongside AIMs ratings and samples were collected for analysis of striatal GLUT, DA and 5-HT. Post-mortem SNc and DRN were analyzed for DA lesion verification via HPLC and VGLUT3 protein expression via ELISA, respectively. Behaviorally, VGLUT3-KD in DRN-originating 5-HT neurons exacerbated LID trajectory and severity. Prospectively, we anticipate analysis of striatal neurochemistry may reveal VGLUT3-KD-induced changes in DA and GLUT, commensurate with LID. Taken together, such results may reflect novel convergence of presynaptic GLU, 5-HT, and DA from raphe-striatal 5-HT neurons and underscore the complexity of 5-HT hyperconnectivity as a mode of maladaptive compensation, driving aberrant striatal signaling.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.16/D47

Topic: C.03. Parkinson's Disease

Support: Endlyz Therapeutics

Title: Overexpression of ATP13A2 rescues lysosomal and metabolomic disruptions in GBA-Parkinson's induced pluripotent stem cell derived dopamine neurons

Authors: *A. MEHTA^{1,2}, W. MCGUINNESS^{1,2}, N. AHUJA^{1,2}, E. BRITTI^{1,2}, H. WATERS^{1,2}, T. DIVINEY^{1,2}, F. LICITRA³, J. WOLAK³, C. LANG^{1,2}, R. WADE-MARTINS^{1,2};

¹Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; ²Kavli Inst. for Nanoscience Discovery, Univ. of Oxford, Oxford, United Kingdom; ³Endlyz Therapeut., Oxford, United Kingdom

Abstract: Parkinson's disease (PD) is characterised by the progressive loss of dopamine neurons (DANs) in the brain, but the mechanism of this neurodegeneration remains to be elucidated. Lysosomal dysfunction is a key player in DAN susceptibility in PD. Mutations in the *GBA* gene encoding lysosomal enzyme β -glucocerebrosidase (GCase) are an important genetic risk factor in PD. GBA-PD contributes to lysosomal dysfunction by loss of GCase activity, enlarged lysosomes, and alkalinised lysosomes. Similarly, mutations in ATP13A2, a lysosomal polyamine transporter, has been implicated in mediating lysosomal dysfunction in Kufor-Rakeb Syndrome, a rare form of PD. In this study, we investigated ATP13A2, and GBA-PD related lysosomal and metabolomic abnormalities. In GBA-PD iPSC-derived DANs we observed enlargement and accumulation of lysosomes. Knockdown of ATP13A2 in control iPSC-DANs resulted in enlargement, accumulation and alkalinisation of the lysosomes. To understand the association of the two PD-associated lysosomal proteins, GBA and ATP13A2, we looked for the endogenous expression levels of ATP13A2 in GBA-PD patients iPSC-DANs and observed decreased ATP13A2 levels in GBA-PD compared to controls. Additionally, we also observed polyamine perturbation in GBA-PD iPSC-DANs and GBA-PD mice transgenic for human mutant GBA. Overexpression of ATP13A2 in GBA-PD iPSC-DANs led to the rescue of lysosomal number and lysosomal size, reacidification of lysosomes, and correction of polyamine perturbations. Overall, our findings indicate that the loss of ATP13A2 in GBA-PD leads to disruption of lysosomal and polyamine homeostasis. Restoration of lysosomal and polyamine dysfunction by ATP13A2 overexpression highlights the therapeutic potential of ATP13A2 for GBA-PD.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson's Disease

Support: ASAP-024297

Title: Lysosomal Polyamine Sequestration Drives Epigenetic and Neurotoxic Changes in Parkinson's Disease Astrocytes

Authors: ***E. COCCIA**¹, G. PARFITT², J. W. BLANCHARD¹;

¹Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Genentech, San Francisco, CA

Abstract: Loss-of-function (LOF) variants in ATP13A2 cause juvenile-onset Parkinson's disease (PD), a rare and severe form with early onset of motor and cognitive symptoms. ATP13A2 is a lysosomal polyamine exporter, and its dysfunction induces a sequestration of polyamines in lysosomes. However, the mechanisms linking these metabolic defects to neurodegeneration remain unclear. Using isogenic iPSC-derived midbrain organoids, we show that ATP13A2 LOF in astrocytes induces dopaminergic neurotoxicity. Mechanistically, ATP13A2-deficient astrocytes exhibit lysosomal dysfunction and accumulation of α -synuclein, alongside depletion of cytosolic polyamines. This triggers compensatory upregulation of polyamine biosynthetic enzymes, diverting S-adenosyl methionine (SAM) from DNA and histone methylation. The resulting global hypomethylation and increased chromatin accessibility drive epigenetic reprogramming of astrocytes toward a senescent, pro-inflammatory state. These altered astrocytes release cytotoxic vesicles that further exacerbate dopaminergic neuron death. Importantly, both chemical and genetic inhibition of SAM utilization in polyamine biosynthesis prevent these epigenetic changes and preserve neuronal viability. Our findings establish a mechanistic link between polyamine metabolism, lysosomal dysfunction, α -synuclein accumulation, epigenetic dysregulation, and accelerated neurodegeneration in ATP13A2-related PD. This work identifies new therapeutic targets and provides an innovative strategy to model brain aging in vitro by manipulating polyamine biology. Together, these results offer key insight into how metabolic and epigenetic changes in astrocytes drive early neurodegeneration and highlight the potential for targeting polyamine pathways to mitigate age-related and genetic forms of PD.

Disclosures: **E. Coccia:** None. **G. Parfitt:** None. **J.W. Blanchard:** None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Topic: C.03. Parkinson's Disease

Support: MJFF Grant MJFF-024454
MJFF Grant MJFF-025414
MJFF Grant MJFF-024056

Title: Novel Antibodies to Isolate Intact Organelles for Parkinson's Disease Research.

Authors: *N. K. POLINSKI¹, P. LIS², M. VISKONTAS², J. W. HIRSCHFELD³, A. AIELLO³, S. COVENEY³, E. CLARK¹, G. THAKURIA¹, R. W. CHO⁴, H. CHENG⁵, M. MUQIT², D. ALESSI², J. L. EBERLING⁶;

¹The Michael J. Fox Fndn. for Parkinson's Res., New York, NY; ²Univ. of Dundee, Dundee, United Kingdom; ⁴Develop., ³Cell Signaling Technology, Inc., Danvers, MA; ⁵Yurogen Biosystem, Worcester, MA; ⁶The Michael J. Fox Fndn. For Parkinson's Res., New York, NY

Abstract: Identifying robust biomarkers and pathway readouts for Parkinson's disease remains a significant challenge, in part because molecular including protein lipids and metabolic changes may be concentrated within specific organelles. The development of the Lyso-IP system using HA-tagged TMEM192 to isolate intact organelles for downstream analyses demonstrated that isolating intact organelles can reveal disease-relevant changes in the lysosome that are not detectable without organelle isolation. While this system and the expansion of this system to HA-tag other organelles is aiding our understanding of organelle-biology, it relies on genetic tagging, limiting its use in non-transgenic systems and patient samples. To address this gap, The Michael J. Fox Foundation (MJFF), in collaboration with the University of Dundee and antibody developers, generated reagents to support isolation of intact organelles in untagged samples. As many antibodies for candidate organelle-IP targets were already commercially available, we first evaluated the ability of multiple commercial clones to immunoprecipitate organelles. In doing so we identified antibodies to TMEM192 to immunoprecipitate lysosomes, EEA1 to immunoprecipitate endosomes, and synaptophysin antibodies to immunoprecipitate synaptic vesicles. All clones showed significant enrichment of the organelles in cell lines and mouse brain, with additional testing in patient samples ongoing. For isolation of lysosomes, Golgi, and mitochondria, we developed novel antibodies in partnership with ABclonal and Cell Signaling Technology. Through ABclonal, two novel TMEM192 antibodies were developed which successfully isolate mouse and/or human lysosomes with high efficiency. In partnership with Cell Signaling Technology, promising antibody clones have been developed to isolate lysosomes through TMEM192 antibodies, mitochondria through OMP25 antibodies, and Golgi through TMEM115 antibodies using HA-tagged cell lines to compare the tagged vs tagless isolation methods. Ultimately, these antibodies will enable the isolation of tag-free organelles from diverse systems, including patient bio-samples, thereby accelerating biological understanding and development more sensitive biomarkers for Parkinson's disease.

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Poster

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Topic: C.03. Parkinson's Disease

Support: NIH Grant R35HG011959-03

Title: Variant-to-gene mapping identifies candidate effector genes for Parkinson's disease, including the lysosomal transporter gene ABCB9

Authors: *J. FARYEAN¹, S. LEWKIEWICZ², N. TULINA², A. CHESI¹;
²Pathology, ¹Univ. of Pennsylvania, Philadelphia, PA

Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by the accumulation of misfolded α -synuclein protein within neurons. While a combination of aging, genetic predisposition, and environmental exposures is thought to contribute to PD pathogenesis, the precise molecular mechanisms remain incompletely understood. Increasing evidence from Genome Wide Association Studies (GWAS) points to genetic dysregulation as a key contributing factor. To date, GWAS have identified 90 genetic loci associated with PD containing single nucleotide polymorphisms (SNPs) located in intergenic or intronic regions. We hypothesize that these SNPs lie within cis-regulatory elements (cREs), where they influence the expression of effector genes involved in PD, and that such genes may serve as potential targets for therapeutic intervention. To identify these effector genes, we employed a variant-to-gene mapping strategy based on 3D genomics. We generated ATAC-seq, RNA-seq and Hi-C datasets from the human neuronal cell line ReNcell VM. By integrating these data, we prioritized SNP-gene pairs in which the SNP resides in open chromatin and forms a three-dimensional contact with the open promoter of an expressed gene. This approach identified 47 SNPs within candidate cREs that interact with 45 gene promoters. Pathway analysis revealed that these genes are enriched in biological processes relevant to PD, including lysosomal trafficking, inflammation, and calcium signaling. We elected to functionally dissect one of these candidate pairs, SNP rs10847864 which showed a contact with the promoter of ABCB9, a lysosomal gene. Using a luciferase reporter assay, we confirmed that the 1 kb region containing rs10847864 functions as an enhancer, significantly increasing transcription of the reporter gene. Using CRISPR interference (CRISPRi), we showed that transcriptional repression of this enhancer region results in downregulation of ABCB9 expression, supporting a functional regulatory interaction. Ongoing work is focused on developing assays to assess lysosomal function and oxidative stress in neuronal cells, which will help elucidate how ABCB9 dysregulation contributes to PD pathophysiology.

Disclosures: J. Faryean: None. A. Chesi: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Support: NIEHS F31ES036890
NIEHS 1R01ES034846
NIEHS R00ES029986
Parkinson's Foundation 1064207
DoD Toxin Exposure Research Program TX220241
American Parkinson's Disease Association 1058111

Title: Inhalation of the Parkinson's disease risk factor trichloroethylene induces blood brain barrier dysfunction as a mechanism for cognitive impairment

Authors: *A. ADAMSON¹, T. WALTERS³, A. M. SMITH⁴, B. R. DE MIRANDA²;
²Neurol., ¹Univ. of Alabama at Birmingham, Birmingham, AL; ³Neurol., The Univ. of Alabama at Birmingham, Birmingham, AL; ⁴Dept. of Neurobio., Univ. Of Mississippi Med. Ctr., Jackson, MS

Abstract: The organic solvent trichloroethylene (TCE) is a ubiquitous environmental contaminant that is associated with Parkinson's disease (PD) risk (odds ratio [95% CI]; 1.7 [1.39-2.07]) and poor long-term cognitive performance (2.11 [1.78-2.50]). Recently published research from our lab showed that 12 weeks of TCE exposure (100 ppm) via inhalation in adult mice induces parkinsonian pathologies (i.e., dopaminergic neurodegeneration in the substantia nigra, accumulation of phosphorylated α Syn, and neuroinflammation) as well as spatial memory impairment. In addition, we observed an increase of senescent cell populations and a loss of adult neurogenesis within TCE-exposed animals, suggesting that brain aging phenotypes may be a potential driver of TCE-induced neurodegeneration and cognitive dysfunction. Many pathologies associated with brain aging, such as blood brain barrier dysfunction, cellular senescence and loss of adult neurogenesis, have been observed in cases of Parkinson's disease and dementia. Interestingly, substantial research demonstrates that many environmental toxicants that influence the risk for Parkinson's disease all convey toxicity onto the blood brain barrier. Therefore, we hypothesize that TCE disrupts the blood brain barrier as a mechanism for cognitive impairment. To investigate this, we employed the p16-3MR mouse line that allows reporting of senescence by driving RFP expression upon p16 promoter binding. The trimodal transgene can also induce clearance of senescent cells through expression of a ganciclovir (GCV)-sensitive element. We exposed 3-month-old p16-3MR mice to 100 ppm TCE inhalation with 25 mg/kg GCV treatment (or saline control) over 12 weeks. We observed that TCE exposed mice had significant gait impairments ($p = 0.0014$), and this effect was lost in TCE mice treated

with GCV. Mice exposed to TCE also displayed increased depressive-like behavioral despair ($p < 0.0001$) that was reduced by GCV treatment. In brain tissue, we observed a significant reduction in adult neurogenesis in the subgranular zone ($p = 0.0003$) in TCE-exposed mice that was rescued by GCV administration ($p = 0.0007$). We also observed an increase in senescence in the endothelial cells of cortical vessels in TCE-exposed mice ($p = 0.0048$) that was reduced by GCV administration ($p = 0.0009$). Together, these data show that inhaled TCE exposure influences cognitive impairment and behavioral deficits, at least in part, by inducing cellular senescence in the blood brain barrier. These data highlight a role for environmental exposures in influencing the heterogeneity of cognitive impairment in PD with potential impacts on neurologic aging.

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Poster

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Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS075321

Title: Cholinergic levels and hallucinations in Parkinson disease

Authors: *J. MEHTA^{1,3,2}, J. O'DONNELL³, S. GROSSEN³, A. M. EID³, P. T. KOTZBAUER³, J. S. PERLMUTTER^{3,4,5,6,7}, M. C. CAMPBELL^{3,7};
²Harvey A. Friedman Ctr. for Aging, ¹Washington Univ. in St. Louis, Saint Louis, MO; ³Neurol., ⁴Program in Physical Therapy, ⁵Program in Occup. Therapy, ⁶Neurosci., ⁷Radiology, Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: A substantial proportion of individuals with Parkinson disease (PD) will eventually develop hallucinations. These hallucinations tend to progress from minor hallucinations (passage, presence, illusions) to fully formed visual hallucinations (VHs), to multisensory hallucinations. Existing research suggests that PD participants with hallucinations demonstrate increased internally directed attention and low-frequency thalamocortical oscillations. However, the mechanism(s) behind these differences and the connection to neuropathology remains unknown. The purpose of this study was to investigate the relationship between the presence/development of hallucinations in PD and cholinergic denervation. PD participants (N = 70) completed baseline vesicular acetylcholine transporter (VAT) PET scans to measure cholinergic function and longitudinal psychiatric evaluations for up to 6 years to assess hallucinations. Baseline VAT PET binding was compared between 22 PD participants with VHs in the past 6 months and 41 PD without current or previous hallucinations. Correlation analyses assessed the relationship between regional VAT PET binding and the Psychosis and

Hallucinations Questionnaire (PsychH-Q), evaluating the severity/frequency of minor to multisensory hallucinations (N = 29). Baseline regional VAT PET binding was compared between those who did (N = 15) and did not (N = 16) develop hallucinations at follow-up. Results demonstrate that, after controlling for age, sex, and duration since motor onset, PD participants with VHS had less VAT binding than those without VHS in the pons, thalamus, and ventral diencephalon as well as the lateral occipital, precuneus, fusiform, insular, middle temporal, precentral, and caudal middle frontal cortices ($p < 0.05$, 10% FDR correction). More severe/frequent hallucinations were associated with lower VAT binding in the thalamus and regions of the occipitoparietal and middle frontotemporal cortices ($p < 0.05$, 10% FDR correction). Finally, lower VAT binding in the ventral diencephalon and fusiform, insular, temporal, precentral, and middle frontal cortices was correlated with the development of VHS 2-6 years later ($p < 0.05$, 10% FDR correction). These findings implicate multifocal loss of cholinergic input and suggest that cholinergic deficits relate to the presence and development of hallucinations in PD. Interestingly, these regions overlap with several large-scale brain networks, including dorsal attention, ventral attention, and default mode networks. Future research would benefit from coupling the present results with EEG or fMRI to delineate the functional impact of cholinergic deficits in PD.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.22/D53

Topic: C.03. Parkinson's Disease

Title: Characterisation of TMEM175 wild type, gain-of-function and loss-of-function lysosomal coding variants expressed in recombinant HEK-293 cells using automated electrophysiology

Authors: D. PAU, S. RICE, R. FINOCCHIARO, E. BLACK, *L. HUTCHISON, D. DALRYMPLE, I. MCPHEE;
Sygnature Discovery, Glasgow, United Kingdom

Abstract: TMEM175 is a constitutively active ion channel with dual functionality, acting as both a potassium channel (K^+) and a proton-activated proton channel (H^+). It plays a key role in the regulation of lysosomal pH, membrane potential and autophagy. Mutations in this gene have been shown to impair normal lysosomal pH homeostasis, which may result in reduced hydrolytic enzyme activity and increased aggregation of insoluble proteins such as phosphorylated α -synuclein, a hallmark of Parkinson's Disease (PD). Such protein aggregation can lead to cell toxicity and death, contributing to the degenerative physical and cognitive symptoms typical of PD. One such mutation is the Loss-Of-Function (LOF) p.M393T, which has been associated with an increased risk of PD in humans. By contrast, Gain-of-Function (GOF) mutations such as the p.Q65P have been linked with a reduced risk of developing PD in humans. Consequently, there is significant potential in the role of TMEM175 as a druggable therapeutic target, and an avenue to further our understanding of the pathology and treatment of PD.

Modulation of channel activity may provide a mechanism to help control pH homeostasis, promoting a favourable lysosomal environment resulting in a reduction in the build-up of toxic aggregates such as α -synuclein. Characterization of TMEM175 and its coding variants may provide important insight into how alterations in channel structure relate to wider lysosomal functionality, and by extension the downstream pathological processes related to PD. For this reason, we have developed robust, high quality recombinant HEK-293 cell lines expressing TMEM175 Wild Type (WT), M393T (LOF) and Q65P (GOF) coding variants.

We detail the electrophysiological characterization of these TMEM175 variants, including proton-activated-proton conductance and pharmacological assessment of known TMEM175 activators and inhibitors, performed using a high-throughput automated electrophysiology platform (SyncroPatch 384i, Nanion). This assay can be used to identify and characterize novel pharmacological tools for TMEM175 drug discovery, furthering the investigation of this target and its involvement in PD and other neurodegenerative diseases.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.23/D54

Topic: C.03. Parkinson's Disease

Title: Evaluating TMEM175 mutation phenotypes in human iPSC-derived dopaminergic neuron models for Parkinson's disease

Authors: S. SCHACHTELE¹, R. FIENE¹, D. MAJEWSKI¹, M. CURTIS¹, S. DONATO², T. SORBO², F. RICCI², A. MONDINI², S. HILCOVE¹, C. B. CARLSON¹, J. ROLLAND², S. CAINARCA², *E. M. JONES¹;

¹FUJIFILM Cell. Dynamics, Madison, WI; ²Axxam S.p.A., Milan, Italy

Abstract: Genome-wide association studies have associated Parkinson's Disease (PD) risk with mutations in TMEM175, a cation channel important for maintaining lysosomal pH. Specifically, the TMEM175 heterozygous M393T mutant has reduced pH and is associated increased protein aggregates in PD. The role of TMEM175 on lysosomal function has increased its potential as a druggable target for modulating protein degradation in both PD and across neurodegenerative diseases hallmarked by protein aggregates. While TMEM175 overexpressing cell lines were important for initial channel characterization, studies in human-relevant dopaminergic neurons are needed to link channel physiology with cellular biology and PD disease pathology. This study establishes and characterizes a panel of human induced pluripotent stem cell (iPSC)-derived midbrain dopaminergic neurons containing PD-relevant TMEM175 mutations. To this end, dopaminergic neurons (iCell DopaNeurons) were differentiated from isogenic iPSC donor lines containing various TMEM175 mutations, including the PD-relevant heterozygous M393T variant, an apparently healthy normal M393M control, a loss-of-function T393T mutation, or the Q65P gain-of-function mutation. The reproducibility of the differentiation protocol, licensed from Memorial Sloan Kettering Cancer Center, is demonstrated by the robust and consistent expression of characteristic midbrain dopaminergic neuron markers (FoxA2, TH, and BIII-tubulin), with levels comparable across mutations indicating neurons of similar purity and quality. iPSC-derived dopaminergic neurons should develop spontaneous and synchronized network bursts in culture. Indeed, using microelectrode array (MEA), all mutants achieved synchronized bursting with preliminary data suggesting similar firing patterns. Lysosomal patch-clamp experiments were performed to evaluate the effects of TMEM175 mutations lysosome function. Compared to M393M neurons, the M393T mutation showed a slight reduction in conductance, while conductance was significantly reduced in the T393T loss-of-function mutant. Conversely, conductance in the Q65P mutant was significantly increased. These changes align with results reported in previous literature using TMEM175 overexpressing cell lines. Using cell-based assays to evaluate lysosomal and mitochondrial function, our preliminary data also showed phenotype variation across mutations. This study shows that iPSC-derived dopaminergic neurons containing TMEM175 mutations exhibit expected channel physiology and offer unique phenotypic differences, making them a useful addition for PD drug discovery research.

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Poster

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Program #/Poster #: PSTR325.24/D55

Topic: C.03. Parkinson's Disease

Title: Developmental programmed cell death in substantia nigra: A plausible predisposition risk to Parkinson's disease

Authors: *Y. HAOREI¹, V. D J², P. A. ALLADI³;

¹Zoology Department, Maitreyi Col., Univ. of Delhi, Delhi, India; ²Neurosci. and Neurol., Rosalind Franklin Univ. of Med. & Sci. (RFUMS), North Chicago, IL; ³Clin. Psychopharmacology and Neurotoxicology, Natl. Inst. of Mental Hlth. and Neurosci., Bangalore, India

Abstract: Parkinson's disease has differential prevalence among ethnic groups. Molecular mechanisms of susceptibility are unknown. Similarly, different mice strains have varying susceptibility to neurotoxin MPTP. Strains with lesser basal DAergic neurons are more susceptible to MPTP than to those with higher neuron numbers in substantia nigra. We compare nigral DAergic neurons in mouse strains C57BL/6J, CD-1 and their F1 crossbreds with respect to developmental apoptosis in the nigra and to assess MPTP-induced degeneration at adulthood. Biphasic wave of peak apoptotic period was observed at postnatal day P2 and P14 in all the strains. The DAergic neurons in the C57BL/6 undergo more protracted apoptosis compared to CD-1 or crossbreds. Cell death markers caspase-3 and AIF were evaluated at P2 and P14, the two peak periods of apoptosis. AIF expression was highest at P14 whereas caspase-3 expression went on to peak at P22. The increased expression of apoptotic factors appears to be associated with the corresponding timing of maximum neuronal apoptosis during development. In contrast to what we saw an upregulation of GDNF in the striatum during development, its expression in the nigra reduced significantly as developmental time progresses. On attaining adulthood, the evaluation of mice treated with MPTP revealed maximal neuronal degeneration, altered Bcl-2 family, up-regulation of AIF, ultrastructural changes and decreased mitochondrial complex activity in the susceptible C57BL/6 as compared to CD-1 or the crossbreds. Thus, we provide amongst the first conclusive evidences of the existence of strain specific differences in mice strains which may reflect the heterogeneity amongst healthy individuals to develop PD and the phenomenon of ethnicity-based differential prevalence of the disease. The differences in the basal nigral neuronal numbers in the adult may be a consequence of differential developmental apoptosis in these mice strains. The vulnerability to a neurodegenerative disease may arise early during development. Our experimental model can be used to further evaluate the tandem molecular events of differential PD prevalence.

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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

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Program #/Poster #: PSTR325.25/D56

Topic: C.03. Parkinson's Disease

Title: Mechanistic insights and therapeutic strategies targeting lysosomal dysfunction in Parkinson's Disease

Authors: *N. CALLIZOT¹, G. CULLEY^{2,3}, C. BOTTO³, L. ROUVIERE³, V. DE CONTO³, A. HENRIQUES⁴, P. POINDRON³;

¹Neuro-Sys, Inc., Gardanne, France; ²Univ. of Gothenburg, Goeteborg, Sweden; ³Neuro-Sys, Gardanne, France; ⁴Neuro-Sys, GARDANNE, France

Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons and the accumulation alpha-synuclein (asyn) aggregates. Lysosomal dysfunctions are now well known to be key elements in pathogenesis. Neuronal health is highly dependent of lysosomal health any change (lysosomal enzyme dysfunction, lipid accumulation, protein burden, etc.) contribute to neuronal toxicity and finally cell death. Several key mechanisms underlie lysosomal dysfunction in PD: a) Lysosomal Enzyme Deficiency i.e. glucocerebrosidase (GCase), b) Lysosomal Membrane Permeabilization leading to the release of lysosomal contents into the cytoplasm and subsequent cellular damage, c) autophagy-Lysosome Pathway Impairment, d) Lysosomal Trafficking Defects. Understanding the mechanisms of lysosomal dysfunction in PD opens new avenues for therapeutic intervention. Strategies aimed at enhancing lysosomal function hold promise for the treatment of PD. Here, using in vitro models of PD (primary dopaminergic neurons, GBA1 mutated cells lines and IPS cells all injured with asyn protofibrils (PFF)), we carefully investigated the asyn PFF impact on the lysosomal health (enzyme function, membrane integrity, autophagosome formation and trafficking) over time. Additionally, we tested the impact on the neuronal survival of small molecules that stabilize lysosomal membranes, improve lysosomal trafficking or restoring the trafficking. We showed that, depending on targeted pathways, their impact on survival is not equivalent. They can be protective or, on the contrary, deleterious, accentuating neuronal stress. In conclusion, restoration of lysosomal homeostasis is an obvious strategy in the fight against neuronal death. However, repair pathways must be manipulated with caution, as they cannot all be activated at the same time and may exacerbate the disease if treatment windows are not adjusted appropriately.

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Poster

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Program #/Poster #: PSTR325.26/D57

Topic: C.03. Parkinson's Disease

Support: ZIA DA000618

Title: Increased KDEL receptor expression in rat midbrain alters dopaminergic phenotype

Authors: *L. K. GREER, K. E. SAVELL, A. F. HOFFMAN, B. T. HOPE, C. R. LUPICA, B. K. HARVEY;

NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD

Abstract: Dopaminergic neurons (DANs) are key regulators of reward and movement. This neuronal population is uniquely sensitive to degeneration in Parkinson's disease (PD), the second most common neurodegenerative disease. While the causes of dopaminergic neuron degeneration in PD are unknown, a common feature found in many PD animal models and human post-mortem PD tissue is the prevalence of endoplasmic reticulum (ER) stress and protein misfolding, representing disruptions in homeostasis. The ER is the primary intracellular site of protein folding, protein processing, and calcium storage. ER resident proteins such as chaperones and isomerases localize to the ER lumen and mediate these homeostatic functions. KDEL receptors (KDELRs) are transmembrane proteins localized to the cis-golgi and play a crucial role in retaining ER resident proteins, particularly during cellular stress. Our work has shown that KDELRs are responsive to ER stress, with their expression increasing to enhance the retention of ER proteins and maintain protein homeostasis. Given that ER resident proteins are central to protein processing, changes to KDEL receptor expression could impact protein homeostasis through controlling the localization of ER resident proteins and contribute to pathologies where ER stress is implicated. Despite this, KDEL receptors are understudied in the brain, and their function in DANs and resulting dopamine dynamics is unknown. In the current study, we increased expression of KDEL receptor in the rat midbrain and examined effects on dopaminergic neurons at the transcriptional and functional level. We isolated midbrain tissue and performed single nucleus RNA-sequencing to characterize the transcriptional landscape after KDEL receptor modulation. Additionally, we characterized the functional consequences of increasing KDEL receptor expression by fast scanning cyclic voltammetry to assess dopamine release and uptake. This project provides new insight into the contribution of KDEL receptors to neuronal phenotype and may provide important information into mechanisms of neuronal protein homeostasis under both normal and pathophysiological conditions.

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Poster

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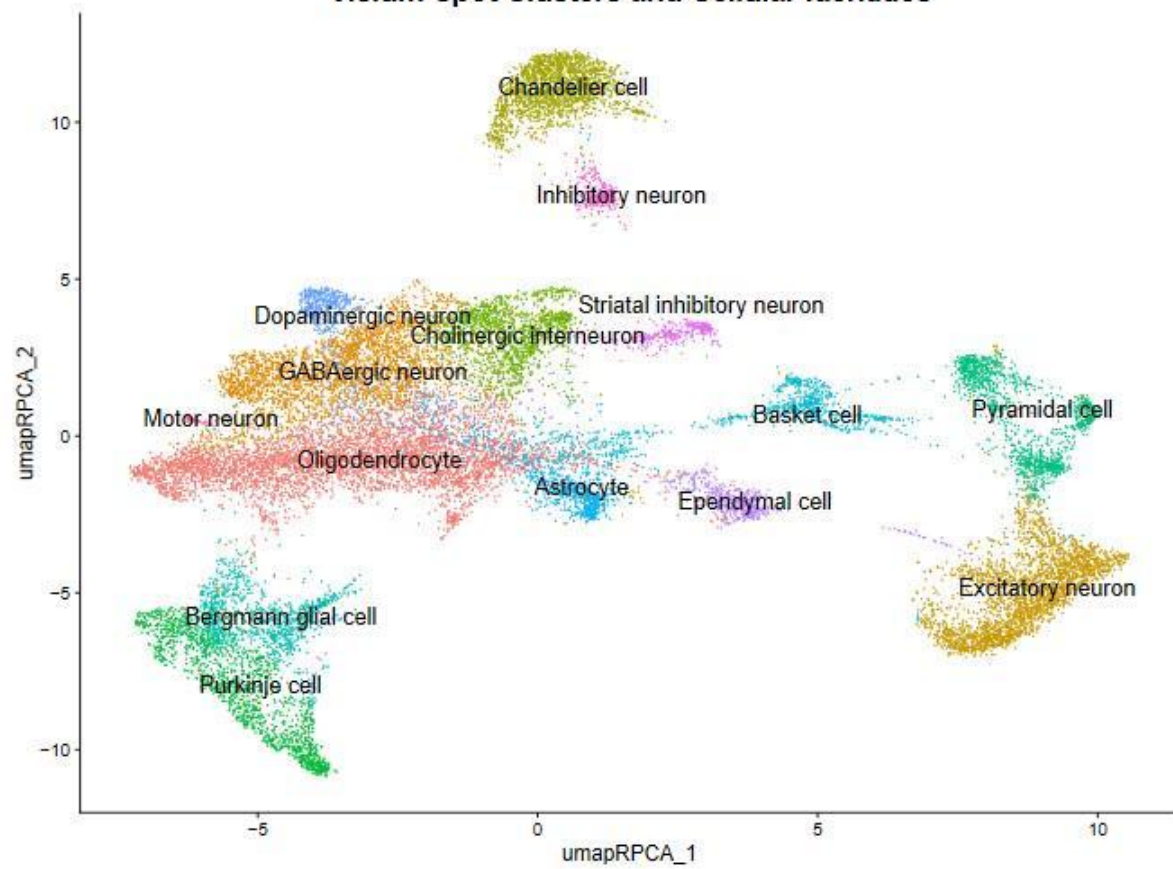
Support: NIH Grant P20GM104360
NIH Grant P20GM113123

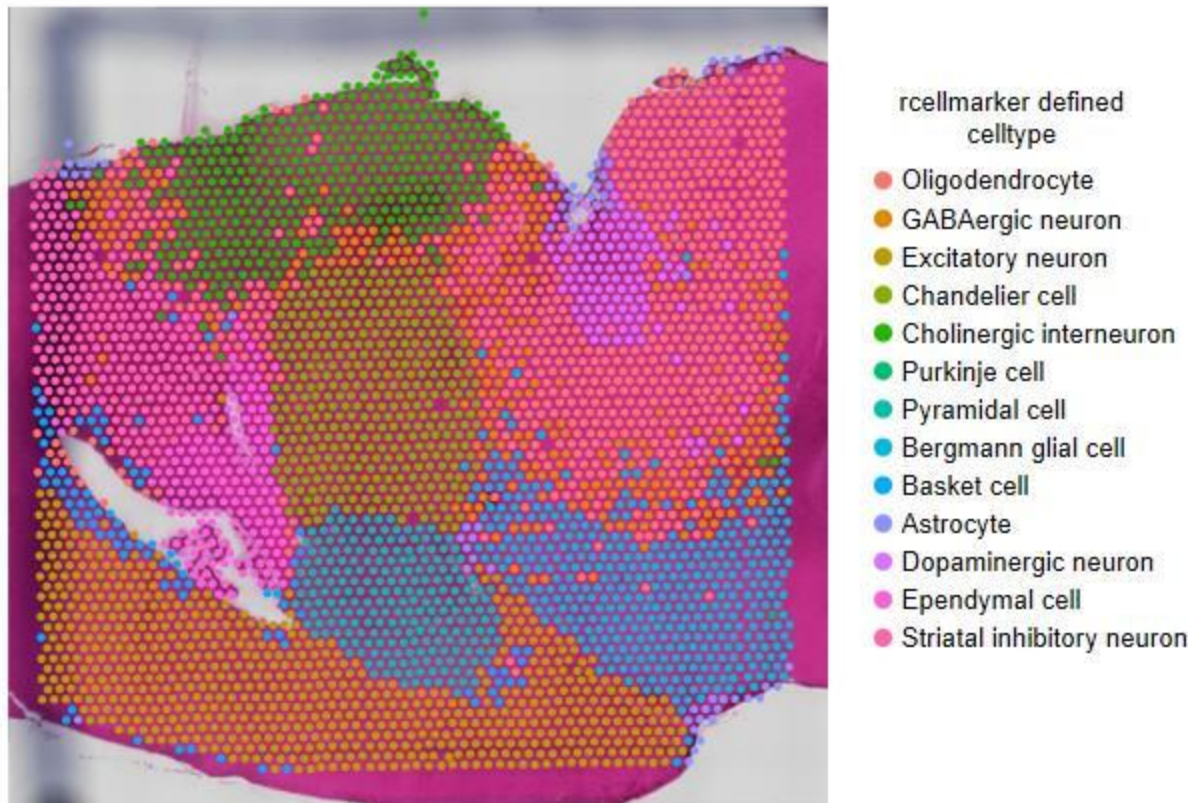
Title: Midbrain spatial gene expression in the thyl- α -synuclein mouse model of Parkinson's disease

Authors: *B. A. MCGREGOR¹, M. RAIHAN³, K. GUO⁴, J. PORTER², J. HUR²;
²Biomed. Sci., ¹Univ. of North Dakota, Grand Forks, ND; ³Pharmaceut. Sci., Chicago State Univ. Sch. of Pharm., Chicago, IL; ⁴Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by aggregated α -synuclein, loss of dopaminergic neurons, and Lewy body formation. This study used a transgenic mouse model of early-stage PD to examine spatial gene expression in the midbrain at critical points associated with behavioral and pathological changes. Brain tissues were collected from three wildtype and transgenic male mice at two and seven months to capture early and progressive disease stages from our total cohort of twelve animals. To define our region of interest, sequential midbrain slices were stained for tyrosine hydroxylase to identify regions containing vulnerable dopaminergic neuron populations. Sample tissue sections were collected from the corresponding depth for H&E staining and Visium CytAssist spatial transcriptomics analysis. Our initial analysis revealed diverse neuronal populations, including dopaminergic neurons, as well as astrocytes and oligodendrocytes, across samples. We observed evidence of a reactive glial phenotype, highlighting a complex relationship between α -synuclein accumulation and neuroinflammatory responses. Ongoing analyses aim to further characterize cell type-specific expression profiles within brain regions affected by synuclein pathology. These findings offer crucial insights into cellular responses to pathological α -synuclein aggregation and how these responses may evolve throughout disease progression.

Visium Spot Clusters and Cellular Identities





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Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.28/D59

Topic: C.03. Parkinson's Disease

Title: Toxic effects of pesticides and environmental chemicals on dopaminergic neurons and their molecular mechanisms in parkinson's disease

Authors: *C. WU¹, T.-C. YANG²;

¹Taipei Med. Univ., Xinyi Dist., Taipei City, Taiwan; ²Dept. of Anat. and Cell Biol., Taipei Med. Univ., Taipei, Taiwan

Abstract: Sodium chlorate (NaClO₃) is a strong oxidizing agent widely used as a non-selective herbicide and more commonly for chlorine dioxide (ClO₂) production in water treatment and

food processing. However, its impact on brain health remains poorly understood, particularly in relation to Parkinson's disease (PD). Recent genome-wide association studies have revealed a potential link between NaClO₃ exposure and increased PD risk, though the underlying mechanisms are still unclear and underexplored. Well-established environmental toxins such as rotenone and paraquat are known to induce PD-like pathology through oxidative stress, mitochondrial dysfunction, and α -synuclein aggregation, ultimately leading to neuronal death. To investigate whether NaClO₃ exerts similar neurotoxic effects, this study employs human induced pluripotent stem cell (hiPSC)-derived dopaminergic neurons as a model to systematically examine its effects. Our preliminary data reveal that NaClO₃ induces dose-dependent cell death and senescence. This project will further investigate whether NaClO₃ mediates neurodegeneration through pathways involving cell cycle inhibitors (p16, p21), apoptotic regulators (BCL-2, Caspases), and aggregation of α -synuclein and tau proteins. This is the first study to comprehensively assess the neurotoxic effects of NaClO₃ on dopaminergic neurons. The findings are expected to provide new insights into the potential neurological risks posed by oxidizing agents used in pesticides, water disinfection, and food processing—especially their relevance to the pathogenesis of Parkinson's disease.

Disclosures: C. Wu: None. T. Yang: None.

Poster

PSTR325: Parkinson's Disease: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR325.29/D60

Topic: C.03. Parkinson's Disease

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ICMR SENIOR RESEARCH FELLOWSHIP
CSIR FOR FELLOWSHIP

Title: Pharmacological activation of transcriptional factor EB (TFEB) by a novel small molecule restores mitochondrial and lysosome crosstalk in Parkinson's disease

Authors: *J. MOHAMED AMEER¹, S. ASMI T³, D. SANTHOSHKUMAR⁴, G. CHANDRA²;

¹Ctr. for development and aging research (cell biology), Inter-university centre for biomedical research and super speciality hospital, KOTTAYAM, India; ²Ctr. for development and aging research (cell biology), Inter-university centre for biomedical research and super speciality hospital, Kottayam, India; ³Inter-University Ctr. for Biomed. Res. and Super Speciality hospital, Kottayam, Kerala, Kottayam, ; ⁴Neuropathology, Natl. Inst. of Mental health and neurosciences, Bengaluru, India

Abstract: Mitochondrial dysfunction and lysosomal impairment are two converging pathological features of Parkinson's disease (PD), yet the mechanistic link between these

organellar deficits remains incompletely understood. In this study, we investigated how mitochondrial stress contributes to lysosomal dysfunction in a cellular model of PD. Differentiated SH-SY5Y neuroblastoma cells exposed chronically to 1-methyl-4-phenylpyridinium (MPP⁺, 500 μ M) exhibited pronounced mitochondrial fragmentation and elevated reactive oxygen species (ROS) production. These mitochondrial alterations were accompanied by lysosomal deficits, including aberrant pH, impaired cathepsin maturation, and disrupted proteostasis. Notably, such lysosomal disturbances were observed only following chronic, but not acute, MPP⁺ exposure, highlighting a temporal threshold for stress-induced lysosomal pathology. Mechanistically, we identified the formation of ROS-enriched mitochondrial-derived vesicles (MDVs) marked by TOMM20, which trafficked toward lysosomes. This vesicular communication coincided with a downregulation of Parkin and the accumulation of Sorting Nexin 9 (SNX9), suggesting a Parkin-SNX9-dependent pathway mediating mitochondrial-to-lysosomal stress signaling. Importantly, overexpression of transcription factor EB (TFEB), a key regulator of lysosomal biogenesis, restored lysosomal function, preserved mitochondrial integrity, and significantly attenuated MPP⁺-induced neurotoxicity. Furthermore, we discovered a natural compound derived from *Withania somnifera*, interacts with TFEB and enhances its expression, offering a pharmacological avenue for restoring mitochondrial-lysosomal homeostasis. Collectively, our findings uncover a novel Parkin-SNX9-mediated mechanism by which chronic mitochondrial stress compromises lysosomal function and establish TFEB activation as a promising therapeutic target. The identification of a novel small molecule as a TFEB activator further supports the potential of targeting mitochondrial-lysosomal crosstalk in the treatment of Parkinson's disease.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.01/D61

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's (Grant No ASAP-020505)
Spanish Ministry of Science and Innovation (Grant No PID2023-127802OB-I00)

Title: Use of a BBB-penetrant AAV9 capsid variant to develop a novel pigmented mouse model of Parkinson's disease

Authors: J. CHOCARRO^{1,2,4}, A. J. RICO^{2,1,4}, G. ARIZNABARRETA^{1,2,4}, M. CHILLON^{5,6,7}, M. VILA^{8,2,4,7}, A. M. GARCIA-OSTA¹, M. CUADRADO-TEJEDOR¹, *J. L. LANCIEGO^{1,3,4};
¹CNS Gene Therapy Program, CIMA - Univ. of Navarra, Pamplona, Spain; ³CNS Gene Therapy

Program, ²CiberNed, Madrid, Spain; ⁴Aligning Sci. Across Parkinson's, Chevy Chase, MD; ⁵Biochem. and Mol. Biol., Univ. Autònoma Barcelona, Bellaterra, Spain; ⁶Val d'Hebron Res. Inst., Barcelona, Spain; ⁷Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain; ⁸Vall d'Hebron Res. Inst., Barcelona, Spain

Abstract: There is a pressing need for the development, characterization, and standardization of animal models of Parkinson's disease (PD) that properly mimic the cardinal features of this disorder, comprising both the motor phenotype and neuropathological signatures. In the past few years, animal modeling has moved from neurotoxin-based approaches toward viral vectors carrying a given genetic payload of interest. Here, to induce pigmentation of the mouse brain upon systemic delivery, we took advantage of a modified adeno-associated viral vector capsid engineered to bypass the blood-brain barrier and coding for the human tyrosinase gene (AAV9-P31-hTyr). Obtained results revealed an ongoing pigmentation of catecholaminergic centers related to the pathophysiology of PD, such as the substantia nigra pars compacta, ventral tegmental area, and locus coeruleus. Moreover, pigmented dopaminergic neurons exhibited Lewy body-like intracytoplasmic inclusions, a progressive nigrostriatal degeneration, and a time-dependent PD motor phenotype. The bilateral pigmented mouse model of PD generated this way is highly reproducible, does not require stereotaxic surgery for viral vector deliveries, and opens unprecedented possibilities for preclinical testing of therapeutic candidates designed to reduce disease progression rates.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.02/D62

Topic: C.03. Parkinson's Disease

Support: Olle Engkvist's Foundation
The Royal Physiographic Society of Lund
MultiPark – a Strategic Research Area at Lund University
Anna-Lisa Rosenberg Foundation

Title: Metabolic conditions of Type 2 Diabetes as a risk factor for Parkinson's disease

Authors: *C. FRYKLUND, M. SWANBERG;
Lund Univ., Lund, Sweden

Abstract: Epidemiological studies have identified Type 2 Diabetes (T2D) as a risk factor for Parkinson's disease (PD), yet the underlying mechanisms remain unclear. T2D is characterised

by both insulin resistance and insufficient insulin secretion, leading to chronic hyperglycaemia. However, the relative contributions of insulin resistance and hyperglycaemia, individually or in combination, to PD pathogenesis remain to be elucidated. In this study, we use a prodromal mouse model of PD combined with metabolic interventions that mimic hyperglycaemia, insulin resistance, and T2D to investigate how these metabolic conditions influence PD-like pathology and motor function. We use C57BL6/J mice hemizygous for Engrailed-1 (*En1*^{+/-}), a model that recapitulates early pathology of PD, but without dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc). *En1*^{+/-} and wild-type littermates were divided into four experimental groups: (1) control (chow diet), (2) insulin resistance (high-fat diet, HFD), (3) hyperglycaemia (streptozotocin, STZ), and (4) T2D (combination of HFD and STZ). The dietary intervention was maintained for 25 weeks, with STZ administered from week 7 to mimic the natural onset of T2D, i.e. insulin resistance followed by insufficient insulin secretion and hyperglycaemia. Metabolic status was monitored via fasting blood glucose and glucose tolerance tests. Motor function was evaluated using open field testing, and nigrostriatal neurodegeneration was assessed post-mortem through immunohistochemistry. Metabolic profiling confirmed the successful induction of insulin resistance, hyperglycaemia, and combined T2D phenotypes in both *En1*^{+/-} and WT mice. Open field testing revealed that *En1*^{+/-} mice exhibited reduced locomotion relative to WT controls, a phenotype that was further exacerbated in the STZ-induced hyperglycaemic group. Our preliminary findings suggest that hyperglycaemia, rather than insulin resistance alone, exacerbates motor deficits in genetically predisposed *En1*^{+/-} mice. These results suggest that chronic hyperglycaemia may represent a key pathological link between T2D and the increased risk of PD. However, open field results are only indicative of neurodegeneration. Ongoing analyses, including quantification of dopaminergic neuron loss in the SNpc, are essential to fully assess how each metabolic condition influences PD-related neurodegeneration.

Disclosures: C. Fryklund: None. M. Swanberg: None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.03/D63

Topic: C.03. Parkinson's Disease

Title: Investigating Neuro-Cellular Stress Responses to Boston Tunnel Particulate Matter in *Caenorhabditis elegans*

Authors: *R. ASAOLU;
Worcester Polytechnic Inst., Worcester, MA

Abstract: Air pollution, particularly fine particulate matter (PM_{2.5}), is a growing global health concern implicated in the onset and progression of neurodegenerative diseases such as

Parkinson's disease. Despite epidemiological associations, the mechanistic underpinnings of PM2.5-induced neurotoxicity remain poorly defined. Our lab employs *Caenorhabditis elegans*, a genetically tractable in vivo model, to investigate how PM2.5 collected from the Boston Tunnel affects neuro-cellular function, behavior, and disease relevant pathology.

Our preliminary findings reveal sex-specific suppression of aversive behavior in PM2.5 exposed hermaphrodites, with less pronounced effects in males, mirroring observed sex disparities in Parkinson's incidence. Following that one of the key mechanisms by which air pollutant causes neurodegeneration is through oxidative stress. We observed that oxidative stress reporter strains show elevated fluorescence following PM2.5 exposure compared to the unpolluted strain. This indicates that the air pollution is causing oxidative stress. We are currently assessing dopaminergic neuron integrity and α -synuclein aggregation using Parkinson disease model strains.

Overall, our findings highlight the neuro-cellular stress responses elicited by real-world PM2.5, an environmental pollutant and its link to neurodegeneration. By leveraging *C. elegans* as a whole-organism model, this research provides critical insight into the environmental determinants of Parkinson disease pathology and may guide future preventative or therapeutic strategies.

Disclosures: R. Asaolu: None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.04/D64

Topic: C.03. Parkinson's Disease

Support: T32GM144856
R35ES035043-01

Title: Aldehyde dehydrogenase 1 and 2 double knockout protects against dopaminergic neuron loss and immune activation induced by sub-chronic dieldrin exposure

Authors: *O. A. YANOURI¹, S. M. ROCHA², A. M. BRIGGS³, R. STRONG⁴, J. A. DOORN⁵, R. B. TJALKENS³;

¹Cell. and Mol. Biol., ²Microbiology, Immunol. and Pathology, ³Envrn. and Radiological Hlth. Sci., Colorado State Univ., Fort Collins, CO; ⁴Pharmacol., Univ. of Texas Hlth. Sci. Cntr, San Antonio, TX; ⁵Pharmaceut. Sci., Univ. of Iowa, Iowa City, IA

Abstract: Parkinson's disease (PD) is the second most prevalent and fastest growing neurodegenerative disease globally. Environmental toxins, including the organochlorine pesticide dieldrin, have been linked to an increased risk of PD. The mechanism of dieldrin's dopaminergic (DAn) neurotoxicity remains unclear; however, in vitro studies report an increase

in the reactive metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) in dieldrin-treated cultures, linking the neurodegenerative effects of dieldrin to perturbed dopamine (DA) metabolism. We hypothesize ablation of aldehyde metabolism in dieldrin-exposed mice will result in the accumulation of reactive DA metabolites, including DOPAL, that will exacerbate DAn neurotoxicity. To interrogate this hypothesis, 8 week old wildtype (WT) C57Bl/6 and aldehyde dehydrogenase 1 and 2 (ALDH1/2) knockout (KO) mice were dosed daily with dieldrin (0, 1, 3 mg/kg) for 6 weeks via oral gavage. Quantitative pathological examination was performed using immunofluorescence labeling of microglia (IBA1) and DAn (TH) cells, as well as hematoxylin and eosin staining, on sections spanning the striatum (ST) and ventral midbrain, encompassing the substantia nigra pars compacta (SNpc) and pars reticulata (SNpr). High-content microscopy was coupled with unbiased deep learning-based cell identification for the stereological quantification of total and TH⁺ nigrostriatal neurons. Additionally, cell counts and morphometric analysis of IBA1⁺ cells was performed. We identified dose- and genotype-mediated effects on dieldrin toxicity, including a dose-dependent loss of TH⁺ and total neurons in the SNpc in male, but not female, mice. Moreover, male ALDH1/2 KO mice displayed less neurodegeneration compared to WT counterparts. Dieldrin treatment resulted in a dose-dependent decrease in ST TH intensity, as well as a sex and genotype interaction effect. There was a decrease in IBA1⁺ cells in the ST of male WT mice treated with 1 mg/kg dieldrin, in contrast to an increase in female KO mice, associated with a statistical main effect and interaction between dose and genotype. Phenotypic characterization of SNpr IBA1⁺ cells demonstrated dose-, sex-, and genotype-specific variation, with a dose-dependent shift towards an ameboid, disease-associated phenotype in male WT mice. These findings suggest ALDH1/2 KO protects against dieldrin-induced pathological changes in male mice. While the disruption of ALDH1/2 is postulated to drive the neurodegenerative effects of dieldrin, it may be that the loss of ALDH1/2 results in induction of compensatory aldehyde metabolic processes which are less susceptible to disruption by dieldrin.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.05/E1

Topic: C.03. Parkinson's Disease

Title: From Clinic to Bench: Transgenic Mouse Model of Infantile Parkinsonism Based on a Human SLC18A2 Mutation

Authors: *R. ALKHATER^{1,2}, N. STEINER³, F. BEN-RACHED⁴, H. FIUMELLI⁶, P. J. MAGISTRETTI⁵;

¹Johns Hopkins Aramco Healthcare-KAUST, Dhahran, Saudi Arabia; ²King Abdullah Univ. for

Sci. and technology, Thuwal, Saudi Arabia; ³Dept. of Physiol., Lausanne, Switzerland; ⁴King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia; ⁵BESE, King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia; ⁶Biol. and Envrn. Sci. & Engin. Div., KAUST, Thuwal, Saudi Arabia

Abstract: Parkinson's Disease (PD) is the second most prevalent neurodegenerative disorder, typically affecting individuals in their 60s. However, several inherited and young age onset forms are described. Among these is Parkinsonism Dystonia type 2 (PKDYS2), an infantile onset form of parkinsonism caused by mutations in the *SLC18A2* gene. This disorder is defined by developmental regression at age 4 months, dystonia and autonomic dysfunction (OMIM 618049; Rilstone et al., NEJM, 2013). This mutation results in loss of function of the vesicular monoamine transporter 2 (VMAT2), an essential protein involved in packaging monoamines for synaptic transmission. Over the past decade, novel mutations in *SLC18A2* have been reported, expanding the phenotypic spectrum and showing heterogeneous responses to treatment (Saida et al., 2023; Zhai et al., 2023, Al Moqbil et al., 2024). To explore the molecular underpinnings of PKDYS2, we engineered the first viable transgenic mouse model of the disease. This model recapitulates key aspects of the human disorder and provides a unique tools for mechanistic exploration. We are currently completing comprehensive transcriptomic analysis of this model to gain deeper insights into the disorder's pathophysiology and inform the design of targeted therapeutic approaches. Our results explain VMAT2's pivotal role in maintaining monoaminergic homeostasis and map the downstream transcriptional changes caused by *SLC18A2* mutations. This work lays the foundation for future research into disease-modifying therapies that extend beyond symptom management and may open new therapeutic avenues for the treatment of Parkinson's Disease and other related neurodevelopmental disorders.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.06/E2

Topic: C.03. Parkinson's Disease

Support: AHW seed grant Award no- 9520851

Title: Reversibility of Rotenone-Induced Parkinsonism Phenotypes in Mice

Authors: *P. JOSHI^{1,2}, F. FAN², X. LOU²;

¹Cell biology, ²Dept. Cell Biology, Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Parkinson's disease (PD) has long been linked to the exposure of pesticides including rotenone, a mitochondrial toxin that causes irreversible neurodegeneration and progressive motor deficits. This view primarily originates from epidemiological studies showing the correlation between pesticide exposure history and PD diagnosis, which is further supported by preclinical models showing dopamine neuron loss following rotenone treatment. However, none of the two lines of evidence demonstrates a direct, causative role of rotenone in human PD, and rigorous longitudinal studies supporting this view is missing. We address this gap by evaluating the long-term effect of pesticide exposure on PD development in mice. Male mice (C57BL/6 strain, 2-month-old) received either oral (30 mg/kg/day×28 days) or intraperitoneal (IP; 2.5 mg/kg/day×21 days) rotenone, with vehicle-treated mice serving as controls. These mice developed severe Parkinsonism-like deficits as revealed by behavioral assays at 1-month after rotenone treatment. Open field tests revealed significant reduction of travel distance (Oral: 28.3±32.6 m, $p < 0.001$; $n=10$; IP: 40.4±19.8 m, $p < 0.024$; $n=9$) and speed decline (Oral: 18.2±7.9 mm/s, $p < 0.001$; IP: 22.6±10.9 mm/s, $p < 0.024$), with significant anxiety-like behaviors (measured as the reduction of center zone distance travel, Oral: 17.7±10.7 m, $p < 0.001$; IP: 21.7±11.7 m, $p < 0.006$) and immobility (increased by 1.9-fold, oral; 1.6-fold, IP). Notably, rotarod performance declined sharply at 1 month (latency to fall: 82.03±16.4 sec for oral; $p < 0.001$, 58.7±31.9 sec for IP administration; $p < 0.001$) and continued worsening until 4 months post-exposure. Surprisingly, rotenone-treated mice (both oral and IP) exhibited progressive recovery from acute toxicity and became indistinguishable from controls 12-months after the treatment. In open field tests, motor function improved over time, reaching 70-80% of normal levels. Notably, while rotarod deficits stabilized after 4 months (with no further decline), control mice continued to deteriorate. By 12-month after treatment, complete behavioral normalization occurred regardless of administration route, although the oral group recovered faster from greater initial impairment. These longitudinal observations were in contrast to the traditional pesticide neurotoxicity paradigm for PD. In our longitudinal study, rotenone effect followed three distinct phases: (1) acute toxicity (month 1), (2) fast recovery phase (month 2-4), and (3) slow recovery and stabilization phase (month 5- 2). Cellular and molecular investigation is underway to identify the key factors and signals underlying this recovery.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.07/E3

Topic: C.03. Parkinson's Disease

Title: Pink1/Parkin double knockout model of Parkinson's disease in rats - a characterization study

Authors: J. TOIVANEN¹, V. LAZARI², R. RUTTER², L. RAUHALA¹, T. BRAGGE¹, R. IMMONEN¹, J. ROKKA¹, J. K. UHARI-VÄÄNÄNEN¹, A.-M. PENTTINEN¹, **J. RYTKÖNEN¹**, *S. BÄCK¹;

¹Charles River Discovery Res. Services, Kuopio, Finland; ²NRG Therapeut., Stevenage, United Kingdom

Abstract: Mutations in genes coding for PTEN-induced kinase 1 (PINK1) and Parkin RBR E3 ubiquitin-protein ligase (PARKIN) have been linked to familial Parkinson's disease (PD). When functional, these enzymes work in concert in mitophagy, clearing dysfunctional mitochondria from cells. The objective of this study was to observe disease biomarkers in the Pink1/Parkin double knock-out (dKO; Envigo/Inotiv) rat model of Parkinson's disease until approximately 6 months of age.

Pink1/Parkin dKO and wild-type (WT) littermate (n=12/group) male rats were subjected to open field and kinematic gait analysis testing at 2, 3 and 6 months of age. Neurofilament light chain (NfL) and soluble glial fibrillary acidic protein (GFAP) levels were analyzed from CSF and plasma. Brain volumetric and metabolite analysis were performed by magnetic resonance imaging and spectroscopy (MRI and MRS) at 3 and 6 months in addition to DAT-PET imaging at 5 months. Weakness symptoms were registered by cage-side observations. At 6 months, TH-positive cells, Iba-1 and GFAP were analyzed by IHC from the substantia nigra (SN), and dopamine and metabolites were analyzed from the striatum using HPLC.

All Pink1/Parkin dKO rats manifested moderate to severe hind-leg weakness by 6 months of age. Also, rearing frequency and total distance travelled on the open field were decreased but movement velocity increased at 6 months. The gait overall score was significantly different between the WT and Pink1/Parkin dKO at 2, 3 and 6 months. Notable differences were, e.g., compromised inter-limb coordination, increased movement jerkiness and reduced hip height. Whole brain volume was increased at 6 months, as well as cortex volume at 3 and 6 months in Pink1/Parkin dKO rats. Glucose levels were reduced in the striatum at 2 and 6 months and taurine at 3 and 6 months. Creatine (Cr) and, Cr+phospho-Cr and glutamine levels were significantly lower only at 3 months. There was a statistically non-significant trend towards reduced dopamine transporter levels at 5 months as measured with PET imaging. NfL and GFAP levels in the CSF were elevated at 6 months of age. Striatal dopamine levels and TH-positive cell counts or Iba-1 or GFAP staining in the SN were not altered at 6 months.

In conclusion, Pink1/Parkin dKO rats showed evidence of early brain hypertrophy and altered brain energy metabolism prior to symptoms onset. Gait changes seem to start at a young age. However, there was no evidence of significant pathology of the dopaminergic system or signs of neuroinflammation by 6 months.

Disclosures: **J. Toivanen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **V. Lazari:** A. Employment/Salary (full or part-time): NRG Therapeutics. **R. Rutter:** A. Employment/Salary (full or part-time): NRG Therapeutics. **L. Rauhala:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **T. Bragge:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **R. Immonen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **J. Rokka:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **J.K. Uhari-Väänänen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **A. Penttinen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **J. Rytkönen:** A. Employment/Salary (full or part-

time): Charles River Discovery Research Services. **S. Bäck:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.08/E4

Topic: C.03. Parkinson's Disease

Support: MR/W006820/1

Title: Sleep and Neurodegeneration in Parkinson's: Unravelling Pathological Changes

Authors: G. DEEGAN¹, K. ILIC¹, L.-S. DAMOC¹, E. KIM¹, M. B. MESQUITA², Ç. GELEGEN VAN EIJL³, D. CASH¹;

¹BRAIN Ctr., Neuroimaging, King's Col. London, London, United Kingdom; ²Medscopix SL, Mallorca, Spain; ³Birkbeck Col., London, United Kingdom

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterised by many motor and non-motor symptoms including tremor, rigidity, cognitive impairment, and sleep disturbances. Different rodent models of PD exist, however; none recapitulate all aspects of the disease. Here, we characterise brain structure and function, and sleep architecture in a genetic (Gba1 D427V heterozygote), early-stage PD, and toxin-based (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)), advanced-stage PD mouse model. Sixty animals, mixed sex, 13.5-month-old C57Bl/6 mice (n=21 Gba1 D427V heterozygotes; n=21 wild type (WT) littermate controls; n=18 MPTP-lesioned mice) were scanned using structural and functional magnetic resonance imaging (MRI). Following MRI and to record sleep phases, all mice were video-EEG recorded for 24 hours following cortical electrode implantation in somatosensory and visual cortices. Vigilance states were scored manually, with 10 second epochs being assigned wake, non-rapid eye movement (REM) or REM scores. Mice were subsequently sacrificed and their brains harvested for biochemical analysis by high-performance liquid chromatography (HPLC), and for further immunohistochemical staining. The results confirmed unique alterations in sleep macrostructure between both Gba1 D427V heterozygotes and MPTP-lesioned mice, relative to controls. We also observed differences in catecholamine metabolite levels between MPTP and Gba1 D427V heterozygotes, with both groups demonstrating alterations in dopamine and other metabolite levels relative to WT controls. Structural and functional MRI comparison of all groups is ongoing, as well as detailed characterisation of EEG changes. This study provides evidence of distinct differences between genetic (early-stage) and toxin-based (advanced-stage) PD models, which suggests the deterioration of neuronal network integrity over the course of disease progression. These findings highlight the utility of these models in capturing diverse aspects of PD pathology and advancing our understanding of its progression. Overall, our

investigation provides insight into the pathophysiology and sleep architecture of early and advanced-stage models of PD and offers potential avenues for future therapeutic exploration.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.09/E5

Topic: C.03. Parkinson's Disease

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2022.12292.BD
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Title: Abrogation of Adult Neurogenesis Could Contribute to Dopaminergic Degeneration and Motor Decline in Parkinson's Disease

Authors: *B. ARAÚJO^{1,2,3,4}, R. CARIDADE^{1,2}, C. FERREIRA⁵, J. MARTINS-MACEDO¹, C. TEIXEIRA^{1,2}, C. GUEDES^{1,6}, P. SVENNINGSSON⁷, L. PINTO^{3,4}, F. TEIXEIRA^{1,2};
¹Inst. for Res. and Innovation in Hlth. (i3S), Porto, Portugal; ²Sch. of Hlth. (E2S), Polytechnic Univ. of Porto, Rua Dr. António Bernardino de Almeida, Porto, Portugal; ³Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Medicine, Univ. of Minho, Braga, Portugal; ⁴ICVS/3B's Associate Lab, PT Government Associated Lab, Braga/Guimarães, Braga, Portugal; ⁵Dept. of Pediatrics, Univ. of Oxford, Oxford, United Kingdom; ⁶RISE-Health, Ctr. for Translational Hlth. and Med. Biotech. Res. (TBIO), E2S, Polytechnic of Porto, R. Dr. António Bernardino de Almeida, Porto, Portugal; ⁷Dept. of Clin. Neuroscience; Karolinska Institutet, Stockholm, Sweden

Abstract: Depressive disturbances, symptoms of prodromal stages of Parkinson's Disease (PD), are prevalent in 40% to 50% of clinical cases of PD, alongside a typical reduction in adult hippocampal neurogenesis observed in both PD and its related conditions. Indeed, the neurogenesis deficit may affect the clinical course of the disease and possibly be a target for delaying or preventing PD onset. The glial fibrillary acidic protein-thymidine kinase (GFAP-Tk) transgenic rat model allows for the induction of abrogation of neurogenesis after administration of the antiviral Ganciclovir (GCV) for 21 days. GCV induces apoptosis on neural stem cells. This project involved a combination of the GFAP-Tk model and the 6-hydroxydopamine (6-OHDA)-induced unilateral model of PD in the striatum. Hence, the animals were divided into

WT-control animals (n=4); Tk - animals with reduced neurogenesis (n=4); WT 6-OHDA - animals with dopaminergic degeneration (n=9); Tk 6-OHDA - animals with reduced neurogenesis and dopaminergic degeneration (n=8). Motor behavior tests were conducted - Staircase and Rotameter. The number of neuroblasts (DCX+) and proliferative cells (Ki67+) were quantified, as well as the dopaminergic density (TH+) on the substantia nigra, striatum, and other brain regions innervated by dopamine. The new survival cells were decreased in the hippocampus (DCX dorsal region: $p<0.001$; DCX ventral region: $p=0.040$) and sub-regions of the subventricular zone (SVZ) in Tk and Tk 6-OHDA groups compared with the WT (SVZ lateral region DCX: $p=0.008$; SVZ dorsal region Ki67: $p=0.002$). The dopaminergic neurons and their innervations were reduced throughout the nigrostriatal pathway in the 6-OHDA animals. Similar results were obtained in other innervated dopaminergic regions (nucleus accumbens, olfactory tubercle, hypothalamus, globus pallidus). Interestingly, in the anterior amygdalar area, Tk 6-OHDA animals showed a significant reduction compared to the Tk group (Tk vs. Tk 6-OHDA, $p=0.044$), but not with WT 6-OHDA. In the olfactory bulbs, no significant differences were detected. The animals treated with 6-OHDA exhibited less fine motor coordination. Tk 6-OHDA animals appeared to have more severe impairments than WT 6-OHDA. Therefore, our results may suggest that neurogenesis and dopaminergic degeneration are interrelated through the possible modulation of limbic regions, which are also involved in the dopaminergic pathway and contribute to severe motor impairments. Investigating this potential regional interconnection may augment our understanding of behavior dimensions in PD pathophysiology and the development of therapeutic strategies for the early stages of PD.

Disclosures: B. Araújo: None. R. Caridade: None. C. Ferreira: None. J. Martins-Macedo: None. C. Teixeira: None. C. Guedes: None. P. Svenningsson: None. L. Pinto: None. F. Teixeira: None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.10/E6

Topic: C.03. Parkinson's Disease

Support: AMED-Moon Shot Grant

Title: Ubl3 as a genetic and molecular modulator of synucleinopathies in parkinson's disease mouse models

Authors: *M. SETOU^{1,2}, M. ISLAM³, R. FERDOUS³, C. ZHANG³, S. ARAMAKI³, S. HIRAYAMA³, T. KAHYO^{3,4};

¹Cell. and Mol. Anat., Hamamatsu Univ. Sch. Med., Hamamatsu, Japan; ²Intl. Mass Imaging and Spatial Omics Center, Inst. of Photonics Med., ³Cell. and Mol. Anat., ⁴Quantum Imaging

Laboratory, Div. of Res. and Develop. in Photonics Technol., Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan

Abstract: Ubiquitin-like protein 3 (UBL3) is a posttranslational modification factor involved in sorting proteins into small extracellular vesicles and has been implicated in the regulation of alpha-synuclein (α -syn), a key pathogenic protein in Parkinson's disease (PD). Previous work from our laboratory (Chen *et al.*, 2023; Yan *et al.*, 2024) demonstrated that UBL3 interacts with α -syn in cellular models—an interaction modulated by Osimertinib treatment and MGST3 silencing, respectively. While these findings point to a complex UBL3- α -syn relationship, its role in animal models remains poorly understood. To investigate this, we examined the genetic interplay between UBL3 and α -syn using multiple transgenic mouse models: *Ubl3*-knockout (*Ubl3*-KO), SNCA-Tg, SNCA-A53T, *Ubl3*-KO/SNCA-Tg, and *Ubl3*-KO/SNCA-A53T, alongside wild-type (WT) controls. A combination of immunohistochemistry (IHC), immunofluorescence (IF), western blotting of sequentially extracted α -syn, tyrosine hydroxylase (TH) neuron quantification, and behavioral assays were employed to characterize the phenotypes. Additionally, stereotaxic delivery of adeno-associated virus encoding UBL3 (AAV-UBL3) into the striatum of SNCA-A53T mice was performed to assess the effects of UBL3 overexpression in vivo. IHC and IF analyses revealed elevated phosphorylated α -syn (p-S129) in the substantia nigra (SN) of *Ubl3*-KO, *Ubl3*-KO/SNCA, and *Ubl3*-KO/SNCA-A53T mice compared to WT counterparts. These findings were corroborated by western blot analysis and aligned with prior cellular observations. Quantifying TH-positive neurons indicated a significant neuronal loss in the SN of *Ubl3*-deficient mice, suggesting a neuroprotective role for UBL3 against α -syn-induced toxicity. Notably, AAV-UBL3 overexpression markedly reduced p-S129 α -syn levels in the SN of SNCA-A53T mice compared to a truncated UBL3 control (AAV-UBL3-CAAX), highlighting its therapeutic relevance. Behavioral assessments mirrored molecular findings, with knock-out strains exhibiting reduced locomotor activity compared to WT, further supporting the role of UBL3 in mitigating α -syn-associated dysfunction. Collectively, these findings identify UBL3 as a key regulator of α -synuclein pathology *in vivo* and underscore its potential as a therapeutic target in synucleinopathies such as Parkinson's disease.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.11/E7

Topic: C.03. Parkinson's Disease

Support: NIH NIA Intramural Research Program

Title: Reconfiguration of patchy striatal neurons following developmental loss of nigrostriatal dopaminergic neurons

Authors: *V. M. MARTINEZ SMITH¹, J. DONG², B. SULLIVAN³, H. CAI⁴;
¹LNG/TS, NIA/NIH, Bethesda, MD; ²NIH, Bethesda, MD; ⁴Neurogenetics, ³Natl. Inst. on Aging, Bethesda, MD

Abstract: Parkinson's Disease (PD) is associated with the preferential degeneration of Aldehyde dehydrogenase 1A1-positive (ALDH1A1⁺) dopaminergic neurons (DANs) in the *substantia nigra pars compacta* (SNc). The loss of these ALDH1A1⁺ DANs leads to the progressive motor deficits that characterize PD. Pituitary homeobox 3 (Pitx3)-deficient mice, specifically those with the aphakia (ak) mutation, were expected to model these characteristics, as we found that the mutated mice (*Pitx3^{ak/ak}*) displayed a 60% loss of ALDH1A1⁺ DANs compared to control (*Pitx3^{ak/+}*) mice. However, locomotor testing revealed that the *Pitx3^{ak/ak}* mice displayed significantly higher velocity than the *Pitx3^{ak/+}* mice in an open field chamber. We hypothesized that this inversion of expectations might be attributed to a compensatory reconfiguration of dopamine-responsive striatal projection neurons (SPNs) during development, more specifically patchy SPN reconfiguration. Patchy SPNs, defined as clusters of SPNs that are biochemically distinct from those in the vast surrounding matrix, include both dopamine receptor D1 (Drd1)-expressing direct-pathway excitatory SPNs (dSPNs) and dopamine receptor D2 (Drd2)-expressing indirect-pathway inhibitory SPNs (iSPNs). Our lab recently found that patchy SPNs regulate locomotion differently from SPNs in the matrix. Patchy dSPNs inhibit locomotion and patchy iSPNs promote locomotion. Therefore, to explore if our seemingly paradox locomotor results were, in fact, due to a reconfiguration of these patchy SPNs, we first performed RNAscope in-situ hybridization. This assay revealed that in SPNs overall, the dSPN/iSPN ratio showed a non-significant increase in the experimental mouse (1.5 to 1.6), while the patchy dSPN/iSPN ratio significantly dropped in the experimental mouse, with the *Pitx3^{ak/+}* ratio being around 1.75 and the *Pitx3^{ak/ak}* ratio being around 0.7. To investigate the behavioral effects of these reconfigurations, we performed optogenetic stimulation and found that during patchy SPNs stimulation, there was a significant 1cm/s increase in the velocity of *Pitx3^{ak/ak}* mice while the velocity of *Pitx3^{ak/+}* mice was significantly decreased by 2cm/s. In summary, our findings suggest that the developmental loss of ALDH1A1⁺ DANs in the SNc of the mutated *Pitx3* mice causes a reconfiguration of patchy SPNs, which leads to an increase in locomotion rather than the expected decrease. The discovery and investigation of this inversion provides novel insights to the classically defined D1/D2 pathway and sheds light on a mechanistic circuit rearrangement that could lead to previously unexplored clinical strategies.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.12/E8

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS122805

Title: The anti-dyskinetic and the anti-parkinsonian effects of sub-anesthetic ketamine are not sex specific

Authors: ***R. PARMAR**¹, S. SINGH², M. J. BARTLETT⁴, S. RAGSDALE³, S. J. SHERMAN⁵, T. FALK²;

²Dept. Of Neurol., ³Dept. of Neurosci., ¹Univ. of Arizona, Tucson, AZ; ⁴Dept. of Surgery and Neurosurg., Col. of Med., Tucson, AZ; ⁵Dept. Of Neurol., The Univ. of Arizona, Tucson, AZ

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder that, in addition to its cardinal motor symptoms, often also presents with depression and sleep disturbances. Levodopa (L-DOPA) effectively improves motor symptoms but not non-motor symptoms, and chronic use can lead to L-DOPA-induced dyskinesia (LID), affecting > 90% of patients. We previously showed that sub-anesthetic ketamine treatment (20 mg/kg, 5 doses 2-hrs apart over 10-hrs) reduces established LID and attenuates its development in male PD rats with 6-OHDA hemi-lesions. Ketamine also exhibited acute anti-PD effects. Since ketamine's effects can vary by sex in other disease models (e.g., depression, alcohol dependence), we tested its effects in female PD rats. This study assessed whether ketamine can prevent or reduce LID in female Sprague-Dawley rats. To test ketamine's effects on established LID, females with unilateral 6-OHDA lesions and LID (after three weeks of daily L-DOPA treatment) were divided into vehicle (n = 5) and ketamine (n = 5) groups. Rats received ketamine (20 mg/kg) or vehicle over a 10-hr period (5 i.p. injections, 2-hrs apart), followed immediately by L-DOPA (7 mg/kg). Abnormal involuntary movements (AIMs) were scored every 3-7 days over a 49-day period. Results indicated that 20 mg/kg ketamine effectively alleviated established LID acutely, with a sustained reduction in LID observed 49 days post-treatment mirroring the long-term efficacy seen in male LID rats. To test whether ketamine can prevent or reduce the development of LID, we used an attenuation model. Female PD rats received daily L-DOPA (6 mg/kg) and weekly ketamine (20 mg/kg; n = 10) or vehicle (n = 10), following the same 10-hr treatment protocol. AIMs were monitored every 3-4 days for 14 days. Ketamine significantly attenuated LID development, consistent with the findings in males. We also tested the Forelimb Adjusting Steps Test in female unilateral 6-OHDA rats (vehicle: n = 12, ketamine: n = 12) to assess anti-PD effects. Rats were moved laterally across a smooth surface using only the unrestrained forepaw, and steps were counted. Our results show that in female PD rats, ketamine (20 mg/kg) does not interfere with L-DOPA's anti-PD effectiveness in this test of akinesia and produces an acute anti-PD effect by itself, as was shown prior in male PD rats. Post hoc analyses of striatal tyrosine hydroxylase (TH) and mTOR levels are currently underway. In summary, both the anti-dyskinetic and the anti-PD activities of ketamine are not sex specific. This work further supports the repurposing of sub-anesthetic ketamine for individuals with LID.

Disclosures: **R. Parmar:** None. **S. Singh:** None. **M.J. Bartlett:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MJB owns stock in PharmaTher Inc. **S. Ragsdale:** None. **S.J. Sherman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS has a patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, licensed to

PharmaTher Inc., and consulted for PharmaTher. **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TF holds a patent (licensed to PharmaTher Inc.) for using ketamine to treat levodopa-induced dyskinesia in Parkinson's disease, has consulted for, and received travel support from PharmaTher.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.13/E9

Topic: C.03. Parkinson's Disease

Support: Summer Scholars and Artists Program
Center for Development and Behavioral Neuroscience at Binghamton University

Title: Antidyskinetic effects of novel serotonergic combination drug therapy in a hemiparkinsonian rat model of levodopa-induced dyskinesia

Authors: ***J. JODLOWSKI**¹, E. SARINICK², B. J-ELMI², C. BUDROW², C. R. BISHOP¹;
¹Psychology, ²Behavioral Neurosci., Binghamton Univ., Binghamton, NY

Abstract: Parkinson's Disease (PD) is a neurodegenerative movement disorder characterized by the progressive loss of nigrostriatal dopamine (DA) neurons producing symptoms of rigidity, tremors, and bradykinesia. While levodopa (L-DOPA) remains the gold-standard therapy, long-term use often leads to adverse effects including abnormal involuntary movements (AIMS) known as L-DOPA induced dyskinesia (LID). Previous studies show serotonin (5-HT) 1A receptor (5-HT_{1A}R) agonists, such as vortioxetine (VTX) and 5-hydroxytryptophan (5-HTP) alleviate LID when individually paired with L-DOPA. However, with chronic use, the doses required to achieve these effects may lead to a progressive narrowing of the therapeutic window, diminishing long-term effectiveness. In this study, male and female Sprague-Dawley rats (N = 53) received coadministration of VTX and 5-HTP at lower doses to explore a synergistic effect on LID, with the goal of retaining motor efficacy while also preserving the therapeutic window. Subjects were rendered hemiparkinsonian via a unilateral 6-hydroxydopamine lesion into the medial forebrain bundle. Following recovery, equally lesioned rats--determined by the forepaw adjusting steps (FAS) test--were rendered dyskinetic through a 2-week period of daily L-DOPA (6 mg/kg, s.c.) treatment, and were assessed via AIMS testing on days 1, 7, and 14. In Experiment 1, VTX (1 mg/kg, s.c.) and 5-HTP (10 mg/kg, i.p.) were counterbalanced and acutely injected in a within-subjects design just prior to L-DOPA. For Experiment 2, VTX (1 mg/kg, s.c.) and 5-HTP (10 mg/kg, i.p.) were co-administered before L-DOPA daily for 2 weeks in a between-subjects design. For both experiments, AIMS and FAS were implemented to assess alterations in LID and motor efficacy, respectively. Results revealed coadministration of

subthreshold doses of VTX and 5-HTP with L-DOPA attenuated dyskinesia while maintaining the motor benefits of L-DOPA, suggesting a potential synergistic mechanism and supporting it as a translational therapy for PD patients to improve long-term treatment. Ongoing post-mortem assessment of striatal DA and 5-HT levels and gene expression for the 5-HT transporter (SERT) and 5HT-1AR should uncover therapeutic brain mechanisms for this promising combination therapy.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

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Topic: C.03. Parkinson's Disease

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Title: Hemolysin A from *Proteus mirabilis* Triggers Gut α -Synuclein Aggregation and Neurodegeneration

Authors: ***J. KIM**, E. HUH, J. CHOI, Y. CHOI, S. LEE, J. PARK, D. KIM, M. OH;
Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Recent studies underscore the critical role of the gut microbiome in Parkinson's diseases (PD), particularly through the aggregation of intestinal alpha-synuclein (α -syn) and its potential spread to the brain. *Proteus mirabilis*, a gram-negative bacterium, has been linked to neurodegeneration, but the underlying mechanisms remain unclear. In this study, we demonstrate that hemolysin A, a pore-forming toxin produced by *P. mirabilis*, induces α -syn aggregation in the gut and contributes to neurodegenerative pathology. Using vagotomised and antibiotic-treated mouse models, we found that hemolysin A disrupts membrane integrity and activates mTOR-mediated autophagy in intestinal neuroendocrine cells, thereby linking bacterial activity in the gut to α -syn-driven brain pathology. These findings suggest that hemolysin A may act as initiators of gut-originated neurodegeneration and offer new insights into microbiome-related mechanisms in PD.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.15/E11

Topic: C.03. Parkinson's Disease

Support: NIH F31NS137783
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Title: L-dopa induced iron accumulation in the ventral midbrain: the roles of sex and iron repletion

Authors: ***R. O. SERPA**¹, K. PALSA³, E. TUFANO², T. HELMUTH⁴, S. MILLS-HUFFNAGLE⁵, J. R. CONNOR³;

¹Penn State Col. of Med. Neurosci. Grad. Program, Hershey, CA; ²Penn State Col. of Med. Neurosci. Grad. Program, Skyline View, PA; ³Neurosurg., ⁴Penn State Col. of Med., Hershey, PA; ⁵Penn State Col. of Med. Neural and Behavioral Sci., Central Pennsylvania Chapter, Hershey, PA

Abstract: This study aims to investigate the impact of L-3,4-dihydroxyphenylalanine (L-DOPA) on ventral midbrain (VMB) iron levels under iron deficiency (ID) and repletion (IR) conditions in male and female rats. Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons due to iron accumulation in the substantia nigra (SN) located in the VMB. Antiparkinsonian medications, L-DOPA, and a monoamine oxidase-B (MAO-B) inhibitor, selegiline are commonly used to treat PD. However, the effect of L-DOPA on VMB iron levels during ID and IR conditions remains unclear in males and females. To investigate the effects of L-DOPA and selegiline on VMB iron homeostasis, we used post-natal day (PND 21) 21 male and female Long-Evans rats. The rats were initially assigned to two groups based on dietary iron levels, 35mg/kg diet for iron adequate (IA) group (n=18 males and females), 3.5mg/kg for ID group (n=36 males and females) for 5 weeks. After 5 weeks, half of the rats on ID diet (n=18 males and females) were repleted with IA diet for 3 weeks and concurrently injected with Antiparkinson treatments. Vehicle (0.1% ascorbic acid), carbidopa+L-DOPA (5/20 mg/kg), or selegiline (0.3mg/kg) was administered via subcutaneous injection in all groups. We measured the iron levels, and iron homeostasis proteins transferrin receptor (TfR1), ferroportin-1 (FPN1), and H & L ferritin in the VMB of all groups by atomic absorption spectroscopy and immunoblotting respectively. Our findings demonstrate that L-DOPA treatment induced iron accumulation in the VMB of male IR rats but does not significantly alter iron levels in ID or IA diet groups. Notably, the L-DOPA-induced iron accumulation in IR rats was sex-specific, with males exhibiting greater susceptibility than females. Iron homeostasis proteins showed significant alterations, with increased TFR1 and FPN1 expression in L-DOPA treated IR group compared to vehicle and ID in males, however in females it was not altered. Additionally, LCN2 and GFAP levels were elevated compared to the vehicle and ID groups in L-

DOPA-treated IR males versus females. These findings suggest that L-DOPA administration under iron-depleted-repleted conditions disrupts iron homeostasis by promoting iron uptake and reducing iron storage capacity, particularly in males. This sex-specific vulnerability to iron accumulation highlights the need for personalized PD therapies that consider both iron status and biological sex.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

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Program #/Poster #: PSTR326.16/E12

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS122805

Title: Reduction of L-DOPA-induced dyskinesia by optogenetic inhibition of PV interneurons in motor cortex

Authors: *T. FALK¹, A. ARISTIETA², S. L. COWEN²;

¹Neurol., ²Dept. of Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Parkinson's disease (PD) is the 2nd most common neurodegenerative disease. Although dopamine replacement with L-DOPA is the gold-standard therapy for PD, it results in debilitating side effects, the most serious being L-DOPA-induced dyskinesia (LID). In a preclinical LID model, we showed prior that low-dose sub-anesthetic ketamine treatment led to both acute and long-term reduction of abnormal involuntary movements (AIMs). Ketamine is a N-methyl-D-aspartate receptor (NMDAR) antagonist that has a particularly high affinity for NMDARs on parvalbumin (PV)-expressing neurons. NMDARs are abundantly expressed on PV neurons in cortex, providing a means to powerfully activate these neurons in response to coincident input. Hyperlocomotion is a common behavioral result of NMDAR antagonism, and a recent study found that hyperlocomotion was due to NMDARs on cortical PV neurons and not principal neurons. According to the cortical disinhibition hypothesis for both the antidepressive and the anti-dyskinetic activity of ketamine, it causes a burst of glutamate due to reduced cortical GABA PV interneuron (PV-IN) activity in either prefrontal cortex (PFC) or motor cortex (M1), respectively; the firing of PV-IN is driven by NMDARs, and the active, open-channel state allows ketamine to enter and block activity. Here we investigated whether PV-neuron suppression in M1 is sufficient to reduce LID. PV-cre transgenic rats (LE-Tg(Pvalb-iCre)2Otc (RRRC); n = 6) received uni-hemispheric 6-hydroxydopamine lesions (20 micrograms 6-OHDA injected in the medial forebrain bundle) and were injected with AAV5-CAG-Flex-ArchT-tdTomato (UNC Vector Core) targeting the DA-lesioned M1. Two optical fibers were placed

above M1 for light delivery. LID was first established with daily L-DOPA injections for 10 days (i.p., 12 mg/kg + 15 mg/kg benserazide). On testing days LID was induced via L-DOPA injection and the LED (7.5 mW of green light at the tip of the optic fiber) was turned on for 1 min every 20 min. AIMs scores were collected before, during, and after each stimulation bout. PV-neuron inhibition significantly reduced AIMs ($p < 0.05$; Wilcoxon rank sum test), and the effect was strongest for Axial AIMs. Thus, this pilot study provides evidence that M1 PV-IN suppression is sufficient to reduce LID, in line with the hypothesis that PV-INs in M1 are involved in the anti-dyskinetic activity of ketamine.

Disclosures: **T. Falk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent for the use of ketamine as a novel treatment for levodopa-induced dyskinesia associated with Parkinson's disease, licensed to PharmaTher Inc., consulted for and received travel support. **A. Aristieta:** None. **S.L. Cowen:** None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

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Topic: C.03. Parkinson's Disease

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NIH T32 NS095775-08

Title: Exploring increased PARylation as a mechanistic moderator of Parkinson Disease risk at the CD38 locus

Authors: A. COLAFRANCESCO¹, M. S. SIMMONS², K. NIEBUHR², S. A. ANDRABI², K. L. GAMBLE³, M. L. OLSEN⁴, ***R. M. COWELL**⁵;

¹Neurol., UAB Neurosci. Grad. Programs, BIRMINGHAM, AL; ³Psychiatry & Behavioral Neurobio., ²Univ. of Alabama at Birmingham, Birmingham, AL; ⁴Virginia Tech., Blacksburg, VA; ⁵Neurol., Univ. Alabama at Birmingham, Birmingham, AL

Abstract: Homeostasis of brain metabolism has been implicated as an important factor in the initiation of age-related diseases, like Parkinson's Disease (PD), but little is known about how glial cells of the brain contribute to the metabolic alterations seen in diseased states. Compelling studies suggest that astrocytes are responsible for maintaining the energy balance for neurons in their local environment through energetic coupling and that dysfunction contributes to disease pathogenesis. To prioritize PD-associated genes based on their enrichment in glia, we performed a principal axis factoring approach to stratify GWAS-implicated genes affected at the eQTL and sQTL level by cell type. This revealed a PD-linked single nucleotide polymorphism (SNP) in the astrocyte-enriched gene *CD38*, where homozygous carriers of the risk allele exhibit a 50%

reduction in CD38 mRNA throughout the brain. As CD38 is responsible for ~90% of nicotinamide adenine dinucleotide (NAD⁺) conversion, we propose that the CD38-linked increase in PD risk could be due to impairments in astrocyte-derived provision of metabolic substrates to neurons. Supporting this, we identified CD38 as a key regulator for NAD⁺ balance, where *Cd38*^{-/-} mice have elevated NAD⁺/NAM levels. Further, we found a robust increase in poly-ADP-ribosylated (PAR) modified proteins, and decreased expression of PAR eraser and writer enzymes in *Cd38*^{-/-} mice suggesting dysregulation of metabolic pathways. To explore differences in proteins that are PAR modified, we performed an affinity purification of PAR and mono-ADP-ribosylated (MAR) modifications and used mass spectrometry to identify proteins with increased PAR/MAR modifications in *Cd38*^{-/-} mice. Gene ontology analysis revealed proteins that were more PARylated in *Cd38*^{-/-} mice are involved in regulating cellular responses to stress-activated signaling pathways and protein phosphorylation which could impair astrocytic state and their ability to maintain energetic homeostasis. Altogether, these data indicate that CD38 deficiency associated with variations at the *CD38* locus could prime the brain for metabolic dysregulation, enhancing vulnerability of neurons to oxidative stress, inflammation, and disease pathology. Future studies will evaluate how CD38 deficiency influences dopaminergic neuron vulnerability to age and/or PD-related pathogenic stimuli.

Disclosures: **A. Colafrancesco:** None. **M.S. Simmons:** None. **K. Niebuhr:** None. **S.A. Andrabi:** A. Employment/Salary (full or part-time): University of Alabama at Birmingham. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health. **K.L. Gamble:** A. Employment/Salary (full or part-time): University of Alabama at Birmingham. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health. **M.L. Olsen:** A. Employment/Salary (full or part-time): Virginia Tech. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health. **R.M. Cowell:** A. Employment/Salary (full or part-time): University of Alabama at Birmingham. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health, Michael J. Fox Foundation for Parkinson's Research. F. Consulting Fees (e.g., advisory boards); Acelot, Inc., C-Path Institute, Trialtus Bioscience.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.18/E14

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS107512
NIH T32AG0496688
MJFF 022867

Title: Interrogating early sex-specific abnormalities in cholinergic innervation and behaviorally-evoked ACh transients in mPFC of Lrrk2-G2019S mice

Authors: *A. MAGEE¹, S. GUPTA², D. L. BENSON³, G. W. HUNTLEY⁴;
²Neurosci., ¹Icahn Sch. of Med. at Mount Sinai, New York, NY; ³Neurosci., Mount Sinai, New York, NY; ⁴Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Cognitive impairment is a prevalent non-motor symptom in hereditary and idiopathic Parkinson's (PD). Among PD patients, male sex elevates risk of early cognitive symptoms and accelerates conversion to Parkinson's dementia, but the basis for early cognitive symptoms generally and sex-specific vulnerability specifically is unknown. Cholinergic projections to prefrontal cortex modulate top-down circuits controlling attention and other executive functions, and their loss has been implicated in dementia in humans. Previous work has shown mild deficits in visuospatial attention and corticostriatal-dependent instrumental learning in young adult male mice carrying a G2019S knockin mutation in leucine-rich repeat kinase 2 (Lrrk2-G2019S). Here, we interrogated young adult male and female wildtype and Lrrk2G2019S mice anatomically, to assess cholinergic innervation density in area PL, and functionally, using a ACh biosensor, and fiber photometry to investigate behaviorally-evoked ACh transients. Cholinergic axons were identified by crossing P60-P100 wildtype (WT) and Lrrk2-G2019S (GS) mice with ChAT-Cre:tdTomato reporter mice and by immunofluorescent labeling for vesicular acetylcholine transporter. Confocal images were acquired from area PL and analyzed with ImageJ. An AAV-GRAB-ACh3.0 biosensor was targeted to area PL. Signals were reported by fiber photometry during a modified 2-day instrumental learning task. In males, the density of cholinergic innervation of GS mice was significantly sparser across all layers compared to that in WT mice. In contrast, in females, the density of cholinergic innervation in GS mice was significantly denser in comparison with WT mice. Despite significant genotype- and sex-dependent anatomical differences in cholinergic innervation, there were no within-sex, genotype-dependent differences in behavioral performance. Similarly, in both males and females, there were no significant genotype-differences in the magnitude of behaviorally-evoked ACh transients during the ITI, when attentional load is expected to be maximal. There were, however, subtle genotype-dependent differences in how the behaviorally-evoked signals evolved temporally. Despite significant sex- and genotype-dependent differences in cholinergic innervation of mPFC, behavioral performance and magnitude of evoked ACh transients appeared largely similar to WT mice. These outcomes may reflect early functional compensation for stark anatomical differences during tasks that do not pose strong attentional demand.

Disclosures: A. Magee: None. S. Gupta: None. D.L. Benson: None. G.W. Huntley: None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.19/E15

Topic: C.03. Parkinson's Disease

Title: Aav-human a53t-mutated synuclein disrupts nigro-striatal system in mice

Authors: *A. BROWN¹, M. PROKESCH², E. AUER³, T. LÖFFLER³, M. DAURER⁴, L. BREZNIK⁴, S. FLUNKERT³;

¹Scantox ApS, Ejby, Denmark; ²Discovery, Scantox Neuro, Graz, Austria; ³Scantox Neuro GmbH, Grambach, ; ⁴Scantox Neuro GmbH, Grambach, Austria

Abstract: Phosphorylation and aggregation of α -synuclein (α -syn) plays a crucial role in Parkinson's disease (PD) and other neurodegenerative diseases. The A53T point mutation in α -syn, which has been identified in rare forms of familial PD, is reported to increase α -syn oligomerization and aggregation. Thus, we generated a mouse model recapitulating core features of PD that can be used to research A53T α -syn-related pathologies, downstream effects and ultimately be a valuable tool for the development of new therapeutics. To this end, the substantia nigra (SN) of 13 weeks old C57BL/6J mice was unilaterally injected with AAV-human A53T mutated α -syn (AAV-hA53T). The contralateral SN was treated with an AAV-empty control vector. After 8 weeks, brains were collected and analyzed by biochemical and histological methods. Analyses revealed that human α -syn levels were highly increased in the ipsilateral SN as determined using a Mesoscale Discovery (MSD) immunosorbent assay and quantitative immunofluorescence, suggesting that the α -syn expression driven by the AAV vector was successful. Additional analysis showed that murine levels of α -syn in the striatum were not affected. However, further quantitative rater-independent immunofluorescence analyses demonstrated significantly elevated levels of α -syn phosphorylated at serine 129 in the SN. Also, a significant loss of TH-positive soma and fibers in the SN and CPu, respectively were detected - indicating a loss of dopaminergic neurons. Signal of dopamine transporter (DAT) was significantly lower in the AAV-hA53T ipsilateral compared to the contralateral SN. Evaluating inflammatory processes showed that not only microgliosis was evident after AAV-hA53T injection but also CD3-positive T-cells and more specifically cytotoxic CD8-positive T-cells infiltrated the brain. In addition, analysis of the newly identified Parkinson's disease (PD)-relevant biomarkers—NOD2, OGA, TMEM175, TRPML1, TFEB and ATP13A2—is currently ongoing. In summary, the AAV-human A53T α -syn mouse model recapitulates several core features of PD and enables studying A53T α -syn-related pathologies and downstream signaling. The model might be further valuable for the analysis of molecular mechanisms underlying synucleinopathies and pave the way for the development of novel therapeutic interventions.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.20/E16

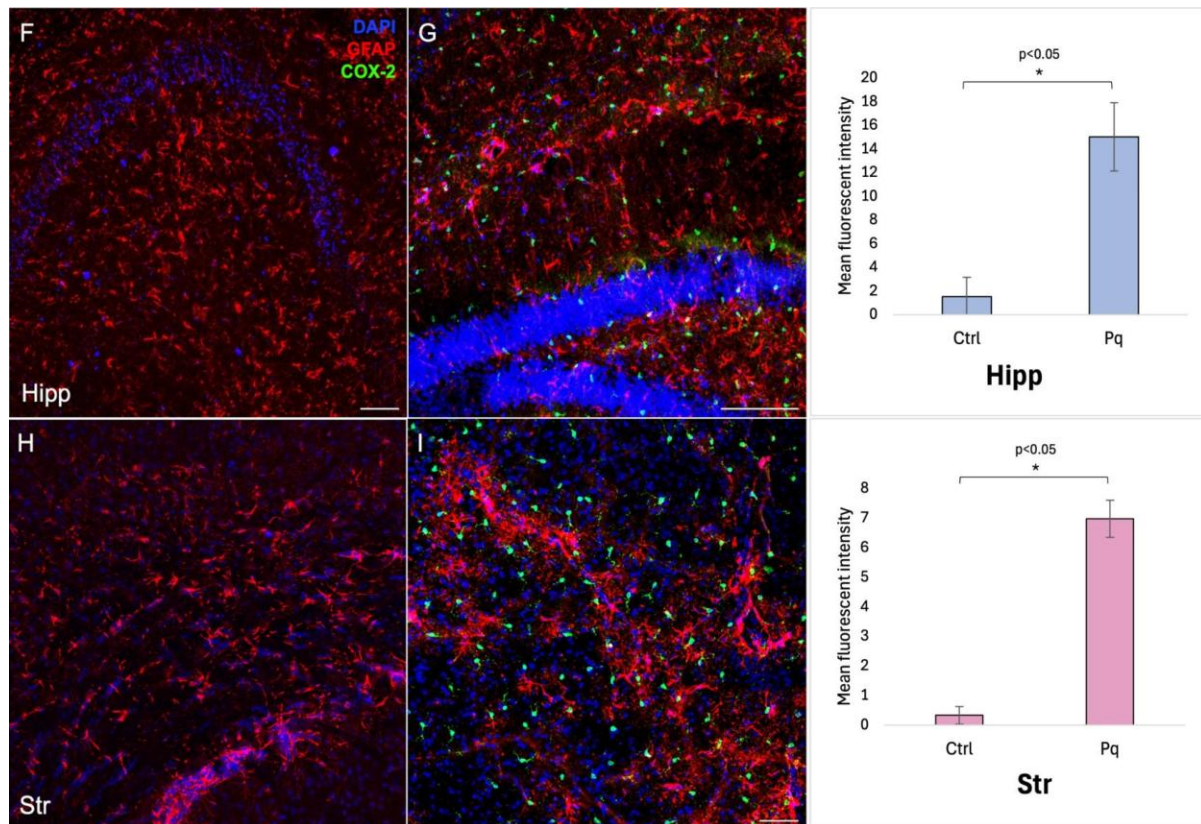
Topic: C.03. Parkinson's Disease

Title: Astrocytic COX-2 Expression in a Parkinson's Mouse Model: Implications for Blood-Brain Barrier Damage

Authors: *A. L. RUMBAUT¹, H. BROWN², J. J. RIERA³;

¹Col. of Medicine/Biomedical Engin., Florida Intl. Univ., Miami Springs, FL; ²Col. of Arts, Sci. and Education/College of Publ. Hlth. and Social Work, ³Biomed. Engin., Florida Intl. Univ., Miami, FL

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disorder, yet many aspects of its pathology remain unresolved, including vascular implications. Growing evidence suggests chronic neuroinflammation may disrupt the blood-brain barrier (BBB), contributing to disease progression. Astrocytes, key regulators of BBB integrity, undergo reactive changes in PD that may impair vascular function. Cyclooxygenase-2 (COX-2), an inflammation-induced enzyme, produces prostaglandin E2 (PGE2), a vasodilator that may destabilize the BBB. We conducted a systematic review of 793 experimental studies on vascular changes in PD, retrieved from PubMed, FIU Database, Web of Science, and Scopus, and screened via PRISMA methodology. Five major vascular changes were identified from the final 25 sources, with increased BBB permeability being most common across animal, human, and in vitro studies. To explore these findings experimentally, we analyzed hippocampal tissue from paraquat (PQ)-treated and control mice using immunofluorescence and confocal microscopy. COX-2 was stained with SP21, a synthetic monoclonal antibody. ImageJ was used for fluorescent signal quantification and cell count. Colocalization coefficients assessed overlap between GFAP-positive astrocytes and COX-2. PQ astrocytes showed hypertrophy, irregular morphology, and thickened processes. COX-2 localized to astrocytic endfeet and somata. SP21 signal intensity was significantly elevated in PQ mice. These findings suggest astrocytic COX-2 may mediate BBB disruption in PD via chronic inflammation, supporting its exploration as a therapeutic target.



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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.21/E17

Topic: C.03. Parkinson's Disease

Title: Temporal characterization of alpha-synuclein pathology in a bilateral AAV-alpha-synuclein rat model of Parkinson's disease

Authors: C. SKEITH, *T. H. JOHNSTON, P. A. HOWSON, D. AREF, M. P. HILL, J. M. BROTHIE;

Atuka Inc., Toronto, ON, Canada

Abstract: One common feature of Parkinson's Disease (PD) is the presence of pathological forms of alpha-synuclein (aSyn) throughout the central nervous systems of patients. The established AAV-aSyn rat model of PD which utilizes viral vectors to mediate the overexpression of aSyn, produces neurodegeneration within the injection site with limited spread

to other brain regions. In this study, we aimed to assess the temporal characteristics of pathological aSyn in a bilateral rat model of PD to better understand its advantages and limitations in recapitulating the human disease. The nigra of female Sprague-Dawley rats were bilaterally injected with AAV1/2 expressing hA53T-aSyn or empty vector. A battery of behavioural assessments were performed at 3-week and 6-week post injection to assess development of motor and non-motor deficits (including Open Field Assay (OFA)). Tissue was collected at 3- and 6-weeks and used to quantify total transgenic and aggregated aSyn in the nigrostriatal pathway via ELISA and to evaluate the level of neurodegeneration. This bilateral rodent model of PD exhibited signs of motor dysfunction as indicated by a reduction in the time animals were mobile in the OFA test. In addition, while levels of transgenic and aggregated aSyn continued to rise over the 6-week period in the substantia nigra (injection site), levels of pathological aSyn plateaued within the striatum at 3-weeks. This work helps define the development of synucleinopathy and the emergence of motor and non-motor symptoms in this rodent model of PD.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.22/E18

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS124037
Parkinson's Foundation Summer Student Fellowship

Title: Astrocyte heterogeneity in the substantia nigra revealed using a novel model for Parkinson disease risk

Authors: *N. GULLAPALLI¹, A. COLAFRANCESCO², M. S. SIMMONS¹, S. BASAK³, M. L. OLSEN⁴, R. M. COWELL⁵;

¹Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; ²Neurol., UAB Neurosci. Grad. Programs, BIRMINGHAM, AL; ³Virginia tech, Blacksburg, VA; ⁴Virginia Tech., Blacksburg, VA; ⁵Neurol., Univ. Alabama at Birmingham, Birmingham, AL

Abstract: More than 10 million people worldwide are diagnosed with Parkinson's disease (PD), a progressive neurodegenerative movement disorder characterized by dopaminergic neuron (DAN) loss in the substantia nigra pars compacta (SNc). Pathological evidence implicates glial dysfunction and inflammation in idiopathic PD progression, yet there is a lack of direct evidence linking inflammation, glial dysregulation, and the DAN loss in PD. Our lab investigates glial cell roles in maintaining DAN viability. Using human GWAS and eQTL data, we identified a single

nucleotide polymorphism in the gene locus for cluster of differentiation (*Cd38*), in which homozygous carriers of the PD risk allele exhibit a 50% reduction in *Cd38* mRNA in the brain. CD38, a protein involved in maintaining intra- and extracellular metabolism, is highly enriched in astrocytes, a non-neuronal cell type that could be responsible for PD-associated brain dysfunction. To further investigate how *Cd38*'s deficiency influences astrocyte state and/or neuronal viability, we performed RNA-sequencing and found that *Cd38*^{-/-} mice have changes in genes involved in inflammation (*C4b* [0.888]), glycogen metabolism (*Pygb* [0.498]), and senescence (*Cdkn1a* [-0.637]). To determine how these changes could affect dopaminergic neurons, we explored the expression of these genes in the substantia nigra pars compacta. Interestingly, we found that there was astrocyte heterogeneity in the SN, with three types being identified using single-cell transcriptomics. Using fluorescent *in situ* hybridization (FISH), we localized three types of astrocytes (*Myoc*⁺, *Cst3*⁺, *Igfbp2*⁺) in the SN; while *Myoc*⁺ astrocytes were localized primarily to the ependyma, *Cst3*⁺ and *Igfbp2*⁺ were localized to astrocytes within the SNc. Images were collected on a confocal microscope and analyzed using Cell Profiler with statistical analyses performed in GraphPad Prism. These findings highlight the need for considering astrocyte heterogeneity when determining how astrocyte-enriched genes associated with PD risk give rise to disease.

Disclosures: N. Gullapalli: None. A. Colafrancesco: None. M.S. Simmons: None. S. basak: None. M.L. Olsen: None. R.M. Cowell: None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.23/E19

Topic: C.03. Parkinson's Disease

Support: MJFF #16964
MJFF #021180
NIH RO1 NS101958

Title: Using ATP6V0A1 Genetics and Biology to Understand Dopaminergic Neuron Vulnerability in Parkinson's Disease

Authors: *G. O. SAJDAK¹, S. M. BOAS⁴, S. N. FOX², L. A. VOLPICELLI-DALEY⁵, D. J. MOORE⁶, K. L. GAMBLE³, R. M. COWELL⁷, M. S. SIMMONS², K. G. NIEBUHR², A. BERGSMA⁶;

²Neurol., ³Psychiatry, ¹Univ. of Alabama at Birmingham, Birmingham, AL; ⁴CDIB, Univ. of Michigan, Ann Arbor, MI; ⁵Neurol., UAB, Birmingham, AL; ⁶Dept. of Neurodegenerative Sci., Van Andel Inst., Grand Rapids, MI; ⁷Neurol., Univ. Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic (DAergic) neurons and accumulation of phosphorylated α -synuclein, but the underlying mechanisms of cell type-specific vulnerability remain unresolved. To explore the potential contributors to dopaminergic vulnerability in idiopathic PD (iPD), we used a novel statistical approach to explore the cell type-specificity of genes affected at the expression or splicing level by variation in PD-associated risk alleles. Based on a number of criteria including high enrichment in neurons which project from the substantia nigra to the dorsolateral striatum, we prioritized the gene ATP6V0A1 for studies focused on identifying novel mechanisms of dopaminergic neuron dysfunction and loss in iPD. ATP6V0A1 encodes the proton-transporting subunit of the vacuolar ATPase (v-ATPase) and is involved in multiple processes important for dopaminergic neurons including lysosome acidification and clathrin-mediated endocytosis. However, very little is known about the impact of changes in ATP6V0A1 abundance on dopaminergic neurons in vivo. To determine how changes in ATP6V0A1 expression influence DAergic neuron viability and function, we used cre-mediated deletion and adeno-associated virus-mediated overexpression models to manipulate ATP6V0A1 expression in dopaminergic neurons in adult mice in vivo. We found that dopaminergic neurons are acutely vulnerable to ATP6V0A1 deletion, with behavioral impairments and reductions in tyrosine hydroxylase expression and dopamine content in the striatum at one month post-deletion. However, overexpression is well tolerated, suggesting that overexpression could potentially be used to drive lysosomal acidification for clearance of aggregated proteins (e.g. synuclein). Future studies will explore how manipulation of ATP6V0A1 expression influences dopaminergic neuron viability and vesicular trafficking, informing how variation at the ATP6V0A1 locus increases risk for PD and whether overexpression and/or overactivation of the v-ATPase could be used as a potential neuroprotective strategy.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

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Program #/Poster #: PSTR326.24/E20

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS127037
the Postma Family Pilot Award

Title: Mitochondrial homeostasis in a model of early Parkinson's disease

Authors: *R. ROMAY-TALLON¹, C. GONZALEZ¹, A. BROWN¹, T. NAPIER², A. E. KIRBY¹;

²Dept. of Psychiatry and Behavioral Sci., ¹Rush Univ. Med. Ctr., Chicago, IL

Abstract: Rationale. Fusion and fission are dynamic mitochondrial processes necessary for maintenance of neuronal circuits as well as synaptic plasticity. In Parkinson's disease (PD), mitochondrial malfunction contributes to dopaminergic (DA) degeneration in the substantia nigra pars compacta (SNpc). Thus, mitochondrial dynamics are potential therapeutic targets for PD particularly in early stages of the disease. However, these mechanisms are poorly understood. Here we test the hypothesis that mitochondrial dysregulation is present in a rat model of early-stage PD. We evaluated the levels of mitochondrial fusion (OPA1) and fission (DRP1) protein markers in the nigrostriatal system. **Methods.** Adult Sprague Dawley male rats received bilateral injections of (i) 6-OHDA (7.5ug/2uL) (n=16) into the rostral dorsolateral striatum (rdlSTR) or (ii) vehicle (n=16). Following recovery, forelimb stepping quantified PD-like akinesia. In half of the animals (cohort 1; n=8 per treatment group), rostral striatum (rSTR), containing 6-OHDA-induced lesions, was dissected out. Caudal striatum (cSTR), unaffected by 6-OHDA, was also collected to test lesion specificity. Mitochondrial and cytosolic fractions were isolated and expression of OPA1 and DRP1 were determined using Western blotting. In the other half of the animals (cohort 2; n=8 per treatment group), brains were fixed, and immunohistochemistry was used for densitometric analysis of DRP1 and OPA1 in the rdlSTR, using medial striatum (mSTR) as the lesion control. In all rats, stereological analysis of tyrosine hydroxylase (TH)-positive SNpc neurons was performed. **Results.** Intra-rdlSTR 6-OHDA decreased forelimb stepping by ~70% and increased latency to first step by 4-fold, paralleled by ~20% decrease in the number of TH-positive neurons in the SNpc, confirming a PD-like phenotype. Western blot analysis (cohort 1) did not show any significant differences between treatment groups in DRP1 and OPA1. Densitometry (cohort 2) showed a ~23% increased expression of DRP1 and a ~38% decreased expression of OPA1 in rdlSTR in 6-OHDA-treated group (p<0.05, Student's *t*-test). No changes were detected for either protein in the mSTR. **Conclusions.** These results suggest that a disbalance in the mitochondrial homeostatic mechanisms is detectable in early stages of PD. The findings indicate that these proteins may serve as biomarkers for early diagnosis as well as potential therapeutic targets. *Supported by NIH NS127037 and the Postma Family Pilot Award. The intellectual contributions of Amanda L. Persons, PhD are gratefully acknowledged.*

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

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Program #/Poster #: PSTR326.25/E21

Topic: C.03. Parkinson's Disease

Support: NIH NS 127037
Cohn Family Foundation
Postma Family Pilot Award

Title: Deep brain stimulation of the subthalamic nucleus engages limbically oriented subfields of the substantia nigra pars reticulata in a rat model of early Parkinson's disease

Authors: R. ROMAY-TALLON¹, C. M. GONZALEZ¹, A. D. BROWN¹, T. NAPIER², *A. E. KIRBY¹;

¹Neurolog. Sci., ²Psychiatry and Behavioral Sci., Rush Univ., Chicago, IL

Abstract: Rationale. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) improves motor deficits in Parkinson's disease (PD), but changes in motivation also occur in some patients. Here, we tested the hypothesis that STN-DBS that improves motor function engages motor regions that connect with the limbic system. Optogenetic stimulation was used in a rat model of early PD and activated neurons were quantified in STN terminal regions.

Methods. To model early-stage PD, male Sprague Dawley rats (n=11) received bilateral injections of 6-OHDA (7.5ug/2uL) into the dorsolateral striatum. To target STN excitatory outputs, a viral vector (rAAV5-CamKII-(T159C/E123T)ChR2-EYFP) was injected into the STN, resulting in expression of a channelrhodopsin modified for fast neuronal activation in glutamatergic neurons. An optic fiber was permanently implanted above the STN. Following recovery, forelimb stepping quantified PD-like akinesia. Behavioral assessments were performed before and after surgery. At post-surgery days 28-35, the rats underwent two DBS sessions (465 nm, 125 or 130Hz) per day. On the last study day, rats received DBS and were killed 90min later. Placement of optic fiber tips and staining for opsin expression (GFP) were verified immunohistochemically. Activated neurons (cFos) were quantified in substantia nigra pars reticulata (SNr), substantia nigra pars compacta (SNc) and globus pallidus (GP) using unbiased stereology. SNr sections were aligned to an atlas for comparison of topography of activated neurons.

Results. Striatal 6-OHDA decreased forelimb stepping from 14.4 to 5.2 steps and increased latency to first step by threefold, confirming a PD-like phenotype. Based on improvement in stepping in one limb after DBS, the contralateral brain hemisphere was assigned to responder (>10 steps after DBS and optic fiber terminating over STN; N=8) or non-responder (<6 steps after DBS; N=5) groups. The number of activated neurons was higher in the responders (Mann-Whitney test, SNr, p<0.01; SNc, p<0.01; GP, p<0.05). Within the SNr, there were hotspots of activated neurons in responders which were located within rostral medial and mid dorsal aspects of the nucleus, whereas activated neurons were sparse in non-responders.

Conclusions. Optogenetic STN-DBS that improves parkinsonian motor deficits engages SNr subfields that project to limbic and motor areas. Ongoing studies of STN DBS are determining the relationship between brain activation and changes in motivated behavior.

The intellectual contributions of Amanda L. Persons, PhD are gratefully acknowledged.

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Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.26/E22

Topic: I.04. Executive Functions

Support: NIH Grant NS100849
VA grant I01RX002987

Title: Brain structural correlates of midfrontal delta/theta rhythms in Parkinson's Disease

Authors: *C. HUNTER^{1,2}, B. E. YEAGER², A. I. ESPINOZA^{1,2}, V. MAGNOTTA¹, W. G. DARLING³, C. WEBER¹, J. DAWSON¹, E. UC², J. F. CAVANAGH⁴, N. S. NARAYANAN²; ²Dept. of Neurol., ³Dept. of Hlth. and Human Physiol., ¹Univ. of Iowa, Iowa City, IA; ⁴Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative condition known for its motor features, yet it can also cause significant cognitive impairments, including deficits in executive function, attention, and memory, with some individuals developing dementia. Although the motor effects of PD have been well-studied, the cognitive effects are less well understood and have vanishingly few treatments. Understanding the neural circuits that contribute to cognitive control is critical for identifying neurophysiological markers and finding new therapeutic targets. We have previously demonstrated that low-frequency delta (1-4 Hz) and theta (5-8 Hz) rhythms within the brain are a reliable marker of cognitive control and can be measured via scalp electroencephalography (EEG). However, the structural origin of these rhythms is unknown.

We recruited individuals with PD from the University of Iowa Hospitals and Clinics. Participants completed an auditory oddball task, a well-established task of cognitive control, while scalp EEG was recorded. Structural MRI scans were also obtained and the Freesurfer program was used to quantify cortical volumes. We then tested whether individual differences in delta/theta power were associated with specific cortical structure volumes. This work enhances our understanding of the structure-function relationship underpinning cognitive control. It provides a potential pathway for the development of neurophysiological markers of cognitive impairment and may inspire future cognitive neuromodulation strategies for patients with PD.

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Poster

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Program #/Poster #: PSTR326.27/E23

Topic: C.03. Parkinson's Disease

Support: DP1-AT011991
K. Lisa Yang Brain–Body Center
Lore Harp McGovern Fellowship

Title: Unravelling the environmental origins of gut-to-brain propagation of Parkinson's disease pathology

Authors: *S. SELVARAJI¹, K. PANG², P. ANIKEEVA³, R. MONDAL², J. MOSBERGER⁴,
¹Res. Lab. of Electronics, ²RLE, ³Brain and Cognitive Sci., MIT, Cambridge, MA; ⁴Neurosci., Wellesley Col., Boston, MA

Abstract: Parkinson's disease (PD), the most common neurodegenerative motor disorder worldwide, affects over 10 million people. Characterized by dopaminergic neuron loss and alpha synuclein (α -syn) aggregates in the midbrain region, PD results in motor symptoms. Current treatments for PD patients are entirely symptomatic, necessitating early-stage, disease-modifying treatments. Emerging evidence suggests that PD pathology may originate in the gut, preceding disease onset by decades. Gastrointestinal dysfunctions in PD patients, α -syn accumulation in the gut and exposure to environmental factors like pesticides and smoking support the gut-to-brain propagation hypothesis via neural circuits governing bidirectional communication. These environmental factors modulate the epigenome although their mechanistic role in PD progression via gut-brain axis remain elusive. Here we investigate the nexus between the disease progression of PD via the gut-brain axis and epigenetic interactions in an environmental mouse model of PD. We demonstrate through a 5-day transient exposure to rotenone (environmental toxin), followed by a 9-month observation period that the gut dysfunctions manifested without any observable motor symptoms. About 48% reduction in dopaminergic neurons were observed in rotenone-treated mice compared to controls, without significant behavioural changes. Strikingly, the rotenone-treated mice exhibited a 46% increase in whole gut transit time, reduced colonic length and colonic contractions, elongated colonic crypt depth (134 μ m vs 84 μ m in controls) and elevated number of acid mucin goblet cells (20.7 vs 2.4 in controls) indicative of constipation and compromised mucus barrier function. This chronic environmental model captures the prodromal phase of the pathophysiology of PD, providing access to a therapeutic window for early intervention. To dynamically monitor and map the physiological progression of PD in real-time, we recently developed a multifunctional gut-brain neurotechnology device. We implanted it in healthy mice with LED-embedded fiber probes in the proximal colon enabling localised optogenetic stimulation. A 20Hz, 10ms pulse width stimulation for 45min led to epigenetic alterations, with increased global DNA methylation levels. Following a successful 2-month long implant, we are now deploying this device in rotenone-induced PD models to establish a timeline correlating motor and gastrointestinal dysfunction. Our findings identify key physiological and epigenetic signatures driving early-stage PD progression, reinforcing gut as the entry point of environmental modifiers of gene expression and neural activity.

Disclosures: S. Selvaraji: None. K. Pang: None. P. Anikeeva: None. R. Mondal: None. J. Mosberger: None.

Poster

PSTR326: Parkinson's Disease in Animal Models: Circuits, Behavior, and Mechanistic Insights

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR326.28/E24

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: NSTC 113-2320-B-002-067-
NSTC 113-2923-B-002-005-MY2

Title: Modeling MPAN: C19orf12 loss leads to motor and emotional dysfunction in mice

Authors: *H. WANG¹, I.-S. YU², C.-H. LIN⁴, Y.-T. CHEN^{3,1};

¹Genome and Systems Biol. Degree Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan; ²Lab. Animal Ctr., ³Grad. Inst. of Med. Genomics and Proteomics, Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; ⁴Dept. of Neurol., Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: Neurodegeneration with brain iron accumulation (NBIA) is a rare neurodegenerative disease affecting 1-3 per million people, characterized by iron accumulation in the globus pallidus and substantia nigra. Mitochondrial membrane protein-associated neurodegeneration (MPAN), a subtype of NBIA caused by germline mutations in *C19orf12*, accounts for 10-30% of NBIA cases. MPAN onset typically ranges from childhood to early adulthood, with symptoms including progressive dystonia-parkinsonism, ataxia, motor axonal neuropathy, optic atrophy, cognitive decline, psychiatric symptoms, and premature death. MPAN is the second or third most prevalent subtype of NBIA; however, the function of C19orf12 and its role in MPAN pathogenesis remain poorly understood. To investigate MPAN pathogenesis in vivo, we generated a *C19orf12* knockout (KO) mouse model and conducted comprehensive neurological assessments. Using the rotarod test, grip strength measurement, hind limb clasping test, and modified-SHIRPA test, we observed that KO mice exhibited marked weight loss, as well as motor and emotional deficits. These findings support a causal link between C19orf12 mutations and neurological disease.

Disclosures: H. Wang: None. I. Yu: None. C. Lin: None. Y. Chen: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.01/E25

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

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Title: Interleukin signal disruption contribute to oligodendrocyte dysfunction in PLP-150Q HD mice

Authors: *X. LI^{1,1}, H. TONG^{1,2}, X. LI¹, S.-H. LI¹;

¹GHM Inst. of CNS Regeneration, Jinan Univ., Guangzhou, China; ²Dept. of Neurosurg., The First Affiliated Hosp. of Jinan Univ., Guangzhou, China

Abstract: Huntington's disease (HD) is a neurodegenerative proteinopathy characterized by the ubiquitous expression of mutant huntingtin (mHTT), which drives pathogenesis through both cell-autonomous neuronal dysfunction and non-cell-autonomous glia-mediated toxicity. Despite these insights, the mechanisms underlying oligodendroglial dysfunction and their intercellular communication during HD progression remain elusive. This study utilized PLP promoter-driven transgenic mice that specifically express N-terminal truncated mHTT (150Q) fragment in oligodendrocytes to investigate the oligodendrocyte-autonomous effects of mHTT on HD progression. The PLP-150Q HD mice exhibited age-dependent mHTT aggregation compared to PLP-23Q mice and progressive demyelination in the corpus callosum, striatum, and cortex. This pathology paralleled three key hallmarks: activation of pro-inflammatory glia (GFAP⁺ astrocytes and IBA1⁺ microglia), elevated levels of inflammatory mediators (IL-1 β , TGF- β , and NF- κ B) in the corpus callosum, and late-stage degeneration of striatal medium spiny neurons. To explore the mechanisms underlying this neuroinflammatory phenotype, we performed bulk RNA sequencing analysis of the striatum and corpus callosum in PLP-150Q mice during the early disease stage in comparison with PLP-23Q mice. Gene enrichment analysis revealed significant dysregulation of transcripts related to myelin proteins and immune-inflammatory factors, with certain interleukins showing the most pronounced alterations. Cross-species validation via RT-qPCR confirmed these molecular changes, showing over 50% alterations in both white matter and striatum of PLP-150Q mice, which were corroborated in HD knock-in pigs. These changes were further validated through western blotting and immunofluorescence staining. Notably, overexpression of the same factors in early-stage PLP-150Q mice rescued motor function deficits in behavioral assays during the later stages, while DAB immunohistochemistry and quantitative western blot analysis confirmed the restoration of myelin protein expression. We are currently performing single-cell sequencing to delineate the mechanistic events by which immune-inflammatory factors contribute to demyelination and neurodegeneration.

Disclosures: X. Li: None. H. Tong: None. X. Li: None. S. Li: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.02/E26

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: The role of galectin-9 in huntington's disease

Authors: *T. HSU, Y. CHERN;

Inst. of Biomed. Sci. (IBMS), Academia Sinica, Taipei, Taiwan

Abstract: Galectin-9 (Gal-9), a tandem-repeat type lectin with two carbohydrate-recognition domains, has been implicated in multiple cellular processes including immune modulation and lysosomal regulation. Recent findings indicate that Gal-9 is highly expressed in microglia, the resident immune cells of the central nervous system, and plays a critical role in modulating their functional properties. While Gal-9 has been shown to maintain lysosomal integrity via LAMP2 binding under homeostatic conditions in peripheral cells, its role in neuroinflammation and microglial behavior remains unclear. In this study, we report that Gal-9 physically interacts with GAPDH, a glycolytic enzyme with emerging non-metabolic functions. Using SM826 microglial cells, co-immunoprecipitation and immunofluorescence revealed Gal-9-GAPDH colocalization in the cytosol. Functionally, pharmacological inhibition of GAPDH impaired microglial phagocytosis, as did Gal-9 knockout. Notably, dual perturbation of Gal-9 and GAPDH did not produce additive effects, suggesting a shared regulatory pathway. However, Gal-9 deletion did not affect GAPDH enzymatic activity, indicating non-catalytic interaction. Additionally, Gal-9 deficiency impaired microglial migration and enhanced motor performance in a Huntington's disease (HD) mouse model, though no significant histological differences were observed at late disease stage. These findings uncover a novel Gal-9-GAPDH axis involved in regulating microglial immune functions, potentially offering new insights into therapeutic strategies for neurodegenerative diseases such as HD.

Disclosures: T. Hsu: None. Y. Chern: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.03/E27

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: Behavioral, brain metabolic and retinal structural changes in cQ20 mice after conditional HTT loss

Authors: *T. HEIKKINEN¹, R. IMMONEN¹, T. BRAGGE¹, J. KORKALAINEN¹, T. MIETTINEN¹, M. YACOUN¹, **J. RYTKÖNEN**², M. CERRADA-GIMENEZ³, R. CHEN⁴, D. S. HOWLAND⁴, D. M. MARCHIONINI⁵;

¹Charles River Discovery Res. Services, Kuopio, Finland; ²Charles River Discovery Res. Services, Kuopio, ; ³Experimentica Ltd., Kuopio, Finland; ⁴CHDI Management, Inc. for CHDI Foundation, Inc., New York, NY; ⁵CHDI Management, Inc. for CHDI Foundation, Inc., New York, NY

Abstract: Huntingtin (*HTT*) lowering is currently one of the major treatment strategies for Huntington's disease (HD). *HTT* is expressed in all cells in the body and its function has been implicated in numerous mechanisms. Several reports from rodent studies have suggested safety issues after *HTT* loss.

In this study we used a mouse model with conditional loss of *HTT*. LoxP sites were inserted at 1356 bp 5' from the start of exon 1 and 2150 bp 3' from the end of exon 1 (in intron A) in the Q20 knockin mouse to generate cHtt-Q20_{3'}LoxP mice, which were crossed to UBC-Cre^{ERT2} to generate Htt-Q20^{lox/lox};UBC^{cre/+} progeny (cQ20). Young adult mice were treated with vehicle or tamoxifen (TM) at 12 weeks of age to induce cre-mediated recombination and global excision of *HTT*. In these mice there is no measurable unintended cre recombination in the absence of TM, and over 70% loss of *HTT* after TM administration.

After 4 weeks of *HTT* loss, the cQ20 mice showed significantly increased percentage of the 10-20 Hz tremor, and decreased percentage of the higher frequency, over 20 Hz tremor. At the same age, the *HTT* loss in cQ20 mice led to significantly decreased thalamic concentrations of alanine, GABA, glutamate, glycerophosphocholine, NAA and combined concentrations of NAA+NAAG, and elevated thalamic concentrations of creatine, glutamine and combined concentrations of creatine + phosphocreatine, as revealed by in vivo MRS measurements. Similar brain metabolic changes have been observed in R6/2 and zQ175 mouse striatum. In contrast, we did not detect changes in any metabolites in the striatum after *HTT* loss. In addition, OCT imaging revealed that *HTT* loss induced retinal thinning in certain cell layers of the eye, similar to what has been reported in the R6/2 mouse model. Altogether, total *HTT* loss led to observable changes, some of which resemble those seen in mice with expression of mutant *HTT*.

Disclosures: **T. Heikkinen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **R. Immonen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **T. Bragge:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **J. Korkalainen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **T. Miettinen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **M. Yacoub:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **J. Rytkönen:** A. Employment/Salary (full or part-time): Charles River Discovery Research Services. **M. Cerrada-Gimenez:** A. Employment/Salary (full or part-time): Experimentica Ltd. **R. Chen:** A. Employment/Salary (full or part-time): 3CHDI Management, Inc. for CHDI Foundation, Inc. **D.S. Howland:** A. Employment/Salary (full or part-time): 3CHDI Management, Inc. for CHDI Foundation, Inc. **D.M. Marchionini:** A. Employment/Salary (full or part-time): CHDI Management, Inc. for CHDI Foundation, Inc..

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.04/E28

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: Abnormal home cage climbing behaviour in a mouse model for Huntington's Disease.

Authors: R. SILLITO¹, M. CAMILLERI², R. S. BAINS⁴, S. WELLS⁵, ***D. ARMSTRONG**³;
¹Actual Analytics Ltd, Edinburgh, United Kingdom; ²Sch. of Informatics, ³Univ. of Edinburgh, Edinburgh, United Kingdom; ⁴Mary Lyon Ctr. at MRC Harwell, Oxfordshire, Harwell, United Kingdom; ⁵MRC Harwell, Harwell, United Kingdom

Abstract: Mice in home cages explore their environment to the full, climbing all over the available surface, particularly at night. In IVC cages with typical wire cage lids and food hoppers, healthy mice will show a complex mix of behaviours during these activities such as climbing up onto the food hopper and cage lid, hanging from the lid using front paws only, hanging inverted from the lid with all four paws and crawling around the lid (sometimes chasing each other). Previous work has indicated a progressive deficit in home cage climbing and locomotor behaviour with age in a N171-82Q mouse model of Huntington's disease (HD) when compared to wild-type littermate controls. Here we developed a set of algorithms to track individual mice within small groups as they explore their cage in 3D. A 2D array of RFID readers on the floor of the cage picks up individual identity and course location data for each tagged mouse. Mice were video recorded 24h per day using infra-red lighting and, critically, a side-mounted IR camera that gives an optimal perspective on climbing activity. By combining video tracking with RFID, we can identify clear periods where we have high confidence in the position and identity of each individual animal. Moreover, we can extract pose information to determine whether they are hanging vertically from two paws or exploring horizontally along the lid of the cage. We recorded N171-82Q and matched littermate controls at age 8 weeks, 13 weeks and again at 15-16 weeks (n>24 males and n>24 females per genotype). We observed a series of changes in climbing activity between HD model and control groups which get more pronounced as the animals age. In line with previous reports, at 15-16 weeks the effects are obvious with decreased climbing in addition to an overall decrease in locomotor activity in the HD group. However, we also see pronounced effects in younger animals when the phenotype is much less obvious using classical approaches. At both 13 weeks and even as young as 8 weeks old we see clear significant changes in climbing behaviour. In the young animals, the effects during the day/inactive phase are negligible but are significant towards the end of the active night phase. By quantifying different aspects of climbing behaviour, we have identified new biomarkers and captured their relative importance in revealing early disease progression for HD. These insights may provide a useful basis for considering other disease models that entail complex locomotor deficits.

Disclosures: **R. Sillito:** A. Employment/Salary (full or part-time): Actual Analytics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actual Analytics Ltd. **M. Camilleri:** A. Employment/Salary (full or part-time): Actual Analytics Ltd. **R.S. Bains:** None. **S. wells:** None.

D. Armstrong: A. Employment/Salary (full or part-time): Actual Analytics Ltd (Edinburgh UK). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actual Analytics Ltd (Edinburgh UK).

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.05/E29

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: R01NS127344
R01NS124084

Title: Impact of Huntington's Disease pathology on meningeal bridging veins and immune cells

Authors: *Y. ZHOU¹, Y. JIANG¹, Y. OUYANG¹, H. LIU¹, Y. SHI¹, L. DU¹, W. DUAN²;
¹Johns Hopkins Univ., Baltimore, MD; ²Psychiatry, Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Mutant huntingtin (mHtt) aggregation in neurons is a pathological hallmark of Huntington's disease (HD), and glymphatic clearance is impaired in HD; therefore, enhancing glymphatic clearance may alleviate mHtt accumulation. Cerebral fluid exchange between the brain parenchyma and meninges relies on arachnoid cuff exit (ACE) points, where bridging veins penetrate the arachnoid barrier to reach the dura, serving as essential gateways for cerebrospinal fluid (CSF) drainage. However, their role in HD pathogenesis remains unexplored. Using the zQ175-DN mouse model of HD, we assessed ACE morphology and immune infiltration under HD conditions. HD mice exhibited disrupted ACE architecture, impaired arachnoid barrier continuity, abnormal extracellular matrix deposition, decreased leptomeningeal arterial-associated parenchymal border macrophages (PBMs), and increased immune cell infiltration compared to age-matched wild-type mice. These HD-related ACE impairments were associated with reduced AQP4 polarization, indicative of disrupted glymphatic function. Enhancing lymphatic vessel angiogenesis restored ACE structural integrity and improved glymphatic drainage, whereas inhibiting lymphatic vessel growth worsened ACE deterioration and inflammation. Our findings highlight arachnoid bridges as vulnerable sites in HD pathology, where barrier failure and impaired lymphatic drainage exacerbate immune infiltration and fluid clearance dysfunction. Thus, preserving arachnoid integrity and lymphatic function may represent effective therapeutic strategies for mitigating neuroinflammation and slowing HD progression.

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Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR327.06/E30

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: NIH Grant 1F99NS141400
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UTHealth Dr. John J Kopchick Fellowship Award
UTHealth Presidents Research Excellence Award

Title: Huntingtin maintains neuronal homeostasis through endolysosomal trafficking, a process disrupted in Huntington's disease

Authors: *S. M. FARMER, Jr¹, X. YE¹, S. XU¹, D. COVARRUBIAS², B. RIOS¹, S. ZHANG¹;

¹Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; ²Rice Univ., Houston, TX

Abstract: Huntington's disease (HD) is a fatal autosomal-dominant neurodegenerative disease (ND) characterized by involuntary movement (chorea), cognitive decline, and behavioral disturbances. HD is caused by an abnormal expansion of the polyglutamine (polyQ) tract in Huntingtin (HTT), inducing both a gained toxicity and a disruption of HTT's normal physiological functions that together lead to the progressive loss of striatal and cortical projection neurons within the corticostriatal and cortico-basal ganglia-thalamocortical circuits. While emerging evidence suggests that HTT regulates neuronal homeostasis, the exact mechanism in a physiologic system remains unknown. To address this, we leveraged *Drosophila*, where HTT (dHtt) is fully conserved. Systematic characterization of dHtt-knockout (dHtt-KO) flies revealed abnormally small endolysosomal units with increased luminal acidity. Whole-genome deficiency screens revealed that dHtt genetically interacts with regulators of endolysosomal trafficking, a critical process that controls receptor cargo internalization, recycling and degradation, and most importantly, is found commonly disrupted in NDs including HD. Follow-up depletion assays showed that dHtt specifically interacts with Rab5, the master regulator endocytosis, and two downstream Rab5 effectors, Rabenosyn-5 (Rbsn5) and Rabankyrin-5 (Rank5). Consistent with prior cell-based studies showing that HTT binds to membrane-bound Rab5 via HAP40, HTT's essential binding partner, we found that dHtt/dHap40 complex localizes on Rab5-positive compartments, suggesting a conserved mechanism across species. Further, while rab5 mutants showed less dendritic complexity, phenocopying mutant Htt138Q-expressing animals, we found that dHtt/dHap40-KO had more complexity, suggesting that dHtt/dHap40 might negatively interact with Rab5 and/or its effectors. Finally, genetic depletion of Rbsn5 and Rank5 was found to ameliorate lethality associated with mutant Htt138Q toxicity, and resolve aggregation, indicating a direct interplay between HTT/HAP40 and Rab5's downstream effectors in HD. Future work will specifically test the impacts of HTT/HAP40/Rab5-mediated trafficking or turnover of neuromodulatory receptors, and the viability of therapeutically targeting this pathway in HD.

Disclosures: S.M. Farmer: None. X. Ye: None. S. Xu: None. D. Covarrubias: None. B. Rios: None. S. Zhang: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.07/E31

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: NIH R01NS127344
NIH R01NS124084

Title: Critical roles of meningeal lymphatics in Huntington's Disease

Authors: *L. DU¹, Y. ZHOU¹, Y. SHI², P. LU⁴, H. LIU¹, Y. OUYANG¹, H. WANG¹, S. DA MESQUITA⁵, J. THOMAS⁶, W. DUAN³;

²Biol., ³Psychiatry, Neurosci., ¹Johns Hopkins Univ., Baltimore, MD; ⁴Psychology, Cornell Univ., Ithaca, NY; ⁵Dept. of Neurosci., Mayo Clin., Jacksonville, FL; ⁶Paris Brain institute, Inserm, Paris, France

Abstract: Recent discoveries have revealed the presence of lymphatic vasculature within the meninges, underscoring its pivotal role in draining cerebrospinal fluid (CSF) and metabolic waste from the central nervous system (CNS) to peripheral lymph nodes. Meningeal lymphatic vessels (MLVs) facilitate the clearance of waste products, antigens, and fluids, as well as trafficking immune cells. Dysfunction of MLVs impairs CSF drainage and contributes to the accumulation of neurotoxic substances in the brain. In this study, we investigated the functional roles of MLVs in brain waste clearance using zq175DN knock-in mouse model of Huntington's Disease (HD). We found that ablation of MLVs by VEGF-C/D trap accelerated the onset of HD-like behavioral symptoms and increased mutant huntingtin (mHtt) aggregates in the brain. Conversely, enhancing lymphangiogenesis by overexpressing VEGF-C improved mHtt clearance and modestly ameliorated HD symptoms. Furthermore, pharmacological activation of Piezo1 with Yoda-1 improved CSF drainage to cervical lymph nodes in HD mice, identifying Piezo1 as a potential therapeutic target for HD. These findings highlight a role of meningeal lymphatic drainage in the progression of HD pathology and support further exploration of therapeutic interventions that target this brain-draining lymphatic system to prevent the progression of brain pathology and neurodegeneration in HD.

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Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR327.08/E32

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Contracts from the CHDI Foundation (S.A.A., J.B.C.)
Discovery Award from the Maryland Stem Cell Research Fund (S.A.A.)

Title: Multi-omic cellular trajectories in the striatum of Huntington's disease knock-in mice reveal risks of a HTT-lowering antisense oligonucleotide gene therapy

Authors: *A. SANTOSH KUMAR¹, E. WILDERMUTH⁴, M. E. CORTES-GUTIERREZ⁵, K. PUCHA², B. HERB³, S. A. AMENT⁶;

¹Human Genet. and genomic Med., ³IGS, ²Univ. of Maryland Baltimore, Baltimore, MD; ⁴Univ. of Maryland Sch. of Med., Baltimore, MD; ⁵Univ. of Maryland, Baltimore, MD; ⁶Dept. of Psychiatry and Inst. for Genome Sci., U Maryland Sch. of Med., Baltimore, MD

Abstract: Early pathogenic events by which Huntington's disease (HD) mutations lead to neurodegeneration remain incompletely characterized. Here, we describe these early effects of the HD mutation in unprecedented detail via single-nuclei RNA sequencing (snRNA-seq) and single-nuclei chromatin accessibility profiling (snATAC-seq) in the striatum of six-month-old *Htt*^{Q111/+} knock-in mice. Differentially expressed genes were detected primarily in medium spiny neurons (MSNs), with the greatest vulnerability observed in a *Drd2*-expressing sub-population whose marker genes indicate localization to striosomes. Structured decomposition analyses enabled us to map the progression of HD molecular signatures in MSNs along pseudotemporal trajectories leading from healthy to diseased states. Gene regulatory network modeling predicted key regulators of these transitions, involving the de-repression of enhancers inactive in healthy MSNs. We leveraged these models to enhance the pre-clinical evaluation of a non-allele-specific HTT-lowering antisense oligonucleotide (ASO) gene therapy. Surprisingly, this HTT-lowering ASO failed to rescue HD molecular signatures and produced aberrant neuroinflammatory effects, suggesting risks of this therapeutic strategy. Network modeling in our multi-omic data nominated novel therapeutic targets.

Disclosures: A. Santosh Kumar: None. E. Wildermuth: None. M.E. Cortes-Gutierrez: None. K. Pucha: None. B. Herb: None. S.A. Ament: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.09/E33

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Huntington's Disease Society of America

Title: Dysregulation of huntingtin interacting protein networks in human juvenile Huntington's disease brain

Authors: *S. PODVIN¹, C. MOSIER², V. Y. HOOK³;

¹UC San Diego, La Jolla, CA; ²Skaggs Sch. of Pharm. and Pharmaceut. Sci., Univ. of Calif., San Diego, La Jolla, CA; ³Pharm. Sci. and Neurosciences, UCSD, La Jolla, CA

Abstract: Human Huntington's disease (HD) is a genetic neurodegenerative disorder caused by the mutant HTT gene containing CAG repeat expansions, resulting in motor dysfunction and behavioral deficits. CAG repeats of 40-53 occur in adult HD and 60-120 repeats occur in early onset juvenile HD, differing from the normal range of 5-35 repeats. The HTT gene is translated to the huntingtin (Htt) protein that interacts with proteins in the development of HD. There have been few studies of Htt interactors in human HD brain. Therefore, this study evaluated the hypothesis that dysregulation of Htt protein interactors occurs in human juvenile HD brains. The strategy of this study was to analyze proteomic data of human juvenile HD brain putamen and cortex regions for dysregulation of Htt interacting proteins, using a database that we compiled of Htt interactors identified in HD model systems from yeast to HD mice. Results showed significant dysregulation of Htt protein interactors of mitochondria, signal transduction, RNA splicing, chromatin organization, translation, membrane trafficking, endocytosis, vesicle, protein modification, granule membrane, and macroautophagy pathways. The majority of downregulated and upregulated Htt interactors occurred in the putamen region compared to cortex. Dysregulation displayed downregulation of mitochondria and signal transduction interactors, combined with upregulation of RNA splicing, chromatin organization, and translational interactors. Network analysis revealed interactions among clusters of Htt interactors. These findings demonstrate prevalent dysregulation of Htt protein interactors in human juvenile HD brain, especially in the putamen region that controls movement deficits in HD. (This research was supported by a grant from the Huntington's Disease Society of America (HDSA) to S. Podvin, C. Mosier, and V. Hook.)

Disclosures: S. Podvin: None. C. Mosier: None. V.Y. Hook: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

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Program #/Poster #: PSTR327.10/E34

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Hereditary Disease Foundation, Research Grant

Title: Role of CDKN1A in Disease-Stage-Specific Neurodegeneration in Huntington's Disease

Authors: *Y. OH;

Mercer Univ. Sch. of Med., Columbus, GA

Abstract: Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by the selective loss of striatal medium spiny neurons (MSNs), caused by a CAG trinucleotide repeat expansion in the HTT gene on chromosome 4. Despite advances in our understanding, the precise molecular mechanisms underlying MSN vulnerability in HD remain unclear.

Historically, studying MSNs from HD patients at different disease stages has been challenging due to the inaccessibility of live neuronal tissue. Recent advances in direct neuronal conversion have enabled the generation of MSNs from patient-derived fibroblasts, providing a novel platform to examine disease-stage-specific cellular phenotypes. In this study, we examined MSNs converted from fibroblasts of symptomatic HD patients, pre-symptomatic HD patients (pre-HD), and healthy controls (HC). Our results highlight a neuroprotective role of CDKN1A (p21), a cell cycle regulator. Transcript analysis revealed that CDKN1A expression is significantly downregulated in symptomatic HD MSNs compared to pre-HD MSNs. To determine whether CDKN1A modulates neurodegeneration through DNA damage or autophagic processes, we quantified levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), 53BP1, and p62 via immunofluorescence. We observed increased DNA damage and p62 accumulation in HD MSNs, which correlated with reduced CDKN1A levels. These findings suggest that CDKN1A contributes to neuroprotection in HD by modulating DNA repair mechanisms and autophagic flux. Altogether, our data support a critical role for CDKN1A in regulating the onset and progression of HD, and further highlight its potential as a therapeutic target.

Disclosures: Y. Oh: A. Employment/Salary (full or part-time): Mercer University School of Medicine.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.11/E35

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: Activity-dependent local protein synthesis is altered in Huntington's Disease using human iPSC-derived neurons

Authors: *T. A. SAROVICH^{1,2}, J. WEI^{1,2};

¹Biomed. Sci., Florida Atlantic Univ., Boca Raton, FL; ²Stiles-Nicholson Brain Inst., Florida Atlantic Univ., Jupiter, FL

Abstract: Activity-dependent local protein synthesis is altered in Huntington's Disease using human iPSC-derived neurons

Huntington's Disease (HD) is a fatal, inherited neurodegenerative disorder marked by motor impairment, cognitive decline, and psychiatric symptoms. While the genetic cause, a CAG

expansion in the *huntingtin* (HTT) gene, is well defined, the cellular mechanisms driving early neuronal dysfunction remain unclear. A hallmark of HD is the accumulation of mutant huntingtin protein (muHTT), which disrupts key cellular processes, including synaptic function. Since changes in neuronal activity and synaptic plasticity underlie early HD symptoms, we investigated how muHTT affects synaptic functions and protein homeostasis, particularly local protein synthesis in this study. Using proximity labeling combined with mass spectrometry, we identified an association between HTT and seven ribosomal subunits. Notably, muHTT exhibited increased affinity for certain ribosomal proteins (e.g., RPS23, $p = 0.1$), suggesting altered translational regulation. To explore this, we used iPSC-derived forebrain neurons from HD patients (71 CAG repeats) and matched controls. Puromycin incorporation was used to label newly synthesized puromycin-peptides in live neurons. To visualize nascent protein production, we co-stained neurons with puromycin and pre/post-synaptic markers. HD neurons showed significantly fewer puromycin-labeled puncta in synaptic compartments than to controls, indicating impaired local translation. We further quantified puromycin in dendrites by normalizing the density of the puromycin signal to that of Map2-positive dendritic processes. At baseline, HD neurons exhibited a 22% reduction in puromycin incorporation into dendrites compared to healthy neurons, indicating impaired basal local translation. Upon treatment with picrotoxin, a GABA receptor antagonist that enhances neuronal activity, this impairment was exacerbated. HD neurons showed roughly 60% less puromycin incorporation relative to controls. Together, these findings suggest that muHTT disrupts activity-dependent local protein synthesis at the synapse, potentially through aberrant interactions with ribosomal machinery. This impairment in synaptic translation may contribute to early synaptic dysfunction and the progressive decline observed in HD.

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Disclosures: T.A. Sarovich: None. J. Wei: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.12/E36

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: Characterisation of patient iPSCs-derived striatal neurons with >140 CAG repeats

Authors: J. TILMAN¹, A. PITERA², Y.-F. WANG², A. BILLINTON², A. PAREDES-REDONDO¹, S. BARRETO¹, A. DALE¹, S. VYAS¹, *S. HUMPHREYS¹, T. BRISTON², A. BARNES¹;

¹Axol Biosci., Cambridge, United Kingdom; ²Harness Therapeut., Cambridge, United Kingdom

Abstract: Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder characterised by a profound loss of striatal neurons in the basal ganglia. Patients suffer

from a range of motor, cognitive and psychiatric symptoms, with no cure to date. Therefore, the generation of reliable physiologically relevant functional striatal neurons *in vitro* is fundamental to advance in the research of the HD field.

At Axol Bioscience, we have developed human induced pluripotent stem cell (hiPSC)-derived striatal neurons from healthy individuals and a 125 CAG HD patient. The current bank of HD iPSCs now has 143 CAG repeats because of its time in culture. We confirmed that both lines express key striatal neuron markers at the RNA and protein level after 20 days maturation. In collaboration with Harness Therapeutics, we observed morphological differences between the neuronal cultures, HD striatal neurons showed smaller cellular bodies and an increase in neurite branches compared to WT, which appeared with larger cell clusters. In addition, longitudinal culture of the HD striatal neurons revealed trinucleotide instability, mimicking the somatic instability seen in patients. In addition, modulation of FAN1 expression is being investigated to assess impact on trinucleotide instability over time. Functionally, HD striatal neurons are more active than control neurons when assessed by Multi Electrode Array (MEA) and differences in expression of HTT and HTT1a have been assessed by immunocytochemistry.

Other patient-derived iPSC lines with different CAG repeat lengths, including 39 CAG, 42 CAG, 38 CAG (unaffected at time of sampling) and 66 CAG, have also been matured to striatal neurons. Further characterisation and longitudinal studies of these cell lines are currently ongoing. This will allow the development of physiologically relevant cellular models with clear disease phenotypes for target identification and drug screening.

Disclosures: **J. Tilman:** A. Employment/Salary (full or part-time): Axol Bioscience. **A. Pitera:** A. Employment/Salary (full or part-time): Harness Therapeutics. **Y. Wang:** A. Employment/Salary (full or part-time): Harness Therapeutics. **A. Billinton:** A. Employment/Salary (full or part-time): Harness Therapeutics. **A. Paredes-Redondo:** A. Employment/Salary (full or part-time): Axol Bioscience. **S. Barreto:** A. Employment/Salary (full or part-time): Axol Bioscience. **A. Dale:** A. Employment/Salary (full or part-time): Axol Bioscience. **S. Vyas:** A. Employment/Salary (full or part-time): Axol Bioscience. **S. Humphreys:** A. Employment/Salary (full or part-time): Axol Bioscience. **T. Briston:** A. Employment/Salary (full or part-time): Harness Therapeutics. **A. Barnes:** A. Employment/Salary (full or part-time): Axol Bioscience.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.13/E37

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Fisher Center
Cure Huntington's Disease Initiative

Title: Deep Molecular Profiling of the Human Thalamus in Neurodegeneration

Authors: *R. MCLELLAN¹, C. PRESSL², M. SIANTOPUTRI¹, D. OH¹, N. HEINTZ¹;
¹Rockefeller Univ., New York, NY; ²Lab. of Mol. Biol., The Rockefeller Univ., New York, NY

Abstract: The thalamus is a central communication hub responsible for integrating and relaying information between cortical and subcortical brain regions. Historically viewed as a passive relay station for sensorimotor information, the thalamus is now recognized as playing a key role in a variety of higher order cognitive processes. Enhanced appreciation for diverse functional roles of thalamus has been accompanied by increased attention to its involvement in various neurological disorders, including Alzheimer's Disease (AD) and Huntington's Disease (HD). Despite these advancements, the cellular and molecular architecture of the human thalamus remains poorly understood, and little is known about which thalamic cell types are vulnerable to degeneration. To bridge this gap, we are exploring cell type specific molecular alterations in the thalamus occurring in AD and HD. Utilizing fluorescence-activated nuclear sorting (FANS), we isolated distinct thalamic cell types from postmortem brain tissue of healthy individuals and those diagnosed with AD or HD. We then performed comprehensive transcriptional profiling of thalamic cell types to characterize the molecular landscape of the adult human thalamus and interrogate cell type specific alterations associated with neurodegeneration. Ultimately, we hope to identify vulnerable thalamic cell populations and the underlying mechanisms driving this vulnerability. Moreover, this project presents a rare opportunity to study a structure implicated in two different neurodegenerative diseases, enabling us to uncover similarities and differences between them.

Disclosures: R. McLellan: None. C. Pressl: None. M. siantoputri: None. D. Oh: None. N. Heintz: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.14/E38

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: HDF Research Grant

Title: Identification of age-associated upstream regulator to promote neuronal resilience in HD patient-derived neurons

Authors: *S. LEE;
Mercer Univ. Sch. of Med., Columbus, GA

Abstract: Huntington's Disease (HD) is a dominantly inherited neurodegenerative disease with adult-onset clinical symptoms. Striatal medium spiny neurons (MSNs) are the primary cell type that degenerates in HD pathology, but the mechanism of neurodegeneration in HD patients is unclear. Directly converted MSNs from HD patient-derived fibroblasts (HD-MSNs) have been used to model HD pathologies, such as neuronal cell death and DNA damage. Using this method

in previous studies, three upstream regulator candidates were defined as key factors that can rescue HD-MSNs from neuronal death; NF- κ B1, IRF3, and SOD1. This study aims to determine which of these has the strongest neuro-protective effect in HD pathology when downregulated in HD-MSNs. To this end, we analyzed the levels of Sytox green-positive (neuronal death) and 53BP1-positive (DNA damage) cells in HD-MSNs treated with these factor-specific shRNAs. We found that NFKB1 knockdown most significantly reduced neuronal death and DNA damage in HD-MSNs, suggesting that targeting NFKB1 can confer neuronal resilience against HD pathology.

Disclosures: S. Lee: A. Employment/Salary (full or part-time): Mercer University School of Medicine.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.15/E39

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: CHDI

Title: Epigenetic mechanisms governing cell type specific somatic expansion and toxicity in Huntington's disease

Authors: *M. BAFFUTO, N. HEINTZ;
The Rockefeller Univ., New York, NY

Abstract: Huntington's disease (HD) is characterized by neuronal dysfunction and degeneration that varies markedly by brain region and cell type. We previously showed that CAG repeat expansion in exon 1 of the *mHTT* gene correlates with increased expression of the mismatch repair genes *MSH2* and *MSH3* in striatal medium spiny neurons¹, and demonstrated that, in the striatum and cerebral cortex of individuals with HD, hundreds of genes are dysregulated in neuronal cell types carrying somatically expanded CAG repeat in *mHTT*^{1,2}. Here we employ comprehensive epigenetic profiling in specific neuronal and glial cell types from the human striatum, cerebral cortex, hippocampus and cerebellum of control and HD donor samples to identify cell type- and species-specific transcriptional control mechanisms in the mismatch repair genes *MSH2*, *MSH3* and *FAN1* that can explain the specificity of somatic CAG expansion in the first stage of HD. In the second, toxic phase of HD we identify two distinct epigenetic mechanisms that disrupt regulation of hundreds of genes in the majority of HD MSNs, including several that cause haploinsufficient neurological disorders. Our data support a mechanistic model of HD pathogenesis in which regulation of mismatch repair gene transcription determines the selectivity of somatic expansion, and DNA methylation stabilizes the toxic effect of mutant huntingtin on HD-modifying proteins MED15 and TCERG1, which regulate enhancer function and transcription elongation.

Disclosures: M. Baffuto: None. N. Heintz: None.

Poster

PSTR327: Huntington's Disease: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR327.16/E40

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: R21NS111202
R01EB025819

Title: Characterization of spontaneous and chemically evoked activities in iPSC-derived neurons with Huntington's disease

Authors: *G. A. CANNESTRO^{1,2}, T. A. SAROVICH^{1,3}, J. GIORDANO^{4,2}, J. WEI^{1,2,3};
¹Biomed. Sci., ²Ctr. for Complex Systems, Florida Atlantic Univ., Boca Raton, FL; ³Stiles-Nicholson Brain Inst., Florida Atlantic Univ., Jupiter, FL; ⁴Florida Atlantic Univ., Boca Raton, FL

Abstract: Huntington's disease (HD), caused by an expansion of CAG repeats in the huntingtin (HTT) gene, is a hereditary neurodegenerative disorder that impairs cognitive, motor, and emotional functions. While considerable research has focused on understanding the molecular functions of both normal and mutant huntingtin, the ways in which these molecular mechanisms disrupt neuronal activity and lead to disease phenotypes remain unclear. Advancing our understanding of HD pathogenesis and developing effective therapies requires models that can bridge molecular insights with readouts of neuronal activities. Here, we present a multi-electrode array (MEA)-based platform to characterize spontaneous and induced activities of induced pluripotent stem cells (iPSC)-derived forebrain neurons from a healthy control and a HD patient. MEA enables functional assessment across spiking, burst, and network burst levels. Immunostaining and Western blot confirmed no significant differences in cell composition, allowing for valid activity comparisons. Developmental profiling revealed that while healthy neurons exhibited steady increases in activities from DIV10-40, HD neurons showed a biphasic pattern: initial hyperactivity, a transient quiescent phase, and re-emergence of activity. This irregular maturation may reflect disease-relevant mechanisms. At DIV40, baseline comparisons showed minimal differences across the spiking, burst and network burst parameters analyzed. However, upon application of picrotoxin (PTX), a GABA receptor antagonist, to induce neuronal activities, significant group differences were observed. Healthy neurons responded with higher increases in mean firing rate (MFR, fold change: 3.64 in healthy vs. 1.42 in HD), burst rate (MBR, 6.89 vs. 2.35), and burst duration (MBD, 1.44 vs. 0.75). HD neurons also showed fewer spikes participating in bursts and reduced burst spike frequency. Network burst analysis revealed a diminished response in HD neurons, with lower spike participation and shorter network burst durations. Principal component analysis (PCA) of 23 spiking and bursting parameters post-PTX showed clear separation between healthy and HD neurons, largely driven by burst-related

features. These findings suggest that while HD neurons show normal baseline activity, their network responsiveness to excitation is significantly impaired, highlighting functional deficits potentially relevant to disease pathology.

Disclosures: G.A. Cannestro: None. T.A. Sarovich: None. J. Giordano: None. J. Wei: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.01/E41

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Japan Agency for Medical Research Development (AMED) Grant 17ek0109201h0001
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Kanae Foundation for the Promotion of Medical Science
Hamaguchi Foundation for the Advancement of Biochemistry
Pharmacological Research Foundation

Title: Ceramide kinase causes neurodegeneration through cholesterol accumulation in niemann-pick disease type C

Authors: *H. NAKAMURA;

Grad. Sch. of Pharmaceut. Sciences, Chiba Univ., Chiba, Japan

Abstract: Niemann-Pick disease type C (NPC) is a lipid storage disorder caused by mutations in *NPC1* or *NPC2* genes. NPC is typically presented in the childhood with visceral lipid accumulation and complex progressive neurodegeneration characterized by cerebellar ataxia, dysphagia, and dementia, resulting in a shortened lifespan. While cholesterol is well known to be accumulated in NPC, multiple species of sphingolipids accumulate as well. In the present study, we found that ceramide-1-phosphate (C1P), a phosphorylated ceramide (Cer) produced by ceramide kinase (CERK), levels were increased in *Npc1*^{-/-} cells, *Npc1*^{-/-} mouse brain, and the plasma from the patients with NPC. CERK inhibition decreased free cholesterol accumulation in *Npc1*^{-/-} cells and patient-derived induced pluripotent stem cell neurons. C1P accumulation in *Npc1*^{-/-} cells negatively regulated the transport of LDL-derived free cholesterol to the endoplasmic reticulum. Rab9 overexpression in *Npc1*^{-/-} cells reduced free cholesterol accumulation, which was recovered by C1P treatment. Thus, C1P accumulation causes defects in the NPC1-independent and Rab9-dependent vesicular cholesterol trafficking in *Npc1*^{-/-} cells. Genetic deletion of *Cerk* in *Npc1*^{-/-} mice attenuated cholesterol accumulation in the cerebellum,

improved Purkinje cell survival, and prolonged lifespan. Moreover, in *Npc1^{-/-}Cerk^{-/-}* mice, oral administration of an approved drug for NPC “Miglustat”, a glucosylceramide synthase inhibitor, had a synergistic therapeutic effect. These results suggest that CERK is a novel therapeutic target for NPC.

Disclosures: H. Nakamura: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.02/E42

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Research Grants Council of Hong Kong 14102220
CUHK Gerald Choa Neuroscience Institute 8425035

Title: Upregulation of FasII underlies synergistic neuropathological and behavioral defects in a *Drosophila* model of myotonic dystrophy

Authors: *E. CHAN;

Sch. of Life Sci., The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Myotonic dystrophy type 1 (DM1) is a multisystemic disorder that has been extensively studied for decades, yet our understanding of its neuropathological aspect remains rudimentary. In this study, we characterized a novel model of DM1 neuropathology by expressing untranslated expanded *CUG* repeats at the *Drosophila* larval neuromuscular junction. In this model, both pre- and postsynaptic expression of *CUG* repeats participate to induce reduction of synaptic boutons, increase of arbor disassembly and impairment of larval locomotor activity. We found that the expression of *CUG* repeats caused an upregulation of the cell adhesion molecule, FasII (NCAM1 in mammals), in both the motor neurons and the body wall muscles. Knockdown of *fasII* was sufficient to rescue bouton numbers and locomotor impairment in this model. Further analyses identified the upregulation of the FasII-C isoform as a major contributor of these phenotypes. Remarkably, overexpressing the FasII-A-PEST+ isoform rescued the synaptic and behavioral defects, likely by outcompeting the upregulated FasII-C. Our study provided the foundation for a basic mechanism of synapse dysregulation in DM1.

Disclosures: E. Chan: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.03/E43

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: NINDS 3R15NS095317-02A1S1

Title: From Fragmentation to Dysfunction: TDP43's Role in ALS and FTD

Authors: *W. M. MIREMBE¹, E. NA², C. S. BROWER¹;

¹Biol., Texas Woman's Univ., Denton, TX; ²Texas Woman's Univ., Denton, TX

Abstract: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are associated with the cleavage and aggregation of the TAR DNA-binding protein 43 (TDP43). While loss of function due to cleavage is thought to contribute to disease, it remains unclear whether the resulting protein fragments also cause toxicity through a gain-of-function mechanism. To address this, we studied two specific TDP43 fragments, TDP43²¹⁹ and TDP43²⁴⁷, commonly found in aggregates from human FTD brains, in contexts where full-length TDP43 remains intact. We expressed these fragments in primary mouse neurons to assess neurodegenerative features such as protein aggregation and activation of apoptotic markers. We also expressed them in the upper motor cortex of mice with normal endogenous TDP43 levels and evaluated motor function using behavioral assays. Our results show that these TDP43 fragments increase protein aggregation, trigger apoptotic pathways, and worsen motor deficits, supporting a toxic gain-of-function role in neurodegeneration. Interestingly, the fragments differed in their effects, with TDP43²⁴⁷ showing greater aggregation and more pronounced toxicity. This suggests that different TDP43 cleavage products may contribute uniquely to disease progression.

Disclosures: W.M. Mirembe: None. E. Na: None. C.S. Brower: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.04/E44

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: RS-2025-00521565

Title: Mutations of NEK1 cause ciliary dysfunction as pathogenic mechanism in Amyotrophic Lateral Sclerosis (ALS)

Authors: *S. CHA;

Dementia Res. Group, Korea Brain Res. Inst., Daegu-gwangyeoksi, Korea, Republic of

Abstract: Neuronal primary cilia play a critical role in maintaining neuronal identity as a regulator of cell signaling and cell-cycle. Recently, while a link between primary ciliary defects and neurodegenerative diseases is emerging, the underlying pathological mechanisms remain poorly understood. In this study, we analyzed whole-exome sequencing of 920 Korean ALS patients to investigate the genetic contribution of *NEK1* to Amyotrophic Lateral Sclerosis (ALS) pathogenesis. *NIMA-related kinase 1 (NEK1)* encodes a serine/threonine kinase involved in cell cycle control and has been identified as a risk gene for ALS. Through the analysis, we identified 17 *NEK1* variants in 24 patients. Among these, two novel variants, p.E853Rfs*9 and p.M1?, reduced NEK1 expression, resulting in loss-of-function (LOF). Additionally, a synonymous splicing variant (p.Q132=) exhibited an aberrant isoform lacking exon 5. These *NEK1* variants consistently disrupted primary ciliary structure, impaired sonic hedgehog signaling, and altered cell-cycle progression. Furthermore, the ALS-linked variants induced intracellular calcium overload, which activated the AurA-HDAC6 axis, resulting in ciliary disassembly. These defects were rescued by treatment with the intracellular Ca^{2+} chelator, BAPTA. We also found that *NEK1* variants led to reduced α -tubulin acetylation, mitochondrial abnormalities, and defective DNA damage response. Notably, pharmacological inhibition of HDAC6 effectively recovered the NEK1-related defects in patient fibroblasts, and these findings were successfully replicated in iPSC-MNs model. Taken together, our findings suggest that NEK1 contributes to ALS pathogenesis through the LOF mechanism, and HDAC6 inhibition may provide a promising therapeutic strategy for ALS patients carrying *NEK1* variants.

Disclosures: S. Cha: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.05/E45

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: Identification of ATXN2-Regulated Genes as Biomarkers for ALS Progression and Therapy

Authors: E. L. MA, *M. LU;

iLab Res. Inst., Mountain View, CA

Abstract: Identification of ATXN2-Regulated Genes as Biomarkers for ALS Progression and Therapy Eddie Ma, Matthew Lo, Mason Lu, Qian Wang iLab Research Institute Amyotrophic Lateral Sclerosis (ALS) is the most common adult-onset motor neuron degenerative disease, shown by the progressive loss of both upper and lower motor neurons in

the brain and spinal cord. ALS is steadily being recognized to be a multisystem disorder, however 90-95% of ALS cases are sporadic with no known cause despite advances in understanding of its pathogenesis. The current lack of an ALS cure, points to the need for deeper molecular investigations. A hallmark of ALS pathology is the formation of insoluble cytoplasmic aggregates of transactive response DNA-binding protein 43 (TDP-43), encoded by the *TARDBP* gene. Notably, overexpression of wild-type TDP-43 alone can induce neurotoxicity and mimic ALS-like features. In non-human models, knockout of the *ATXN2* gene has shown protective effects against TDP-43-induced toxicity, but its neuroprotective mechanisms in human motor neurons remain poorly understood. To address this, we analyzed bulk RNA sequencing data from dataset GSE261875, downloaded from the NCBI Gene Expression Omnibus. The dataset comprises isogenic human motor neuron lines under eight experimental conditions: two negative controls, Empty Vector (EV) and YFP-only; wild-type TDP-43 overexpression (wTDP-43); and cytoplasmically localized TDP-43 (cTDP-43)-all of which were analyzed with or without *ATXN2* knockout generated via CRISPR. In total, 32 samples were analyzed. Differential gene expression analysis revealed significant transcriptomic changes between wTDP-43 and cTDP-43, in which cTDP-43 overexpression recapitulated a more severe ALS phenotype. However, in the *ATXN2* knockout background, the transcriptional differences between wTDP-43 and cTDP-43 were reduced, suggesting a potential neuroprotective role of *ATXN2* deletion. After comparing differentially expressed genes in our *ATXN2* knockout affected genome set to genes showing no significant change with *ATXN2* knockout, we highlighted a set of candidate genes modulated by *ATXN2* that could potentially protect motor neurons from cTDP-43 related toxicity and ALS progression and serve as a early diagnostic biomarker or therapeutic target for ALS. This work supports further investigation into *ATXN2*'s neuroprotective mechanism and their application in ALS treatment development.

Disclosures: E.L. Ma: None. M. Lu: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.06/E46

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: High-throughput phenotyping of fibroblast-derived induced neurons (FiNs) in SPG4-HSP

Authors: *K. METZNER^{1,2}, T. BÖRSTLER², J. WINKLER³, B. WINNER², F. KRACH²;
¹Univ. Hosp. Erlangen, Erlangen, Germany; ²Dept. of Stem Cell Biol., ³Dept. of Mol. Neurol., Friedrich Alexander Univ. Erlangen-Nuernberg, Erlangen, Germany

Abstract: Hereditary spastic paraplegia type 4 (SPG4), caused by mutations in the *SPAST* gene, is characterized by progressive neurodegeneration, particularly affecting corticospinal motor neurons. The disease typically presents in early adulthood with spasticity and weakness of the

lower limbs, progressing over time. Here, we aim to identify phenotypic abnormalities in a fibroblast-derived induced neuron (FiN) model of SPG4 that could serve as measurable readouts for future drug testing. This approach is particularly compelling as age- and disease-related epigenetic signatures of the donor fibroblasts are preserved in FiNs. We established a direct transdifferentiation protocol to generate FiNs from SPG4 patient and age- and sex-matched control fibroblasts. This method bypasses the induced pluripotent stem cell (iPSC) stage to retain the epigenetic and age-related characteristics of the donor cells. Using imaging-based high-throughput phenotypic screens, we identified significant deficits in SPG4 FiNs, including reduced neurite lengths and elevated neurite disintegration indices compared to controls. These findings indicate neurite damage in SPG4 FiNs. This platform establishes a robust disease model to investigate SPG4 pathophysiology and provides a basis for high-throughput drug screening to identify compounds that restore neurite integrity and length in SPG4-affected neurons.

Disclosures: K. Metzner: None. T. Börstler: None. J. Winkler: None. B. Winner: None. F. Krach: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR328.07/E47

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: NIH
Medical Biotechnology Program of UICOMR

Title: Uncovering Plasma Biomarkers and Potential Therapeutic Targets in SPG11-linked Hereditary Spastic Paraplegia

Authors: *A. CHENNAI RAVIKKUMAR¹, Z. CHEN², G. THAKUR³, X.-J. LI⁴;
¹Univ. of Illinois Col. of Med. Rockford, Rockford, IL; ²Univ. of Illinois at Chicago, Rockford, IL; ³Univ. of Illinois, Chicago, Rockford, IL; ⁴Dept. of Biomed. Sci., Univ. of Illinois, Rockford, IL

Abstract: *SPG11*-associated hereditary spastic paraplegia (SPG11-HSP) is a progressive neurodegenerative disorder characterized by corticospinal tract degeneration and lipid metabolism dysfunction caused by mutations in the *SPG11* gene resulting in loss-of-function of the protein Spatacsin. Although the genetic basis is known, the mechanisms underlying disease progression remain poorly understood, and no validated plasma biomarkers currently exist to support non-invasive, real-time monitoring of disease progression or therapeutic evaluation. To address this gap, we used human embryonic stem cell (hESC)-derived cortical projection neurons (PNs) with biallelic *SPG11*-mutation (40X, 651X) to model disease phenotypes *in vitro* and a novel *SPG11* knockout mouse model for *in vivo* exploration. *SPG11*-deficient neurons

displayed reduced axonal length and focal axonal swellings. The *SPG11* knockout mice exhibited significant motor impairment on rotarod testing (reduced latency to fall), accompanied by elevated plasma NFL levels compared to wild-type controls, suggesting its utility as a blood-based biomarker of neurodegeneration. Ongoing work includes profiling additional lipid metabolism markers and finding a correlation between these proposed neurodegenerative and lipid biomarkers upon Liver X Receptor (LXR) activation. The *in vivo* therapeutic potential of the LXR agonist drug candidates would also be assessed. These findings highlight the NFL as a promising biomarker for SPG11-HSP and lay the groundwork for therapeutic intervention strategies targeting lipid metabolism and axonal integrity.

Disclosures: A. Chennai Ravikkumar: None. Z. Chen: None. G. Thakur: None. X. Li: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.08/E48

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: PNR-RMR1-2022-12376284 CUPJ83C22002280007
ERP-2023-23684289 - ERP-2023-EJPRD-GNAO1-EU

Title: Synaptic dysfunction in cellular and rodent models of G protein-related disorders

Authors: *M. IONTA^{1,2}, A. RENNA^{1,2}, R. DE MORI², I. EL ATIALLAH², M. MONTANARI², G. PONTERIO², A. TASSONE², M. BENEDETTI³, V. KATANAIEV⁴, A. ROSA^{3,5}, P. BONSI²;

¹Univ. of Rome "Roma Tre", ROME, Italy; ²Fondazione Santa Lucia IRCCS, Rome, Italy; ³Sapienza Univ. of Rome, Rome, Italy; ⁴Univ. of Geneva, Geneva, Switzerland; ⁵Fondazione Inst. Italiano di Tecnologia, Rome, Italy

Abstract: Synaptic dysfunction underlies a group of genetic disorders known as synaptopathies, associated with severe neurological phenotypes characterized by a variable association of hyper- and hypo-kinetic movement disorders, neurodevelopmental disorders, and epilepsy.

Synaptopathies include disorders caused by mutations in GNAL and GNAO1 genes, encoding for the α_{olf} and α_o subunits of G proteins, respectively, which participate in G-protein coupled receptors (GPCRs)-mediated cAMP signaling pathway. In the striatum, stimulatory $G\alpha_{olf}$ is associated with adenosine A2A receptor (A2AR) and dopamine D1R, whereas inhibitory $G\alpha_o$ mediates dopamine D2R signaling. Notably, our previous work on a GNAL rat model showed alterations in both A2AR and D2R striatal protein levels, as well as an impairment of D2R-dependent synaptic plasticity, which was rescued by A2AR antagonism. Aim of the present work was to further characterize the alterations in the striatal GPCR-cAMP signaling pathway caused by GNAL and GNAO1 mutations in rodent models and in human iPSCs-derived neuronal cells,

in particular addressing the impact on striatal A2AR-D2R interplay. Firstly, we investigated the effect of a chronic *in vivo* treatment with a non-selective A2AR antagonist, caffeine, on the behavioral, biochemical and electrophysiological alterations observed in the GNAL rat model, and found that caffeine treatment rescued D2 receptor levels. The analyses on behavior and synaptic plasticity are being completed. Western blotting and electrophysiology analyses on a GNAO1 mouse model carrying the pathogenic C215Y mutation (C215Y-GNAO1) are ongoing. Interestingly, we found that, contrary to what observed in the GNAL model, the D2R striatal levels are not altered in C215Y-GNAO1 mice. To further investigate the impact of GNAO1 mutations on striatal function, we differentiated striatal projection neurons (SPNs) from iPSCs and their isogenic controls. Electrophysiological recordings from G203R-GNAO1 and E246K-GNAO1 iPSC-derived SPNs at 58-72 days did not disclose significant alterations in membrane properties and excitability. Immunofluorescence staining with antibodies against GSX2, DARPP-32, β -tubulin III, Isl1 and met-enkephalin showed no difference in the ability of control and GNAO1 lines to differentiate in SPNs. Accordingly, the expression levels of both GABA subunits and striatal-specific genes, measured with RT-PCR at different time points, showed no differences in the ability of mutant cells to differentiate into SPNs. Further analyses at earlier time-points are ongoing to confirm these findings.

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Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.09/E49

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: NIH Grant UH3NS095553

Title: Tolerability of closed-loop DBS in essential tremor patients

Authors: *N. GEIGEL¹, J. WONG^{1,3}, A. GUNDUZ^{2,3};

²Biomed. Engin., ¹Univ. of Florida, Gainesville, FL; ³Fixel Neurolog. Inst., Gainesville, FL

Abstract: Essential tremor (ET) is a neurodegenerative movement disorder characterized by an action-intention tremor of the bilateral upper extremities during voluntary movement. Up to 50% of cases are medication refractory. In these cases, deep brain stimulation (DBS) of the ventralis intermedius nucleus (VIM) of the thalamus has been shown to be an effective alternative for tremor suppression.

Currently, stimulation is delivered continuously, which can lead to unnecessary stimulation during tremor-free periods. Closed-loop DBS (CL-DBS) for ET has been developed as an

alternative to continuous stimulation. CL-DBS operates by responsively stimulating only during voluntary movement when tremor is present. To capture the transition from rest to movement and rapidly suppress tremor, stimulation must be ramped up at a steep rate. While CL-DBS may decrease the likelihood of stimulation-induced side effects overall, the abrupt onset of stimulation may cause recurring paresthesias.

During CL-DBS programming, we optimized the ramp rate to reach the therapeutic amplitude rapidly while accounting for the tolerability issues. Here, we report a sub-analysis of a larger prospective clinical trial of CL-DBS for ET. We describe our experiences in the programming optimization process of four subjects. We characterize the tolerability of a closed-loop stimulation paradigm across various parameters, including stimulation amplitude, pulse width, frequency, ramp rate, percentage of stimulation outside of the VIM, and their correlations with stimulation-induced paresthesias.

We created a binary logistic regression to predict the presence of paresthesias during CL-DBS using the aforementioned variables and found that higher ramp rates and a higher percentage of stimulation outside of the VIM significantly predicted the presence of paresthesias triggered at stimulation onset across subjects. We found Odds Ratios of 254.9 and 1.789, respectively, with an area under the curve for the model of 0.98.

We conclude that those patients stimulated beyond the posterior border of the VIM may be more likely to experience paresthesias, and this effect can be mitigated by lowering the ramp rate.

Disclosures: N. Geigel: None. J. Wong: None. A. Gunduz: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.10/E50

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Cala Health

Title: Unraveling How Transcutaneous Afferent Patterned Stimulation Provides Noninvasive Treatment of Refractory Essential Tremor Through Modulation of VIM Thalamus

Authors: J. RANUM¹, J. PERRAULT¹, B. M. KRAUSE², M. I. BANKS³, K. LUDWIG⁴, W. LAKE¹, *A. J. SUMINSKI¹;

¹Univ. of Wisconsin-Madison, Madison, WI; ²Anesthesiol., Univ. of Wisconsin - Madison, Madison, WI; ³Dept. of Anesthesiol., Univ. of Wisconsin, Madison, WI; ⁴Bioengineering and Neurolog. Surgery, Univ. of Wisconsin Madison, Madison, WI

Abstract: Essential tremor (ET), the most common movement disorder in adults, presents with involuntary shaking of the upper extremities during postural hold and kinetic tasks linked to dysfunction in the cerebello-thalamo-cortical network. Recently, transcutaneous afferent

patterned stimulation (TAPS), applied through a wrist-worn device, has emerged as a non-invasive treatment for medication-refractory ET. However, its mechanism remains unclear. We hypothesize that TAPS reduces tremors through modulation of the VIM thalamus in the cerebello-thalamo-cortical network. Employing refractory pure ET patients seeking VIM deep brain stimulation (DBS), we quantified clinical tremor improvement following TAPS treatment in a pre-operative setting, followed by intra-operative, microelectrode recording of the contralateral thalamus with concurrent TAPS treatment on and off. After one preoperative session, TAPS significantly reduces upper limb tremor average (0.61, $p=0.002$), with an asymmetric effect favoring the treated limb ($p=0.047$) and the greatest improvement tending to kinetic tremor ($R^2=0.943$, $p=0.002$). The magnitude of TAPS-related tremor reduction demonstrates a positive correlation with the modulation of alpha ($R^2=0.213$, $p<0.001$) and beta band LFPs ($R^2=0.255$, $p<0.001$) in the VIM. TAPS also suppressed spiking activity in the VIM ($R^2=0.104$, $p=0.029$), though it was uncorrelated with the degree of tremor reduction. Of note, TAPS-related modulation of LFPs and spiking activity was greatest near the optimal placement location for the DBS lead in treating ET ($R^2=0.122$, $p=0.006$). In sum, TAPS likely reduces tremor in ET by modulating the VIM and connected nodes in the cerebello-thalamo-cortical pathway.

Disclosures: J. ranum: None. J. Perrault: None. B.M. Krause: None. M.I. Banks: None. K. Ludwig: None. W. Lake: 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.; Cala Health. A.J. Suminski: 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.; Cala Health.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.11/E51

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Singapore Ministry of Education Research Fund, MOE2018-T2-2-103
Singapore Ministry of Health Research Fund, MOH-000207 and 000212
National Medical Research Council Open Fund

Title: Modeling MT IVNS1ABP-Induced neurodegeneration by human iPSCs derived Motor Neurons in a newly identified Progeria Syndrome

Authors: *F. YUAN¹, S.-C. ZHANG²;

¹Sanford Burnham Prebys, San Diego, CA; ²Ctr. for Neurologic Dis., Sanford Burnham Prebys, La Jolla, CA

Abstract: We have recently encountered a family whose teenagers display progeria symptoms, including dyschromatosis, gray hairs, progressive motor deficits, and intellectual developmental delay. Whole genomic sequencing revealed a homozygous mutation in the locus of IVNS1ABP gene. As an influenza virus nonstructural protein-1 binding protein, IVNS1ABP functions as a modifier of the aryl hydrocarbon receptor pathway, as well as involving in pre-mRNA splicing and F-actin organization. Structurally, IVNS1ABP is a kelch protein, belongs to the family of adaptor proteins of the E3 ligase. Unlike other KLHL proteins, it has been reported regulate proteasome system through KLHL20 instead of directly binding with Culin3. The association of IVNS1ABP and ageing or neurodegeneration has never been reported. How IVNS1ABP mutation leads to the severe neuronal symptoms remains unknown. Here, we acquired dermal fibroblasts from the patients and their family members, generated and created isogenic iPSCs from the fibroblasts and then differentiated the isogenic iPSCs to spinal cord motor neurons (MNs) as patients showed severe movement deficits. IVNS1ABP mutation (MT) ips derived MNs presented phosphorylated neural filament aggregates along the axon, Tau accumulation, increased DNA damage and neuronal death, suggesting the accelerated neuron degeneration. Transcriptomics of MNs revealed neuronal activity alteration on the molecule function. Functional analysis of MNs co-culture with muscle fibers showed reduced acetylcholine receptors in forming neural muscle junction (NMJ). Meantime, MT MNs also presented a reduced calcium transients frequency, suggesting the ionic signal alteration. Actin co-sedimentation and bioactivity analysis suggested IVNS1ABP mutation altered actin interaction and polymereization, which may be responsible for the MN degeneration phenotypes. Our findings established an *in vitro* model to mimic the motor neuron degeneration for this undiagnosed disease and suggested a potential mechanism underlying the ageing symptoms of the patients.

Disclosures: F. Yuan: None. S. Zhang: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.12/E52

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: The Collaborative Center for X-linked Dystonia Parkinsonism
Maryland Stem Cell Research Fund

Title: Retrotransposon expression causes R-loop formation, driving neurodegenerative phenotypes in X-linked dystonia-parkinsonism ventral forebrain organoids

Authors: L. D'IGNAZIO¹, *A. RODRIGUES LORENZETTI^{2,1}, E. B. PENNEY^{3,4}, T. A. EVANS^{1,5}, B. QAMAR¹, A. S. FELTRIN¹, A. ROCHA BARBOSA¹, B. SHEEHAN¹, B. TORRES¹, H. H. GILES^{1,6}, Y. WANG¹, T. SAWADA¹, M. ZABOLOCKI^{8,9}, M. G.

MURCAR^{3,4}, E. MUNOZ¹⁰, M. ANG¹⁰, C. BARDY^{8,9}, C. BRAGG^{3,4}, A. C. PAQUOLA^{1,5}, J. A. ERWIN^{1,5,7};

¹Lieber Inst. for Brain Develop., Baltimore, MD; ²Gage Lab., Salk Inst. for Biol. Studies, La Jolla, CA; ³Dept. of Neurol., Massachusetts Gen. Hosp., Charlestown, MA; ⁴The Collaborative Ctr. for X-linked Dystonia-Parkinsonism, Charlestown, MA; ⁵Dept. of Neurol., ⁶Dept. of Genet. Med., ⁷Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ⁸SAHMRI - South Australian Hlth. and Med. Res. Inst., Adelaide, Australia; ⁹Flinders Hlth. Med. Res. Inst., Adelaide, Australia; ¹⁰Dept. of Pathology, Univ. of the Philippines, Manila, Philippines

Abstract: Retrotransposon activation is emerging as a significant component in neurodegenerative diseases. X-linked dystonia-parkinsonism (XDP), a genetic neurodegenerative movement disorder, is caused by the insertion of a SINE-VNTR-Alu (SVA) retrotransposon within the *TAF1* gene, located on the chromosome X, and follows an X-linked recessive pattern of inheritance. The disease primarily affects males and manifests in adulthood, with age at onset inversely correlated with the length of a hexameric short tandem repeat (STR) contained in the XDP-causing SVA (XDP SVA). This association suggests a role for these STRs in pathogenesis. Currently, there is no cure for XDP, and only a few treatments can improve symptoms (e.g., deep brain stimulation). Learning more about the impact of the XDP SVA on pathogenesis may lead us to the identification of targets for new therapeutics. To explore this, we developed ventral forebrain organoids (VFOs) derived from patient induced pluripotent stem cells (iPSCs), a model of the striatum in vitro. We generated isogenic lines in which the XDP SVA was deleted using genome editing, and performed an extensive comparative characterization with non-edited lines, including but not limited to, transcriptional profiling, electrophysiological assays, and immunostaining. The presence of the XDP SVA in mature VFOs led to a transcriptional profile compatible with neurodegeneration hallmarks, delayed maturation in network development, and increased DNA repair and apoptosis. We identified a repetitive RNA species transcribed from the XDP SVA hexameric STR, which shows increased expression over VFO maturation, and is also detected in postmortem brain tissue of donors. The colocalization of this STR-derived RNA and a G4-forming hexameric STR ([AGAGGG]_n) favors the development of R-loops, which were detected in both the patient-derived VFOs and postmortem brain, potentially causing DNA damage and somatic instability. We designed antisense oligonucleotides (ASOs) targeting these repeat transcripts and successfully rescued apoptosis in mature VFOs, suggesting their contribution to pathogenesis. Our results open a new avenue for prospective therapeutics to target XDP SVA-derived repetitive transcripts and ameliorate cell death, and highlight the need for further investigation of retrotransposon expression in XDP.

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Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.13/E53

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Collaborative Center for X-linked Dystonia Parkinsonism
MD Anderson Neurodegeneration Consortium
The National Human Genome Research Institute Centers of Excellence in Genomic Science Program

Title: Mouse model of X-linked Dystonia Parkinsonism implicates oligodendrocytes in disease pathology

Authors: *P. PRAKASH¹, W. ZHANG², Y. ZHAO², K. LIMBERG³, C. F. LABORC³, A. C. MAR³, R. BROSH², J. BOEKE², S. A. LIDDELOW³;

²Inst. for Systems Genet., ³Inst. for Translational Neurosci., ¹NYU Sch. of Med., New York, NY

Abstract: X-linked Dystonia Parkinsonism (XDP) is an X-linked recessive neurodegenerative movement disorder characterized by the preferential loss of striatal medium spiny neurons (MSNs). XDP is caused by a disease-specific SINE-VNTR-Alu (SVA) retrotransposon insertion in the ubiquitous *TAFI* gene, which encodes the TATA-binding protein associated factor 1, known for its indispensable role in transcription and cell viability. Two factors have limited our understanding of XDP pathology. First, since *TAFI* is expressed in all cells, the contribution of individual cell types to the disease is not defined. Second, since SVAs are found solely in primates (including humans), we have not been able to replicate disease phenotypes using a relevant *in vivo* rodent model. To address these challenges, we have engineered a humanized model of XDP (*TAFI*^{PC-XDP}), which has a conditional hybrid mouse/human version of the *TAFI* locus. This engineered locus contains exons 1-24 of mouse (m) *TafI* and exons 25-38 of the human (h) *TAFI* gene. A single XDP-causing SVA insertion is included in the intron 32 of the human *TAFI* region. When crossed with cell-type specific Cre-expressing mice, these unique XDP model mice replace exons 25-38 of m*TafI* and replace it with the corresponding SVA-containing h*TAFI* region. This unique mouse can be used to study the contribution of individual cell types to XDP phenotype. We found that expressing the mutated *TafI* in Nestin⁺ neural stem cells induces a disease phenotype in mice, with features similar to human XDP, including striatal atrophy, progressive motor dysfunction, and reduced lifespan. RNA sequencing highlighted a dramatic downregulation of mature oligodendrocyte (OL)-associated genes but not oligodendrocyte precursor cell (OPC) genes, suggesting that OL differentiation and myelination in XDP brains are disrupted. Immunohistochemistry revealed aberrant MBP⁺ myelination in the striatum and corpus callosum (CC), along with a significant reduction of SOX10⁺ OL-lineage cells in the CC of male XDP mouse brains. While we observed changes in myelin integrity and mature OLs, we did not detect significant changes in the numbers of PDGFRA⁺ OPCs in male XDP brains, consistent with the findings of our RNA sequencing experiment. Human postmortem tissue from XDP patient brains also shows pathology similar to that of XDP mouse brains, including a notable reduction in MBP⁺ myelin. Our ongoing work focuses on understanding how the disruption of OL function contributes to XDP pathology. Investigating the underlying mechanism of OL dysfunction in XDP may provide new mechanistic insights into the drivers of disease pathology.

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Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.14/E54

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Title: A new murine model of X-linked dystonia parkinsonism (XDP)

Authors: ***K. L. PLOENSE**¹, **W. ZHANG**², **R. BROSH**³, **J. BOEKE**², **M. E. EHRLICH**⁴; ¹Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Biochem. and Mol. Pharmacol., ³Pathology, NYU Sch. of Med., New York, NY; ⁴Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Problem: X-linked dystonia Parkinsonism (XDP) is an X-linked recessive movement disorder affecting approximately 5 in every 100,000 men in the Philippine island of Panay. The causative XDP mutations likely the insertion of a SINE-VNTR-Alu (SVA) retrotransposon in the 32nd intron of the *TAF1* gene, a crucial element of the ubiquitously expressed TFIID transcription factor complex. Affected patients develop dystonia and parkinsonism and the disease is fatal. Methods/ approach: Our group sought to model XDP through the use of a humanized model, (courtesy of Dr. Boeke). Female C57Bl/6J mice homozygote for a conditional, humanized *Taf1/TAF1* gene are crossed with male mice expressing a Synapsin cre-recombinase-transgene. Double het males and Cre(negative) control male mice were subjected to a panel of locomotor tests, including the hindlimb clasping test, accelerating rotorod, vertical pole test, balance beam test, and a locomotor activity assay. Using standard IHC/IF techniques we assayed the size of the striatum and and percentage surface area occupied by striosomes, astrogliosis, microgliosis, and numbers of cholinergic interneurons (ChINs). Results: At 7 months, the hTaf1/Synapsin-cre+ mutant mice exhibited a large difference in body mass (~7 g less overall) and significantly decreased brain mass and size. These mice also displayed increased hindlimb clasping as well as deficits in accelerating rotorod behavior. Furthermore, these mice exhibited freezing-like symptoms, possibly seizures, and a lack of control over muscle movements at the end of life. The hTaf1/ Synapsin-cre+ mutants exhibited massive astrogliosis and activation of microglia as well as decreased striosome number and area within the striatum. In the cortex, there was increased

astrogliosis at 7 months, but no observed difference in the number of activated microglia in the hTaf1/ Synapsin-cre⁺ mutants Conclusion: The above-mentioned motor abnormalities and anatomical findings provide support for using the hTaf1/ Synapsin-cre mouse to model aspects of XDP. Their 7-month course is also amenable to pharmacologic testing.

Disclosures: K.L. Ploense: None. W. Zhang: None. R. Brosh: None. J. Boeke: None. M.E. Ehrlich: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.15/E55

Topic: C.04. Movement Disorders Other Than Parkinson's Disease

Support: Del Monte Institute for Neuroscience Pilot Award OP213039

Title: Mix23 contributes to the onset of motor and cognitive impairment and links m-aaa and i-aaa complexes in the mitochondria

Authors: *L. SHEPPARD¹, J. RANDOLPH², A. MACHERLA³, J. W. HAMMOND⁴;
¹Univ. of Rochester Neurosci. Grad. Program, Rochester, NY; ²Neurol., ³Univ. of Rochester, Rochester, NY; ⁴Neurol., Univ. of Rochester, Penfield, NY

Abstract: MIX23, also known as CCDC58, is a small protein residing in the mitochondrial intermembrane space. As an “orphan protein” of the mitochondria IMS, there is limited information regarding MIX23's function. Interestingly, a recent study revealed that humans with a *Mix23* variant display chorea-athetosis and intellectual disability, suggesting a neurological impact. To investigate the role of MIX23 in these symptoms, we analyzed a mouse line with an exon 4 deletion in the *Mix23* gene (*Mix23*^{-E4}). Motor and cognitive assessments were conducted at 4, 8, and 12-14 months of age. *Mix23*^{-E4} female mice begin to display signs of disturbed mobility at 4 months of age. By 12-14 months, both male and female *Mix23*^{-E4} mice show significant motor impairments in rotarod, beam walk assays, and via gait analysis. Additionally, *Mix23*^{-E4} mice show reduced freezing in contextual fear conditioning, indicating deficits in associative learning. A *Mix23* knockout HeLa cell line was utilized to elucidate the protein's functional impact in the mitochondria. Co-immunoprecipitation and mass spectrometry analysis revealed MIX23's strongest binding partner, SMIM12, loses association with the Prohibitins/m-AAA complex in *Mix23* knockout cells compared to WT cells. However, loss of MIX23 does not change SMIM12's binding with the SLP2/i-AAA complex. This suggests MIX23 and SMIM12 link the two AAA protease complexes together. This linkage may downregulate their protease activity, as SMIM12 is partially degraded in the absence of MIX23. Finally, Seahorse MitoStress assays revealed no significant differences in mitochondrial respiration between wild-type and *Mix23* knockout HeLa cells. In conclusion, MIX23 and SMIM12 associate with the

Prohibitin/m-AAA and SLP-2/i-AAA complexes and likely contribute to protein quality control pathways of the mitochondria. Loss of this MIX23 function triggers motor and cognitive impairments in mice.

Disclosures: L. Sheppard: None. J. Randolph: None. A. Macherla: None. J.W. Hammond: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.16/Web Only

Topic: C.06. Neuromuscular Diseases

Support: KAKENHI JP 23K06976

Title: Hsp990 promotes the degradation of disease-causing proteins via autophagy in models of neurodegenerative diseases

Authors: *H. ADACHI, Z. HUANG, E. BAT-ERDENE;
Dept. of Neurol., Univ. of Occup. and Envrn. Health, Japan, Kitakyushu, Japan

Abstract: [Objective] Polyglutamine (polyQ) diseases are inherited neurodegenerative diseases caused by the expansion of a polyQ tract within the causative genes. In chronic neurodegenerative diseases commonly observed phenotypes include the abnormal accumulation of disease-causing proteins and the formation of nuclear and cytoplasmic inclusions. Under pathologic conditions, the accumulated level of such misfolded and toxic proteins may exceed the protective ability of the proteolytic machinery; the inability to either maintain misfolded proteins in a soluble form or degrade them, results in their accumulation and the formation of inclusions. HSP990 has been used as a compound involved in stress responses as an inhibitor of the molecular chaperone Hsp90. However, the precise cellular mechanism of action of HSP990 in neurodegeneration is elusive. [Materials and Methods] We examined the effects of HSP990 in cultured cell models of neurodegenerative diseases. Cells were transfected using Lipofectamine 2000 with plasmids encoding mutant androgen receptor, huntingtin and ataxin-1. [Results] HSP990 preferentially decreased the expression of those causative proteins in the neuronal cell models ($p < 0.01$). The expression levels of the autophagic marker LC-3 II were significantly elevated in the cells after treatment with HSP990 ($p < 0.01$). HSP990 also decreased the expression of p62 ($p < 0.01$). Alpha serine/threonine kinase (Akt) is a crucial mediator of mTOR, and increases mTOR activity through the phosphorylation of mTOR at serine 2448, and further regulates autophagy. HSP990 dose-dependently suppressed the phosphorylation of mTOR, and both total-P70S6K and phospho-P70S6K (Thr389), suggesting that HSP990 inhibits mTOR activity. [Conclusions] These findings demonstrated that HSP990 induced autophagosome formation and enhanced the preferential degradation of the disease-causative proteins, and

promoted therapeutic effects in cellular models. HSP990 is a potential therapeutic reagent for the prevention of neurodegenerative diseases associated with the accumulation of polyQ-expanded proteins.

Disclosures: H. Adachi: None. Z. Huang: None. E. Bat-Erdene: None.

Poster

PSTR328: Additional Movement Disorders: Cellular and Molecular Mechanisms in Animal and Cellular Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR328.17/E56

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Elucidating the role of mitochondrial dysfunction in beta-propeller associated neurodegeneration (bpan)

Authors: *L. RAMOS-RODRIGUEZ¹, X. R. ORTIZ-GONZALEZ²;

¹Biomed. Grad. Studies; Neurosci. Grad. Group, Children's Hosp. of Philadelphia; Univ. of Pennsylvania, Philadelphia, PA; ²Neurol., CHOP/UPenn, Philadelphia, PA

Abstract: Beta-Propeller Associated Neurodegeneration (BPAN) is a rare genetic disorder characterized by a distinct progression from static infantile encephalopathy to a progressive adult Parkinsonian movement disorder. Models of BPAN have shown a substantial mitochondrial dysfunction phenotype. These phenotypes include abnormal mitochondrial morphology, decreased ATP production, increased lipid peroxidation, and protein peroxidation. While several mechanisms have been associated with neurodegeneration, there is no clear mechanism that explains the progression from encephalopathy to degeneration. BPAN is caused by mutations in the gene WDR45, which encodes the protein WIPI4, a protein involved in autophagy initiation. Autophagy is a cellular process that removes damaged organelles and proteins via the formation of autophagosomes, double-membrane structures that engulf cytosolic cargo and deliver it to lysosomes for degradation. In WIPI4-deficient cell lines, autophagosome formation is severely impaired, resulting in an accumulation of immature and unclosed autophagic structures. These cells also fail to respond to autophagy inducers like rapamycin, suggesting that the initiation of autophagy is disrupted. Making us question whether there is an autophagy induction failure in WIPI4-deficient cells that might impact mitochondrial quality control (mitophagy). Our models show decreased mitochondrial membrane potential, ATP production, and accumulation of mitochondrial DNA. However, we have not seen clear signs of oxidative damage, suggesting that there are adaptive mechanisms in our cell model that can help reduce ROS production. Given WIPI4's role in autophagy, we hypothesize that impaired mitophagy leads to progressive neurodegeneration in BPAN. We differentiated WIPI4-deficient iPSCs into neurons via Neurogenin2 overexpression to explore whether mitophagy is disrupted. Western blot of mitophagy regulators in WIPI4-deficient iNeu revealed increased BNIP3 and PARKIN levels compared to WT iNeu. Immunofluorescence analyses of BNIP3 also showed an increased

overlap with mitochondrial marker TOM20, in WIPI4-deficient iNeu, comparable to a mitochondrial stress drug (FCCP). These results suggest that mitophagy is activated, but it is unclear whether progression and maturation of mitophagosomes still occur. Our future work aims to determine whether the BNIP3 increase reflects a compensatory response or a failure of mitophagy turnover. Understanding the mechanism by which BNIP3 is accumulated in WIPI4-deficient iNeu could provide insight into the progression of mitochondrial dysfunction in BPAN models.

Disclosures: L. Ramos-Rodriguez: None. X.R. Ortiz-Gonzalez: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.01/E57

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01NS103981
NIH Grant R01CA273586
AARG-NTF-21-849207 (CRW)

Title: Revealing microglia heterogeneity in tuberous sclerosis complex: a transcriptomic and cellular phenomic analysis

Authors: *A. NAGAYACH¹, Z. CHENG², E. S. WOHLEB³, G. YU⁴, C. WANG⁵;

¹Dept. of Radiation Oncology, OSUCCC-James Cancer Hosp. and Solove Res. Institute, The Ohio State Univ., Columbus, OH; ²Bradley Dept. of Electrical and Computer Engin., Virginia Polytechnic Inst. and State University, Arlington, VA; ³Dept. of Pharmacol. & Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; ⁴Bradley Dept. of Electrical and Computer Engin., Virginia Polytechnic Inst. and State University, Arlington, VA; ⁵Dept. of Radiation Oncology, OSUCCC-James Cancer Hosp. and Solove Res. Institute, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Tuberous sclerosis complex (TSC) is an autosomal genetic disorder caused by the *TSC1* or *TSC2* gene mutations which led to the over-activation of the mammalian target of rapamycin complex 1 (mTORC1). TSC patients are afflicted with intellectual disability, autism, epilepsy, and other neuropsychiatric conditions. However, the derivation and progressions of the neurological aspects of TSC are still not well understood. Microglia are the resident immune cells of the brain that exhibit structural and functional plasticity. Evidently, microglia heterogeneous traits are allied with specific microglia functional and structural phenotypes that orchestrate the paradigm of different neurodegenerative diseases. We investigated the microglia cells in *Tsc1*^{GFAP} cKO mice at postnatal day 21 (P21). scRNA-seq analysis revealed a remarkable divergence in the microglia activation in the *Tsc1*^{GFAP} cKO cortex. Interestingly, a subset of microglia in the *Tsc1*^{GFAP} cKO expresses a unique set of genes that are analogous to the

previously identified disease associated microglia (DAM) and also possess certain genes that are expressed precisely in response to the TSC1 ablation. This novel subset of genes expression gets noticeably abolished after rapamycin (an mTORC1 inhibitor) treatment. We further verified all these changes allied morpho-functional traits by flowcytometry, qPCR, immunofluorescence, and phagocytic assay. Conclusively, the microglia heterogeneity in terms of structural and functional transitions in the *Tsc1*^{GFAF} cKO brain will not only provide a better understanding of the neurological events and modules during TSC but also open a novel window for developing effective therapeutic targets.

Disclosures: A. Nagayach: None. Z. Cheng: None. E.S. Wohleb: None. G. Yu: None. C. Wang: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.02/E58

Topic: B.09. Glial Mechanisms

Title: Mitochondrial dysfunction in microglia: a novel pathogenesis of POLG disease

Authors: *J. WANG¹, S. W. NOVAK², E. WANG³, N. G. COUFAL⁴;

¹Univ. of California San Diego, La Jolla, CA; ²Salk Inst., San Diego, CA; ³UCSD, La Jolla, CA;

⁴Pediatrics, UCSD, San Diego, CA

Abstract: Mitochondrial diseases arise from pathogenic variants in nuclear or mitochondrial genes, often presenting as progressive neurological disorders. Mutations in polymerase gamma (POLG), the catalytic subunit of DNA polymerase necessary for mitochondrial DNA replication, have emerged as one of the most common causes of mitochondrial disease. POLG mutations lead to a range of phenotypes from early-onset mtDNA depletion syndromes to later neurodegenerative diseases with symptoms like stroke-like episodes, epilepsy, and neurodegeneration. While peripheral immune dysfunction has been observed in POLG mouse models, contributing to premature aging-like phenotypes, little is known about cell-type specific mechanisms in the brain. Microglia, the resident innate immune cells of the brain, are key players in brain development and age-related decline. Our data show that POLG is highly expressed in primary human microglia relative to other cortical cells. We hypothesize that mitochondrial dysfunction in microglia contributes significantly to POLG-related neurodegeneration. To test this, we generated microglia (iMGs) from human iPSCs carrying patient specific POLG mutations, along with isogenic and unrelated controls. POLG-deficient iMGs exhibited clear mitochondrial defects, including reduced mtDNA copy number, impaired membrane potential, and disrupted oxidative phosphorylation. These dysfunctions were associated with enhanced mitophagy, increased mitochondrial ROS, and elevated lysosomal activity, pointing to excessive oxidative stress and hyperactive autophagy. We also observed inflammatory dysregulation, with POLG-mutant iMGs showing an ameboid, activated

morphology and heightened phagocytosis. To extend these findings in vivo, we xenotransplanted iHPCs from isogenic and POLG-mutant lines into a humanized mouse model lacking murine microglia. These xenografted human microglia (xMGs) recapitulated inflammatory morphology, with reduced branching, increased CD68, astrogliosis, and decreased myelination. In sum, our results suggest that microglia mitochondrial dysfunction is a key driver of POLG-associated neurodegeneration, with mtDNA depletion possibly triggering neuroinflammatory cascades and disease progression.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

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Program #/Poster #: PSTR329.03/F1

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01 NS110707

Title: Ngly1 deficiency perturbs microgliosis and neuroinflammation modeled by human pluripotent stem cells

Authors: *H. ZHANG¹, H. XUE², Y.-C. WANG⁴, Y. LIU³;

¹Ctr. for translational science, ²Florida Intl. Univ., PORT SAINT LUCIE, FL; ³Florida Intl. Univ., Port St. Lucie, FL; ⁴Applied Biomed. Sci. Inst., San Diego, CA

Abstract: N-glycanase 1 (NGLY1) is a cytosolic enzyme that removes *N*-glycans from retrotranslocated misfolded glycoproteins to facilitate their proteasome-mediated degradation. Biallelic loss-of-function mutations in the *NGLY1* gene result in NGLY1 deficiency, a rare genetic disorder characterized by a range of neurological symptoms, including microcephaly, abnormal brain activity, peripheral neuropathy, and hyperkinetic movement disorder. A hallmark of NGLY1 deficiency is the accumulation of ubiquitinated protein aggregates in cells, indicating disrupted ER-associated degradation (ERAD) and proteostasis. While the impact of NGLY1 deficiency on neuronal cells has been reported, how the function of microglia, the resident immune cells with a role in supporting brain development, may be altered in response to defective NGLY1 remains unclear. Here, we use microglia differentiated from patient-derived induced pluripotent stem cells (iPSCs), their gene-corrected isogenic clones, and NGLY1-knockout (KO) WA09 hESC clones along with their control cells, to investigate how NGLY1 deficiency may impact microglial development and function. Quantitative PCR and RNA sequencing analyses showed an upregulation of myeloid differentiation genes (*CDKN1C*, *HOXB7*, *RUNX1*) in all NGLY1-functional and -deficient primitive macrophage progenitors (PMPs). However, compared to isogenic controls, NGLY1-deficient PMPs appeared to hinder microglial maturation and disturb type-I interferon signaling, likely causing impaired proliferation of PMPs, accumulation of immature microglia, and dysregulated

neuroinflammation. Our findings suggest a previously unrecognized role for NGLY1 in regulating the development and neuroimmune function of human microglia. Elucidating this glial contribution to the neurodevelopmental abnormalities caused by NGLY1 deficiency could generate novel mechanistic insights into the pathogenesis of NGLY1 deficiency and offer potential targets for therapeutic intervention.

Disclosures: H. Zhang: None. H. Xue: None. Y. Wang: None. Y. Liu: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.04/F2

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

Title: From neuroinflammation to behavior: uncovering the role of microglia in perinatal stroke

Authors: *E. BERETTA^{1,3}, M. D'URSO⁵, G. CUBONI^{6,4}, L. VIGNOZZI², G. DEIDDA⁷, M. ALLEGRA⁸;

¹Univ. of Padua, PADOVA, Italy; ²Biomed. Sci., Univ. of Padua, Padua, Italy; ³Fondazione di Ricerca Pediatrica (IRP), Padua, Italy; ⁴Fondazione di Ricerca Pediatrica (IRP), Padova, Italy; ⁵Padova Neurosci. Ctr. (PNC), PADOVA, Italy; ⁶Univ. of Padua, Dept. of Biomed. Sci., Padua, Italy; ⁷Fac. of Med. and Surgery, Univ. of Malta, Msida, Malta; ⁸Neurosci. Inst., CNR, Padua, Italy

Abstract: Perinatal ischemic stroke, occurring between the 20th gestational week and the 28th postnatal day, affects about 1 in 2,300 live births and overlaps with critical time windows of heightened plasticity. The injury, potentially disrupting neurodevelopmental trajectories, causes neuronal death and triggers the resident immune cells in the brain, or microglia, within minutes. Upon activation, microglia contribute to both post-ischemic neuroinflammation and repair mechanisms, including plastic remodeling. While microglia-mediated inflammatory response has been associated with detrimental outcomes in adulthood, its role and adaptation in early life remain still an unmet need. To tackle this challenge, we induced a cortical lesion by permanent middle cerebral artery occlusion (MCAO) on postnatal (P) day 14. We assessed spontaneous recovery by behavioral tasks at multiple time points (days 2, 9, 23, 37, 51, 65, 79 post-injury), revealing motor deficits in the grid walk test, associated with a core lesion of about 3mm³ and a reduction in NeuN+ cells at D79. To dissect the specific contribution of microglia, we depleted this cell population via a 5-day treatment using the CSFR-1 inhibitor, PLX-5622, reducing Iba-positive cells by 85%. Beyond their immune function, microglia also regulate synaptic pruning and circuit organization, crucial for excitatory-inhibitory (E/I) balance. Whether these functions persist after early-life brain injury remains unclear. Thus, we investigated the molecular landscape of excitatory (vGLUT-PSD95) and inhibitory (vGAT-Gephyrin) pre- and post-synaptic markers, and perineuronal nets (PNNs), to evaluate stroke-induced reorganization under microglia modulation. This analysis will support future circuit-level investigations, including

electrophysiology. Finally, although stroke-induced inflammation arises centrally in the nervous system, growing evidence suggests a parallel systemic inflammatory reaction. To integrate central and systemic aspects of post-stroke inflammation, we collected peripheral blood from the same animals, to correlate systemic inflammatory markers with central microglial activity, and functional outcomes. Altogether, our findings will shed light on microglia role in neuroinflammation, synaptic remodeling and neuroimmune crosstalk in the developing brain, providing a foundation for potential therapeutic strategies targeting neuroinflammation after perinatal stroke.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.05/F3

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

Support: CIHR487354

Title: Single-cell transcriptomic insights into disrupted cerebellar microglia pathways in a murine model of perinatal brain injury

Authors: *S. TREMBLAY¹, M. MENGUS²;

²Univ. de Montréal, ¹Univ. de Montréal, Montréal, QC, Canada

Abstract: Background: Premature infants born at extreme preterm stages often face multiple inflammatory challenges, such as perinatal cerebellar hemorrhage (CBH) and postnatal infections—both of which are significant contributors to neurodevelopmental impairments. Microglia, with their dual roles in immune defense and neurodevelopment, are thought to play a pivotal role in the progression of cerebellar injury. This research explores microglial function using a murine model of perinatal brain injury caused by hemorrhage and systemic inflammation, with the goal of identifying potential therapeutic strategies to enhance neurodevelopmental outcomes. **Methods:** At postnatal day 2 (P2), cerebellar hemorrhage was induced via intracerebellar injection of collagenase (0.5 U/μL) into the right hemisphere, followed by systemic inflammation induced by intraperitoneal injection of lipopolysaccharide (LPS, 300 μg/kg). Single-cell RNA sequencing was performed on cerebellar cells harvested at P15 from injured (Coll-LPS, n=5) and control (Veh-Veh, n=5) mice. Graph-based clustering identified transcriptionally distinct microglial populations. Differential expression and gene ontology enrichment analyses were used to characterize injury-associated transcriptional changes. **Results:** Five transcriptionally distinct microglial clusters were identified. Based on the top 10 enriched genes in the control group, clusters were annotated as follows: Cluster 1 (Mature microglia), Cluster 2 (Homeostatic microglia), Cluster 3 (Immediate early response microglia),

Cluster 4 (Resolving microglia), and Cluster 5 (Activated inflammatory microglia, or DAM). Focusing on Cluster 1, hierarchical clustering of differentially expressed genes revealed a significant downregulation of biological processes related to neuron projection development, neuronal differentiation, and neurogenesis in the double injury group compared to controls. Enrichment analysis identified key disrupted pathways including axon guidance, neurotrophin signaling, PI3K-Akt, and TGF- β signaling, highlighting impairments in neuronal maturation and circuit formation following perinatal injury. **Conclusions:** Perinatal insults profoundly disrupt microglial pathways crucial for neuronal circuit development. This study highlights a persistent molecular signature of altered microglial identity resulting from neonatal cerebellar injury, emphasizing the significant effects of early-life inflammation and hemorrhage on cerebellar development, as revealed through single-cell resolution analysis.

Disclosures: S. Tremblay: None. M. Mengus: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.06/Web Only

Topic: B.09. Glial Mechanisms

Support: NIH Grant R15NS139138

Title: Traumatic brain injury induces changes in microglial chirality at the acute phase post injury

Authors: J. FAN, M. SERHAN, I. NASSER, F. EL SAGHIR, *Z. ZHANG;
Natural Sci., Univ. of Michigan-Dearborn, Dearborn, MI

Abstract: Cell chirality, the intrinsic handedness of cells, is crucial for various biological processes, including cytoskeletal dynamics, intercellular junction formation, cell migration, and tissue morphogenesis. Changes in cell chirality are often triggered by external stimuli or cytotoxicity and can consequently lead to physiological changes in tissues or organs. Traumatic brain injury (TBI) in children disrupts normal brain development and function, triggering a cascade of effects including cellular damage, neuroinflammation, and neurodegeneration. Microglial cells are key cellular mediators in the pathophysiology of TBI and play a critical role in the neuroinflammatory response following injury. However, it is still unknown whether microglial cells exhibit chiral bias and how this affects their behavior during TBI. The main goal of this study is to investigate the impact of TBI on the chirality of microglia at the acute phase post-injury in the immature brains. Male (M) and female (F) mouse littermates were randomized on postnatal day 20-21 into Sham and TBI groups (n=20 animals per group, 10M/10F). Animals in the TBI groups underwent TBI procedure, while animals in the sham groups underwent anesthesia without injury. Animals in all groups underwent behavioral testing before injury (baseline) and at 4-hour (h) post-injury and were euthanized after behavioral tests. The personnel

were blinded to the group assignments. Microglia were isolated from the injured brain regions in the TBI animals (or the matching regions in the sham). One cohort of microglial cells were seeded and cultured on in-house fabricated ring-shaped micropatterns (200 μ m inner diameter and 500 μ m outer diameter) for 24-48 h to reach confluency. Chirality analysis was performed on high-resolution phase-contrast images using a custom-written MATLAB (MathWorks) program, and cell chirality was determined as clockwise (CW), counterclockwise (CCW), or non-chiral (NC), as previously described. The second cohort of microglial cells was used for gene expression analysis. We found that Sham microglia exhibited NC-dominated chirality with a weak CCW bias (chiral factor = -0.113), whereas after TBI, the microglial chirality changed to strong CCW (chiral factor = -0.512). Additionally, the change in microglial chirality was accompanied with differential expression of cell chirality-associated genes that regulate the crosslinking of actin filaments and cellular kinases, such as protein kinase C alpha (PKC α). These results suggest that targeting microglial chirality could provide novel opportunities to evaluate TBI induced brain damage and improve therapeutic outcomes ultimately.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.07/F4

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

Support: NIH Grant R21 MH124294
NARSAD Young Investigator
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Partnership in Genomics (CZIF2022-007046)

Title: Neuroprotective role of microglial adapter protein Iba1 in acute ischemic stroke

Authors: *O. S. FAVORODE, A. MILLER, S. NANDI;
Anat., Howard Univ. Col. of Med., Washington, DC

Abstract: Stroke is a leading cause of mortality and disability, with approximately 90% of cases classified as ischemic in nature. Ischemic stroke occurs when the blood supply is cut off from a part of the brain, subsequently resulting in neuronal injury. Strategies aimed at neuronal protection have largely failed. Microglia, the brain's resident immune cells, are among the first responders to injury. Allograft Inflammatory Factor-1 (AIF1, also known as Iba1) is an actin-binding, proinflammatory protein in microglia and macrophages with demonstrated roles in microglial activity and in normal brain function. However, Iba1's role in an injured brain is unclear. Interestingly, Iba1 is acutely upregulated in microglia following cerebral infarctions. To investigate microglial Iba1's role in acute ischemic stroke, we compared germline Aif1-deficient

(Aif1^{-/-}) mice to wild-type (WT) controls subjected to photothrombotic stroke, a cortical model for ischemic stroke. Nissl staining revealed that Aif1^{-/-} mice displayed an increase in lesion areas compared to WT mice (WT: 5.1±0.56 %, n=30 slices from 3 mice Vs. Aif1^{-/-}: 19±3.2 %, n=37 slices from 3 mice). Immunohistochemistry of brain sections further demonstrated elevated numbers of C1q-expressing microglial (NeuN⁺ C1q⁺) cells in the ischemic core (WT: 56.6±3.7 cells/mm², n=7 ROIs from 3 mice Vs. Aif1^{-/-}: 110.3±11.6 cells/mm², n=7 ROIs from 3 mice), peri-infarct cortex (WT: 69.7±4.5 cells/mm², n=30 ROIs from 3 mice Vs. Aif1^{-/-}: 99.2±5.8 cells/mm², n=30 ROIs from 3 mice), and contralateral hemisphere (WT: 79.0±4.7 cells/mm², n=31 ROIs from 3 mice Vs. Aif1^{-/-}: 107.5±5.5 cells/mm², n=29 ROIs from 3 mice) of Aif1^{-/-} mice. Additionally, Aif1^{-/-} mice exhibited a significant reduction in non-microglial phagocytic cells (Tmem119⁺ CD68⁺) within the peri-infarct area (WT: 19.8±4.0 cells/mm², n=20 ROIs from 3 mice Vs. Aif1^{-/-}: 8.9±1.8 cells/mm², n=24 ROIs from 3 mice) and ischemic core (WT: 25.6±4.9 cells/mm², n=27 ROIs from 3 mice Vs. Aif1^{-/-}: 9.6±2.1 cells/mm², n=38 ROIs from 3 mice) compared to WT mice. Together, these data suggested that the absence of Iba1 exacerbates ischemic injury, potentially via microglial-derived C1q-mediated complement activation, presumably enhancing neurodegeneration. A diminished non-microglial phagocytic cell recruitment to injury sites upon Iba1 loss, could also promote damage expansion. Our results reinforce the emerging concept that proinflammatory pathways can confer neuroprotection in acute stroke through diverse mechanisms. Thus, targeting Iba1 or inflammatory mediators in general may represent a promising strategy for acute ischemic stroke therapy.

Disclosures: O.S. Faborode: None. A. Miller: None. S. Nandi: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.08/F5

Topic: B.09. Glial Mechanisms

Support: NIH Grant NS126987

Title: Glia and perineuronal net alterations in a genetic knockout model of chronic neuropathic pain

Authors: *E. N. WILLERSON^{1,3}, G. LI³, J. M. LUTCHMAN³, H. LATIFF⁴, G. CATALDO³, J. C. BRUMBERG^{3,2};

¹Psychology, ²The Grad. Center, CUNY, New York, NY; ³Psychology, ⁴Computer Sci., Queens College, CUNY, Flushing, NY

Abstract: Glia have been implicated as key players in the development and maintenance of chronic neuropathic pain. Previous research in mice involves employing invasive procedures to induce pain (broken bones, nerve ligations, chemical injections). This introduces tissue damage, impacting glial function. Here, a genetic knockout (KO) mouse is used to explore the

morphology and development of glia in chronic pain. Prrxl1 is a homeodomain transcription factor necessary for the proper development of the spinal cord dorsal horn (DH) and brainstem trigeminal nuclei (TN). Prrxl1 KO results in dorsal root and trigeminal ganglia neurons being unable to find their targets in the DH and TN (respectively). Using von Frey mechanical stimulation, we determined that Prrxl1 KO results in simultaneous bodily hypoalgesia and orofacial hyperalgesia. Anatomically, microglia morphology was assessed in the DH and in two TN - the Principal Sensory Nucleus (PrV) and the Spinal Trigeminal Nucleus (SpV). Immunohistochemical staining was performed using a monoclonal antibody for Ionized Calcium Binding Adapter Molecule 1 (IBA-1) to reveal microglia. Microglial reconstructions were followed by measures of complexity - number of nodes, branches, soma size, branch length - and compared between nuclei, mouse strain (Prrxl1 KO and wildtype [WT] controls), age, and sex. In many pathologies, including chronic pain, microglia shift morphology from a complex, highly-branched phenotype to one with reduced complexity. This is accompanied by upregulation of phagocytic activity and increased production of pro-inflammatory factors. Here, we found microglia in both the PrV and SpV of adult Prrxl1 KO mice (P60-P150) show significant reduction in complexity compared with WT mice. Interestingly, in all mice, microglia morphology was significantly different between the SpV tract and the PrV. A second non-neuronal component involved in chronic pain, perineuronal nets (PNNs), were also examined. PNNs are net-like structures of the extracellular matrix that primarily surround inhibitory synapses. In chronic pain, these have been reported as degraded in effected regions. Here, PNNs were stained using wisteria floribunda agglutinin (WFA). Density was assessed via luminance (average brightness per pixel), and found to be significantly reduced in the PrV of Prrxl1 KO mice when compared with WT, demonstrating a decrease in PNN density. Our results point to the Prrxl1 KO mouse as a model for studying non-neuronal components of chronic pain, and indicate microglia's involvement in pain arising sans external input. Taken together, this affirms glia and PNNs as promising targets for treatment of chronic pain.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.09/F6

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

Support: Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India

Title: Hippocampal microglial activation and behavioural deficits via the kynurenine pathway in the rat model of neuropathic pain

Authors: *S. K. JHAJHRIA¹, H. SHARMA², K. SARAVANAN³, M. BISHNOI⁴, D. KUMAR²;

¹Anat., ²All India Inst. of Med. Sci., New Delhi, India; ³Saveetha Med. Col., Chennai, India;

⁴Santosh Med. Col., Noida, India

Abstract: Neuropathic pain is a complex and debilitating condition that presents significant challenges for effective management. We anticipated a direct correlation between immune activation and neuroinflammation mediated by the kynurenine pathway (KP) following peripheral nerve injury. This study aimed to explore the relationship between neuropathic pain and microglial activation in the nervous system, particularly in the hippocampus, and investigate the associated biochemical and behavioral manifestations in a rat model of neuropathic pain. The present study intends to determine the effects of neuropathic pain on the hippocampus through behavioural, immunohistological, and biochemical analyses. We used male Sprague Dawley rats (n=6 per group) after getting approval from the Institutional Animal Ethics Committee. We divided the rats into two groups: Sham and Partial Sciatic Nerve Ligation (PSNL), observing them for 28 days. Behavioral assessments included von Frey tests for mechanical allodynia, open field tests for exploratory behavior, and novel object recognition tasks to evaluate memory and learning. Partial sciatic nerve ligation (PSNL) was performed to induce neuropathic pain. After 28 days, animals were sacrificed through perfusion fixation, and the brain was procured. Fresh brains and serum were collected from another group of animals, which were processed and stored for LC-MS (Liquid chromatography-mass spectrometry) analysis to measure KP metabolites. The PSNL group exhibited a reduced paw withdrawal threshold, indicative of mechanical allodynia, and showed decreased locomotor activity and exploratory behaviour compared to the Sham group. Immunohistochemical analysis revealed increased expression of activated microglia in the hippocampus of the PSNL group. Quantitative analysis confirmed a higher number of microglial cells and larger cell volume in the PSNL group. Additionally, circulating serum levels of tryptophan (TRP) and kynurenine (KYN) were significantly decreased in the PSNL group compared to the sham controls. Notably, a significant increase in the ratio of quinolinic acid (QUIN) to TRP in the hippocampus was observed in the PSNL group when compared to sham controls. The observed behavioral changes, along with increased microglial activation in the hippocampus, suggest that neuroinflammatory processes are involved in neuropathic pain. Overall, these findings provide preliminary evidence of alterations in both behavioural and biochemical parameters associated with neuropathic pain.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

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Program #/Poster #: PSTR329.11/F8

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

Support: NIA Grant R01AG062716 (LKF)

Title: Microglia depletion with CSF1R inhibitor alters the aged rat neuroimmune environment

Authors: *O. CANO¹, A. CAMPANILE², E. R. CARLSON³, L. SINGLETON⁴, C. AGUILAR⁵, S. A. COLLINS⁷, K. NIXON⁶, L. K. FONKEN⁵;

¹The Univ. of Texas at Austin, Pflugerville, TX; ³Pharmacol. & Toxicology, ²The Univ. of Texas at Austin, Austin, TX; ⁴Pharmacol. and Toxicology, ⁶Pharmacol. & Toxicology, ⁵Univ. of Texas at Austin, Austin, TX; ⁷Univ. of Texas, Austin, Austin, TX

Abstract: Microglia, the brain's resident immune cells, are implicated in the development of many neurological conditions. In aged animals, microglia can shift towards a pro-inflammatory phenotype and demonstrate impairments in maintaining homeostasis. These dysregulated microglial responses are considered to be a key contributor to neurodegenerative diseases like Alzheimer's disease. Microglia depend on colony-stimulating factor 1 receptor (CSF1R) signaling for their survival, and CSF1R inhibitors can induce microglia depletion. Microglia depletion and repopulation can reset microglia phenotype, as suggested by some, though not all, work in mice. This led us to test the efficacy of CSF1R-induced microglia depletion in aged rats. We first administered the CSF1R antagonist Plxixikon 5622 in diet (PLX, 1200 ppm) for 10 days in aged (~25 months) male Fisher 344 x Brown Norway (F344xBN) rats, but this did not result in microglia depletion. Next, aged F344xBN male and female rats received twice-daily subcutaneous 15 mg injections of PLX for one week. Twelve hours after the last PLX dose, rats were transcardially perfused and brains were collected to examine microglial number via immunohistochemical analysis of Iba1 (a cytoskeletal protein found in microglia), and changes in gene expression of neuroimmune markers.

Microglia in the hippocampus and entorhinal cortex of PLX-treated rats were depleted by 60-75% compared to controls, with greater microglia depletion in the entorhinal cortex than in the hippocampus. Interestingly, male rats also exhibited enhanced depletion compared to females, despite receiving a lower relative dose (when correcting for body mass). Microglia depletion was associated with increased expression of several astrocyte markers, including GFAP (a canonical astrocytic marker) and AQP4 (a water-channel protein found on astrocytes). Further, while some "pro-inflammatory" markers were decreased (e.g., IL-6) in PLX-treated rats, others remained unchanged (e.g., TNF- α). These results suggest that PLX can partially deplete microglia in aged rats. Future work will explore whether repopulating microglia following PLX treatment can reset the age-associated inflammatory environment.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

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Program #/Poster #: PSTR329.12/F9

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

Support: R01AG062716

Title: Long-term consequences of binge-like ethanol exposure on microglia reactivity in aged male rats

Authors: *L. SINGLETON, A. CAMPANILE, E. R. CARLSON, L. K. FONKEN, K. NIXON;
Divison of Pharmacol. and Toxicology, Univ. of Texas at Austin, Austin, TX

Abstract: Aging populations are growing as is alcohol consumption in aged individuals. Alarming, both aging and alcohol misuse can induce neuroimmune dysfunction. The commonalities among alcohol and age-related changes in neuroimmune function suggest alcohol misuse with advanced age may elicit synergistic disruptions within the neuroimmune system. Here we sought to determine if latent impairments in hippocampal-dependent learning and memory in aged ethanol-treated rats are accompanied by changes in microglia. Aged (F344BN; 24-26 months; $n = 21$) male rats were gavaged with ethanol (25% w/v in Vanilla Ensure Plus®) or dextrose-isocaloric control diet once every 8 hours for two days. Ethanol dosing was determined using a modified Majchrowicz model, with rats receiving an initial 5 g/kg dose and subsequent doses ranging from 0-4 g/kg depending on behavioral intoxication state. Rats received a daily average dose of $7.7 \text{ g/kg} \pm 0.4$ which resulted in $391.3 \pm 11.5 \text{ mg/dl}$ blood ethanol concentration. Brain tissue was collected 38-39 days after binge-like exposure. Immunohistochemistry was used to visualize Iba1+ microglia as well as CD68 co-localization with Iba1 to indicate phagolysosomal activity. Aged rats that received ethanol had significantly more Iba1+ cells within hippocampal subfields including the CA1 (15% increase; $p = 0.04$) and dentate gyrus molecular layer (MLDG; 18% increase; $p = 0.003$). Aged ethanol and control rats showed comparable co-localization of Iba1 and CD68, as there was no effect of ethanol treatment on the number of colocalized voxels ($p > 0.1$) or the percentage of CD68 positive microglia ($p > 0.1$) within the MLDG. Morphological analyses of Iba1+ cells in the MLDG revealed that, compared to aged controls, aged ethanol-treated rats had reduced microglia branching ($p < 0.05$), indicative of a reactive state. Alterations in branching were accompanied by only modest changes in soma size: aged ethanol-treated rats trended toward larger somas compared to aged controls ($p = 0.07$). Collectively our data provide novel insight into the prolonged microglial reactivity following aged alcohol exposure. Ongoing research aims to explore potential sex differences in neuroinflammatory responses to alcohol misuse during advanced age.

Disclosures: L. Singleton: None. A. Campanile: None. E.R. Carlson: None. L.K. Fonken: None. K. Nixon: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.13/F10

Topic: B.09. Glial Mechanisms

Support: NIH grant (NIA) RO1 AG075909-01
The Brain Research Foundation Grant BRFSG-2021-01

Title: Lysosome status as a key driver of microglial aging phenotype and cognitive decline

Authors: *F. ETIENNE^{1,2}, K. HOPE³, D. T. GRAY^{1,2}, A. GUTIERREZ^{1,2}, M. LEE^{1,2}, L. M. DE BIASE^{1,2};

¹UCLA, Los Angeles, CA; ²Physiol., David Geffen Sch. of Med., Los Angeles, CA; ³Neurosci., Case Western Reserve Univ. Dept. of Neurosciences, Cleveland, OH

Abstract: During aging, microglia proliferate, produce inflammatory factors, altered interactions with synapses, and prolonged responses to CNS insults, positioning these cells to have profound impact on nearby neurons functions. We recently showed that microglial proliferation in the ventral tegmental area (VTA), where disease-vulnerable dopamine neurons reside, is significantly correlated with worse reward-based cognition in aging mice. VTA microglial proliferation was also linked to changes in microglial lysosome status, suggesting that changes to these organelles during aging will provide critical insight into mechanisms of cognitive decline. In these follow-up studies, we leveraged novel transgenic mice, additional analyses in aging mice, confocal imaging, FACS, and behavior to probe causal links between microglial lysosomes, microglial aging, and cognition. During aging, we found that autofluorescent aggregates (lipofuscin) in VTA microglia were larger and filled more lysosomes than those in nucleus accumbens (NAc) microglia. Using Cln3 knockout (KO) mice to genetically induce accumulation of lipofuscin in lysosomes, independent of aging, we found that this was sufficient to cause proliferation of VTA microglia, similar to what we found during aging. RNAseq-based analysis of microglia from middle aged mice that have either high- or low-levels of lipofuscin aggregates revealed 2000+ differentially expressed genes between microglia with distinct lysosome status. RNAseq analysis of microglia from Cln3 KO and Cln3 WT showed a similar, surprisingly high number of differentially expressed genes, even in mice at 3mo of age. Both of these RNAseq datasets highlighted significant differences across brain region in how microglia were responding to lysosome challenge, indicating key brain-region specificity of links between lysosomes and microglial phenotypes. To further explore how microglial lysosome status impacts microglial function during aging, we genetically induce overexpression (OE) of TFEB (master regulator of lysosome biogenesis) specifically in microglia. TFEB OE in microglia led to upregulation of numerous lysosome-relevant genes and, in aged mice (18mo), led to an increase of lysosomes in microglial cell processes. Moreover, mice with microglial TFEB OE from 3-18mo of age exhibited improved working memory in a T-maze test, compared to untreated 18mo controls. Collectively, we identify lysosomal overload as a key driver of regional differences in microglial aging and highlight the therapeutic potential of targeting microglial lysosomal function to modulate aging-related microglial responses and preserve cognitive function.

Disclosures: F. Etienne: None. K. Hope: None. D.T. Gray: None. A. Gutierrez: None. M. Lee: None. L.M. De Biase: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.14/F11

Topic: B.09. Glial Mechanisms

Title: High-throughput screening of fda-approved compounds identifies modulators of phagocytic activity in ipsc-derived microglia

Authors: *A. MANTEY, T. TANAKA, B. FRAHER, C. TICAS RODAS, L. AGBOR;
Elixirgen Scientific Inc., Baltimore, MD

Abstract: Microglia, the resident immunocompetent cells of the central nervous system, play vital roles in neuronal protection and neural circuit development. Recent studies suggest that microglia contribute to the progression of Alzheimer's disease (AD), and enhancing their selective phagocytic activity may help clear pathological protein aggregates. Thus, identifying compounds that increase microglial phagocytic function has therapeutic relevance. Using Elixirgen Scientific's proprietary Quick-Tissue™ technology, we established a protocol for the differentiation and cryopreservation of human induced pluripotent stem cell (hiPSC)-derived microglia. We developed a targeted approach to screen FDA-approved compounds for their effects on phagocytic activity in these cells. A high-throughput screen of 892 FDA-approved compounds was conducted at 10 µM on day 7 of in vitro culture. Cellular metabolic activity was assessed using PrestoBlue™, and Z-scores were used to identify primary hits. Eleven candidate compounds were selected for a secondary screen. Notably, many of these compounds shared structurally similar functional groups, supporting the reproducibility of the screen. One lead compound was identified that significantly enhanced phagocytosis of Zymosan-conjugated pHrodo™ particles by hiPSC-derived microglia. Application of this compound in a human neuron-astrocyte-microglia triculture model will be discussed.

Disclosures: A. Mantey: None. T. Tanaka: None. B. Fraher: None. C. Ticas Rodas: None. L. Agbor: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.15/F12

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

Title: A systems biology platform that models patient-relevant neuroinflammation signature of progressive glaucoma

Authors: C. NODIN¹, L. T. AGHOLME¹, A. SKILJE¹, *J. SVENSSON DALÉN¹, B. MA², P. KARILA¹, M. C. PIZARRO PEÑARANDA³, Y. NAM³, A. AKHLAQ³, T. V. JOHNSON, III³,

J. PIHL¹, J. M. LEVENSON²;

¹Cellectricon AB, Mölndal, Sweden; ²FireCyte Therapeutics, Inc., Boston, MA; ³Wilmer Eye Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Dysfunction of microglia has been linked to a variety of CNS disorders including neurodegenerative and neuropsychiatric diseases and optic neuropathy. We developed an in vitro neuroinflammation platform that integrates human iPSC-derived microglia into rodent primary neuron-astrocyte co-cultures. The co-culture system recapitulates many aspects of chronic neuroinflammation reported in the literature and enables high-capacity studies that are relevant to neuroinflammation and microglial biology.

A number of functional and endpoint readouts have been developed, such as high content imaging quantification of the cellular phenotype of microglia and other cell types, multiplexed analysis of pro- and anti-inflammatory cytokines, and neuronal excitability analysis. Using commercially available compounds, the role of 10 different targets implicated in neuroinflammatory responses has been characterized.

Our results show differential activity of many targets on microglial-mediated cytokine release and microglial morphology, where many compounds suppress cytokine release but fail to shift microglia back into a morphological state characteristic of neuroprotection. Application of machine learning to diverse screening data has enabled creation of a multi-component screening score that identifies several classes of compounds with distinct effects on microglial functional state.

A major issue in development of translational platforms is validation that the biological perturbations modeled by the platform mimic disturbances occurring in patients. Recent advances in proteomics enable rapid and comprehensive profiling of fluid biomarkers. Microglia effect coordinated inflammatory responses through use of a robust secretome. Proteomic analysis of aqueous humor from patients with glaucoma has identified a biomarker signature of neuroinflammation associated with progressive neurodegenerative disease. Using this panel, we validated that the in vitro neuroinflammation platform exhibits proteomic signatures of inflammation that are more closely aligned with patients than simple monoculture. Collectively, these results support utilization of a screening workflow that incorporates multiple orthogonal endpoints of microglial functional state to fully characterize pharmacology of compounds of interest.

Disclosures: **C. Nodin:** A. Employment/Salary (full or part-time): Cellectricon AB. **L.T. Agholme:** A. Employment/Salary (full or part-time): Cellectricon AB. **A. Skilje:** A. Employment/Salary (full or part-time): Cellectricon AB. **J. Svensson Dalén:** A. Employment/Salary (full or part-time): Cellectricon AB. **B. Ma:** A. Employment/Salary (full or part-time): FireCyte Therapeutics, Inc. **P. Karila:** A. Employment/Salary (full or part-time): Cellectricon AB. **M.C. Pizarro Peñaranda:** None. **Y. Nam:** None. **A. Akhlaq:** None. **T.V. Johnson:** None. **J. Pihl:** A. Employment/Salary (full or part-time): Cellectricon AB. **J.M. Levenson:** A. Employment/Salary (full or part-time): FireCyte Therapeutics, Inc..

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.16/F13

Topic: B.09. Glial Mechanisms

Support: JSPS Kakenhi 24K02339
JSPS Kakenhi 24K12805

Title: Transpupillary in vivo two-photon imaging reveals enhanced surveillance of retinal microglia in diabetic mice

Authors: N. SOTANI^{1,2}, S. KUSUHARA², H. KUNO¹, T. TAKUMI¹, M. NAKAMURA², *Y. TACHIBANA¹;

¹Dept. of Physiol. and Cell Biol., ²Div. of Ophthalmology, Dept. of Surgery, Kobe Univ. Grad Sch. Med., Kobe, Japan

Abstract: The retina, located outside the cranium, is an ideal structure for investigating information processing in the central nervous system due to its well-organized neurovascular unit composed of diverse cell types, including neurons, glial cells (such as microglia, Müller glia, and astrocytes), pericytes, and vascular endothelial cells. Disruption of retinal homeostasis contributes to various ocular diseases, including diabetic retinopathy, age-related macular degeneration, glaucoma, and retinitis pigmentosa. *In vivo* real-time retinal imaging using two-photon microscopy is a powerful tool for visualizing retinal components in both animal models and humans, offering insights into the pathogenesis of these diseases. Although several recent studies have applied two-photon microscopy to image the mouse retina, technical challenges persist, such as limited resolution due to the small numerical aperture of objective lenses and the complexity of implementing adaptive optics. To address these issues, we developed a two-photon microscopy technique for real-time, transpupillary *in vivo* imaging of the retinal neurovascular unit in mice. Our approach integrates systematic head fixation, a custom-made polymethyl methacrylate contact lens, and a glycerin-immersion objective lens with an extended working distance and a higher numerical aperture, optimized for two-photon imaging. To validate the utility of this method, we investigated the behavior and morphological changes of microglia in a mouse model of diabetes. Microglia, the resident immune cells of the CNS derived from yolk sac progenitors, play key roles in immune surveillance and responses to infection, injury, and inflammation. Our imaging system enabled visualization of dynamic microglial process activity surrounding retinal blood vessels. Our results revealed that retinal microglia exhibit enhanced surveillance under diabetic conditions, which is undetectable by static confocal microscopy. Furthermore, we demonstrated that liraglutide, a glucagon-like peptide-1 receptor agonist commonly used for the treatment of diabetes and obesity, reversed these diabetes-induced microglial changes. Our straightforward yet robust imaging approach mitigates the need for advanced optical systems such as adaptive optics, providing a practical tool for real-time retinal imaging. This technique offers significant value for visual neuroscience research and holds strong potential for clinical applications, particularly in the early diagnosis, intervention, and monitoring of retinal diseases.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.17/F14

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

Title: Diet-induced Manipulation of Gut-Microbiome to Evaluate Microglial Activation in a PD-Genetic Mouse Model

Authors: *A. RODRIGUEZ¹, G. CUI²;

¹Neurobio. Lab., Natl. Inst. of Envrn. Hlth. Sci., Raleigh, NC; ²Neurobio. Lab., Natl. Inst. of Envrn. Hlth. Sci., RTP, NC

Abstract: Parkinson's disease (PD) is the most common movement disorder and the second most prevalent neurodegenerative disease. Prodromal PD symptoms, such as gastrointestinal issues linked to gut microbiome dysbiosis (GMD), can appear up to twenty years before motor onset. Disease progression may be accelerated by dietary habits that affect the gut microbiome (GM). While the gut-immune axis likely contributes to neuroinflammation, the connection between GMD and PD-related neurodegeneration remains unclear. Therefore, we sought to evaluate GM mediation of dopamine neuron degeneration by examining the impact of a high-fat diet (HFD) on the MitoPark mouse model of PD (DATIRESc^{re/+}; TFAMflox/flox). MitoPark mice, which lack the TFAM gene in dopamine neurons, display age-dependent dopaminergic loss and progressive PD-like symptoms, starting at 15 weeks. To test the impact of HFD from the prodromal stages to end-of-life, 6-week-old mice were assigned to regular diet or HFD. A three-pronged approach was used to assess impact: microbial 16S sequencing of stool samples, open field test (OF), and neuropathological analysis of dopamine neurons (Th) and microglia (Iba1). We expected that HFD would induce microbial dysbiosis, associated with a more severe loss of dopaminergic neurons and unique characterizations of microglia within the mouse mid-brain, examined at 6 to 30 weeks of age. DAB staining of Th⁺ neurons and confocal-acquired microglial counts in 30-week-old mice showed strong genotype effects. These changes were correlated with a diet-associated shift in the gut-microbiome. Further analysis of the microbiome revealed that while genotype had some small effect, diet amplified microbial volatility and reduced microbial richness. Thus, this model highlights how peripheral factors influence neuroinflammation and aging, offering a framework for environmental and dietary contributions to PD and potentially advance methods for early-detection of PD-associated prodromal symptoms.

Disclosures: A. Rodriguez: None. G. Cui: None.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.18/F15

Topic: B.09. Glial Mechanisms

Title: Parkinson's disease (PD) model composed of an iPSC-derived tri-culture system of neurons, astrocytes, and microglia stimulated with α -synuclein.

Authors: *H. KOBAYASHI, S. ENDOH-YAMAGAMI;
Bio Sci. & Engin. Labs., FUJIFILM Corp., Kanagawa, Japan

Abstract: Parkinson's disease is a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra. In dopaminergic neurons, the pathology is characterized by the aggregation of α -synuclein, known as Lewy bodies, and α -synuclein has been extensively studied as a causative agent of the disease. α -Synuclein has been reported to play a role in inflammatory responses mediated by the NLRP3 inflammasome, as well as in synaptopathy and dopaminergic neuronal death. However, the pathological mechanisms mediated by α -synuclein have not been fully elucidated. In this study, we established a tri-culture system comprising neurons, astrocytes, and microglia derived from human iPSCs to investigate α -synuclein-induced neuroinflammation, synaptopathy, and dopaminergic neuronal death. We established a tri-culture system using iPSC-derived dopaminergic neurons, astrocytes, and microglia, and compared it with a monoculture system of microglia. The microglia in the monoculture system showed amoeboid morphology, whereas in the tri-culture system, the microglia exhibited a branched morphology with extended processes. This suggests that the tri-culture system is in a more homeostatic state compared to the monoculture system. Furthermore, stimulation of these culture systems with α -synuclein resulted in a more pronounced increase in proinflammatory cytokines in the tri-culture system compared to the monoculture system. These findings suggest that reproducing a homeostatic environment is important for recapturing neuroinflammation induced by α -synuclein oligomers stimulation. Moreover, α -synuclein is known to take various forms, including monomers, oligomers, and pre-formed fibrils (PFFs), and these variations resulted in different induction of proinflammatory cytokine expression. Currently, the involvement of the inflammasome in inflammatory responses remains under investigation. Additionally, we are assessing the effects of α -synuclein on synaptopathy, mitophagy, and dopaminergic neuronal death. Our tri-culture system successfully reproduced the neuroinflammatory state by stimulation with α -synuclein oligomers, thereby replicating certain features of Parkinson's disease. This model is expected to contribute to our understanding of the molecular mechanisms of Parkinson's disease and facilitate the development of new therapeutic agents.

Disclosures: H. Kobayashi: A. Employment/Salary (full or part-time): FUJIFILM Corporation. S. Endoh-Yamagami: A. Employment/Salary (full or part-time): FUJIFILM Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FUJIFILM Corporation.

Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.19/F16

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

Support: NIH/NINDS grant R01 NS124226
MJFF000860

Title: Plx5622 mitigates rotenone and lipopolysaccharide-induced synucleinopathy in rotenone- and lipopolysaccharide-induced neuroinflammation model of PD via modulation of cathepsin b, pkc δ , and autophagy pathways

Authors: *M. SHARMA, A. KUMAR, S. GOVINDHASAMY PUSHPAVATHI, H. JIN, G. ZENITSKY, V. ANANTHARAM, A. G. KANTHASAMY, A. KANTHASAMY;
Isakson Ctr. for Neurolog. Dis. Research, Univ. of Georgia, Athens, GA

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by chronic neuroinflammation, accumulation of aggregated α -synuclein (α Syn) in Lewy bodies and neurites, and pronounced loss of nigrostriatal dopaminergic neurons. While microglia have been implicated in PD pathophysiology, the contribution of microglia-driven cell signaling events in PD-like neuropathology remains poorly understood. Recent studies indicate a role for cathepsin-B (CathB) in neuroinflammation-associated neurodegeneration in response to environmental toxin exposure. Herein, using in vitro microglial cell culture studies, we show that rotenone (ROT) (200 nM) and lipopolysaccharide (LPS) (100 ng/mL) treatment in tandem induces CathB activation concomitant with NLRP3 inflammasome activation, autophagosomal-lysosomal (ALS) impairment, iNOS generation, accumulation of pathological α Syn aggregates in a CathB/CSF1R-dependent manner and that pharmacological inhibition of either CathB via OMe CA0374 or CSF1R via PLX5622 ameliorated microglial activation response via suppression of CathB, iNOS, and the NLRP3 inflammasome activation response. Finally, mice treated with ROT and LPS in tandem mirrored our in vitro findings, whereby elevated CathB proteolytic cleavage was found to coincide with NLRP3 and ALS dysfunction as well as α Syn pathology, and generation of proinflammatory mediators in PD-vulnerable DAergic brain regions. Additionally, these changes were accompanied by nigral dopaminergic neurotoxicity and neurobehavioral deficits as compared to ROT-treated mice. Intriguingly, the CSF1R inhibitor PLX5622 ameliorated not only CathB activation but also the generation of proinflammatory mediators, α Syn pathology and nigrostriatal DAergic neurodegeneration in ROT/LPS-treated mice. Collectively, these results indicate that environmental exposure to a DAergic neurotoxicant followed by inflammogen exposure may aggravate microglia-mediated neuroinflammation and ensuing nigral DAergic neurodegeneration via a microglia-mediated CathB-NLRP3 signaling axis and lysosomal impairment and that inhibition of CSF1R may serve as a useful strategy for treating environmental neurotoxicant-associated PD and other neurodegenerative diseases that share prominent microglia-mediated neuroinflammation.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

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Program #/Poster #: PSTR329.20/G1

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

Support: Supported by NIH grant R01AG074968 (B.H.S).
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Title: Transcriptomic profiling reveals the combination of sepsis and amyloid increases endothelial cell, microglial inflammation and astrocyte reactivity

Authors: *K. GIFFIN¹, M. NEWSTEAD², B. H. SINGER³;

¹Neurosci. Grad. Program, ³Div. of Pulmonary and Critical Care Medicine, Dept. of Intrnl. Medicine, Univ. of Mic, ²Univ. of Michigan, Ann Arbor, MI

Abstract: Sepsis is associated with acute and long-term brain dysfunction such as cognitive decline and mental health disorders. Neuroinflammation involving multiple cell types may be an underlying factor and is not fully characterized. We developed a single-nucleus RNA sequencing (snRNAseq) technique to enrich for non-neuronal cells to transcriptionally profile cells during sepsis.

The elderly are especially vulnerable to brain dysfunction following sepsis. Amyloid accumulates in the brains of cognitively normal older adults. We found that early amyloid pathology (5-6-month-old 5xFAD mice) synergistically heightens behavior change and microglial synaptic phagocytosis after abdominal sepsis.

To better understand the mechanisms driving these findings, we investigated the transcriptional profile of non-neuronal cells during acute sepsis in 5xFAD and wildtype mice. To overcome the difficulty of sequencing small populations of glial, we enriched for non-neuronal nuclei using flow cytometric sorting on the marker NeuN. We sequenced NeuN- nuclei from mouse hemibrains 72hrs after sepsis from pneumonia (*Klebsiella pneumoniae*).

We used hdWGCNA, a method to determine unbiased gene co-expression networks, to identify gene expression modules that correlated with both 5xFAD and sepsis. While differential expression analysis revealed an inflammatory phenotype in microglia specific to 5xFAD sepsis, there were too few microglia to detect gene expression modules. In astrocytes, one of 4 identified modules correlated with sepsis and 5xFAD. This module was defined by genes regulating vasculature, cell projection, and chemotaxis, indicating a role of astrocytes in regulating blood-brain-barrier and cell signaling. Within endothelial cells, one of 9 modules significantly correlated with sepsis and 5xFAD. It was an inflammatory module defined by cytokine signaling

pathways. There were 5 modules identified in oligodendrocytes, but none correlated with both 5xFAD and sepsis. One module containing homeostatic and myelin sheath genes significantly correlated with saline and wildtype, indicating a reduction of oligodendrocyte homeostatic function during sepsis. Experiments are in progress to determine transcriptional phenotypes after recovery from sepsis.

Overall amyloid pathology increased the neuroinflammatory processes that may underlie previously observed behavior change and increased microglial synaptic phagocytosis after sepsis. Transcriptional profiles showed roles for each cell type during acute sepsis, with astrocytes regulating blood-brain-barrier and cell signaling while microglia and endothelial cells drive neuroinflammation.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.21/G2

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

Support: NIH Grant DC022073
NIH Grant MH139024

Title: Noise-induced hearing loss is associated with an altered immune response in the hippocampus of the ApoE4 mouse model

Authors: E. PALTJON¹, W. SESE¹, L. KUSUMO², A. HORNAK¹, E. G. VICHAYA³, *D. D. SIMMONS⁴;

¹Biol., ²Psychology & Neuroscience., ³Psychology & Neurosci., ⁴Baylor Univ., Waco, TX

Abstract: Noise-induced hearing loss (NIHL) is strongly correlated with memory deficits, with hearing loss reliably preceding the onset of memory impairment. Although NIHL triggers hippocampal inflammation, the mechanisms are poorly understood. We hypothesize that high levels of noise exposure alter the glial phenotype and induce synaptopathy in the hippocampus. Further, these features may be exacerbated in the apolipoprotein E4 (ApoE4) knock in mouse model known for its late onset cognitive impairments. We used sixteen 3-month-old male C57BL/6 wild type (WT) and ApoE4 knock-in mice (n = 3 - 5 per group). Mice were exposed to broadband noise (BBN) at 120 dB SPL for 2 hours to cause NIHL; controls were untreated. Hearing function was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). Brains were harvested 14 days after noise exposure and sectioned for immunofluorescence. We focused on the dentate gyrus since it encodes auditory information and is a structure highly involved in learning and memory. We used ionized calcium binding adaptor protein 1 (Iba1) to label microglia and postsynaptic density protein 95 (PSD95) to label postsynaptic sites. Microglial morphology and PSD95 puncta were analyzed. Mice had

40 dB SPL threshold shifts 14 days after noise exposure, indicating permanent NIHL. ApoE4 mice had altered microglial morphology with fewer and thicker branches. Additionally, noise-exposed ApoE4 mice showed increased microglia-engulfed PSD95 puncta, indicating greater synaptic phagocytosis compared to WT. These results show that after high levels of noise exposure (1) WT and ApoE4 mice experience similar permanent hearing loss, (2) ApoE4 mice have a higher level of microglial activation, and (3) ApoE4 show increased microglial synaptic engulfment. Our preliminary data suggest increased microglial activation and synaptic phagocytosis as a potential mechanism linking high levels of noise exposure to impaired hippocampal synaptic function. Ongoing studies in the dentate gyrus are examining related changes in mRNA expression, astrocyte morphology, astrocyte-engulfed PSD95 puncta, and cognitive performance.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

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Program #/Poster #: PSTR329.22/G3

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

Support: Diana D Spencer Foundation Grant
Brian Nickerson Alzheimer's Disease Fund

Title: Uncovering the role of microglia states in complement-mediated synapse loss

Authors: *E. M. PIZZI¹, M. MACLEAN², C. DIEMLER², E. BLOSS², G. R. HOWELL²;
¹Neurosci., The Jackson Lab. and Tufts Univ., Bar Harbor, ME; ²The Jackson Lab., Bar Harbor, ME

Abstract: Background: Microglia possess a dual role of neuroprotection and neurotoxicity in Alzheimer's disease (AD). They are thought to exert neuroprotection through phagocytosing amyloid beta plaques, yet they can also become neurotoxic through excessive cytokine production and synaptic pruning. Single-cell transcriptomics have revealed distinct microglial activation states in AD such as Disease-Associated Microglia (DAM) and Interferon-Responding Microglia (IRM), which have been linked to amyloid pathology and potentially synapse loss. Here we aim to determine the relationship between DAMs and IRMs, and their function in plaque deposition and synapse loss. To investigate the role of DAMs and IRMs we utilized the APP^{SAA} allele in two genetic contexts - the widely used C57BL/6J (B6) and the New Zealand Obese (NZO) that develops hallmarks of metabolic syndrome, a combination of conditions including hyperlipidemia, hyperglycemia. MetS is a known risk factor for AD, and we expect chronic MetS to alter microglial responses in the context of amyloid pathology to exacerbate AD-relevant outcomes. **Methods:** scRNAseq was performed on CD11B+ brain cells from 12-

month-old B6.*APP^{SAA/SAA}* and NZO.*APP^{SAA/SAA}* and wild-type controls (Miltényi, 10X Genomics). Microglia states were also localized using spatial transcriptomics (Xenium) and proteomics (CODEX). Immunohistochemistry (IHC) was performed on contralateral hemispheres to assess homeostatic microglia (IBA1, TMEM119), DAM (CLEC7A), IRM (STAT1), complement factors (C1Q, C3), and plaques (X-34). Synapses were quantified with IHC near and away from X-34+ plaques. **Results:** scRNAseq revealed elevated IRM in NZO compared to B6 in response to amyloid pathology. Both DAM (CLEC7A) and IRM (STAT1) expression increased, particularly near X-34+ plaques. Additionally, NZO.*APP^{SAA/SAA}* mice have a different DAM program, and they possess novel clusters potentially associated with vascular health. Multiple states of microglia also expressed initiating factors of the classical pathway of the complement cascade. Preliminary analyses reveal strain and genotype differences in synapse density near X-34+ plaques. **Conclusions:** Amyloid-specific transcriptional states of microglia are modulated by MetS, further highlighting the value of incorporating genetically diverse mouse strains to model the complexity of AD. Ongoing work using lineage tracing and conditional gene knockouts aims to uncover the role of IRM and DAM in AD and related dementias.

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Poster

PSTR329: Microglia and Neuroinflammation in CNS Disease

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR329.23/G4

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

Title: Microglia-mediated gdnf delivery for neuroprotection in als

Authors: *Q. WANG¹, G. ZHANG²;

¹Yale Univ., New Haven, CT; ²Med., Univ. of Texas Hlth. San Antonio, San Antonio, TX

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease with progressive motor neuron loss. Current treatments offer modest benefits but lack robust neuroprotection. Glial cell line-derived neurotrophic factor (GDNF) potentially protects motor neurons, yet its delivery to the central nervous system (CNS) is hindered by the blood-brain and blood-spinal cord barriers. Postmortem bulk analysis of spinal cords from ALS patients highlights impaired lipid metabolism as a key feature of disease progression. However, specific lipid profile alterations in motor neurons of the lumbar spinal cord in ALS remain poorly understood. We utilized a novel microglia replacement-mediated CNS GDNF (MRMC-GDNF) delivery approach to evaluate its therapeutic efficacy in SOD1-G93A mice, a model of ALS, and to examine its impact on lipid profiles in motor neurons of the lumbar spinal cord, employing spatial metabolomics for comparative lipid analysis. Our preliminary results demonstrated that preconditioning with the colony-stimulating factor 1 receptor (CSF1R) inhibitor PLX5622, followed by transplantation of lentiviral vector-enhanced hematopoietic stem cells (HSCs),

facilitated robust microglia replacement and efficient GDNF delivery, safeguarding motor neurons in the lumbar spinal cords of ALS mice. Additionally, we observed ~90% microglia replacement in PLX5622-treated SOD1-G93A mice and a median lifespan increase to 176.5 days in the GDNF group compared to 167 days in controls. Spatial metabolomics revealed notable ceramide alterations in motor neurons of the lumbar spinal cord in untreated SOD1-G93A mice compared to wild-type controls, with PLX5622 treatment effectively reversing these changes in SOD1-G93A mice. Our findings demonstrate that MRMC-GDNF therapy delays ALS symptom onset, slows disease progression, and prolongs survival by targeting ceramide metabolism in motor neurons of the lumbar spinal cord and other CNS regions.

Disclosures: Q. Wang: None. G. Zhang: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.01/G5

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

Title: Agitated behavior in a depression model is associated with prefrontal cortex neuronal and glial alterations

Authors: *M. BAUTISTA-CARRO^{1,2}, D. JUÁREZ SERRANO³, A. DIAZ³, G. FLORES³, J. MORALES-MEDINA^{4,2};

¹Physiology, Biophysics and Neurosciences, Ctr. de Investigación y de Estudios Avanzados del Inst. Politécnico Nacional, CDMX, Mexico; ²Ctr. de Investigación en Reproducción Animal, Tlaxcala, Mexico; ³Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; ⁴Ctr. de Investigación y Estudios Avanzados, Tlaxcala, Mexico

Abstract: Major depressive disorder (MDD) is a prevalent psychiatric illness worldwide. One of its clinical subtypes is agitated depression, characterized by the coexistence of affective symptoms and psychomotor agitation. The latter typically presents as increased motor activity, including hyperlocomotion and repetitive, purposeless movements. Due to the severity of their symptoms, specific management recommendations should be developed and new therapeutic options considered. Olfactory bulbectomy (OBX) is one of the most representative animal models for studying depression with agitated features. OBX involves bilateral ablation of the olfactory bulbs, which causes changes in the endocrine, immune, and neurotransmitter systems similar to those observed in patients with MDD. The open field test (OFT) is commonly used in rodents to measure exploratory behavior and rearing or grooming frequency, in order to predict their emotional state. OBX rodents exposed to OFT show hyperlocomotion and repetitive behaviors. We used the prefrontal cortex (PFC) of 75 adult male Wistar rats (*Rattus norvegicus*) with OBX to determine the influence of astrocytes, microglia, pyramidal neurons, nitric oxide (NO), and neuronal activity on the behavior observed in the OFT. We found that OBX generated hyperlocomotion and increased rearing frequency. Furthermore, it increased the number of cells

labeled with glial fibrillary acidic protein (an astrocyte marker), c-Fos (a marker of neuronal activity), and NO levels, while decreasing the density of dendritic spines in pyramidal neurons in the PFC. In conclusion, OBX induces agitated behavior, mediated by modifications in astrocytes of the PFC, which trigger oxidative stress linked to neuronal activation, along with other neurobiological mechanisms. Our findings should be considered to optimize financial resources for the evaluation of potential antidepressant, anxiolytic, and neuroprotective drugs.

Disclosures: **M. Bautista-Carro:** None. **D. Juárez Serrano:** None. **A. Diaz:** None. **G. Flores:** None. **J. Morales-Medina:** None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.02/G6

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

Title: Effect of olfactory bulbectomy on allodynia and glia in female Wistar rats one-week post-surgery

Authors: ***L. M. CUTIÑO-VIÑALS**¹, **G. GALINDO**², **J. MORALES-MEDINA**³, **G. FLORES**⁴;

¹Doctorado en Ciencias Biológicas, Univ. Autónoma de Tlaxcala; Ctr. de Investigación en Reproducción Animal, Tlaxcala, Mexico; ²Ctr. de Investigación en Reproducción Animal, Tlaxcala, Mexico; ³Ctr. de Investigación y Estudios Avanzados, Tlaxcala, Mexico; ⁴Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico

Abstract: Major depressive disorder (MDD) frequently coexists with chronic pain, particularly in women. A widely utilized model of depression is olfactory bulbectomy (OBX). Previous research in male rats has shown that this model leads to neuroinflammation driven by glial cells in the hippocampus and amygdala, key regions involved in MDD and chronic pain. This study utilized the OBX model in two-month-old female Wistar rats (*Rattus norvegicus*) to investigate the relationship between MDD and chronic pain. The primary objective was to evaluate mechanical and cold allodynia, alongside the density and morphology of astrocytes and microglia in the basolateral amygdala (BLA), central amygdala (CeA), and CA1 region of the dorsal hippocampus one-week post-surgery. A total of 18 animals were used, randomly assigned to either the OBX group or a Sham control group (n = 9 per group). Behavioral assessments included mechanical allodynia measurement via the von Frey test and cold allodynia assessed by the acetone test, both conducted before and one week after surgery. Brain tissue samples were collected for immunohistochemical analysis, using anti-GFAP (Glial Fibrillary Acidic Protein) and anti-Iba1 (Ionized Calcium-Binding Adaptor Molecule 1) to study astrocyte and microglia density and morphology. Results indicated that OBX resulted in lower tactile thresholds (indicating mechanical allodynia) and increased paw withdrawal frequency (signifying cold allodynia). A decrease in astrocyte density was observed in both the BLA and CeA, while

microglia density decreased in the BLA. Astrocytes displayed hypertrophy characterized by increased filament length and branching in the BLA and CeA, whereas microglia exhibited hypotrophy with decreased filament length and branching in the BLA. These findings suggest that OBX effectively models the comorbidity of pain and MDD in females, highlighting the role of neuroinflammation in this relationship, and underscore the active contribution of astrocytes and microglia in mediating these pathophysiological processes.

Disclosures: L.M. Cutiño-Viñals: None. G. Galindo: None. J. Morales-Medina: None. G. Flores: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.03/G7

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

Support: CINESTAV Funding

Title: Microglial and astrocytic alterations in the amygdala emerge before nociceptive deficits in a rat model of depression

Authors: *J. MORALES-MEDINA¹, M. BAUTISTA-CARRO², D. L. CORONA QUINTANILLA³, G. FLORES⁴;

¹Ctr. de Investigación y Estudios Avanzados, Tlaxcala, Mexico; ²Ctr. de Investigación y de Estudios Avanzados del Inst. Politécnico Nacional, Tlaxcala, Mexico; ³Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; ⁴Univ. Autónoma de Puebla / Inst. de Fisiología, Puebla, Mexico

Abstract: Major depressive disorder (MDD) is a highly prevalent psychiatric condition often comorbid with chronic pain. Olfactory bulbectomy (OBX) is a well-known preclinical model for depression-related behavior, with most deficits emerging approximately four weeks post-surgery. Previously, our group observed that OBX induces mechanical and cold allodynia, accompanied by activation of glial fibrillary acidic protein (GFAP)-positive astrocytes and ionized calcium-binding adapter molecule 1 (Iba1)-positive microglia in the basolateral (BLA) and central (CeA) amygdala. As these changes were observed during the maintenance phase of glial activation, we sought to determine whether glial alterations occur at earlier stages. In the present study, we assessed glial and behavioral changes one week after OBX. At this time point, OBX rats did not exhibit significant changes in mechanical thresholds or acetone-induced cold responses. However, notable glial alterations were detected. In the BLA, there was an increase in both the number and process complexity of GFAP-positive astrocytes, along with a reduction in Iba1-positive microglial processes. Similarly, in the CeA, astrocytic process complexity increased, while microglial processes decreased. These findings suggest that glial activation in the amygdala precedes the development of nociceptive behavioral alterations. Early targeting of

glial cells may thus represent a potential strategy to prevent the emergence of pain-related symptoms associated with MDD.

Disclosures: J. Morales-Medina: None. M. Bautista-Carro: None. D.L. Corona Quintanilla: None. G. Flores: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.04/G8

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

Support: NIH grant 5R01NS126680-03

Title: Innate and adaptive immune responses are activated during AAV-induced hippocampal neurodegeneration in the adult mouse brain

Authors: *D. ENTERRIA-MORALES, A. KARVE, A. DOAN, X. YU, E. DUONG, B. L. TSE, E. W. AL-DELAIFY, J. SCHLACHETZKI, M. SHTRAHMAN;
Neurosciences, UC San Diego, La Jolla, CA

Abstract: Adeno-associated virus (AAV) vectors are widely used for gene delivery in neuroscience research and clinical therapies. However, growing evidence indicates that AAV can trigger significant local inflammatory responses and neurotoxicity. Here, we characterized the AAV-induced immune activation and neuronal loss in the adult mouse hippocampus. Young adult mice (2 months old) were unilaterally injected in the right hippocampus with 1 μ L of AAV1-CAG-Flex-GFP (Addgene #51502) at a concentration of 1E13 genome copies/mL. Control animals received saline or empty AAV1 capsid injections. Brains were collected at 1, 4, 8, or 12 weeks post-injection and processed for immunohistochemistry. We observed a progressive immune response characterized by increased density of Iba1+ microglia, GFAP+ astrocytes, and CD68+ activated microglia, peaking at 8 weeks and persisting at 12 weeks post-injection. CD3+ T cells, including CD4+ helper and CD8+ cytotoxic subtypes, were also detected in the hippocampus. No inflammatory markers were observed at 1 week, although neuronal DNA damage (γ H2AX+) was already present, suggesting that genotoxic stress precedes overt neuroinflammation. Animals injected with saline or empty capsid exhibited no signs of inflammation or neuronal damage, confirming that AAV genomic DNA (not capsid) is necessary to induce toxicity. Genetic and pharmacological experiments revealed the role of immune pathways in modulating this response. Mice lacking TLR9, an innate sensor of viral DNA, showed no difference in hippocampal damage compared to wild-type or heterozygous littermates, indicating a TLR9-independent mechanism. In contrast, Trem2 knockout mice, which have impaired microglial phagocytosis, displayed exacerbated AAV-induced damage. Heterozygous Trem2 mice showed no significant difference from wild-type controls. These findings demonstrate that AAV induces a sustained innate and adaptive immune response.

Importantly, microglia play a neuroprotective role in containing viral-induced damage, highlighting the potential of microglial modulation as a therapeutic strategy in minimizing AAV-induced neurodegeneration and other forms of neurodegeneration.

Disclosures: D. Enterria-Morales: None. A. Karve: None. A. Doan: None. X. Yu: None. E. Duong: None. B.L. Tse: None. E.W. Al-Delaimy: None. J. Schlachetzki: None. M. Shtrahman: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.05/G9

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

Support: NIH Director's Pioneer Award DP-AT011979-01

Title: Unraveling Central Mechanisms Modulating Immune Responsivity and Conditioned Immune-suppression

Authors: *A. STANLEY¹, H. F. RODRIGUES², L. REMÉDIO², A. VICENTE², C. R. SCHMIDT², L. SCARIA², R. M. COSTA¹;
²Allen Inst. for Neural Dynamics, ¹Allen Inst., Seattle, WA

Abstract: The brain is capable of modulating immune responses through peripheral neuronal inputs to immune organs. This modulation can be learned, as demonstrated, for example, by paradigms of conditioned immune suppression. In the acquisition phase of such a paradigm, animals are exposed to a sensory stimulus followed by an immune suppressive drug. Then, in the evocation phase of the paradigm, the presentation of the sensory stimulus by itself is capable of suppressing immune responses to an immune stimulant. We aim at identifying the central brain regions, and cell types within those regions, that orchestrate this learned immune response. Since prior studies exploring conditioned immune effects have primarily used in vitro assessments of immune response, the time-course and magnitude of the in vivo response is not known. To address this, we are first characterizing the time-course of immune responses to immune modulatory substances entirely in vivo (Concanavalin A, ConA, or Lipopolysaccharide, LPS). We successfully demonstrated that immune responses can be temporally assessed in awake mice via implanted catheters that allow for stress free repeated blood sampling. We have combined this method with the Olink target 48 cytokines panel, which allows for simultaneous measurement of 45 immune related biomarkers with minimal blood volume. Using this approach, we characterized with great resolution the cytokine release induced by the stimulants ConA and LPS. We are also using a neural activity dependent labeling approach (Targeted Recombination in Active Neuronal Populations, TRAP2) approach combined with 3D brain imaging (tissue clearing + light sheet imaging) to characterize the brain regions responsive to these stimulants. In addition, we have developed a task with precise delivery of olfactory stimuli

to be used in the conditioned immune suppression paradigm. These foundational experiments will allow us to investigate the critical components of the central nervous system involved in the learning and evocation of immune responses and conditioned immune responses.

Disclosures: A. Stanley: None. H.F. Rodrigues: None. L. Remédio: None. A. Vicente: None. C.R. Schmidt: None. L. Scaria: None. R.M. Costa: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.06/G10

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

Title: Effect of T-cell mediated inflammation on contextual fear memory in mice.

Authors: *C. MISHRA¹, A. KUSNECOV²;

¹Dept. of Psychology, Rutgers University-NB, Piscataway, NJ; ²Psychology, Rutgers Univ., New Brunswick, NJ

Abstract: The T cell superantigen Staphylococcus Enterotoxin A (SEA), induces pronounced circulating cytokine and T cell proliferative responses *in vivo*. Coincidentally, this also activates brain regions involved in mediating memory and emotion-regulation. Interestingly, this is associated with augmented spatial navigation learning, as reflected by increased reconsolidation of Morris water maze training. In the current study, using male 3-month old C57BL/6J mice, the effects of SEA were investigated using a shock-induced fear learning paradigm. This involved three daily 5-min conditioning sessions of a single 3-second 0.5 mA shock (unconditioned stimulus, UCS) in a unique context (conditioned stimulus, CS) fitted with a metal grid floor. In Experiment 1 (N=16 mice), we demonstrated robust freezing after conditioning (N=8), which subsided to baseline levels that did not vary from that seen in unconditioned control mice (N=8). However, a single re-exposure to the shock UCS on Day 8 immediately reinstated the freezing response of conditioned mice, which persisted for several days of exposure to the CS context alone. A second experiment (Exp. 2) was then conducted, to test whether IP treatment with SEA (200 µg/Kg) could modify conditioning and/or reactivate suppressed freezing after extinction of initially strong freezing responses. The dose of SEA used generates strong IL-2 and TNF-alpha responses and T cell proliferation *in vivo*. Male mice (N=32) were conditioned for three days as in Experiment 1 but were given SEA or Saline prior to each conditioning session. Seven days (Days 1-7) of extinction then followed. On Day 8, mice again received SEA or saline in the context (without further shock), followed by three days of testing (context only). The behavioral results of Experiment 2 showed gradual suppression of freezing across extinction sessions, but this was not reinstated by SEA. Moreover, SEA treatment prior to conditioning did not influence the magnitude of conditioned freezing responses, nor the rate of extinction. Additional experiments are ongoing to determine whether SEA treatment that coincides with shock-induced reinstatement augments reactivation of freezing behavior and subsequently prolongs extinction.

Overall, however, the data collected to date are consistent with previous evidence published from our laboratory, that SEA treatment does not modulate acquisition of hippocampal-dependent learning but rather primes animals for improved reconsolidation of learning. This will be assessed further in the current fear conditioning studies.

Disclosures: C. Mishra: None. A. Kusnecov: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.07/G11

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

Support: Grants R01 NS112350 (NHV) and R01 NS112308 (RD, NHV)

Title: Brain-invading monocytes promote seizure-associated cognitive deficits and neurodegeneration

Authors: *S. POURKHODADAD¹, W. WANG², R. J. DINGLEDINE⁴, N. H. VARVEL³;
¹Emory Univ., Decatur, GA; ²Emory Univ., atlanta, GA; ³Pharmacol., Emory Univ., Atlanta, GA; ⁴Dept Pharmacol, Emory Univ. Sch. Med., Atlanta, GA

Abstract: Seizure-associated cognitive co-morbidities can substantially reduce the quality of life in people with epilepsy. Neuroinflammation is an invariant feature of all chronic neurologic diseases, including epilepsy, and acute brain insults, including status epilepticus (SE). The generalized seizures of SE trigger a robust inflammatory response involving astrocytosis, erosion of the blood-brain barrier (BBB), activation of brain-resident microglia, and recruitment of blood-borne CCR2+ monocytes into the brain. We have shown that blocking monocyte recruitment into the brain via global *Ccr2* knockout or systemic CCR2 antagonism with a small molecule alleviates multiple deleterious SE-induced pathologies, including BBB damage, microgliosis, neuronal damage, and monocyte brain invasion in the days following pilocarpine-induced SE. This study aimed to determine if fleeting CCR2 antagonism improves SE-associated cognitive impairments in the long term. Here, we show that the brief antagonism of CCR2 eliminates the profound deficit in working memory in the Y-maze and retention memory in the novel object recognition test but does not attenuate anxiety-like behavior in the open field arena. Microgliosis and astrocytosis were observed in brain sections from SE mice after the behavioral tests, and CA1 hippocampal astrocytosis mildly correlated with performance in the Y-maze. Notably, SE mice exposed to the vehicle showed robust neuronal loss in the cortex and CA1 region of the hippocampus, and mice treated with the CCR2 antagonist showed less neurodegeneration in both the cortex and hippocampus. Our results indicate that blood-borne monocytes are a viable therapeutic cellular target to prevent cognitive co-morbidities and neurodegeneration associated with seizures.

Disclosures: **S. pourkhodadad:** A. Employment/Salary (full or part-time): Emory university medical school. **W. Wang:** None. **R.J. Dingleline:** None. **N.H. Varvel:** None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.08/Web Only

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

Support: National Institute of Mental Health, 7R01MH129732-04

Title: Increased Complement C4 in a Sparse Neuronal Subset Induces Network-Wide Transcriptomic Alterations in the Prefrontal Cortex

Authors: ***S. BOLSHAKOVA**¹, S. CARMEN², R. PHADKE³, A. BRACK⁴, C. M. DIAS⁵, A. CRUZ-MARTIN⁶;

¹Broad Inst., Cambridge, MA; ²Escuela de Genética y Biotecnología, Univ. Nacional Mayor de San Marcos, Lima, Peru; ³Boston Univ., Allston, MA, ; ⁴Boston Univ., Boston, MA, ; ⁵Boston Children's Hosp., Boston, MA, ; ⁶Univ. of Colorado, Anschutz Med. Campus, Denver, CO

Abstract: The complement (C) pathway, a vital part of innate immunity, defends against pathogens and supports tissue surveillance. While local activation in the periphery enhances immune protection, dysregulation can trigger a self-amplifying cascade that spreads beyond the initial site, resulting in tissue injury. In the brain, complement proteins regulate synaptic plasticity and connectivity, raising the possibility that similar mechanisms of maladaptive propagation may disrupt neural circuits under pathological conditions. Although complement dysregulation is linked to neurological and psychiatric disorders, the impact of localized upregulation in a small subset of cells on broader cortical networks remains unclear. To examine this, we overexpressed the schizophrenia (SCZ) risk gene C4 (C4-OE) in approximately 2% of prefrontal cortex neurons in mice using in utero electroporation. Bulk RNA sequencing of microdissected tissue revealed widespread transcriptional changes in a network predominantly comprising untransfected cells. C4-OE induced the upregulation of genes involved in cholesterol biosynthesis, axon guidance, synaptic plasticity, cytoprotection, and neurogenesis—suggesting a response consistent with compensatory remodeling or aberrant plasticity. Co-expression analysis identified a C4b-containing module enriched for dendritic development and cell cycle regulation, indicating alterations in circuit maturation. In contrast, immune and inflammatory genes were broadly downregulated, suggesting homeostatic suppression in response to sustained complement activity. Comparisons with human SCZ proteomic datasets revealed conserved gene signatures, highlighting the potential of our model to capture disease-relevant mechanisms. These findings demonstrate that sparse C4 overexpression can trigger widespread transcriptional changes across neural circuits, suggesting that local complement dysregulation may spread through cortical networks and drive broader functional disruption.

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Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.09/G12

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

Title: Investigating how systemic immune responses affect neuroimmune responses in high-fat diet zebrafish

Authors: *I. SAVOVIC¹, S. S. YEUNG¹, X. YE³, P. XIAO², R. CHANG⁴;

¹Sch. of Biomed. Sci., ²The Univ. of Hong Kong, Hong Kong, China; ⁴Sch. Biomed. Sci., ³Lab. of Neurodegenerative Diseases, LKS Fac. of Medicine, Univ. of Hong Kong, Hong Kong, China

Abstract: One of the most pressing problems in our societies is obesity, a significant health risk impacting over 1 billion individuals. This escalating crisis affects both adults and children and is linked to the deaths of 2.8 million people annually worldwide. Obese men and women exhibit different risks for developing metabolic and cardiovascular diseases. The aim of our study is to investigate the mechanism of how obesity and sterile/non sterile inflammation affects neuroinflammation between genders. In this study, one-year-old female and male wild type AB zebrafishes were fed a tailored made high-fat diet three times a day for 8 consecutive weeks. After 8 weeks of high-fat diet, we performed sterile and non-sterile challenge. After 8 weeks of high-fat diet and 24 hours after tail amputation, we examined the levels of inflammatory cytokines, adipokines and molecules involved in iron metabolism in the brains, blood and livers of AB zebrafishes. The findings showed that after sterile inflammation, the brain, blood and liver of both male and female obese tail amputated zebrafishes had increased levels of messenger RNA for inflammatory cytokines, adipokines and molecules that are involved in iron metabolism, showing different patterns between different genders. Next, after 8 weeks of high-fat diet and 24 and 48 hours after LPS injection there were increased levels of messenger RNA of inflammatory cytokines, adipokines and iron transporters in the brains and livers, revealing gender-specific patterns. In the obese female zebrafish adults, results implicated increased levels of inflammatory cytokines 24 and 48 hours after intraperitoneal LPS injection. In the male obese adults, there were increased levels of inflammatory cytokines 48 h after LPS injection. Finally, the behavioral tests revealed significant differences between the obese and control groups; zebrafish showed decreased cognition and increased anxiety after 4 and 8 weeks of the high-fat diet. The expression of Lipocalin-2 in posterior tuberculum of the brain was higher in high-fat diet fed tail amputated zebrafish. Collectively, the findings indicate that zebrafish can serve as an experimental model to study chronic and acute systemic inflammation caused by a high-fat diet and sterile or non-sterile inflammation, highlighting also differing patterns of neuroinflammation between genders. The findings also imply that IL1- β plays a role in these occurrences.

Disclosures: I. Savovic: None. S.S. Yeung: None. X. Ye: None. P. Xiao: None. R. Chang: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.10/G13

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

Support: Department of Defense (W81XWH-22-1-0565)

Title: Phagocytosis Function, Neuroinflammatory Response and Neurotoxicity of iMicroglia from iPSCs Generated from Veterans with Gulf War Illness

Authors: *S. RAO¹, K. CASE², R. S. BABU³, S. REDDY⁴, L. N. MADHU⁵, M. KODALI⁶, P. W. BAAS⁷, K. SULLIVAN⁸, A. K. SHETTY⁹;

¹Inst. for Regenerative Medicine, Dept. of Cell Biol. and Genetics, Texas A&M Univ. S, College Station, TX; ²Neurosci., Drexel Univ. Col. of Med., Philadelphia, PA; ³Texas A&M Univ., College Station, TX; ⁴Texas A&M, College Station, TX; ⁵Inst. For Regen Med, Texas A&M Univ. Coll Med., College Station, TX; ⁶Inst. for Regenerative Medicine, Dept. of Cell Biol. and Genetics, Sch. of Medicine, Texas A&M Univ., College Station, TX; ⁷Neurobio. and Anat., Drexel Univ., Philadelphia, PA; ⁸Envrn. Hlth. Dept, Boston Univ. Sch. of Publ. Hlth., Boston, MA; ⁹Dept. of Cell Biol. and Genet., Inst. for Regenerative Medicine, Texas A&M Univ. Col. of Medicine, Col. Station, Texas, USA., College Station, TX

Abstract: Epidemiological and animal model studies have suggested that the central nervous system impairments observed in veterans with Gulf War Illness (GWI) are linked to neuroinflammation caused by activated microglia. This study aimed to investigate the characteristics of iMicroglia differentiated from induced pluripotent stem cells (iPSCs) obtained from GW veterans, both with and without GWI, in order to understand the role of microglia in neuroinflammation associated with GWI. We generated iMicroglia using eight iPSC lines: four from veterans without GWI and four from veterans with GWI. These microglia expressed specific markers associated with microglia, including TMEM119, CX3CR1, IBA-1, and CD11b. The functional analysis of these iMicroglia was determined by their proficiency to phagocytose fluorescently labeled A β -42 peptide (FAM-A β 42). The overall percentages of iMicroglia incorporating A β -42 were significantly lower in the GWI veteran group compared to the control veteran group, implying an impaired phagocytosis function in iMicroglia from veterans with GWI. We next examined the inflammatory response of iMicroglia when stimulated with lipopolysaccharide (LPS) or interferon-gamma (IFN- γ). Notably, low doses of LPS or IFN- γ stimulation resulted in increased release of TNF α by iMicroglia from all four iPSC lines in the Gulf War Illness (GWI) veteran group. In contrast, only two iPSC lines in the control veteran group exhibited similar behavior. When evaluating the relative response of iMicroglia from high-responder control veterans and GWI veterans to low doses of LPS or IFN- γ , it was found that, at

baseline and for all doses of LPS or IFN- γ stimulation, iMicroglia from the GWI veteran group released significantly more TNF α compared to those from the control veteran group. Additionally, an assessment of the cytotoxic effects of LPS on iMicroglia from both control and GWI veteran iPSC lines indicated that iMicroglia from the GWI veteran iPSC lines are more resistant to LPS exposure. Additional studies indicated that conditioned medium from iMicroglia primed with LPS from GWI veterans could induce neurodegeneration and convert naive human astrocytes into neurotoxic astrocytes that secrete complement C3. Overall, the findings suggest that iMicroglia derived from GWI veteran-iPSC lines exhibit an enhanced inflammatory response to stimulation with LPS or IFN- γ . However, their ability to perform phagocytosis is impaired. Additionally, these iMicroglia are resistant to high doses of LPS and can promote neurodegeneration while facilitating the transformation of naive astrocytes into neurotoxic astrocytes.

Disclosures: S. Rao: None. K. Case: None. R.S. Babu: None. S. Reddy: None. L.N. Madhu: None. M. Kodali: None. P.W. Baas: None. K. Sullivan: None. A.K. Shetty: None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.11/G14

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

Title: A novel patient-derived pre-clinical mouse model for myasthenia gravis

Authors: K. ZI¹, J. LIANG¹, *R. FENG²;

¹GemPharmatech, Nanjing, China; ²Gempharmatech, Nanjing, China

Abstract: Background/Purpose: Myasthenia gravis (MG) is an autoimmune disease in which the immune system targets muscle acetylcholine receptors, disrupting neuromuscular signal transmission. This impairs muscle contraction, causing fatigue and weakness. Here, we characterize an NCG mouse model using peripheral blood lymphocytes (PBLs) from MG patients to investigate disease pathogenesis and therapeutic strategies. **Methods:** We developed a novel mouse model for MG by intraperitoneal injection of MG patient-derived PBLs into immunodeficient NCG mice, leading to pronounced phenotypic similarities with human MG. Starting from the first week after PBL injection, the peripheral blood was collected every two weeks for flow cytometry analysis of peripheral immune cells. The plasma was separated for ELISA assays of human anti-acetylcholine receptor (anti-AchR) antibodies and total IgG. At the study endpoint, gastrocnemius muscles and tibialis anterior muscles were collected and embedded in paraffin blocks for hematoxylin-eosin (HE) staining. **Results:** In the MG patient-derived PBLs-induced mouse model, reconstituted human immune cells were detectable from 3-7 weeks post-injection. In addition, certain pathological features of MG can be observed, such as increased anti-AchR and total IgG. At endpoint, significant damage was detected in gastrocnemius and tibialis anterior muscles, including inflammatory infiltration, muscle fiber

degeneration/necrosis, atrophy, and fibrous tissue hyperplasia. **Conclusion:** In summary, we developed and validated a novel patient-derived MG model that exhibits pathological features similar to those observed in MG patients. This model serves as a valuable tool for evaluating potential therapeutics for treating MG.

Disclosures: **K. Zi:** A. Employment/Salary (full or part-time): GemPharmatech. **J. Liang:** A. Employment/Salary (full or part-time): GemPharmatech. **R. Feng:** A. Employment/Salary (full or part-time): GemPharmatech.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.12/G15

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

Title: Sex-associated expression patterns in immune, survival, and matrix signaling after optic nerve injury

Authors: ***M. GAJENDRAN**^{1,2};

¹Univ. of Missouri, Kansas City, Kansas City, MO; ²Vision Res. Center, Dept. of Ophthalmology, Univ. of Missouri-Kansas City Sch. of Med., Kansas City, MO

Abstract: Optic nerve crush and microbead-induced ocular hypertension trigger glial inflammation, survival signaling, and extracellular matrix (ECM) remodeling. These processes may be influenced by sex as a biological variable, with potential differences in gene expression patterns between males and females. This gene expression study examines injury-relevant signaling pathways in the retina, unmyelinated optic nerve head, and myelinated optic nerve. Gene-level count data from 36 individual eye samples (18 male, 18 female) were obtained from the public RNA-sequencing dataset GSE241782. Samples spanned three distinct tissue regions and multiple injury conditions (naïve, optic nerve crush, and ocular hypertension) across four recovery intervals (zero days, three days, two weeks, and six weeks). Expression was compared with sexes pooled and also stratified by sex to explore potential sex-associated effects. The analysis focused on two mechanistically defined and hormone-responsive signaling pathways: the JAK/STAT signaling pathway and the NF-κB signaling pathway. This analysis generated region- and time-resolved expression profiles of these pathways and enables assessment of sex-associated variation in gene expression responses to optic nerve injury. These findings contribute to a mechanistic understanding of how immune activation, survival signaling, and astrocyte-matrix interactions may be modulated by sex in optic neuropathies such as traumatic injury and glaucoma.

Disclosures: **M. Gajendran:** None.

Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.13/G16

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

Support: NIA R21 G 08165801
AAR9-NTF-22-974072 to NCT

Title: Subchronic TLR7 Immune Challenge Induces Sex-Specific Memory Impairments and Neuroimmune Dysregulation: Role of Type I Interferons

Authors: *H. CHOI¹, P. KE-LIND¹, H. WHITE², N. C. TRONSON¹;

¹Dept. of Psychology, ²Neurosci., Univ. of Michigan, Ann Arbor, MI

Abstract: Over 770 million individuals worldwide are reported as infected with COVID-19, with up to 40% developing “Long COVID” including persistent cognitive symptoms and memory impairments. Although the SARS-CoV-2 virus rarely invades the brain, innate inflammatory signaling triggered by viruses and other systemic infections has been implicated in mediating cognitive decline and in exacerbating Alzheimer’s disease (AD). We hypothesized that during midlife, peripheral TLR7 activation, mimicking exposure to viral ssRNA, would trigger sex-specific and persistent changes that may prime the brain for neurodegeneration. 10-month-old male and female C57BL/6N mice received a subchronic immune challenge consisting of five intraperitoneal injections of the TLR7 agonist R848 (1000 µg/kg) or vehicle (4% DMSO in PBS) (Tchessalova & Tronson, 2019;2020). Eight weeks after subchronic R848, we observed impairments in novel-object recognition memory in both sexes and disrupted contextual fear memory only in males. Males also exhibited disrupted sucrose preference. Females, but not males, displayed a shift towards passive coping strategies in the forced-swim test. We also observed durable immune reprogramming, where males and females exhibited sex-specific patterns of cytokine tolerance and sensitization in both the periphery and the hippocampus. Males also exhibited elevated hippocampal microglial count, while both sexes displayed sustained microglial activation. Previously, our lab demonstrated that blocking type I interferon (IFN) signaling *via* interferon-alpha/beta receptor 1 (IFNAR1) prevented memory impairments induced by acute immune challenge in male mice only. To determine whether type I IFN signaling mediates long-lasting cognitive and neuroimmune consequences downstream of subchronic immune challenge, we now evaluate whether IFNAR1 blockade can attenuate R848-induced memory impairments and neuroimmune dysregulation. Building on these findings, ongoing studies will determine whether a subchronic immune challenge exacerbates AD progression and pathology in APP/PS1 mice. Collectively, these findings suggest that persistent neuroimmune dysregulation following viral-like immune challenges may represent an important mechanistic link between viral infections, such as COVID-19, and increased vulnerability to cognitive decline and Alzheimer’s disease.

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Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.14/H1

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

Support: Mayo Clinic Foundation
R01NS103212

Title: Mhc class i deletion on mural cells increases cd8 t cell infiltration into the brain during theiler's murine encephalomyelitis virus

Authors: *M. A. MAYNES¹, M. PEDRA SEADY², Z. TIAN¹, M. HANSEN², A. HASSANI², J. THELWELL¹, F. JIN², C. LEWIS¹, A. J. JOHNSON²;
²Immunol., ¹Mayo Clin., Rochester, MN

Abstract: CD8 T cell induced blood-brain barrier (BBB) disruption is an established mechanism of neuropathology. The mural cell is a MHC class I expressing neurovascular unit cell type which is critical for maintaining BBB integrity. However, the importance of mural cell antigen-restricted engagement by CD8 T cells is not fully understood. To determine the function of mural cell MHC Class I antigen presentation, we crossed the *Pdgfrβ*-creER^{T2} mouse to our novel H-2D^b LoxP mouse developed by our laboratory. This transgenic mouse approach enabled us to target mural cells during neuroinflammation. We observed robust knockdown of H-2D^b on *Pdgfrβ*⁺ mural cells during TMEV infection in these animals. The effect of conditional H-2D^b knockdown on this cell type resulted in increased CD4 T cell, CD8 T cell, and B cells infiltration into the brain during TMEV infection and a further increase of antigen specific CD8 T cells during peptide stimulation of viral peptide. This data demonstrates an important role of *Pdgfrβ*⁺ mural cells for immune cell recruitment into the CNS. To our knowledge, this is the first study demonstrating an important role for MHC class I antigen presentation by mural cells in a murine model of neuroinflammation.

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Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.15/H2

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

Support: DOD M2501603
TARCC 2025

Title: Acute and Long-Term Cognitive Outcomes of Controlled Circadian Rhythm Dysregulation

Authors: J. REYES¹, J. M. IANNUCCI², G. ALLEN², M. NEWELL ROGERS¹, D. J. EARNEST², *K. M. SOUZA³;
²Neurosci. and Exptl. Therapeut., ¹Texas A&M Hlth. Sci. Ctr., Bryan, TX; ³Texas A&M Univ., Bryan, TX

Abstract: Aging of the rodent circadian system mirrors key features of circadian decline observed in humans, including sleep-wake disturbances and alterations in circadian rhythm entrainment. Increasing evidence suggests that sleep and circadian rhythm disturbances may precede age-related cognitive impairment, underscoring the need to investigate the link between circadian dysregulation and cognitive function. Building on our previous findings showing long-term B cell expansion in response to an established light-dark (LD) cycle shifting paradigm, we determined whether this controlled circadian dysregulation (CCD) also acutely alters both the adaptive immune system and cognitive performance. Young C57Bl/6J mice (3-6 months old) were subjected to an 80-day CCD paradigm involving repeated 12-hour advances of the light-dark (LD) cycle every 5 days. One week following the final LD shift, mice were tested for cognitive performance using the Barnes maze, along with additional assessments including the three-chamber sociability test, open field test, and novel object/location recognition tasks. We also analyzed immune cell activation in both peripheral tissues and the brain. Our results reveal that CCD induces significant acute alterations in the adaptive immune system in young mice. However, changes in cognitive and social behaviors were present but relatively mild. These findings indicate that circadian desynchronization induces immediate changes in the immune system that may persist over time, laying the groundwork for future studies on the immune-cognitive interface underlying sleep and circadian rhythm disturbances.

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Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.16/H3

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

Support: National Institute on Aging Grant R01AG075440

Title: Neurons Incorporating Intranasally Administered EVs from Human iPSC-Derived Neural stem cells Exhibit Enduring transcriptomic changes in the Aged Brain

Authors: *M. KODALI^{1,2}, L. N. MADHU², S. ATTALURI², G. SHANKAR², S. RAO², R. UPADHYA², Y. SOMAYAJI², V. RAO², C. HUARD², S. KOTIAN², B. SHUAI², S. GUPTA³, A. NAIK², P. SHETTY², G. ZANIRATI², X. RAO², J. CAI³, A. K. SHETTY²;

¹Inst. for Regenerative Medicine, Dept. of Cell Biol. and Genetics, Sch. of Medicine, Te, College Station, TX; ²Inst. for Regenerative Medicine, Dept. of Cell Biol. and Genetics, Sch. of Med., College Station, TX; ³Dept. of Vet. Integrative Biosciences, Texas A&M Col. of Vet. Medicine,, College Station, TX

Abstract: Brain aging, characterized by a progressive decline in cognitive function, is associated with an imbalance in longevity-regulating pathways typified by elevated mechanistic target of rapamycin (mTOR) signaling, diminished autophagy, and disrupted metabolic homeostasis in neurons. Targeting such age-associated changes could preserve cognitive function. This study investigated whether the intranasal administration of human induced pluripotent neural stem cell-derived extracellular vesicles (hiPSC-NSC-EVs) in late middle age would mediate beneficial changes in the transcriptome of neurons. Male C57BL/6 mice, receiving 2 doses of either vehicle or hiPSC-NSC-EVs (12×10^9 EVs per dose, separated by two weeks) intranasally at 18 months of age, were euthanized at 20.5 months and nuclei from forebrains were processed for single-nucleus RNA sequencing. Analysis of 11,854 neuronal nuclei with specific gene expression revealed the presence of nuclei from neuronal subclasses, including glutamatergic (Vglut1) and GABA-ergic (Gad1 and Gad2) neurons, dentate granule cells (Prox1), and CA1 (Slitrk5) and CA3 (Prkcd) pyramidal neurons. KEGG enrichment analysis of differentially expressed genes (DEGs) in glutamatergic neurons between the aged-Veh and aged-EVs groups identified pathways related to autophagy, longevity regulation, neurodegeneration, the FoxO, mTOR, and AMP-activated protein kinase (AMPK) signaling. A similar analysis in GABA-ergic interneurons identified pathways of autophagy, neurodegeneration, and the synaptic vesicle cycle. Pathway analysis of DEGs in dentate granule cells identified cell cycle, oxytocin, Hippo, transforming growth factor beta, axon guidance, thyroid hormone signaling, and circadian entrainment pathways. DEGs in CA1 pyramidal neurons mapped pathways related to neurodegeneration, mitophagy, autophagy, Alzheimer's disease, and the regulation of longevity. Validation of these alterations in the hippocampus confirmed reduced concentrations of proteins linked to mTOR activation and increased levels of proteins associated with enhanced autophagy, mitophagy, longevity, FoxO signaling, and pre-and post-synaptic terminals in EVs-treated mice. These mice also displayed an increased generation of newly born neurons. Such beneficial alterations were also associated with improved pattern separation and mood functions. Thus, hiPSC-NSC-EVs treatment in late middle age can induce beneficial transcriptomic changes in both excitatory and inhibitory neurons and subclasses of hippocampal neurons, thereby lessening the activation of multiple pathways associated with brain aging and cognitive decline.

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Poster

PSTR330: Neuroimmune and Glia Function in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR330.17/H4

Topic: G.02. Neuroendocrine Processes and Behavior

Title: Simulated spaceflight induced cognitive deficits: Insight through the gut-brain-axis

Authors: ***M. R. BURKE**^{1,2}, **S. VILLAPOL**²;

¹Physiol. and Biophysics; Neurorestoration, Weill Cornell Med. College-Houston Methodist, Houston, TX; ²Neurosurg., Houston Methodist Res. Inst., Houston, TX

Abstract: The gut-brain-axis (GBA) is a critical interface of neuroendocrine, immune, gastrointestinal, and cognitive functions. Remarkably, low Earth orbit and Apollo spaceflight missions have presented detrimental effects across these systems, such as cognitive decline, persistent inflammation, gut and blood-brain barrier breakdown (BBB), and alterations in microbial diversity. With neuroendocrine-immune pathways connecting the gut, brain, and several other organs, we aim to investigate the GBA perspective to further inform us of dysfunctional cross-communication exacerbating spaceflight ailments. Using a murine model, we simulated spaceflight stressors by exposing 25-week-old male and female C57BL/6J mice to ionizing radiation (IR; 50 or 100cGy X-rays) and hindlimb unloading (HU) to mimic microgravity-induced cephalad fluid shift. We hypothesize that cognitive and behavioral deficits are partly due to the onset of microbial and enteroendocrine-immune dysbiosis. Representative tissues of the GBA and the immune system were assessed through flow cytometry, immunohistochemistry, and Fluorescence In Situ Hybridization (FISH). Fecal samples were also collected throughout the study for metagenomic sequencing analysis of dynamic microbial changes in the gut. Our histology-based gut morphological analysis reveals inflammation and epithelial damage in the small intestine when exposed to IR and HU. Moreover, small intestine mucin production as a means for immune response exhibits increased production 2-weeks post-HU. Correspondingly, PMA/ionomycin stimulated immune cells present a prolonged inflammatory phenotype through significant increases of myeloid and cytotoxic T cells, while helper T cells are significantly reduced even 2-week post IR and HU. Behavioral analysis depicts increased anxiety and learned-helplessness behaviors in IR-expose mice, along with slowed spatial memory and cognition across individual and combined spaceflight stressors. Thus, our results confirm immune dysfunction in the gut and across the system, which may pass the BBB and induce further cognitive impairments. Future countermeasures are planned to alleviate spaceflight effects on the GBA, reducing neuroinflammation, and improving behavioral outcomes during long-duration missions.

Disclosures: **M.R. Burke:** None. **S. Villapol:** None.

Poster

PSTR331: Viral Infection and Neuroinflammation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.01/H5

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

Title: Dynamic and long-term alterations of mitochondrial single cell transcriptional signature following intranasal administration of SARS-CoV-2 spike protein to Humanized ACE2 K18 transgenic mouse as a model of post COVID Syndrome

Authors: *A. Y. HATEM¹, O. A. ABULSEOUD²;

¹Dept. of Neurosci., Grad. Sch. of Biomed. Sciences, Mayo Clin. Col. of Med., Scottsdale, AZ;

²Psychiatry and Psychology, Mayo Clin., Phoenix, AZ

Abstract: The aftermath of COVID-19 as persistent or emerging manifestations 12 weeks after the resolution of infection is known as post-COVID syndrome, including cognitive and neuropsychiatric manifestations. However, the underlying pathophysiology is unclear. We administered a single dose (600ug/kg) of intranasal SARS-CoV-2 spike protein (S1) to adult male hACE2 K18 transgenic mice. We ran behavioral and single cell RNA sequencing experiments at 2-, 6-, and 12-weeks after intranasal S1 administration to assess longitudinal changes compared to saline controls. Our results revealed a persistent decreased time investigating the novel object in the novel object recognition task, increased freezing time in the fear conditioning, and decreased immobility time in the forced swim test through the three time points. We then ran single cell RNA sequencing and examined five brain cells, including endothelial cells (EC), microglia, astrocytes, oligodendrocytes, and neurons. The mitochondrial transcriptional profile showed long-term, dynamic, and cell-specific alterations. Two weeks post-S1 administration, EC showed upregulation of multiple mitochondrial electron transport chain (ETC). At 6 weeks, EC upregulated ETC complexes genes, however, mt-Atp8 (encodes a subunit of mitochondrial ATP synthase) was downregulated, which implies potential increased ROS production with deficient ATP synthesis. Twelve-week EC downregulated mt-Nd1 and mt-ATP8, affecting both complex I in the ETC and ATP synthesis. Moreover, uncoupling protein-2 (UCP2) upregulation could increase ROS generation and act as an uncoupler of oxidative phosphorylation, suggesting a picture of mitochondrial dysfunction. Oligodendrocytes at 2 weeks showed extensive upregulation of mitochondrial machinery, including up to 51 genes, which were reduced to only 10 upregulated genes at six weeks. However, twelve-week oligodendrocytes showed downregulation of ETC genes. Microglia showed a compromised mitochondrial profile, with downregulated ATP synthase subunits (Atp5g3) at 2 weeks and mt-Atp8 at 6 weeks. At 12 weeks, microglia, downregulated 11 mitochondrial genes, denoting exhaustion. Astrocytes showed upregulation of multiple ETC genes at the three time points. However, neurons showed upregulation of ETC only at 2 and 12 weeks. In conclusion, S1 causes long term behavioral changes in hACE2 K18 transgenic mice, which are reminiscent of post-COVID neuropsychiatric manifestations, with underlying dynamic cell specific alterations in mitochondrial transcriptional profile.

Disclosures: A.Y. Hatem: None. O.A. Abulseoud: None.

Poster

PSTR331: Viral Infection and Neuroinflammation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.02/H6

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

Support: NIH Grant R01NS129407
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NIH Grant P30GM122734
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Title: Thalamic Neurometabolite Levels Reflect Cerebrovascular Recovery Following COVID-19

Authors: *A. VAKHTIN¹, D. COE², N. SHAFF¹, N. HOFFMAN¹, A. BIRG³, H. LIN³, D. QUINN⁴, E. ERHARDT⁵, A. PARADA⁴, S. G. RYMAN⁶;

¹The Mind Res. Network, Albuquerque, NM; ²Mind Res. network, Albuquerque, NM; ³New Mexico VA Healthcare Syst., Albuquerque, NM; ⁴Univ. of New Mexico Sch. of Med., Albuquerque, NM; ⁵Univ. of New Mexico, Albuquerque, NM; ⁶Translational Neurosci., Mind Res. Network, Albuquerque, NM

Abstract: Mild COVID-19 can produce chronic symptoms that are often neurocognitive in nature. Our ongoing broader study aims to examine such post-acute COVID sequelae (PACS) as lasting manifestations of dysfunctional cerebrovascular dynamics following infection. However, fundamental post-infection cerebrovascular characteristics and their effects on neuronal tissue health remain unclear, and are thus the focus of the analysis described herein. We reported elsewhere the presence of cerebrovascular impairments in the sub-acute stage after SARS-CoV-2 infection that subsequently normalize in a time-dependent fashion. Here, we examined the effects that these post-infection functional cerebrovascular dynamics may have on neuronal health. To do so, 23 participants who had prior non-hospitalized SARS-CoV-2 infections underwent semi-adiabatic localization by adiabatic selective refocusing (semi-LASER) magnetic resonance spectroscopy (MRS) as well as a CO₂-induced cerebrovascular reactivity fMRI breathing task (CVR; TR = 0.8 s). Measures of magnitude and delay of the vascular response to CO₂ administration were extracted from the CVR data. The MRS data were used to calculate concentrations of choline (Cho), creatine (Cr), and n-acetylaspartate (NAA) in the thalamic region, which has been shown to exhibit robust vascular reactivity. Data were examined via multiple regression analyses using concentrations of thalamic Cho, Cr, and NAA as the dependent variables, and CVR metrics (response delay and magnitude), time since SARS-CoV-2 infection (TSI), and age as the predictor variables. We detected significant relationships between shorter CVR delay (TSI- and age-corrected) and increased thalamic concentrations of Cr ($p = 0.005$) and NAA ($p = 0.019$), but not Cho ($p = 0.065$). CVR Magnitude was not predictive of any examined neurometabolite concentrations. The results suggest that rapid responsiveness of cerebral vasculature to exogenous stimuli may facilitate improvement of neuronal metabolic health during recovery from COVID-19. Follow-up analyses of interest include delineating the aforementioned association of vascular reactivity with neurometabolite concentrations between

former COVID patients who have persistent neurocognitive symptoms versus those who fully recovered.

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Poster

PSTR331: Viral Infection and Neuroinflammation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.03/H7

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

Support: NIGMS Grant P30GM122734
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NINDS Grant R01NS129407
NINDS Grant R01NS133569

Title: Cerebrovascular Reactivity Dynamics Following Mild SARS-CoV-2 Infection

Authors: *D. COE¹, H. PRATHER¹, N. SHAFF¹, N. HOFFMAN¹, S. G. RYMAN², A. BIRG³, E. ERHARDT⁴, H. LIN⁵, A. PARADA⁵, A. A. VAKHTIN⁶;

²Translational Neurosci., ¹Mind Res. Network, Albuquerque, NM; ³Intrnl. Med., VA Hosp., Albuquerque, NM; ⁴Mathematics and Statistics, university of New Mexico, Albuquerque, NM; ⁵Intrnl. Med., Univ. of New Mexico, Albuquerque, NM; ⁶The Mind Res. Network, Albuquerque, NM

Abstract: Acute moderate-to-severe SARS-CoV-2 infection has been linked to vascular conditions, potentially via ACE2 receptors that are extensively expressed by vascular cells. We examined whether mild COVID-19 is associated with cerebrovascular dysfunction, which may play a role in producing symptoms of long-COVID. Cerebrovascular reactivity (CVR) to hypercapnia was assessed in individuals with varying post-infection timelines. We used BOLD fMRI during a block-design hypercapnia challenge (TR = 0.8 s; 30 s 5% CO₂ alternated with room air) to quantify the delay and magnitude of the cerebrovascular response in whole-brain grey matter. CVR was performed on 58 adults who previously had mild COVID-19 (time since infection [TSI] = 16.4 +/- 12.5 mo.), 26 of whom reported neurocognitive post-acute COVID sequelae (PACS+) and 32 reported making a full recovery (PACS-). A subset of participants completed a follow-up scan (~4 months). CVR amplitude and delay were extracted and analyzed using regression models with age and TSI as predictors. Across all participants, greater TSI was cross-sectionally associated with shorter CVR delay ($\beta = -0.288$, $p = 0.036$), likely reflecting the normalization of cerebrovascular function post-COVID. This pattern was also observed in both PACS- and PACS+ groups longitudinally. However, while amplitude increased in the PACS- group longitudinally, this was not observed in PACS+ participants. Follow-up examination

revealed strong coupling between CVR amplitude and delay in the PACS– group ($p = 0.005$), but not in PACS+ participants ($p = 0.227$). The results collectively suggest that mild SARS-CoV-2 infection is associated with measurable changes in cerebrovascular function that improve over time, and that persistent post-COVID symptoms may be produced by altered vascular dynamics such as disrupted amplitude-delay coupling. Ongoing longitudinal work will further elucidate the described trajectories and clinical relevance of their effects.

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Poster

PSTR331: Viral Infection and Neuroinflammation

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.04/H8

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

Support: HHMI Emerging Pathogens Initiative
Stanford School of Medicine Dean's Postdoctoral Fellowship

Title: Neural-immune interactions following respiratory immune challenges affect the aging brain and neurodegeneration in mice

Authors: *A. IBRAYEVA, T. PHAM, A. NGUYEN, H. XU, K. MALACON, A. GERAGHTY, M. MONJE;
Neurol. and Neurolog. Sci., Stanford Univ., Palo Alto, CA

Abstract: Neural plasticity underlies our ability to maintain cognitive abilities. Such plasticity is known to decline with age in the context of neurodegeneration, and to be impaired by various immune challenges, but how immune challenges earlier in life or in aged individuals influence neurodegeneration in susceptible individuals remains incompletely understood. Diverse non-central nervous system (CNS) immune challenges can cause CNS dysfunction, a point that has been devastatingly illustrated during the COVID-19 pandemic, with a high incidence of lasting cognitive symptoms and ~two-fold increased risk of dementia following even mild COVID. In our study, we utilized viral (Poly(I:C) and Influenza A) and bacterial (LPS) immune challenge models in both genetically engineered Alzheimer's disease (AD) mouse models (5XFAD) and wild-type controls to evaluate histological neuropathology and inflammatory responses. Our findings reveal that aged mice exhibit a distinct region-specific neuroinflammatory response to respiratory challenges, with increased cortical microglial reactivity compared to young adults. Respiratory immune challenge results in persistently elevated microglial reactivity, loss of oligodendrocytes and impaired hippocampal neurogenesis. Furthermore, in the AD mouse model, respiratory immune challenges in young adulthood trigger neuroinflammation prior to the typical onset of neuropathology, resulting in increased microglial reactivity, elevated levels of

cytokines and chemokines in cerebrospinal fluid, and elevated A β ₁₋₄₂ plaque density compared to AD model mice without respiratory immune challenge. Taken together, these findings underscore neural dysfunction caused by infections outside the central nervous system that may accelerate or compound age-related neuropathology and highlight the need for targeted therapeutic strategies to mitigate the neurobiological consequences of respiratory immune challenges in aging and AD populations.

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Poster

PSTR331: Viral Infection and Neuroinflammation

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Program #/Poster #: PSTR331.05/H9

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

Support: R01ES021656
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R35ES035043-01

Title: Intranasal infection with Western Equine Encephalitis Virus induces temporally and spatially distinct patterns of gliosis and progressive neurodegeneration in the hippocampus of C57Bl/6 mice

Authors: *A. BRIGGS¹, A. SCHULLER¹, M. HAGER¹, O. YANOURI¹, C. BANTLE², S. ROCHA¹, R. B. TJALKENS¹;

¹Envrn. and Radiologic Hlth. Sci., ²Envrn. and Radiological Hlth. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Arboviruses, including alphaviruses such as Western equine encephalitis virus (WEEV), are increasing in prevalence across human and animal populations, resulting in significant morbidity and mortality. WEEV infection is associated with acute encephalitic effects and may also be associated with longitudinal neuroinflammation which may contribute to onset or progression of disorders including Parkinson's disease (PD) and PD-related dementias such as Lewy Body Dementia. Our lab has previously demonstrated that sub-lethal WEEV infection is sufficient to elicit a robust astrocyte-mediated complement response associated with gait aberrancies, dopaminergic neurodegeneration, and pathologic protein aggregation in the substantia nigra of outbred CD-1 mice and wild-type C57Bl/6 mice. However, the temporal sequence of glial activation and subsequent neurodegeneration in brain regions relevant to cognitive function and memory have not been described, despite the observed persistence of the virus in the hippocampus long after peripheral clearance. Together, this motivated the assessment of hippocampal glial dynamics and neurodegeneration following WEEV infection across an eight week time course. Six-week-old C57Bl/6 mice were intranasally administered 20 μ L of

1×10⁴ PFU/mL McMillan WEEV and subsequently sacrificed at 1, 2, 4, and 8 week timepoints. Hippocampal sections were immunofluorescently labeled, imaged via high-content scanning microscopy, and automatically quantified using deep learning-based analysis pipelines trained across the dataset. This revealed evidence of substantial astrogliosis (percent GFAP⁺ area) at 1 week post-infection (WPI) followed by microgliosis (number of IBA1⁺ cells/mm²) in hippocampal subregions and dentate gyrus at 2 WPI of WEEV infected mice compared to controls. Additionally, a significant decrease in the number of NeuN⁺ neurons/mm² was observed at 1 WPI in the CA1 hippocampal subregion and dentate gyrus of infected mice compared to controls. We further applied high content imaging and spatial phenomics to interrogate the complex temporal relationship between glial cell activation and neuronal loss across time, infection status, and hippocampal subregion of interest as well as 3D rendering of IBA1⁺ cells to assess disease-associated morphology in infected animals compared to controls. Together, these data highlight the dynamic glial involvement in temporal neurodegeneration following WEEV infection in the hippocampus. Future work will aim to investigate the significance of these pathologic changes on the cognition and executive function.

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Poster

PSTR331: Viral Infection and Neuroinflammation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.06/H10

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

Support: NIH Grant R56AI137264
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Title: Neurotropic alphaviruses induce PARP-1 dependent mechanisms of motor neuron dysfunction and cell death

Authors: *R. E. WILLIAMS^{1,4}, L. PIETERSE^{4,2}, M. J. ELRICK^{5,3};

¹Solomon H. Snyder Dept. of Neurosci., ²Dept. of Biomed. Engin., ³Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ⁴W. Harry Feinstone Dept. of Mol. Microbiology and Immunol., Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD; ⁵Hugo W. Moser Res. Inst., Kennedy Krieger Inst., Baltimore, MD

Abstract: Alphaviruses, including Chikungunya virus (CHIKV) and Venezuelan equine encephalitis virus (VEEV), are emerging global health threats capable of causing severe neurological complications such as encephalomyelitis, seizures, cognitive dysfunction, paralysis, ataxia, and neurodegeneration. These arthropod-borne viruses can directly infect cortical, hippocampal, and motor neurons, triggering cell death and irreversible neurological damage. While our previous work has demonstrated that cortical and hippocampal neurons typically

undergo apoptotic cell death during infection, the mechanisms driving motor neuron loss, a key contributor to virus-induced paralysis, have remained less well defined. This project identifies and characterizes a non-apoptotic, PARP-1-mediated mechanism of motor neuron death during alphavirus infection, marked by metabolic failure, cellular stress, and activation of caspase independent death pathways, such as necrosis. Using both human induced pluripotent stem cell (hiPSC)-derived motor neurons and NSC34 motor neuron-like cells, we demonstrated that CHIKV and VEEV hyperactivate PARP-1, leading to increased poly(ADP-ribose) (PAR) production, significant NAD⁺ and ATP depletion, increased levels of oxidative stress, and subsequent motor neuron death without the translocation of apoptosis inducing factor. Pharmacological and genetic inhibition or degradation of PARP-1 activity significantly improves metabolic homeostasis and cell survival, highlighting its critical role in virus-induced neurotoxicity. These findings provide a foundational understanding of how alphaviruses contribute to neurodegenerative cascades and point to PARP-1 as a potential therapeutic target. By delineating the molecular underpinnings of motor neuron vulnerability, this work may inform future strategies to prevent or mitigate virus-induced neurological dysfunction, paralysis, and other disabling outcomes.

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Poster

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Program #/Poster #: PSTR331.07/H11

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

Support: Neurovirology Core Grant

Title: Proteomics of chikungunya virus infected human microglial C20 cells to understand pathogenesis of chikungunya encephalitis.

Authors: *N. D¹, A. SHRUNGESWARA³, L. P², N. CHOUDHARY³, T. PRASAD³, M. VENKATASWAMY²;

¹Dept. of Basic Neurosciences, ²Dept. of Neurovirology, Natl. Inst. of Mental Hlth. and Neuro Sci. (NIMHANS), Bangalore, India; ³Ctr. for Systems Biol. and Mol. Medicine, Yenepoya Res. Ctr., Yenepoya (Deemed to be University), Mangalore, India

Abstract: Chikungunya fever, caused by Chikungunya virus (CHIKV) and transmitted by *Aedes* mosquitoes, presents with acute fever, rash, and persistent joint pain. Children and the elderly with comorbidities may develop Chikungunya Encephalitis marked by demyelination and long-term cognitive deficits. Chikungunya Encephalitis patients show elevated TNF- α , IFN- α , IL-6, and IFN- γ -induced monokine levels compared to uncomplicated cases, though their role in pathogenesis remains unclear. Understanding microglial involvement, the primary immune cell

in the central nervous system (CNS), is key to uncovering CHIKV-related CNS complications. Employing an *in vitro* model of CHIKV infected C20 human microglial cells, we performed data-independent proteomic analysis on cells harvested at 0, 4, 8, 12 and 24 hours post infection (hpi). A temporal variation was observed in host proteome and pathways. Signal transduction and metabolic pathways, and the cell death and infection pathways were enriched until the 12 hpi. The inflammatory cell response and immune system pathways were enriched at multiple time points, the highest being at 24 hpi. The antiviral process pathway was noted only at 24 hpi. Immune system and inflammatory cell response pathways were identified as the most enriched clusters of pathways in this study, followed by antiviral processes. The summary of Differentially expressed proteins (DEPs) is as follows: ATP6V1B2 was downregulated and is potentially required for activating an immune response. HSPA4, HSPA9, and LAMTOR1 increase with time, while SAMHD1 was downregulated and could be responsible for inflammatory responses. PPP2R5D, PSMA6, and RPABC3 were decreased while STIP1 increased with time and are potentially involved in aiding the viral replication and life cycle. VDAC2, LAMTOR1 and ribosomal proteins, RPL6, RPL23, RPS14, are potentially involved in cellular apoptosis. Future antiviral drugs could be developed to target DEPs involved in antiviral mechanisms such as EIF4G1, IPO5, and MX2, to inhibit CHIKV replication in CNS infections. Based on our findings, we hypothesize that cell death, viral replication and infection pathways are downregulated with time while the antiviral processes increase with time. Along with this, the immune system and inflammatory processes also increase significantly which could potentially lead to inflammation related conditions such as Encephalitis.

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Poster

PSTR331: Viral Infection and Neuroinflammation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.08/H12

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

Title: Host cell metabolic pathways modulate susceptibility to cell death by neuroinvasive California serogroup orthobunyaviruses

Authors: *L. M. FRANCOMACARO, S. SUPATTAPONE;
Biochem. & Cell Biol., Geisel Sch. of Med. at Dartmouth, Hanover, NH

Abstract: The California serogroup (CSG) of orthobunyaviruses is comprised of neuroinvasive viruses with varied pathogenicity: La Crosse virus (LACV) is a leading cause of pediatric arboviral encephalitis (LACV-E) while Inkoo virus (INKV) has a high seroprevalence but a low incidence of disease. Supportive care is the standard of treatment for LACV-E; there is no vaccine or antiviral. Although most CSG viruses are benign, endemic spread and a documented ability to reassort both *in vitro* and *in vivo* threatens the emergence of novel virulent strains. To

identify host cell pathways involved in the cytotoxicity of CSG infection, we conducted whole genome CRISPR-Cas9 knockout screens in neuroblastoma cells using either LACV or INKV survival as the selective pressure. Screens were conducted in paired biological triplicate and analyzed using the MAGeCK pipeline to identify enriched genes and pathways in surviving cells. In a knockout screen, genes that are enriched cells are those that contribute to viral cell death in wild type cells. Interestingly, the genetic hits for both LACV and INKV were overwhelmingly similar -they utilize shared host pathways. The top hit, caspase 9 dependent apoptosis, is in concordance with the CSG literature, validating the screens at a technical level. Also heavily enriched were mitochondrial energy production pathways; cells with impaired energy production survived viral infection longer than wildtype cells. We were able to validate this finding in wildtype cells: cells treated with oligomycin, an ATP synthase inhibitor, survived infection for longer. Environmental manipulations of total energy flux, mild hypothermia and feeding galactose rather than glucose in the media, similarly increased survival of CSG infection. Mechanistic studies showed that both mild hypothermia and feeding galactose, in the absence of viral infection, lower the total reducing power of the cell. Further probing found that mild hypothermia did not reduce caspase 9 dependent apoptosis while feeding galactose did. In sum, modulation of host cell energy pathways improved survival of both INKV and LACV infection for cells in culture providing a promising avenue for development of therapeutics that may be applicable to newly emerging reassorted strains.

Disclosures: L.M. Francomacaro: None. S. Supattapone: None.

Poster

PSTR331: Viral Infection and Neuroinflammation

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

Support: CAPES - Finance Code 001
FAPESP 2024/02895-0
NGO “The Tooth Fairy Project”
FAPESP 2024/22501-6

Title: Measles Virus (MeV) replication involves neuronal budding, accompanied by viral mutation and protein loss of function, as well as neuroinflammation and impairment of synaptogenesis.

Authors: *D. SANTOS¹, P. C. B. BELTRÃO-BRAGA²;

¹Univ. de São Paulo, São Paulo, Brazil; ²Microbiology, Univ. de Sao Paulo, São Paulo-SP, Brazil

Abstract: Measles virus (MeV) is a neurotropic pathogen that can lead to severe complications in the CNS, such as measles inclusion body encephalitis (MIBE) and subacute sclerosing

panencephalitis (SSPE). Despite its clinical relevance, the mechanisms involved in MeV replication, persistence, and adaptation in human neural cells remain poorly understood. In this study, we modeled MeV (vaccine strains Swartz) infection (MOI 1) in three hiPSC cell lines (PD1, PD2, and PD3) (Russo et al., 2018). Neural progenitor cells (NPC) and cortical neurons were evaluated at three time points (1 hpi, 48 hpi, and 72 hpi), considering viral replication, infectivity capacity, synaptic gene modulation, and viral progeny aspects. NPC were highly susceptible and permissive to MeV (PD1 - $t=12.99$; $df=2$; $p=0.0059$ / PD2 - $t=6.535$; $df=2$; $p=0.0226$ / PD3 - $t=4.539$; $df=2$; $p=0.0453$), presenting cytopathic effects, cytoplasmic inclusion bodies, increased apoptosis, and the production of infectious viral particles ($F=112.8$; $p<0.0001$), as confirmed by an infectivity assay. In this assay, the supernatant produced by these cells was collected and used to infect VERO cells. Neurons were also susceptible to MeV and supported viral replication (PD1 - $t=10.36$; $df=2$; $p=0.0092$ / PD2 - $t=47.88$; $df=2$; $p=0.0004$ / PD3 - $t=217.1$; $df=2$; $p=0.0001$); however, they did not release infectious viral progeny as confirmed by an infectivity assay ($F=1.180$; $p=0.3583$), and no viral RNA was detected in the culture supernatant ($F=1.225$; $p=0.3114$). Notably, transmission electron microscopy (TEM) revealed viral particles budding from neurons, representing the first ultrastructural evidence of MeV morphogenesis in these cells. Viral sequencing showed two nucleotide substitutions in the N gene in neuron-derived particles, resulting in an R222I mutation that, according to structural modeling, may disrupt nucleoprotein oligomerization. Moreover, MeV infection altered the expression of synaptic genes (Syn1 and PSD95) in a donor-dependent manner and triggered a strong pro-inflammatory cytokine response in both NPC and neurons. These findings provide novel insights into MeV neurotropism and demonstrate that hiPSC-derived neural models are a valuable tool for investigating viral adaptation, immune responses, and the potential consequences of infection in the human CNS. Russo, FB, et al. (2018). Modeling the Interplay Between Neurons and Astrocytes in Autism Using Human Induced Pluripotent Stem Cells. Biological psychiatry, 83(7), 569-578.

Disclosures: D. Santos: None. P.C.B. Beltrão-Braga: None.

Poster

PSTR331: Viral Infection and Neuroinflammation

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Program #/Poster #: PSTR331.10/H14

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

Support: NIH Grant 5U01DA058527
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Title: Differential endocannabinoid receptors and immune genes following oral CBD administration in SIV infected rhesus macaques

Authors: *L. MAMUN¹, J. ROSADO-FRANCO¹, A. ELLISON³, S. JOYNER⁴, D. W. WILLIAMS²;

²Pharmacol. and Chem. Biol., ¹Emory Univ., Atlanta, GA; ³Mol. Microbiology and Immunology, Mol. and Comparative Pathology, Johns Hopkins Univ., Atlanta, GA; ⁴FSU, Tallahassee, FL

Abstract: Human Immunodeficiency Virus (HIV) is successfully managed with treatment using combined-antiretroviral therapy (cART). However, cART's inability to readily cross the blood-brain-barrier (BBB) reveals a shortcoming in the conventional approach to HIV treatment, as it does not address the buildup of latent virus -and corresponding inflammation- in the brain. Thus, cannabidiol (CBD), was evaluated as a compound of interest due to its ability to freely permeate across the BBB and its anti-inflammatory properties. Limited data exists regarding the expression of the endocannabinoid receptors that CBD interacts with to mediate its anti-inflammatory effects, especially the non-canonical receptors. To investigate this, rhesus macaques were inoculated with SIVmac251 (on week 0), and given increasing oral doses of CBD (10, 20, and 40 mg/kg) on weeks 1, 2, and 3, respectively. On week 4, the study was terminated, necropsy and perfusion with sterile saline performed, and tissue collected from five brain regions -Occipital Lobe, Parietal Lobe, Thalamus, Midbrain, and Cerebellum. Additionally, tissues from six peripheral organs were collected - Heart, Kidney, Spleen, Mesenteric Lymph Node (MLN), Liver, and Adipose Tissue. qRT-qPCR was performed to evaluate canonical and non-canonical endocannabinoid receptors, as well as immune markers between CBD-exposed and SIV-only macaques. We found that following CBD exposure, CB1 was significantly upregulated in all 5 brain regions. We determined that several of the extended family endocannabinoid receptors -PPAR α , PPAR γ , GPR18, TRPV1, TRPV2, FAAH, and 5-HT1R- also showed significant changes across multiple brain regions following CBD exposure. The inflammatory receptor, TRAIL, also significantly increased. While endocannabinoid receptors are well characterized in brain, we were also interested in evaluating the impact of CBD on their expression in peripheral organs. Like brain, we found that CB1 was upregulated in multiple peripheral organs. In addition, significant changes to non-canonical receptors such as PPAR α , PPAR γ , TRPV1, TRPV2, 5-HT1R, ADGRF1, and ADORA2A occurred in several peripheral organs. Of the inflammatory receptors, CCL2, TNF- α , TRAIL, and IL-12a were significantly changed after CBD exposure. Surprisingly, there was no significant change in CB2 in neither brain nor periphery. Our findings suggest that CBD may exert its anti-inflammatory effects through modulation of both the canonical CB1 pathway, but also in part by non-canonical receptor pathways, providing potential mechanistic and therapeutic targets for reduction of HIV-associated inflammation in the brain as well as the periphery.

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Poster

PSTR331: Viral Infection and Neuroinflammation

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Program #/Poster #: PSTR331.11/H15

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

Support: R01DA054534
R01AG072298
T32GM135134

Title: Chimeric human microglial and dual chimeric human microglial/astroglial models of HIV-associated neurocognitive disorders (HAND)

Authors: *S. I. BOSCH¹, D. M. CHANDLER-MILITELLO^{2,3}, C. LONG^{2,4}, J. N. MARIANI^{2,3}, S. J. SCHANZ², S. A. GOLDMAN^{2,3};

¹Microbiology and Immunol., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY; ²Ctr. for Translational Neuromedicine, ³Neurol., ⁴Genomics Res. Ctr., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: In individuals living with HIV-1, a prominent manifestation of viral persistence and associated chronic neuroinflammation is HIV-1 associated neurocognitive disorders (HAND), which affects >40% of HIV-1⁺ subjects despite cART. The pathogenesis of HAND remains poorly understood, in part because of the lack of relevant, humanized experimental model systems that recapitulate key features of this disorder. To investigate how HIV-1 infection drives glial reactivity and neuroimmune dysfunction in the CNS, we have developed both *in vitro* and *in vivo* models for HIV-1 infection of human microglia and subsequent astrocytic reactivity. We have differentiated WA09-line (H9) human embryonic stem cells (hESCs) towards CD140a⁺/CD44⁺ human glial progenitor cells (hGPCs) and CD43⁺/CD235a⁺ human hematopoietic progenitor cells (HPSCs). We then either: (A) engrafted the hGPCs and/or HPSCs into Rag2/IL2 γ $\times$ human mCSF knock-in mice to develop human microglial or dual-chimeric human astroglial/microglial mouse models. Or (B) we further differentiated the hGPCs/HPSCs *in vitro* into GFAP⁺ human astrocytes or CD45⁺/CD11b⁺/CX3CR1⁺/CSF1R⁺ human microglia. *In vivo*, engrafted hGPCs/HPSCs dominate glial chimeric brains, showing competitive dominance of human over mouse GPCs/HPSCs. In addition, to overcome limited endogenous expression of the HIV-1 co-receptor CCR5 in microglia and astrocytes, we have generated a stable CCR5-expressing WA09 hESC line via CRISPR-Cas9-mediated insertion into the AAVS1 safe-harbor locus. Differentiated WA09-CCR5-derived human astrocytes and microglia maintained CCR5 expression across maturation protocols *in vitro* (97.9%⁺/98.4%⁺ by flow cytometry) and *in vivo* (CCR5⁺/HA⁺ by IHC). Accordingly, WA09-derived human microglia were highly susceptible to infection by HIV-1 CCR5-tropic strains ADA and YU2 (6ng/mL p24 at 7D post-infection). Furthermore, we noted *in vivo* microglial infection in humanized mice, as revealed by colocalization of P2RY12⁺/human nuclei (hN⁺)/ and HIV-1 capsid protein (p24⁺). Together, these models provide a robust platform for dissecting the interactions of HIV-infected human microglia with both human astrocytes and host neurons *in vivo*, providing us significant new insight into the mechanisms underlying HAND pathogenesis.

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Poster

PSTR331: Viral Infection and Neuroinflammation

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Program #/Poster #: PSTR331.12/H16

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

Support: 5R01DA052859-02
1F31DA058562-01A1
U01DA058527

Title: Oral cannabidiol decreases inflammatory cytokines and viral load during acute HIV/SIV infection

Authors: *A. ELLISON¹, J. ROSADO-FRANCO¹, L. MAMUN¹, S. KNERLER¹, S. WILLIAMS-MCLEOD³, S. JOYNER⁴, P. J. GASKILL⁵, M. J. CORLEY⁶, E. M. WEERTS³, D. W. WILLIAMS²;

²Pharmacol. and Chem. Biol., ¹Emory Univ. Sch. of Med., Atlanta, GA; ³Johns Hopkins Univ., Baltimore, MD; ⁴Florida State Univ., Tallahassee, FL; ⁵Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; ⁶Dept. of Native Hawaiian Hlth., Honolulu, HI

Abstract: ~40 million people worldwide live with human immunodeficiency virus (HIV), with 1.3 million new diagnoses and >600,000 deaths due to HIV related causes in 2023. Antiretroviral therapy (ART) is extremely effective at reducing plasma HIV viral loads to below detectable levels and has drastically improved mortality and quality of life of those living with HIV. Even so, ART does not address underlying myeloid-derived immune dysfunction and uncontrolled inflammation induced by HIV. Compounds with putative anti-inflammatory properties may complement antiviral properties of ART by addressing HIV-associated inflammation. Cannabidiol (CBD) is one such compound, with immunomodulatory effects on cytokines, including IL-1, IL-6, and TNF α . We investigated immunomodulatory effects of CBD using a rhesus macaque model of simian immunodeficiency virus (SIV) infection and cell culture of primary human immune cells, hypothesizing that CBD will activate the endocannabinoid system to decrease viremia and inflammation. Rhesus macaques were inoculated with SIV and administered single escalating weekly doses of oral CBD (10, 20, and 40mg/kg) starting one week after injection. At four weeks post inoculation samples were collected, including eight tissues, cerebrospinal fluid, and blood. Plasma and CSF viral load were both decreased at week four compared to peak infection, as was seeding of the viral reservoir as determined by reduced proviral copies in peripheral and brain tissue and tissue-specific immune cells at necropsy. Additionally, frequency of peripheral blood mononuclear cells and markers of their activation were reduced, as were soluble cytokines in plasma and CSF and gene expression of tissue cytokines, including CCL2, IL-12, and TNF α . Furthermore, CBD modulated gene expression of 12 canonical and extended endocannabinoid receptors in tissues, with changes dependent on the tissue and receptor evaluated. Of 33 endocannabinoid ligands assessed by LC-MS, all but three remained unchanged by SIV or CBD. Furthermore, monocytes and T cells were isolated from human blood and used for *in vitro* experiments. T cells were activated and monocytes were differentiated into macrophages and microglia before infection with HIV and treatment with CBD (0.1, 1, and 10 μ M). CBD significantly decreased viral replication in macrophages and T cells, but not microglia. CBD also reduced activation of NF κ B and STAT1 in macrophages, and cytokine production was reduced in macrophages, as well as microglia, specifically in donors

whose cytokines were induced after HIV infection. CBD had no effect on cytokine production in donors whose cytokines were not induced after HIV infection.

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Poster

PSTR331: Viral Infection and Neuroinflammation

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Program #/Poster #: PSTR331.13/I1

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

Support: NIH Grant R01DA047157
VA 1I01BX005114

Title: Epigenetic reader, bromodomain-containing protein 4 plays an important role in neuropathic pain induced by HIV glycoprotein 120 with morphine in mice

Authors: X. TONG¹, M. PARDO¹, S. LIU¹, H. YI¹, X. ZHU¹, J. K. LEE², R. C. LEVITT¹, *S. HAO^{3,4},

¹Anesthesiology, ²The Miami Project to Cure Paralysis, ³Univ. of Miami, Miami, FL; ⁴Miami VA Healthcare Syst., Miami, FL

Abstract: People living with HIV have an increased prevalence of conditions associated with opioid analgesic misuse. HIV-1-infected opioid abusers show more severe neuropathology than HIV-positive nondrug users. The exact molecular mechanisms of chronic pain experienced by HIV patients with opioid abuse are poorly understood, for which no effective treatment currently exists. Epigenetic modifications resulting in the changes in the chromatin structure play a critical role in gene expression. Bromodomain-containing protein 4 (Brd4) is a member of the bromodomain and extraterminal domain protein (BET) family and functions as an epigenetic 'reader' that binds acetylated lysines in histones in brain neurons to mediate the transcriptional regulation. Here, we established a neuropathic pain model of interaction of intrathecal HIV envelope glycoprotein 120 (gp120) and chronic morphine use in mice. The combination of gp120 and morphine (gp120/M) induced persistent mechanical allodynia and thermal hyperalgesia. In gp120/M model, we examined effects of knockout of neuron-selective Brd4 using ARC^{CreERT2}::Brd4^{fl/fl} cKO mice. ARC^{CreERT2}::Brd4^{fl/fl} mice were produced by ARC^{CreERT2} mice crossbreeding with Brd4^{fl/fl} mice. Tamoxifen-inducible Cre-mediated recombination resulted in deletion of Brd4^{fl/fl} sequences in the neurons of ARC^{CreERT2}::Brd4^{fl/fl} mice. ARC^{CreERT2}::Brd4^{fl/fl} mice were injected with 75 mg/kg tamoxifen (Tam) or vehicle (corn oil) for 5 day. About twenty days after tamoxifen, mice received gp120/M procedure for 3 days. Tamoxifen treatment significantly reduced mechanical allodynia and thermal hyperalgesia for 3 weeks, suggesting that knockout of neuronal Brd4 reduces neuropathic pain. Western blots

showed that there was significant increase in spinal Brd4 in oil-gp120/M group compared to oil-sham group, and there was significant decrease in Brd4 in Tam-gp120/M group compared to oil-gp120/M group. The results above suggest that conditional knockout of neuronal Brd4 reduces neuropathic pain.

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Poster

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

Support: NIDA Grant DA057337
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Title: Cocaine modulates antiviral and unfolded protein responses to enhance HIV infection in iPSC-microglia via sigma-1

Authors: *O. OTEJU¹, S. MATT², H. S. FOX³, P. J. GASKILL¹;

¹Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; ²Pharmacol. and Physiol., Drexel Univ., Philadelphia, PA; ³Dept. of Neurolog. Sci., Univ. of NE Med. Cente, Omaha, NE

Abstract: Cocaine (Coc) use is highly comorbid among individuals living with HIV, yet the specific pathways through which Coc impacts HIV infection are not fully understood. Our prior research showed that dopamine levels induced by stimulant use increase HIV replication in macrophages and microglia. However, it is uncertain whether Coc acts solely via dopamine or if it has other distinct effects on HIV. To assess this, we inoculated human-induced pluripotent stem cell-derived microglia (iMg) with HIV +/- Coc. We evaluated changes in the percentage of infected iMg as well as p24Gag secretion using high-content imaging and AlphaLISA. Coc increased p24 secretion and the number of infected iMg. These effects persisted even in the presence of antiretroviral therapy. The inhibition of dopamine receptors did not diminish the impact of Coc, but pharmacologic inhibition of sigma-1 receptor (S1R) did block the effect. S1R is a chaperone protein targeted by Coc, and S1R agonists independently increased p24 secretion, suggesting that Coc acts through S1R rather than dopaminergic pathways. Immunofluorescence staining showed increased S1R in p24- cells and reduced S1R in p24+ cells in HIV+Coc cultures, and showed that HIV+Coc promotes S1R movement to the nuclear envelope and endoplasmic reticulum. Single-cell RNA sequencing (scRNAseq) validated by qPCR and Western Blot revealed Coc reduces host antiviral response genes (e.g., Interferon Alpha Response) and enhances XBP1-driven unfolded protein response, suggesting Coc reduces the

host response against viral replication and enhances viral protein folding. Multiplex cytokine panel supported this, showing reduced antiviral cytokines (e.g. IL-12) and increased pro-survival cytokines (e.g. IL-4). To confirm these responses are possibly driven by the bystander cells exposed to HIV and Coc, we segregated our scRNAseq clusters into HIV^{high} vs HIV^{low} clusters and found increased S1R and XBP1 in the HIV^{low} clusters. These data suggest that the Coc-induced changes in S1R may enhance susceptibility to HIV. Future studies in mixed culture systems will assess changes in HIV infection dynamics, neuronal/glial health, and function in response Coc.

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Poster

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

Support: R01 MH087332
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R01DA063181

Title: Microglia mediate neuronal injury by HIV and Methamphetamine in association with release of pro-inflammatory cysteinyl leukotrienes

Authors: *H. SINGH¹, M. KAUL²;

¹Sch. of Med., Univ. of California, Riverside, Riverside, CA; ²Biomed. Sci., Univ. of California Riverside, Riverside, CA

Abstract: Microglia are the resident immune cells of the central nervous system (CNS) and play a crucial role in maintaining brain health. However, in the context of HIV infection glial cells, such as microglia and astrocytes release inflammatory and neurotoxic factors, which appear to cause neuronal damage and lead to neurocognitive impairment (NCI). Despite advances in combined antiretroviral therapies that have extended the lifespan of people living with HIV (PWH), the prevalence of NCI remains largely unchanged. The use of methamphetamine (METH) among PWH can exacerbate NCI, though the underlying mechanisms are not fully understood. Therefore, in this study, we focused on how METH influences HIV infection of microglia and affects neuroinflammation and neurotoxicity. Using HIV-1BaL at a multiplicity of infection (MOI) of 1, we observed that microglia derived from induced pluripotent stem cells (iPSC) produced peak levels of the HIV protein p24 around day 6, followed by a decline through day 12. Interestingly, upon exposure to 100 μ M of METH, p24 production decreased for day 6, although the overall trend remained similar. These findings suggest that METH may interfere with HIV replication. However, this apparent reduction in viral output did not translate to reduced neurotoxicity of cell-free microglial conditioned media (CM). The neurotoxicity assay

revealed that neuronal survival was significantly reduced CM of HIV infected microglia compared to non-infected controls. In fact, METH alone and in combination with HIV significantly reduced neuronal survival, indicating that viral load alone may not fully reflect viral pathogenicity. Analysis of CM from microglia infected with HIV-1BaL at MOIs ranging from 0.01 to 1.0 and METH at 1, 10 or 100 μ M indicated that the psychostimulant increases the release of pro-inflammatory cysteinyl leukotrienes (CysLT), which play a critical role in HIV neurotoxicity. Altogether, our data reveal a complex interplay between HIV infection, METH exposure, and microglial neurotoxic activation.

Disclosures: H. Singh: None. M. Kaul: None.

Poster

PSTR331: Viral Infection and Neuroinflammation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR331.16/I4

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

Support: NIH NIGMS P20AGM109091-10S3
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Title: Microglial viability and cytokine expression in the presence of antiretroviral treatment

Authors: *M. WALKER¹, H. LI¹, M. SHTUTMAN², R. M. BOOZE¹, C. F. MACTUTUS¹;
¹Dept. of Psychology, ²Drug Discovery and Biomed. Sci., Univ. of South Carolina, Columbia, SC

Abstract: Over the past four decades, medications have been developed to help people living with HIV (PLWH) manage symptoms and decrease their viral load; this treatment regimen is known as combined antiretroviral therapy (cART). However, HIV-associated neurocognitive disorders (HAND) still affect up to 50% of individuals on cART. *In vitro* studies have shown that neuroinflammation and microglial activation are two key indicators of HAND pathogenesis in PLWH on cART, though the specific biological processes involved are not well understood. Thus, the present study aims to assess how antiretrovirals interact with microglia to clarify the mechanisms of action that underlie HAND development in individuals undergoing HIV treatment. BV2 cells (immortalized murine microglia) were treated with either BIC/FTC/TAF (Biktarvy), FTC/TFV/DTG, or corresponding vehicle controls. First, a cell viability assay was used to examine how drug treatment for 6, 12, 24, and 48 hours impacted BV2 cell viability. Additionally, a multiplex immunoassay was employed to assess how drug treatment for 2, 4, 6, and 8 days influenced the expression of 32 cytokines, chemokines, and growth factors in BV2 microglial cells. Both drug models produced significant increases in cell viability compared to untreated cells and their corresponding vehicle control. Notably, FTC/TFV/DTG was the only

treatment to generate a time-dependent effect, revealing a significant positive relationship between cell viability and treatment duration. Although we cannot attribute marked elevations in protein expression in the presence of BIC/FTC/TAF to drug treatment alone, we can definitively conclude that significant increases in MIP-1 α and RANTES expression were due to FTC/TFV/DTG treatment. Further, a drug-specific linear relationship between length of treatment and protein expression was only found for IL-17 in the BIC/FTC/TAF-treated cells. These data suggest that, of the two current frontline cART regimens for treatment-naïve PLWH, BIC/FTC/TAF may contribute less to the downstream development of HAND. Additional research is currently underway to assess how well BIC/FTC/TAF can suppress HIV *in vitro*.

Disclosures: M. Walker: None. H. Li: None. M. Shtutman: None. R.M. Booze: None. C.F. Mactutus: None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.01/I5

Topic: D.03. Stroke

Support: NINDS R01NS058784 (GKS)

Title: Neural stem cell transplantation modulates immune response in ischemic mouse brain

Authors: *V. GUPTA, X. LIANG, M. KIM, L. DRUSCHEL, T. BLISS, G. K. STEINBERG; Neurosurg., Stanford Univ., Stanford, CA

Abstract: Intracerebral transplantation of neural stem cells to facilitate stroke recovery is showing promise in ongoing clinical trials. Parallel pre-clinical investigations of associated molecular mechanisms of action are a center of focus, as understanding how the stem cells modulate the brain will help improve the outcomes of this therapeutic intervention. Here, we investigate the molecular mechanism of action using mRNAseq. Adult male C57BL/6 mice were subjected to a 35-minute transient middle cerebral artery occlusion model of stroke, followed one week later by transplantation of human embryonic-derived neural stem cells (NR1), or vehicle, into the ipsilesional hemisphere. Functional recovery was assessed by rotating beam and pellet reach tests. One cohort of mice (n=5 each group) were sacrificed 7 days after transplantation and brains harvested. Brains were dissected to separate the ipsilesional anterior (transplanted) and posterior (non-transplanted) cerebral regions, along with contralesional hemisphere and cerebellum, and transcriptomic analysis performed using bulk mRNA seq. Genes differentially expressed between stem cell- and vehicle-treated animals were determined using DESeq2 analysis. Functional enrichment analyses were performed using Gene Ontology, KEGG pathways and Gene Set Enrichment Analyses (GSEA). All brain regions investigated exhibited differentially expressed genes (DEGs) between the stem cell and vehicle-treated mice, indicating that the transplanted stem cells had a widespread effect. Using a threshold of

log2FoldChange>1 and $p<0.05$, 380, 262 and 245 DEGs were found in the non-transplanted ipsilesional, contralesional and cerebellum regions respectively. As expected, the largest effect of the NR1 cells was near the transplant site which showed 839 differentially expressed genes. GSEA based GO enrichment analyses of the DEGs in the transplanted ipsilesional anterior brain region showed a significant downregulation of several immune mechanisms including macrophage migration and chemotaxis, microglial activation, cytokine receptor interaction and neuroinflammation in the NR1 treated group vs vehicle groups. GSEA based KEGG Pathway analysis of DEGs from this region also demonstrated decreased cytokine mediated inflammation in addition to the complement pathway. In conclusion, these data indicate that transplanted NR1 cells have local and long distant effects, with the most prominent being a potent inhibition of macrophage associated sub-acute inflammation in the transplant vicinity, which was associated with enhanced behavior recovery.

Disclosures: V. Gupta: None. X. Liang: None. M. Kim: None. L. Druschel: None. T. Bliss: None. G.K. Steinberg: None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR332.02/I6

Topic: D.03. Stroke

Support: NIH Grant R01NS120322
NIH Grant K00NS129171

Title: Neuroprotective Near-Infrared Light Therapy to Modulate Secondary Injury Following Transient Ischemic Stroke in Mice

Authors: *A. H. NETZLEY¹, F. J. TORRES TORRES¹, T. H. SANDERSON²;
¹Emergency Med., Univ. of Michigan, Ann Arbor, MI; ²Emergency Med., The Univ. of Michigan, Ann Arbor, MI

Abstract: Ischemic stroke is a major cause of long-term disability, frequently leading to persistent impairments in motor function and cognition. Although reperfusion is necessary to restore oxygen and nutrient supply to affected brain tissue, it can paradoxically exacerbate injury through mechanisms involving oxidative stress, excitotoxicity, and a robust neuroinflammatory response. Microglia, the brain's resident immune cells, are central mediators of post-stroke inflammation, and the intensity and duration of their reactivity can significantly influence outcomes. To investigate potential therapeutic strategies targeting these processes, we are using a mouse model of transient middle cerebral artery occlusion (MCAO), a well-established method for simulating ischemia/reperfusion (I/R) injury. Adult mice undergo 60 minutes of intraluminal MCAO followed by reperfusion, and a subset receive transcranial near-infrared (NIR) light therapy during the acute recovery period. NIR wavelengths 750nm and 950nm have been shown

to modulate mitochondrial activity and reduce the production of reactive oxygen species, with emerging evidence suggesting a role in dampening microglial reactivity. Neuroseverity scoring and behavioral testing is used to evaluate motor and functional deficits. At various time points post-injury, brain tissue is collected for histological analysis of microglial morphology and neuronal integrity, with a particular focus on the peri-infarct cortex and hippocampus. This study aims to define the therapeutic potential of NIR light therapy in attenuating neuroinflammation and promoting recovery after stroke. Findings may inform the development of novel, non-invasive treatments for stroke patients.

Disclosures: A.H. Netzley: None. F.J. Torres Torres: None. T.H. Sanderson: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mitovation.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: PSTR332.03/I7

Topic: D.03. Stroke

Support: NIH Grant R01NS112940
NIH Grant R01NS079691
NIH Grant F31NS135993

Title: A biomaterial strategy promotes glia-associated axonal sprouting into the fibrotic stroke core

Authors: *E. M. RATHBUN¹, S. DODDIPALLI¹, K. TAKESHIMA¹, D. BAHADIR¹, K. ERNING², N. V. PHAN², T. SEGURA², S. CARMICHAEL¹;
¹Neurol., UCLA Sch. Med., Los Angeles, CA; ²Biomed. Engin., Duke Univ., Durham, NC

Abstract: In the U.S., ischemic stroke accounts for 87 percent of stroke cases and is a leading cause of long-term disability in adults, especially if patients receive delayed care. After stroke, an infarct core forms and remains devoid of neurons and axons. Axonal sprouting, the formation of new connections in the nervous system, and angiogenesis, the formation of new blood vessels, are cellular processes indicative of neural repair in both human patients and mouse models of stroke. However, axonal sprouting occurs only in the surrounding region of the healthy peri-infarct, as axons are unable to penetrate through the barrier at the interface of the infarct. However, this void of axons in the infarct can be overcome with the injection of biomaterials into the fibrotic core during the subacute stage of stroke, allowing for discovery of cellular interactions between new blood vessels, axons, and glial cells. To examine cellular responses to a potential therapeutic biomaterial, we induced a stroke in the young male mouse primary motor cortex with photothrombosis and later injected a microporous annealed particles (MAP) hydrogel scaffold with nanoparticle-immobilized vascular endothelial growth factor (VEGF) into the

stroke site, yielding the identification of cell types and processes within the hydrogel that are indicative of neural repair. Primarily, axons sprout into the hydrogel and the remaining infarct tissue over time from the peri-infarct cortex and the corpus callosum. To label the cell bodies and axons of stroke-projecting and hydrogel-projecting neurons, we injected adeno associated viruses with a retrograde serotype (AAVrg) before the stroke and at a chronic timepoint. In combination with *in situ* hybridization and immunohistochemistry, this identified and localized neuronal subtypes that have the potential to make connections within the hydrogel. Finally, we have characterized the association of axons with blood vessels and astrocytes within the MAP scaffold both with and without VEGF, revealing roles of porous hydrogel delivery and VEGF signaling that create a unique cellular niche involved in the formation of new connections after stroke.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.04/I8

Topic: D.03. Stroke

Title: Neural xenografts contribute to long-term recovery in stroke via molecular graft-host crosstalk

Authors: *R. RUST¹, R. WEBER⁴, K. KISLER², M. ZHANG³, C. TACKENBERG⁵;
²Dept. of Physiol. and Neuroscience, Zilkha Neurogenetic Inst., ³Dept. of Physiol. and Neurosci., ¹USC, Los Angeles, CA; ⁴Inst. for Regenerative Med., Inst. for Regenerative Medicine, Univ. of Zurich, Switzerland, Zurich, Switzerland; ⁵Inst. for Regenerative Med., Univ. of Zurich, Schlieren, Switzerland

Abstract: Stroke is a leading cause of disability and death due to the brain's limited ability to regenerate damaged neural circuits. To date, stroke patients have only few therapeutic options and are often left with considerable disabilities. Induced pluripotent stem cell (iPSC)-based therapies are emerging as a promising therapeutic approach for stroke recovery. In this study, we demonstrate that local transplantation of iPSC-derived neural progenitor cells (NPCs) improves long-term brain tissue repair responses and reduces neurological deficits after cerebral ischemia in mice. Using *in vivo* bioluminescence imaging and *post-mortem* histology, we show long-term graft survival over the course of five weeks and preferential graft differentiation into mature neurons without signs of pluripotent residuals. Transplantation of NPCs led to a set of brain tissue repair responses including increased vascular sprouting and repair, improved blood-brain barrier integrity, reduced microglial activation, and increased neurogenesis compared to

littermate control animals receiving sham transplantation. Employing deep learning-assisted behavior analysis, we found that NPC-treated mice displayed improved gait performance and complete fine-motor recovery in the horizontal ladder rung walk, five weeks post-injury. To dissect the molecular graft composition and identify graft-host interactions, single nucleus profiling of the cell transplants and host stroke tissue was performed. We identified graft differentiation preferentially towards neurons with GABAergic and glutamatergic phenotypes in similar proportions, with the remaining cells acquiring astrocyte and NPC-like phenotypes. Interaction between graft and host transcriptome indicated that GABAergic cell grafts were primarily involved in graft-host communication through the regeneration-associated neurexin (NRXN), neuregulin (NRG), neural cell adhesion molecule (NCAM) and SLIT signalling pathways. In conclusion, our study reveals that transplanted iPSC-derived NPCs primarily differentiate into GABAergic neurons contributing to long-term recovery and further delineates the regenerative interactions between the graft and the stroke-injured host tissue.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.05/I9

Topic: D.03. Stroke

Support: R01G082207

Title: Transcranial Near-infrared II Photobiomodulation Promotes Functional Recovery Following Intracerebral Hemorrhage

Authors: ***Y. ZHANG**¹, **Z. HUANG**², **J. K. KOTICHA**^{3,1}, **P. ZOU**⁴, **J. ZHANG**⁵, **X. ZONG**⁶, **Q.-G. ZHANG**⁷;

¹LSUHSC-Shreveport, Shreveport, LA; ²Louisiana State Univ. Hlth. Sci. Center-Shreveport, Shreveport, LA; ³Caddo Parish Magnet High Sch., Shreveport, LA; ⁴LSUHSC-shreveport, SHREVEPORT, LA; ⁵LOUISIANA STATE UNIVERSITY HEALTH SCIENCES CENTER AT SHREVEPORT, shreveport, LA; ⁶Louisiana State Univ. Hlth. Sci. Ctr. Neurol. Dept., Shreveport, LA; ⁷Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA

Abstract: Background: Intracerebral hemorrhage (ICH) is a devastating condition, yet effective treatments to improve neurological outcomes remain limited. Photobiomodulation (PBM) is a non-invasive therapeutic approach that involves exposing cells or tissues to low levels of near-infrared light to modulate biological activity at the cellular or tissue level. There is growing interest in exploring the therapeutic potential of PBM in neurological disorders. Transcranial near-infrared II PBM, in particular, has demonstrated improved light penetration into subcortical regions with reduced tissue scattering. In this study, we sought to investigate whether

transcranial near-infrared II PBM can confer beneficial effects in ICH. **Methods:** Type IV collagenase was stereotactically injected into the striatum of mice to induce ICH. A 1064-nm laser at an irradiance of 50 mW/cm² (measured at the cortical level) or sham treatment was applied for 5 minutes, starting 24 hours after the collagenase injection, and continued daily for 14 days. The horizontal ladder test, adhesive removal test, and hanging wire test were performed at baseline and at various time points following the induction of ICH to assess motor coordination, sensorimotor function, grip strength, and overall coordination. **Results:** A 5-minute 1064-nm laser treatment did not significantly increase local scalp temperature. Furthermore, mice treated with the 1064-nm laser exhibited accelerated functional recovery following ICH, without any effect on survival rate. **Conclusions:** Our findings suggest that near-infrared II PBM, when applied after bleeding has stabilized, can improve functional recovery following ICH. Thus, PBM may represent a non-invasive, non-pharmacological treatment for enhancing neurological recovery after ICH.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Topic: D.03. Stroke

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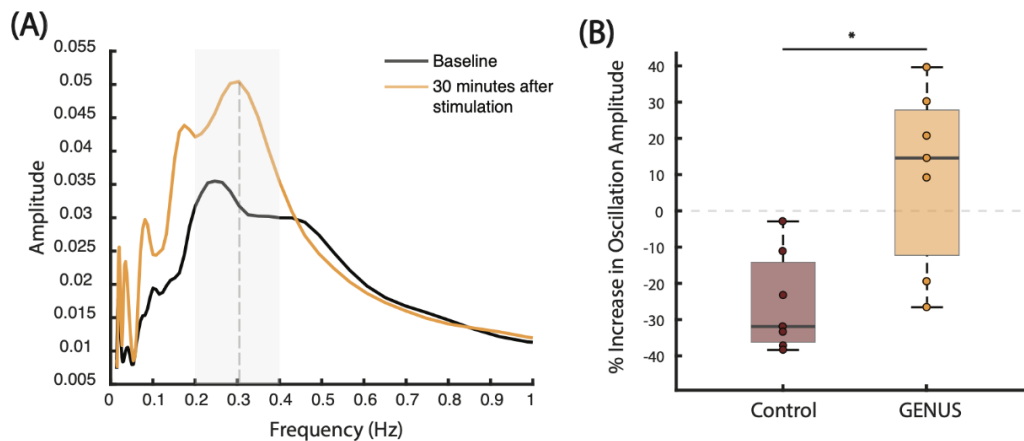
Title: Beneficial effects of 40 Hz visual stimulation on post-ischemic recovery in mice

Authors: *P. R. BRESSAN, R. P. TANG, B. RAUSCHER, J. JIANG, E. LONG, A. DEVOR, D. A. BOAS;

Biomed. Engin., Neurophotonics Center, Boston Univ., Boston, MA

Abstract: Stroke is among the major causes of death and disability worldwide, yet few therapeutic options are available to support recovery, particularly during the subacute phase. Vascular oscillations, rhythmic fluctuations in cerebral blood flow, have been implicated in mediating post-ischemic recovery, with recent studies linking increased low frequency vascular oscillations to better long-term functional and behavioral outcomes following ischemic stroke. Gamma Entrainment Using Sensory Stimulation (GENUS) has been shown to have neuroprotective effects on neurological conditions, especially Alzheimer's disease, which has been attributed to the capacity of GENUS to enhance neurovascular oscillations across different frequency bands. Our research investigates the effects of GENUS on low frequency vascular oscillations in both healthy and post-stroke mice. Using laser speckle contrast imaging, we measured cerebral blood flow and vascular oscillatory dynamics in awake mice exposed to either 40 Hz visual stimulation or constant light. In healthy animals, GENUS significantly increased

low frequency oscillations amplitude compared to control stimulation. In a preliminary photothrombotic stroke model, the effects of GENUS on low frequency oscillations were disrupted 24 hours after ischemia, but were retained at the 48-hour timepoint. These findings provide important evidence that 40 Hz visual stimulation can enhance low frequency vascular oscillations, supporting further investigation into GENUS as a potential non-invasive therapeutic approach for stroke recovery.



Effects of GENUS on Vascular Oscillations. (A) Power spectrum of vascular oscillations from a single representative GENUS trial, shown across timepoints. The low-frequency oscillation band (0.2–0.4 Hz) is highlighted by the shaded region. **(B)** Box-and-whisker plot showing the percent increase in the amplitude of low-frequency vascular oscillations (LFOs) from Baseline to 30 minutes after stimulation. GENUS led to a significant increase in oscillation amplitude compared to control trials ($n = 7$ datasets per group, $p = 0.0111$, Wilcoxon rank-sum test).

Disclosures: P.R. Bressan: None. R.P. Tang: None. B. Rauscher: None. J. Jiang: None. E. Long: None. A. Devor: None. D.A. Boas: None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Topic: D.03. Stroke

Support: NIH grant R01NS058784
CIRM grant CLIN2-12379 (GKS)

Title: Integrative TRAP-Seq and Secretome Profiling Reveal CRISPR Targets for Enhancing Stem Cell Therapy in Chronic Stroke

Authors: *N. JOHNSTON¹, R. AZEVEDO-PEREIRA², J. VU³, X. LIANG⁴, P. HABIB¹, T. BLISS⁵, G. K. STEINBERG²;

¹Neurosurg., ²Stanford Univ., Stanford, CA; ³Neurosurg., Stanford Univ., East Palo Alto, CA;

⁴Dept. Neurol, ⁵Dept of Neurosurg, Stanford Univ., Stanford, CA

Abstract: Stem cell transplantation is showing promise as a much needed therapy to promote recovery in the sub-acute and chronic phases of stroke. The molecular mechanisms underlying stem cell action remain poorly understood, but elucidating these mechanisms is crucial to enhance stem cell efficacy. In this study, we integrate Translating Ribosome Affinity Purification followed by RNA sequencing (TRAP-seq) along with secretome profiling of cell media to identify potential stem cell-secreted factors that could act as molecular targets for improving stem cell-mediated stroke recovery. Our clinically tested human neural stem cells (NR1), modified to express a ribosomal tag, were transplanted into the cortex of adult male Sprague Dawley rats (n=5), one week following stroke induced by distal middle cerebral artery occlusion. Two days following transplantation, brains were harvested and the cortex in the vicinity of the graft site excised. TRAP-seq was performed on the excised tissue (i.e. Translating Ribosome Affinity Purification, followed by RNAseq) to identify genes expressed by the NR1 graft in the stroke-injured brain. This identified 175 NR1 genes encoding for secreted factors. In parallel, to characterize the NR1 secretome in vitro at the protein level, we conducted a 63-plex Luminex Assay on NR1-conditioned cell media. Integrated analysis of these two data sets revealed a subset of stem cell-secreted proteins converging on pathways involved in synaptic plasticity, axonal remodeling, and neuroinflammation: processes known to be involved in stroke brain recovery. From this dataset, we prioritized four novel gene candidates for functional screening using in vitro CRISPR-based knockout strategies. Overall, our study identifies key stem cell-secreted factors and takes the first step towards elucidating their potential roles in enhancing stroke recovery. This lays the foundation for rational enhancement of stem cell therapies via targeted gene manipulation.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR332.08/I12

Topic: D.03. Stroke

Support: Fondation pour la Recherche sur les AVC 2023
Union des Blessés de la Face et de la Tête 2024

Title: Vagus nerve stimulation reverses cortical activity impairment during the recovery phase of photothrombotic stroke

Authors: J. LECLERC, T. BRIAR, C. DEROUCK, C. DUCLOS, E. VERIN, J.-P. MARIE, J. CHUQUET;

Univ. Rouen Normandie, Normandie Univ, GRHVN UR3830, ROUEN, France

Abstract: Stroke is the leading cause of acquired neurological disability. With no effective standardized treatments to repair damaged brain networks, the search for new neuroreparative strategies remains a major challenge. An increasingly popular solution in preclinical and clinical studies is vagus nerve stimulation (VNS). Notably, the delivery of pain-free electrical impulses via this easily accessible cranial nerve can enhance patients' functional recovery, without generating any major side effects. Despite an increased number of studies suggesting VNS could mobilize a reserve of cerebral plasticity, the precise mechanisms underlying its effects on functional recovery remain unknown. We recently discovered that the perilesional cortex, undergoing repair, suffers from a significant chronic deficit in gamma oscillations, which are thought to facilitate neuronal plasticity. Furthermore, the strength of these oscillations correlates with better recovery outcomes during motor relearning. In this work, we tested the hypothesis that VNS, parameterized similarly to those in studies demonstrating its post-stroke benefits, normalizes excitability of the cortex under repair. Thus, we performed in vivo extracellular recordings of the perilesional and contralateral cortices in adult male and female C57BL/6J mice seven days after photothrombotic stroke centered on the caudal forelimb area and applied VNS for 30 minutes, delivering 0.5-second bursts at 30 Hz (200 μ s, 0.8 mA pulses) every 10 seconds. Our results show that this VNS protocol, compared to sham-VNS, favorably modulates post-stroke cortical activity, reversing the oscillatory deficit and increasing the firing rate in the perilesional cortex. These findings provide insights into the mechanisms underlying VNS-driven recovery and suggest that targeting cortical excitability could optimize post-stroke rehabilitation. Further investigations will help refine VNS protocols, paving the way for more effective neurorehabilitation strategies.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.09/J1

Topic: D.03. Stroke

Title: Multimodal platform combining VR and TENS for post-acute stroke rehabilitation - an RCT proposal

Authors: *A. SPARAPANI¹, B. FASCHING², N. PAPARO¹, G. AURUCCI³, A. CIMOLATO¹, S. RASPOPOVIC¹;

¹Ctr. for Med. Physics and Biomed. Engin., ²Department of Neurol., Med. Univ. of Vienna,

Vienna, Austria; ³D-HEST, ETH Zurich: Eidgenössische Technische Hochschule Zurich, ZURICH, Switzerland

Abstract: Stroke is a disabling medical condition that affects 15 million individuals per year, leaving 70% of survivors without functional independence as a consequence, placing a growing strain on healthcare systems already short on trained physiotherapists. The condition results in upper-limb impairments, involving motor and sensory deficits, alongside distortions in body representation. The latter expands from misjudging upper-limb dimensions to a diminished sense of ownership of the affected limb, and alterations in planning and executions of movement. Yet, conventional rehabilitation approaches tend to prioritize motor recovery, often neglecting sensory and body representation deficits. Standard protocols also lack objective, integrated assessments across these domains. In contrast, emerging digital rehabilitation tools enable more personalized, minimally supervised and multimodal interventions, offering a comprehensive approach in stroke rehabilitation.

In this context, we developed a novel multimodal platform integrating fully immersive Virtual Reality (VR) with Transcutaneous Electrical Nerve Stimulation (TENS) to deliver task-oriented upper limb rehabilitation. The system simultaneously captures objective kinematic data and body representation metrics to assess patient recovery. Here, we present preliminary results from the first participants of an ongoing randomized clinical trial with sub-acute stroke survivors. The patients completed 12 sessions of one hour over a two-week period (5 sessions/week) using our platform. Each session consisted of personalized rehabilitation tasks tailored to the individual disability and engagement level.

Clinical assessments revealed meaningful functional improvements in the Action Research Arm Test (ARAT) and in sensory-motor impairments (Fugl-Meyer Assessment Upper Extremity). Objective kinematic measurements captured by our platform during sessions allowed to assess improvements in velocity and smoothness of movement throughout the rehabilitation.

Furthermore, patients improved the number of repetitions and movements. Alongside motor improvements, participants showed reduced upper-limb perceptual distortion by the end of the intervention, suggesting positive effects on body representation in conjunction with motor recovery.

These initial results support the feasibility and potential clinical utility of a multimodal VR-TENS paradigm for early-phase post-stroke rehabilitation and pave the way for a new generation of digital, low-cost and minimal supervision technologies toward patient rehab and assessment.

Disclosures: **A. Sparapani:** A. Employment/Salary (full or part-time): Neuroengineering Lab, Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria. **B. Fasching:** A. Employment/Salary (full or part-time): Neurology Department, Medical University of Vienna. **N. Paparo:** None. **G. Aurucci:** A. Employment/Salary (full or part-time): Laboratory for Neuroengineering, Department of Health Sciences and Technology, Institute for Robotics and Intelligent Systems, ETH Zürich, Zürich, Switzerland. **A. Cimolatto:** A. Employment/Salary (full or part-time): Neuroengineering Lab, Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria. **S. Raspopovic:** A. Employment/Salary (full or part-time): Neuroengineering Lab, Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.10/J2

Topic: D.03. Stroke

Title: A VR rehabilitation system for supporting functional recovery in hemiplegia patients

Authors: *T. OKAMURA, H. YOSHIDA, T. KOHAMA;

Grad. Sch. of Biology-Oriented Sci. and Technol., Kindai Univ., Kinokawa/Wakayama, Japan

Abstract: Hemiplegia is a condition characterized by paralysis on one side of the body, primarily resulting from stroke or brain injury, and significantly affects patients' quality of daily life. Mirror therapy has attracted attention as an effective rehabilitation method for patients with hemiplegia. This therapy aims to enhance motor function by stimulating neural plasticity in the brain through the mirror imitation of movements on the non-paralyzed side of the body. Recently, efforts have been made to enhance the effectiveness of rehabilitation by incorporating Virtual Reality (VR) technology, which offers a greater level of immersion than traditional mirror-based methods. Despite the promise of VR-based methods, they face challenges, such as requiring sufficient space and struggling to capture subtle finger movements accurately. Furthermore, few studies have objectively evaluated the effectiveness of rehabilitation in VR spaces by measuring brain activity. In this study, we aim to develop a more effective VR mirror therapy system by eliminating the limitations of conventional mirror-based methods. The system proposed in this study acquires whole-body joint information using a posture estimation model derived from monocular camera images, and integrates it with finger joint information obtained from the front camera of the head-mounted display. The patient's movements and the avatar in the VR space are synchronized in real-time. Additionally, the proposed system was evaluated based on brain activity measurements obtained using functional near-infrared spectroscopy (fNIRS). A one-handed tapping task was performed as a preliminary test of the proposed system. The effect of VR mirror therapy was examined by comparing brain activity during mirror therapy with that during normal tapping conditions. Standard preprocessing techniques were employed on the measured fNIRS signals, and the statistical significance for each channel was assessed using the Wilcoxon signed-rank test. The results showed no significant differences in the activity of the ipsilateral motor cortex, where the effects of mirror therapy are typically expected. These findings indicate that the existing system may not yet achieve the desired level of effectiveness. The potential causes of this may be insufficient activation of the contralateral motor cortex and the position of the motor area associated with tapping outside the fNIRS measurement channel. In the future, we plan to improve the system to enhance immersion and reality, review the probe position, and optimize the task design to stimulate the motor cortex effectively.

Disclosures: T. Okamura: None. H. Yoshida: None. T. Kohama: None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.11/J3

Topic: D.03. Stroke

Support: George W. Housner Student Discovery Fund

Title: Development of an adaptive acupressure method for facial paralysis prevention and recovery

Authors: *K. WAN;
Caltech, Pasadena, CA

Abstract: Facial paralysis is a prevalent condition, affecting around 45%-60% of stroke survivors. It involves partial or complete loss of voluntary muscle control on one side of the face and can arise from various causes, including Bell's palsy, viral infections, stroke, and trauma. This condition often results in facial asymmetry and complications. Clinical observations suggest that facial acupressure can enhance muscle activity and support the recovery of facial paralysis. However, there is limited research on the underlying mechanisms of acupoint massage, its role in prevention and rehabilitation of facial paralysis, and how massage techniques might be adapted to different levels of the condition. This study aims to address these issues by developing a personalized, adaptive acupoint massage strategy tailored to various levels of facial paralysis, with the goal of improving both prevention and rehabilitation outcomes. We employed image recognition technology on a dataset of 360 facial paralysis images to identify and localize six primary facial acupoints: Yangbai, Zanzhu, Sibai, Yingxiang, Jiache, and Dicang. Based on this analysis, we designed and fabricated a novel soft robotic device capable of delivering variable pressure to both acupoint and non-acupoint regions across the face. The device was also equipped to detect and measure electromyography (EMG) signals, capturing facial muscle activity under different pressure levels and stimulation sites. In total, we collected 1040 datasets, each comprising synchronized pressure and EMG signal recordings. Analysis showed a nonlinear relationship between applied pressure at targeted acupoint location and EMG response: a 60% increase in pressure yielded a 30% increase in EMG signal amplitude. Furthermore, when equal pressure was applied to an acupoint and its symmetrically positioned non-acupoint counterpart, the acupoint stimulation exhibited a 39% higher EMG signal, indicating enhanced muscle activation through targeted acupressure. By comparing the locations of acupoints on both paralyzed and healthy sides of the face, we developed an adaptive massage strategy capable of adjusting both location of acupoint stimulation and magnitude of applied pressure according to various levels of facial paralysis. Our approach deepens the understanding of the mechanisms of facial acupressure and establishes an effective, personalized rehabilitation strategy for the early prevention and recovery of facial paralysis. In addition to offering clinical potential for treating facial paralysis, this method can also reduce the risk of long-term complications and sequelae associated with the condition.

Disclosures: K. Wan: None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.12/J4

Topic: D.03. Stroke

Title: The Effects of Epidural Electrical Stimulation in Upper Limb Function in Participants with Stroke

Authors: *C. WU^{1,2}, S.-T. TSAI⁴, Y.-C. CHEN³;

¹Tzu Chi Univ., Hualien, Taiwan; ³Neurosurg., ²Hualien Tzu Chi Hospital, Buddhist Tzu Chi Med. Fdn., Hualien, Taiwan; ⁴Neurosurg., Hualien Tzu Chi Hosp., Hualien, Taiwan

Abstract: **Motivation** Upper limb motor impairment is a major contributor to long-term disability and reduced quality of life following stroke. Conventional rehabilitation strategies often provide limited functional recovery, particularly in individuals with severe motor deficits. Epidural electrical stimulation (EES) of the cervical spinal cord has recently emerged as a promising neuromodulatory intervention capable of re-engaging spared neural circuits and facilitating motor recovery. This study aims to evaluate the therapeutic potential of temporary cervical EES in improving upper limb motor function in individuals with chronic stroke.

Methods Four individuals with chronic post-stroke hemiparesis underwent surgical implantation of a temporary epidural electrode array spanning cervical spinal segments C3 to T1. Stimulation parameters were individualized based on intraoperative testing and tailored to each participant. Motor function was assessed pre- and post-intervention using standardized clinical outcome measures, including the Fugl-Meyer Assessment for Upper Extremity (FMA-UE), the Modified Ashworth Scale (MAS), and the Action Research Arm Test (ARAT). The stimulation period lasted three weeks, during which participants continued conventional therapy. **Results** The mean age of the participants was 40 ± 7.98 years. After three weeks of cervical EES, all participants exhibited voluntary upper limb movement improvements. Quantitatively, the mean FMA-UE score increased by 3.5 points, and the mean ARAT score increased by 1.75. Muscle tone, as measured by the MAS, also showed favorable changes. No adverse events related to surgery or stimulation were observed. These findings suggest that EES may facilitate neuroplasticity and contribute to functional gains in upper limb control in the chronic phase of stroke recovery.

Conclusion Cervical EES represents a clinically feasible and well-tolerated neuromodulatory intervention for enhancing upper limb motor function in individuals with chronic stroke. Improvements observed over a short intervention period highlight its potential as an adjunctive therapy to conventional rehabilitation. Further studies with larger cohorts and longer follow-ups are warranted to validate these preliminary findings and optimize stimulation protocols. EES may offer a novel avenue for improving motor outcomes and overall quality of life in stroke survivors.

Disclosures: C. Wu: None. S. Tsai: None. Y. Chen: None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR332.13/J5

Topic: D.03. Stroke

Support: Italian Ministry of Health (RF-2021-12373484; CUP: C53C22001570001)
Ricerca Corrente 2025 from Fondazione Policlinico Universitario “A. Gemelli” IRCCS

Title: Long-term effects of transcranial direct current stimulation on motor recovery and brain tissue repair in a photothrombotic stroke mouse model

Authors: *F. D'ALELIO¹, C. FEROLETO¹, C. D'AMELIO², I. PAOLETTI¹, C. CALIGIURI², M. MOROTTI¹, C. CODAZZI², L. LEONE^{2,1}, C. GRASSI^{2,1}, M. V. PODDA^{2,1};

¹Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; ²Univ. Cattolica del Sacro Cuore, Rome, Italy

Abstract: Ischemic stroke represents one of the leading causes of long-term neurological disability worldwide, often resulting in persistent motor deficits. Following ischemic stroke, the abrupt loss of cerebral perfusion triggers a cascade of cellular responses—including neuronal apoptosis, sustained microglial activation and astrocytic glial scar formation—that drive chronic tissue damage and impair long-term recovery. These maladaptive processes interfere with the brain's endogenous repair and plasticity mechanisms. Transcranial direct current stimulation (tDCS) has emerged as a non-invasive neuromodulatory technique capable of inducing changes in cortical excitability and synaptic plasticity, potentially supporting functional recovery after brain injury (PMID:35291824). In this study, we investigated whether bi-hemispheric tDCS could induce long-term functional improvements and promote tissue repair in a mouse model of photothrombotic stroke of the caudal forelimb area of the primary motor cortex. Male C57BL/6J mice were randomized into two groups: the tDCS group received anodal stimulation (250 μ A) over the ipsilesional motor cortex and cathodal stimulation over the contralesional cortex once daily for three consecutive days, starting 72 hours post-stroke. The control group received sham stimulation following the same procedure but without current delivery. Motor recovery was assessed at baseline, 72 hours post-stroke (prior to tDCS), and 56 days post stroke, to evaluate long-term outcomes by using a battery of motor tests evaluating different parameters, such as forelimb strength (Grip Strength Test), paw preference (Cylinder Test), and coordination (Grid Walking Test). Behavioral results revealed significant and sustained motor function improvements in the tDCS-treated group compared to sham group, with motor gain persisting over the long term. Histological analyses revealed a significant reduction of the infarct volume in tDCS mice compared to sham mice at the end of follow-up, accompanied by increased neuronal survival, as indicated by higher NeuN expression levels in the peri-infarct area of tDCS mice. Additionally, tDCS reduced microglial activation, as revealed by decreased expression of the microglial marker Iba1. In conclusion, our findings suggest that tDCS promotes sustained motor recovery and influences post-stroke brain tissue repair, highlighting its potential as a non-invasive rehabilitative strategy for ischemic injury.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR332.14/J6

Topic: D.03. Stroke

Support: JSPS KAKENHI Young Scientist Grant (25K20838)
JSPS KAKENHI Grant-in-Aid for Research Activity Start-up (23K19888)

Title: Effects of rTMS on cortical evoked potentials and motor-related dynamics: a TMS-ECoG study in primates

Authors: *M. MITSUHASHI, R. YAMAGUCHI, L. ZHANG, T. ISA;
Dept. of Neuroscience, Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan

Abstract: Repetitive transcranial magnetic stimulation (rTMS) is increasingly used as a non-invasive treatment for a range of neurological and neuropsychiatric disorders. However, the underlying physiological mechanisms remain incompletely understood. While studies using TMS combined with scalp electroencephalography (TMS-EEG) have been widely employed to assess the effects of rTMS, their interpretation is limited by muscle artifacts, low spatial resolution, and challenges in assessing high-frequency cortical dynamics. To overcome these limitations, we used chronic electrocorticography (ECoG) in a non-human primate model to investigate the physiological effects of rTMS with high temporal and spatial resolution. Subdural ECoG electrodes (28 channels per hemisphere) were chronically implanted over the bilateral upper-limb regions of the primary sensorimotor cortex, including the premotor area in a macaque monkey. High-frequency rTMS (10 Hz, 1200 pulses over 20 minutes) was applied to the hand area of either left or right primary motor cortex (M1), or sham stimulation (click sound without magnetic pulses) was delivered as control. To evaluate the effects of rTMS, we recorded ECoG signals during a reach-and-grasp task performed with the right forelimb, before and after each session of rTMS. In addition, single-pulse TMS was delivered to the left M1 hand area before and after rTMS to assess its effect on cortical evoked potentials (CEPs) recorded using ECoG. Note that single-pulse TMS was always applied to the left M1, regardless of the side of rTMS stimulation. CEPs recorded from the left M1 hand area, which was the center of the single-pulse TMS site, typically exhibited an early positive peak (~5 ms), followed by a negative peak at 10~15 ms, a subsequent positive peak at 20~30 ms, and additional late components. Notably, the amplitude of the negative peak at 10~15 ms significantly increased after rTMS to the left M1. This effect was not observed following sham stimulation or after rTMS to the right M1. Analysis of task-related ECoG signals revealed enhanced event-related desynchronization (ERD) in the beta band during movement after rTMS to the left M1. In contrast, movement-related high-

gamma band activity remained unchanged across conditions. These results suggest that high-frequency rTMS enhances local synaptic transmission and shifts the cortical network toward a more depolarized state, which may facilitate release from inhibitory control during movement, as reflected in enhanced ERD. Our results will contribute to a better understanding of the cortical mechanisms underlying rTMS and may support the development of more targeted therapeutic applications.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: PSTR332.15/J7

Topic: D.03. Stroke

Title: Neurorehabilitation after ischaemic stroke: A synthesis of motor training and adjuvant therapies for forelimb recovery in preclinical models

Authors: *M. J. SMITH¹, J.-L. ZHAO³, A. ANTONIC-BAKER¹, L. J. CHRISTIE⁵, A. N. CLARKSON², D. R. CORBETT⁶, N. DANCAUSE⁴, K. S. HAYWARD⁷, M. WINGFIELD⁸, N. LANNIN¹;

¹Dept. of Neurosci., ²Monash Univ., Melbourne, Australia; ⁴Dept. of Neurosciences, ³Univ. de Montréal, Montreal, QC, Canada; ⁵Allied Hlth. Res. Unit, St Vincent's Hlth. Network Sydney, Sydney, Australia; ⁶Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; ⁷Departments of Physiotherapy and Med., ⁸Departments of Physiotherapy, Univ. of Melbourne, Melbourne, Australia

Abstract: Background/aims: Stroke frequently leads to upper limb impairments. While motor training improves strength, coordination and upper limb use, recovery is often incomplete. Adjuvant treatments combined with motor training may enhance recovery by offering a permissive, neuroplastic milieu. The primary aim of this systematic review is to determine if task-specific forelimb motor training either alone or in combination with other interventions, increases performance in forelimb motor tasks or improves spontaneous recovery of the forelimb after ischaemic stroke. **Method:** Searches were conducted in PubMed, MEDLINE, Embase and Web of Science. Two authors independently conducted both the title/abstract screen and review full-text. **Results:** Sixty-one studies were included. Most were conducted in rats (83.6%) predominantly using only males (80.3%). Stroke models included endothelin-1 (49.2%), photothrombosis (37.7%), middle cerebral artery occlusion (11.5%) and electro-cauterisation (1.6%). Motor training interventions included skilled reaching (78.7%), staircase test (6.6%), robotic rehabilitation (4.9%) or other tasks involving pushing or rotation of a lever (9.8%). Adjuvants were evaluated in 42 studies (66.1%) and included pharmacotherapies, enriched environments, cortical or vagus nerve stimulation, aerobic exercise, dietary interventions, and cell therapy. We are currently conducting a meta-analysis to evaluate intervention effectiveness.

Conclusion: Clinically, there is increasing interest in combining novel pharmacological, behavioural and neuro-modulatory adjuvants with motor training to enhance recovery. While early trials have begun exploring this concept, clinical translation remains limited. This review will identify the most promising interventions and inform future clinical trial design, supporting the implementation of translational research into clinical practice.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR332.16/J8

Topic: D.03. Stroke

Support: JSPS KAKENHI Grant Number: JP24K02797, JP24K22248

Title: Neuroprotective Role of Passive Body Movement in Preserving Motor Function and Limiting Infarct Expansion in dMCAO mouse

Authors: ***R. OKURA**¹, R. KATO¹, K. HAYASHI¹, R. NAKAHARA², Y. OKADA¹, F. NIIDA¹, J. MIYATA¹, N. SAKITANI³, A. ITO¹;

¹Kyoto Univ., Kyoto, Japan; ²Rehabil. Med., Univ. of Washington, Seattle, WA; ³Biotech., Natl. Inst. of Advanced Industrial Sci. and Technol. (AIST), Takamatsu, Japan

Abstract: Walking training in rehabilitation therapy has demonstrated benefits for ischemic stroke patients, facilitating motor recovery through sensory input/output and mechanical loading. However, the effects of this walking after stroke are complex and unclear. In recent studies, treadmill training (TMT) has been shown to reduce the infarct volume and preserve neurological function, largely through improved cerebral edema. Additionally, passive mechanical vibration stimulation, as one of the elements of TMT, has been implicated in physiological brain function enhancement. Given these observations, we hypothesize that passive body movement (PBM) may yield comparable neuroprotective effects. This study aimed to elucidate the impact of PBM intervention on infarct volume and motor function in a distal middle cerebral artery occlusion (dMCAO) mouse model. We performed dMCAO on 30 seven-week-old CB-17 male mice. Excluding 3 due to intraoperative mortality, the remaining 27 mice were randomly assigned to either the PBM intervention group (n = 14) or the non-intervention control group (n = 13), with 5 mice excluded due to minimal infarct volume on images. The intervention group underwent PBM treatment for 7 consecutive days (2 Hz, 5 mm amplitude, 30 min/day). Cerebral infarct volume was quantified using 2,3,5-Triphenyl tetrazolium chloride (TTC) staining at 8 days post-

infarction, revealing a significantly smaller infarct volume in the PBM intervention group compared to controls ($16.7 \text{ mm}^3 \pm 3.03$ vs. $26.5 \text{ mm}^3 \pm 3.03$, respectively; $p = 0.03$). Motor function assessments, including the cylinder test and Parallel Bar Test (PBT), were conducted at five time points: pre-infarction (day -1) and post-infarction days 1, 3, 5, and 7. Split-plot analysis of the right-left (R/L) preference score in the cylinder test showed a significant effect of evaluation day ($p < 0.01$) but not of intervention or interaction. PBT slip rate analysis demonstrated significant differences between groups regarding intervention ($p = 0.03$) and evaluation day ($p < 0.01$), though interaction effects were non-significant. Additionally, infarct volume did not correlate with either slip rate or R/L preference score ($r = 0.15$, $p = 0.62$; $r = 0.39$, $p = 0.07$, respectively). These results suggest that PBM, administered during the acute phase of cerebral infarction, suppresses infarct volume expansion, contributing to improved motor function. PBM's neuroprotective effects could involve edema suppression, as previously observed in studies on TMT. Further investigation is warranted to elucidate the underlying mechanisms of these effects.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.17/J9

Topic: D.03. Stroke

Title: Timing matters: how daily rhythms affect neuroprotection therapy for stroke

Authors: *E. LICASTRO^{1,2}, V. VISCARDI², O. CUOMO², R. PATIL^{1,2}, K. HAYAKAWA^{1,3}, E. H. LO^{1,3}, G. PIGNATARO^{2,3}, E. ESPOSITO^{1,3};

¹Neuroprotection Res. Laboratories, Departments of Radiology and Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; ²Dept. of Neurosci. - Sch. of Med., Univ. of Naples "Federico II", Napoli, Italy; ³Radcliffe Dept. of Medicine, Univ. of Oxford, Consortium Intl. pour la Recherche Circadienne sur l'AVC (CIRCA), Oxford, United Kingdom

Abstract: Despite strong preclinical evidence supporting neuroprotective strategies, clinical translation in stroke therapy has largely failed. Remote limb ischemic postconditioning (RLIP), a non-invasive intervention involving brief occlusion of a limb artery after cerebral ischemia, has shown promise in animal models but limited efficacy in clinical trials. One possible reason is the "circadian mismatch" between rodents and humans: preclinical studies are typically conducted in nocturnal rodents during their inactive (light) phase, whereas human strokes occur during the active (morning) phase. To test whether time-of-day impacts RLIP efficacy, male C57BL/6J mice (8-12 weeks old; $n=7-10/\text{group}$) were housed under either normal or reversed light cycles for three weeks. Transient focal cerebral ischemia was performed (surgery time 8am-12pm) by 60-minute middle cerebral artery occlusion (tMCAO) either during the mice's inactive phase (ZT1-

5) or active phase (ZT13-17), followed by RLIP (10-min femoral artery occlusion). Adequate ischemia was confirmed using continuous Laser Doppler Flowmetry (LDF); animals with LDF $\geq 30\%$ of baseline during MCAO were excluded. Neurological function and sensorimotor deficits were assessed at 1- and 3-days post-ischemia using a standardized scoring system for motor and sensory performance, the adhesive tape removal and foot-fault tests. Infarct volume was measured via TTC staining. Protein expression of PER2, a core circadian clock protein, and neuronal nitric oxide synthase (nNOS), a proposed RLIP mediator, was analyzed by Western blot at 1- and 3-days post-ischemia. All procedures and measurements were randomized and performed blinded. Group comparisons were made using unpaired two-tailed t-test. P-values less than 0.05 were considered statistically significant. RLIP significantly reduced infarct volumes when applied during the rodent inactive phase but failed to confer protection during the active phase. RLIP also improved neurological scores when applied during the mice's inactive phase. During the inactive phase, the time-dependent neuroprotection was associated with reduced PER2 and increased nNOS expression. These findings suggest that diurnal rhythms critically influence RLIP efficacy, potentially mediated by PER2-nNOS signaling. As most strokes in humans occur during the early active phase (morning), which corresponds to the rodent inactive phase, a circadian mismatch may partially explain the gap between animal data and clinical outcomes. Reassessing RLIP protocols in the context of biological timing may enhance translatability and help optimize the therapeutic window for stroke treatment.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Topic: D.03. Stroke

Support: National Institutes of Health (NIH) National Institute of Neurological Disorders and Stroke grant R01NS093057 (GKS)
Bernard and Ronni Lacroute (GKS)

Title: Neuronal optogenetic stimulation modulates cholesterol dynamics in post-stroke recovery

Authors: *N. RADIT¹, H. CHEN², H. UCHINO², T. C. CHIANG², A. KIM², A. G. LEE⁴, M. Y. CHENG², G. K. STEINBERG^{2,3};

¹Neurosurgery, Stanford Sch. of Med., ²Neurosurg., ³Stanford Stroke Ctr., Stanford Univ., Stanford, CA; ⁴Pediatrics, Univ. of California, San Francisco, San Francisco, CA

Abstract: Background: Post-stroke brain stimulation is a promising neurorestorative approach, yet the molecular mechanisms driving recovery remain unclear. Our previous work demonstrated that post-stroke optogenetic stimulation of the ipsilesional primary motor cortex (iM1) enhances

functional recovery. RNA sequencing of the iM1 revealed cholesterol metabolism as a key pathway altered by stimulation. Here we investigated the temporal dynamics of a key cholesterol enzyme, 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), following stroke and how iM1 stimulation alters its expression.

Methods: C57Bl6 male mice (6-7 weeks) underwent stereotaxic surgery to express Channelrhodopsin in iM1 excitatory neurons and optical fiber implantation. After 5-6 weeks, mice underwent transient middle cerebral artery occlusion (30 min). Stimulated mice received optogenetic stimulations from post-stroke days (PD) 5-14. Rotating beam test was performed at pre-stroke baseline, PD4, 7 and 14. Brains were collected from stroke mice at PD1, 7 & 15, and sham mice were processed for immunohistochemistry (n=4-5/group) using antibodies that target cholesterol enzyme HMGCS1 and neurons (NeuN). The expression of HMGCS1 and NeuN were also examined in stimulated and non-stimulated mice at PD15.

Results: iM1 stimulations enhanced recovery at PD14, with longer distance traveled and faster speed on the rotating beam test ($p < 0.05$). Previous RNA sequencing revealed involvement of multiple cholesterol biosynthesis and metabolism pathways at PD15 in iM1-stimulated mice. Immunostaining revealed that HMGCS1 was primarily expressed in neurons in the primary motor cortex, as confirmed by co-staining with NeuN. In the peri-infarct area, HMGCS1 expression was localized in glial cells, including microglia and astrocytes. After stroke, HMGCS1⁺ neurons were decreased in both iM1 and cM1 compared to sham. Timecourse analysis showed that HMGCS1 increased transiently at PD1 in iM1, followed by reduced expression at PD7 & 15. At PD15, stimulated mice exhibited increased neuronal HMGCS1 expression and a higher number of NeuN⁺ neurons in iM1 compared to non-stimulated mice. Stimulated mice also showed elevated glial HMGCS1 in the peri-infarct region compared to the non-stimulated group.

Conclusions: Our transcriptome data revealed important insights into the molecular signaling of optogenetic stimulation-induced recovery, particularly cholesterol metabolism. Ongoing studies include qPCR validation of cholesterol related genes, and co-staining of cholesterol levels with glial markers. These data suggest a role for cholesterol metabolism in stimulation-induced recovery.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.19/J11

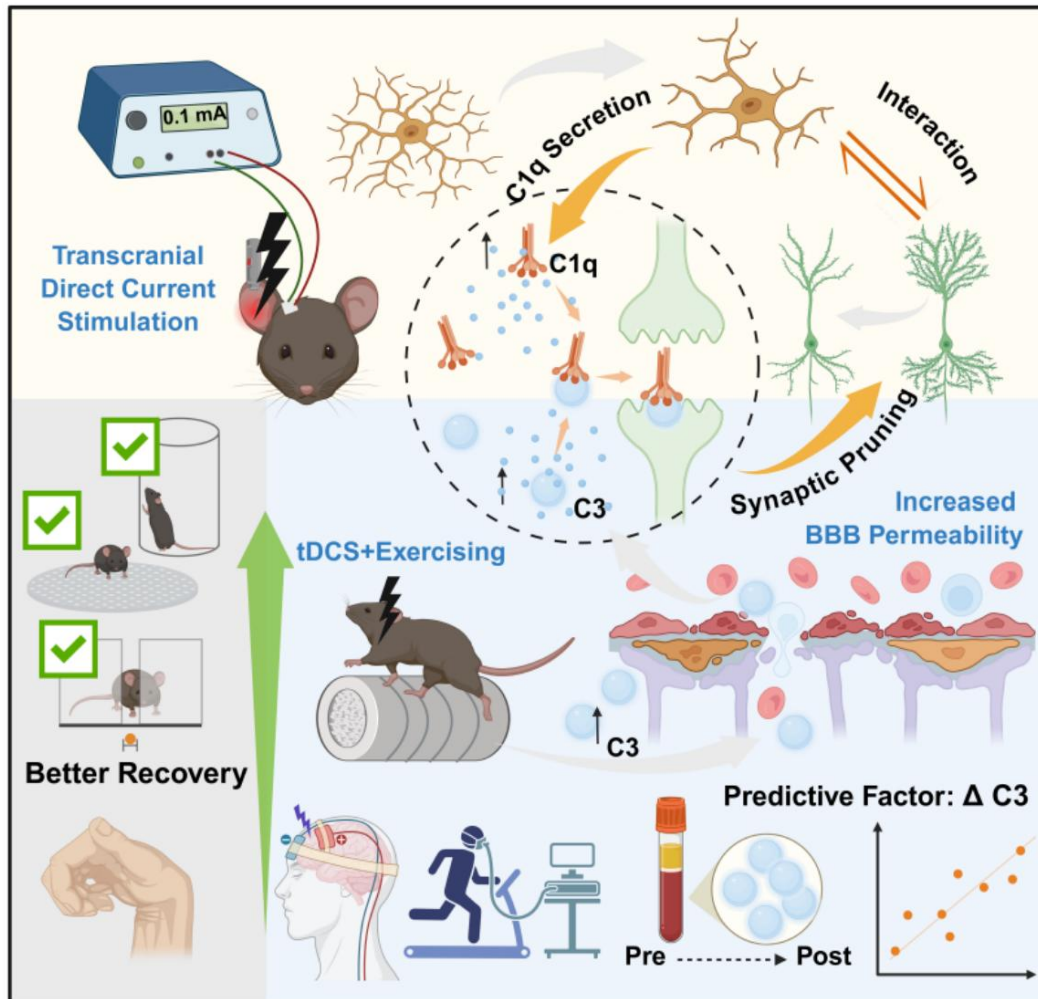
Topic: D.03. Stroke

Support: National Natural Science Foundation of China 82271422

Title: Microglia-mediated synaptic pruning via the c1q-c3 axis underlies transcranial direct current stimulation efficacy in post-stroke recovery

Authors: *Z. GAO;
Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China

Abstract: Transcranial direct current stimulation (tDCS), a novel non-invasive physical stimulation method, has been widely applied to alleviate post-stroke dyskinesia. However, recent research has reported that clinical efficacy of tDCS varies between individuals. This variation can be attributed to its unclear mechanism, which in turn leads to the lack of effective efficacy prediction models. In this study, we discovered that microglia-mediated engulfment of dendritic spines constituted the core mechanism underlying tDCS. Mechanistically, our in vivo 2P imaging, 3D reconstruction, and omics analysis revealed that the C1q-C3 axis mediated tDCS-driven microglial phagocytosis, which promotes the reconstruction of functional circuits during stroke rehabilitation. BBB permeability evaluations discovered that C3 was specifically regulated by tDCS in the brain parenchyma and periphery. On this basis, we proposed an improved rehabilitation protocol of tDCS combined with exercise, whose clinical benefit is generated by its blood-brain transport of exercise-generated C3. Finally, integrating human cohort data with an animal model, our findings reveal a novel prognostic biomarker— Δ C3, which promotes functional recovery of fine motor skills post stroke. Collectively, these findings delineate a distinct neuro-immune interaction pattern mediated by tDCS, explain the clinical potential of an optimised strategy, and suggest a predictive marker for monitoring the functional prognosis during post-stroke recovery.



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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Support: LSU Health Shreveport, Chancellor's Aim High Research Award

Title: Pi3k-akt pathway activation in age- and cell-type specific regulation of neurogenesis and functional recovery after stroke

Authors: *M. BRADFORD, N. AICH, K. BROWN, B. AKHTER, E. WILLIAMS, K. RODGERS;
LSU Hlth. Shreveport, Shreveport, LA

Abstract: Background: Ischemic stroke is a debilitating neurological condition affecting millions globally, often resulting in chronic disability. Stroke is typically thought of as a disease of the elderly, but it can occur at any age. Stroke is often more destructive in the pediatric population due to delays in diagnosis or misdiagnosis, yet it is well documented that the developing brain has remarkable plasticity and promotes increased post-ischemic functional recovery compared with adults. Even so, little is known about the mechanisms underlying post-stroke recovery in the young brain. Using a pediatric model of ischemic stroke (middle cerebral artery occlusion, MCAO) in juvenile mice, we observed a marked repopulation of neurons within the ischemic core of juvenile mice at 30d and improved functional recovery that was not found in adults. In the current work, we found a possible mechanism underlying these age-associated differences involving brain-derived neurotrophic factor BDNF-TrkB (tropomyosin receptor kinase B) signaling, which promotes neuronal survival and neurogenesis via phosphoinositide 3-kinase (PI3K-AKT) pathway activation and regulation of neurite outgrowth, synaptic reorganization, and synaptic plasticity.

Methods: To investigate the role of stroke-induced PI3K-AKT signaling in neurogenesis and functional recovery, we performed experimental stroke (MCAO) in juvenile and adult mice. Neurogenesis and PI3K-AKT pathway activation was examined with immunofluorescence and western blotting, and functional recovery with *in vivo* electrophysiology.

Results: We found elevated BDNF expression at acute (72hr) and chronic (30d) time points post-ischemia in MCAO-injured juveniles, while MCAO-injured adults had low levels of BDNF expression, which has been reported to contribute to poor post-ischemic outcomes. Further, we found neuronal BDNF-TrkB expression in stroke-injured juveniles and astrocytes in adults, which has been associated with pathological and damaging effects. Lastly, PI3K-AKT signaling pathway components were elevated in both groups. However, neuronal activation in MCAO-injured juveniles supported neuroregenerative responses, while astrocytic activation increased apoptosis and reduced neurogenesis in adults.

Conclusions: These results reveal age- and cell-type specific regulation of BDNF-TrkB mediated PI3K-AKT pathway signaling after stroke. In juveniles, neuronal pathway activation supports brain repair and recovery, and astrocytic activation in adults is damaging and pathological.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.21/J13

Topic: D.03. Stroke

Support: College of Charleston Summer Undergraduate Research with Faculty Grant

Title: Elucidating the Role of BDNF in Neonatal Hypoxic Ischemia Recovery

Authors: *A. A. HONG¹, K. CAMBURN², M. AKIRTAVA², S.-K. SIMS³;
¹Biol., ²Psychology, Col. of Charleston, Charleston, SC; ³Col. of Charleston Program In Neurosci., Charleston, SC

Abstract: INTRODUCTION: Neonatal hypoxic ischemia (HI) in premature infants can cause death or life-long functional impairments, with challenges in early diagnosis and limited treatments. The exogenous administration of brain-derived neurotrophic factor (BDNF) is a potential therapeutic option, as seen in adult clinical and animal preclinical studies, having shown to improve synaptic plasticity, reduce infarct size and inflammation, and aid motor recovery, and promotes learning and memory. Intranasal administration of BDNF is a noninvasive method that bypasses the blood-brain barrier, allowing direct access to the brain. This project evaluated BDNF's therapeutic potential in a neonatal HI model and further elucidated its role in recovery through the intranasal delivery of the BDNF/TrkB antagonist ANA-12. Additionally, sex differences in treatment response were explored, as previous research documented the differences in central and peripheral inflammation between female and male rodents after HI. METHODS: HI was induced on post-natal day 6 (PND 6) rats using the modified Vannucci Model and a hypoxic ischemic model by ligating the right carotid artery, followed by a two-hour exposure in an 8% oxygen/ 92% nitrogen enclosed chamber. Male and female rat pups received a 2-hour hypothermia treatment post-HI. PND 7 pups were subjected to 15µg of recombinant human BDNF intranasally from PND 8 to 13, given in small increments and alternating nostrils. Behavioral recovery was assessed on PND 7 via righting reflex, cliff avoidance, and negative geotaxis. On PND 14, brains were harvested for immunohistochemistry and western blotting to measure BDNF levels, TrkB phosphorylation, MAP2, and synapsin, indicating synaptic and dendritic plasticity. Lesion size and neuroinflammation in brain slices were analyzed using TTC staining and GFAP, and THEM 119 markers for astrocytes and microglia, respectively. Additional Nissl staining and multiplex ELISA were performed to measure pro-inflammatory cytokines. RESULTS: Preliminary data suggested a predictive relationship between the intranasal administration of ANA-12 and a reduction in plasticity and recovery compared to HI neonates that received intranasal BDNF. The results further demonstrate that the neonatal rodents with lower BDNF levels developed and performed significantly poorer.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

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Program #/Poster #: PSTR332.22/J14

Topic: D.03. Stroke

Support: NIH Grant T32AG082661
American Heart Association 23PRE1018993
NIH Grant T32NS082145
NIH Grant T32GM113896

Title: Dissecting molecular pathways controlling post-stroke neuronal plasticity

Authors: ***D. BETZ**¹, M. KENWOOD³, A. TODD², J. KAISER⁴, P. RHOTON⁵, A. TUMANOV¹, V. V. SAHNI⁶, M. P. GOLDBERG⁷;

¹UT Hlth. San Antonio, San Antonio, TX; ²UT Hlth. San Antonio, SAN ANTONIO, TX;

³Neurol., UT Health SA, San Antonio, TX; ⁴Sahni Lab., Burke Neurolog. Inst., White Plains, NY;

⁵UT Hlth. SA, San Antonio, TX; ⁶Burke Neurolog. Inst., Weill Cornell Med., White Plains, NY;

⁷Neurol., Univ. of Texas Hlth. Sci. Center, San Antonio, Shavano Park, TX

Abstract: Chronic disability affects over half of stroke survivors, and even with intensive rehabilitation, functional improvement plateaus within 3-6 months. The period for improvement, often called the critical window for spontaneous recovery, demonstrates unprecedented neural plasticity, enabling the formation of new circuits. Understanding mechanisms driving this critical window is essential for revealing therapeutic targets to enhance or extend post-stroke plasticity. Rodent models of stroke display time-limited, incomplete recovery. After motor cortical stroke, robust post-stroke plasticity occurs in multiple regions, including the peri-infarct and contralesional motor (cM1) cortex. To investigate the molecular underpinnings of region-specific plasticity, we performed single-nucleus RNA sequencing across both regions at time points critical for motor recovery. This approach enables the identification of shared and region-specific molecular programs and unique neuronal subtypes with enhanced plasticity.

Analysis of 150,000 nuclei revealed a novel stroke-induced neuronal population that emerges in the peri-infarct cortex by day 3 and persists through day 21 post-stroke. Using a neural network classifier trained on the transcriptional signature of this population, we additionally identified this population in the cM1. These cells arise from multiple cortical subtypes, exhibiting gene expression linked to plasticity, axonal growth, and synapse formation. They downregulate PTEN, a known inhibitor of neuronal repair, and upregulate GAP43, a key driver of neurite outgrowth. Furthermore, transcription factor network analysis identifies Restrictive Element-1 Silencing Transcription Factor (REST), an epigenetic repressor of neuronal plasticity and excitability, as a central upstream regulator of this phenotype.

Together, these findings define a novel, stroke-induced neuronal phenotype and uncover molecular pathways that may be leveraged to promote post-stroke plasticity, offering promising therapeutic targets for post-stroke recovery.

Disclosures: **D. Betz:** None. **M. Kenwood:** None. **A. Todd:** None. **J. Kaiser:** None. **P. Rhoton:** None. **A. Tumanov:** None. **V.V. Sahni:** None. **M.P. Goldberg:** None.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.23/J15

Topic: D.03. Stroke

Support: 1KO8S11788-01A2

Title: Environmental Enrichment Modulates Circuit-Specific Changes in Cell Adhesion Molecules and Improves Somatomotor Recovery after Experimental Ischemic Stroke

Authors: ***R. A. LEON-ALVARADO**¹, **R. AN**², **B. WANG**², **B. HOUSE**⁴, **K. P. SMITH**¹, **T. ABOU-ANTOUN**¹, **A. J. ALBERTSON**³;

¹Neurol., ²Washington Univ. in St Louis, St Louis, MO; ³Neurol., Washington Univ. in St Louis, St. Louis, MO; ⁴St. Louis, MO.

Abstract: Recovery after ischemic stroke occurs for months after the injury and is associated with robust changes in neuronal activity, structure, and connectivity within both local and distant brain circuits. The specific drivers of this plasticity are poorly understood. Environmental enrichment (EE) enhances neuronal plasticity and recovery after experimental stroke. Trans-synaptically expressed cell adhesion molecules (CAMs) are integral to the formation and stabilization of new synapses during cortical plasticity. N-cadherin and associated β -catenin are two linked CAMs essential to this plasticity and are broadly disrupted during acute ischemia. We hypothesized that thalamocortical N-cadherin and β -catenin would be highly regulated during recovery after somatosensory stroke in a circuit-specific fashion. We further hypothesized that EE would modulate this regulation while enhancing stroke recovery. To examine this, we performed behavioral assessments and quantified levels of N-cadherin, and β -catenin at sequential time points (1, 4, & 7 weeks) after photothrombotic stroke in parallel cohorts. 8-12-week-old male C57BL/6 mice (n = 105) were divided into two conditions: standard housing and enriched housing cages. Infarcts were induced within the forepaw somatosensory cortex (sham groups had no infarct). Four regions of interest relative to the stroke (ipsilesional and contralesional cortices and thalami) were dissected. Western blots were performed for each region at each time point per condition to quantify expression of N-cadherin, and β -catenin. In the ipsilesional cortex, we observed a decrease in N-cadherin and β -catenin expression after 1 week in the standard housing group. EE significantly mitigated this decrease and improved somatomotor recovery (cylinder test). Conversely, both proteins were significantly downregulated in the contralesional thalamus in the EE condition but not the standard housing condition. Together, these data suggest that stroke recovery is associated with widespread, circuit-specific regulation of CAMs and that EE may enhance recovery by modulating this regulation. This modulation may reflect regionally specific, transient windows of plasticity. Ongoing work will address the necessity of this regulation for synaptic re-organization after stroke.

Disclosures: **R.A. Leon-Alvarado:** A. Employment/Salary (full or part-time): Washington University in St Louis. **R. An:** None. **B. Wang:** None. **K.P. Smith:** A. Employment/Salary (full or part-time): Washington University in St Louis. **T. Abou-Antoun:** A. Employment/Salary (full or part-time): Washington University in St Louis. **A.J. Albertson:** A. Employment/Salary (full or part-time): Washington University in St Louis.

Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR332.24/J16

Topic: D.03. Stroke

Support: Novo Nordisk
Sino-Danish center (SDC)
William Demant fonden

Title: Exploring RIC-miRNAs as novel protectors in stroke-related inflammation

Authors: *L. MARZIANI¹, K. STENZ², B. MUMM³, H. ZHANG⁴, X. WANG⁵, K. R. DRASBEK⁶;

¹Ctr. of Functionally Integrative Neurosci. (CFIN), Aarhus Univ. (DK), Aarhus C, Denmark;

²Dept. of Clin. Medicin, Aarhus Univ., Aarhus, Denmark; ³Ctr. of Functionally Integrative Neurosci. (CFIN), Aarhus Univ., Aarhus C, Denmark; ⁴Inst. of Genet. and Developmental Biol., Beijing, China; ⁵Inst. of Genet. and Developmental Biology, C, Beijing, China; ⁶Ctr. of Functionally Integrative Neurosci., Ctr. of Functionally Integrative Neuroscience, Aarhus Univ., Aarhus C, Denmark

Abstract: BACKGROUND: Stroke is the second leading cause of death worldwide. Disrupted blood flow deprives cells of oxygen, triggering oxidative stress and subsequent inflammation. Specifically, inflammation can lead to the breakdown of the blood-brain barrier (BBB) by reducing tight junctions between endothelial cells lining the blood vessels allowing harmful substances to enter the brain. Recanalization, the only approved treatment for ischemic stroke, must be administered within 4.5 to 6 hours of symptoms onset. Thus, new treatments offering prolonged neuroprotection are urgently needed. One promising approach is represented by remote ischemic conditioning (RIC), a non-invasive treatment, which hold the potential to improve stroke outcome. Upon RIC treatment, microRNAs (miRNAs) that can regulate protein and cellular homeostasis, are released into the bloodstream. **AIM:** RIC-induced miRNAs (RIC-miRNAs) are hypothesized to protect human brain microvascular endothelial cells (HBMECs) by regulating gene expression and preserving BBB integrity. **METHOD:** To assess the impact of RIC-miRNAs during inflammation, HBMECs were transfected with four RIC-miRNAs: miR-16-5p, miR-144-3p, miR-182-5p, and miR-451a. Following, inflammation was induced *in vitro* by exposing HBMECs to conditioned media from LPS-activated microglia. RNA sequencing was performed to identify differentially expressed genes, and candidate targets for RIC-miRNAs were selected for validation. **RESULTS:** RNA sequencing revealed 700 genes as potential targets of RIC-miRNAs. Dual luciferase assays confirmed two direct targets of two distinct RIC-miRNAs, both involved in angiogenesis, suggesting a potential role of RIC-miRNAs in vascular repair under inflammatory conditions. **FUTURE PERSPECTIVE:** RIC-miRNAs may offer a novel therapeutic approach for stroke treatment by targeting inflammation-related pathways and contributing to BBB protection beyond the limitations of current treatments.

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Poster

PSTR332: Stroke Recovery: Non-Pharmacological Approaches to Therapy

Location: SDCC Halls B-H

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Program #/Poster #: PSTR332.25/K1

Topic: D.03. Stroke

Support: National Research Foundation (NRF) of Korea (RS-2022-NR075830)
Korean government (MSIT) (No. RS-2022-NR070859)

Title: Exercise Preconditioning Alleviates Photothrombotic Ischemic Stroke in Mice by Orchestrating Neutrophils

Authors: *K. TAE YEON, H. MOON;
Seoul Natl. Univ., Gwanak-gu, Korea, Republic of

Abstract: Neutrophils are rapidly recruited to the infarcted brain and exacerbate tissue damage by infiltrating the infarct site and releasing neutrophil extracellular traps (NETs) within 24 to 36 hours after the onset of ischemia. Previous studies have demonstrated that reducing neutrophil accumulation and restricting neutrophil functions in the injured brain mitigates ischemic stroke (IS) severity. Notably, exercise preconditioning (EP) has the potential to modulate neutrophil function, thereby contributing to the amelioration of IS pathology. This study investigated the functional differences between resting and EP-induced neutrophils under IS conditions. Eight-week-old C57BL/6J male mice underwent 8 weeks of voluntary wheel running before photothrombotic ischemic stroke (PTI) surgery. Grip strength and open field tests were conducted 2 days after PTI to verify whether EP facilitates acute recovery and limits ischemic damage. To evaluate whether EP affected neutrophil recruitment and chemotactic signaling, including migration and NET formation, we assessed the expression of Ly6g, Cxcl2, and fMLP in infarcted cortical regions using RT-qPCR. A migration assay was performed in blood-isolated neutrophils using a transwell. Circulating NETs in plasma were quantified by ELISA, while NET presence in the brain was evaluated by IF staining. Statistical analyses were performed using Student's t-test for two-group comparisons (Sed-PTI versus EP-PTI) in the migration assay and IF staining (CitH3), one-way ANOVA for ELISA and PCR, and two-way ANOVA with group (Sed and EP) and time (pre- and post-PTI) as factors to assess behavioral performance. Data are presented as mean $\pm$ SD, and $p < 0.05$ was considered statistically significant. EP-PTI mice exhibited higher grip strength ($p = 0.038$) and general activity ($p = 0.03$) compared to sedentary PTI (Sed-PTI) mice. EP-PTI mice showed significantly reduced Ly6G expression ($p = 0.0016$), indicating decreased neutrophil infiltration into the infarcted brain. Key chemotactic mediators fMLP ($p = 0.0023$) and CXCL2 ($p = 0.013$) were also suppressed in EP-PTI mice compared to Sed-PTI mice. In addition, neutrophils isolated from EP-PTI mice demonstrated diminished migration capacity ($p = 0.0035$) compared to those isolated from Sed-PTI mice. Moreover, EP-

PTI mice showed reduced NET formation in both plasma ($p = 0.0388$) and brain ($p = 0.042$). These findings suggest that EP enhances acute recovery 2 days after PTI and may beneficially orchestrate IS pathology by suppressing neutrophil infiltration and chemotactic signaling, inhibiting neutrophil migration, and reducing NET formation in vivo.

Disclosures: K. Tae Yeon: None. H. Moon: None.

Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.01/K2

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

Support: NEI (P30-EY026877 and R01-EY036028)
Gilbert Vision Restoration Initiative
Research to Prevent Blindness, Inc.

Title: Differential Roles of Endogenous CaMKII Isoforms in Retinal Ganglion Cell Survival Following Optic Nerve Injury

Authors: *X. XIA¹, H. CHANG², Z. LUO², J. LI¹, W. YAN², M. NAHMOU², J. OCHOA², M. BIAN², M. S. KAPILOFF¹, J. L. GOLDBERG²;

²Byers Eye Institute--Ophthalmology, ¹Stanford Univ., Palo Alto, CA

Abstract: Calcium/calmodulin-dependent protein kinase II (CaMKII) plays a critical role in neuronal signaling and survival, yet isoform-specific contributions to retinal ganglion cell (RGC) neuroprotection following injury remain unclear. In this study, we investigated the roles of individual CaMKII isoforms in RGC survival using a conditional knockout approach in mice. We first surveyed CaMKII isoform expression in mouse RGCs using PACBio sequencing and found all 4 genes were expressed, but CaMKII γ and δ showed the most expression and isoform diversity. Floxed alleles for CaMKII α , β , γ , and δ were selectively knocked out in the retina via intravitreal injection of Cre-expressing AAV2-sncg-cre or control AAV2-sncg-luciferase/flag 2 weeks before injury. Two weeks following optic nerve crush (ONC), retinas were flat-mounted and immunostained with RGC-specific marker RBPMs to quantify RGC survival. Deletion of CaMKII γ or CaMKII δ resulted in significantly increased RGC death compared to controls, indicating a critical neuroprotective role for these isoforms. In contrast, knockout of CaMKII α only slightly increased RGC death, and knockout of CaMKII β had no effect on RGC death after ONC. These findings highlight isoform-specific functions of CaMKII in RGC resilience to axonal injury and suggest that CaMKII γ and δ may be potential targets for neuroprotective therapies in optic neuropathies.

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Poster

PSTR333: Axon Protection and Regeneration

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.02/K3

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

Support: R01-EY029739

Title: Loss of extracellular matrix-interacting integrin receptors on retinal ganglion cells reduces inflammation-promoted axon regeneration in vivo

Authors: *M. P. FROST;
UConn Hlth., Farmington, MA

Abstract: Adult mammalian central nervous system (CNS) projection axons fail to regenerate after injury or disease resulting in irreversible loss of function. The ability to actively grow/regenerate CNS axons is lost during developmental maturation. To overcome this unmet clinical challenge, we identified specific integrin receptors that interact with a defined extracellular matrix (ECM) protein which can foster axon growth. We transcriptomically and immunohistochemically characterized expression of these receptors in uninjured and injured retinal ganglion cells (RGCs). We also ablated both receptors in the RGCs using tamoxifen-inducible Thy1-driven Cre recombinase mouse lines crossed to two floxed-receptors mouse lines, and tested the effects on zymosan-promoted optic nerve axon regeneration after optic nerve crush (ONC) injury. We found that the expression of both receptors was enriched in the wildtype RGCs that survived ONC injury, and that conditional ablation of these receptors in the RGCs significantly reduced zymosan-promoted optic nerve axon regeneration compared to wildtype mice. These findings provide important insights into the mechanisms of how neuroinflammation promotes CNS axon regeneration.

Disclosures: M.P. Frost: None.

Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.03/K4

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

Support: Zebra Biologics Inc.
NIH Grant R41EY032011

Title: Prolonged retinal ganglion cell and optic nerve preservation by the TrkB agonist ZEB85 in an experimental glaucoma model

Authors: *S. REINEHR¹, W. QIN¹, R. M. LINDSAY², J. P. ZEHGE¹, C. THEISS³, R. FUCHSHOFER⁴, B. DICK¹, P. DISTEFANO², S. C. JOACHIM¹;

¹Ruhr-University Bochum, Bochum, Germany; ²Zebra Biologics Inc., Concord, MA; ³Dept. of Cytology, Inst. of Anat., Bochum, Germany; ⁴Inst. of Human Anat. and Embryology, Regensburg, Germany

Abstract: Purpose: Glaucoma is a leading cause of blindness worldwide and defined as loss of retinal ganglion cells (RGCs) and their axons. Elevated intraocular pressure (IOP) is the main risk factor of this disease. While IOP-lowering therapies slow disease progression, there are no approaches to protect RGCs and subsequent vision loss, emphasizing the need for disease-modifying therapies. This might be achieved by enhancing the neurotrophic BDNF/TrkB pathway. Hence, we aimed to explore the protective effects of a TrkB agonist antibody (ZEB85) on CTGF high-pressure glaucoma mice. **Methods:** CTGF transgenic and wildtype (WT) female and male mice were injected intravitreally in one eye with either ZEB85 or a control antibody (Co-ab) at 10 weeks of age. Non-injected eyes served as controls (WT, CTGF). IOP was measured regularly (n=8/group). 5 or 10 weeks after treatment, electroretinogram recordings were performed (n=5-8/group). 5 weeks after injection, retinas were prepared as cross-sections (n=6/group), while after 10 weeks, retinas were processed as flatmounts (n=7/group). For both, immunohistology with an antibody against RBPMS (RGCs) was performed. In addition, optic nerve cross-sections were stained with methylene blue at 10 weeks (n=5/group). **Results:** At 15 and 20 weeks of age, IOP was significantly increased in all CTGF groups compared to WT mice (all: p<0.001). At both investigated time points, the a-wave amplitude, which reflects the electrical output of the photoreceptors, was diminished in CTGF and CTGF-Co-ab mice, while injection of ZEB85 preserved amplitude. The b-wave amplitude, which reflects mostly the ON bipolar cell responses, was also reduced in CTGF mice, and amplitudes were restored in CTGF-ZEB85 mice at both time points (all: p<0.05). Additionally, a significant RGC loss was observed in CTGF and CTGF-Co-ab retinas (all: p<0.05), while RGCs counts in CTGF-ZEB85 mice were comparable to the WT situation 5 and 10 weeks after a single injection, respectively. Moreover, the optic nerve damage score was high in non-treated CTGF mice, while intravitreal injection of ZEB85 was able to preserve the optic nerve structure. **Discussion:** Our findings show that intravitreal treatment with ZEB85 preserved electric output of the retina as well as RGCs in CTGF glaucoma mice. 10 weeks after the injection, the optic nerve structure was comparable to WT littermates. Importantly, a single injection of the TrkB agonist antibody ZEB85 showed remarkable durability in retinal and optic nerve preservation in the mouse CTGF high-pressure glaucoma model. This approach could therefore serve as a new therapeutic option for glaucoma patients.

Disclosures: S. Reinehr: None. W. Qin: None. R.M. Lindsay: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zebra Biologics Inc.. J.P. Zehge: None. C. Theiss: None. R. Fuchshofer: None. B. Dick: None. P. DiStefano: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zebra Biologics Inc. S.C. Joachim: None.

Poster

PSTR333: Axon Protection and Regeneration

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.04/K5

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

Support: NIH grant EY031697 (WKJ)
UCSD GTI 2024-014 (WKJ)
P30 EY022589 (Vision core grant)

Title: Therapeutic Potential of AKAP1 Gene Therapy in Preservation of Retinal Ganglion Cells and Visual Function in Glaucoma

Authors: *T. BASTOLA¹, Z. SHEN², M. POUDEL¹, K.-Y. KIM³, S. CHOI⁵, W. JU⁴;
¹Ophthalmology, Univ. of California San Diego, SAN DIEGO, CA; ²Ophthalmology, Univ. of California San Diego, San Diego, CA; ³Natl. Ctr. Microscopy and Imaging, ⁴Ophthalmology, Univ. of California San Diego, La Jolla, CA; ⁵Sch. of Med., UCSD, La Jolla, CA

Abstract: A-Kinase anchoring protein 1 (AKAP1) is a multifunctional mitochondrial scaffold protein that plays a critical role in regulating mitochondrial dynamics, bioenergetics, and calcium homeostasis by anchoring various proteins, including protein kinase A, to the outer mitochondrial membrane. This study investigates the potential of gene therapy-mediated amplification of AKAP1 to protect retinal ganglion cells (RGCs), their axons, and the central visual pathway from glaucomatous damage by preserving mitochondrial function. We utilized two established mouse models of glaucoma: the DBA/2J (D2) genetic model and an inducible model with intracameral microbead (MB) injections. Five-month-old D2 mice in a pre-glaucomatous state received intravitreal gene therapy with recombinant adeno-associated virus serotype 2 (AAV2) constructs—either AAV2-Null or AAV2-AKAP1 for five months, followed by a single intraocular pressure (IOP) measurement at 10 months. Similarly, 12-week-old C57 mice in the MB model were injected with gene therapy three weeks before MB injection, with weekly IOP measurements recorded over 8 weeks. In both 10-month-old D2 mice and MB model mice, elevated IOP led to a significant decrease in AKAP1 expression in the retina, coinciding with RGC loss. *In vivo* intravitreal delivery of AAV2-AKAP1 for AKAP1 overexpression in the retina significantly protected RGCs in both the middle and peripheral retinas compared to glaucomatous Null-D2 mice or Null-MB mice. Importantly, visual function tests showed that AKAP1 amplification preserved pERG and pVEP responses in both glaucomatous D2 mice and MB-model mice. Glaucomatous Null-D2 retinas exhibited increased calcineurin (CaN) and total dynamin-related protein 1 (DRP1) expression, along with reduced DRP1 phosphorylation at serine 637 and optic atrophy type 1 expression. However, AAV2-AKAP1 gene therapy restored the levels of these mitochondrial dynamics-regulating proteins and OXPHOS proteins in the retina of glaucomatous D2 mice. Additionally, AKAP1 amplification via gene therapy significantly enhanced mitochondrial biogenesis by upregulating PGC-1 α and TFAM protein expression. Finally, our results demonstrated that AKAP1 gene therapy successfully restored CTB intensity in the SC of D2 mice compared to glaucomatous Null-D2 mice. These findings indicate that gene therapy aimed at amplifying AKAP1 could be a

promising therapeutic approach to protect RGCs and their axons, thereby preserving the central visual pathway and visual function. This protection is achieved by maintaining the structural and functional integrity of mitochondria in the context of glaucomatous neurodegeneration.

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Poster

PSTR333: Axon Protection and Regeneration

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Program #/Poster #: PSTR333.05/K6

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

Support: NEI P30-EY026877
BrightFocus Foundation
Gilbert Vision Research Initiative
Research to Prevent Blindness, Inc.

Title: Enhancing Optic Nerve Head Axon Guidance of Transplanted Human Stem Cell-Derived Retinal Ganglion Cells

Authors: *Z. LUO, X. XIA, H. CHANG, M. BIAN, J. L. GOLDBERG;
Ophthalmology, Stanford Univ., Palo Alto, CA

Abstract: Donor retinal ganglion cell (RGC) transplant is a promising approach for vision restoration in optic neuropathies including glaucoma. Optimizing donor cell axon growth and guidance remains an important unmet need in neuroscience. The current study investigates how pre-treatment of donor cells and of the recipient tissue influences donor cell axons after transplant. Cells dissociated from retinal organoids (day 40-60) derived from the Brn3b-tdTomato H9 human embryonic stem cell line were grown in floating or adherent cultures. Intravitreal transplant was performed using a low-pressure (10 psi) Picospritzer to promote peripheral retinal engraftment. Three days post-transplant, an absorbable gelatin sponge loaded with 0.05 µg or 0.5 µg Netrin was placed in the superior retrobulbar space. A second dose (0.2 µg or 2 µg, respectively) was administered seven days later. Retinas and optic nerves were collected one week after the second implant for histological analysis. All experiments were independently repeated at least three times. Data were analyzed using *chi*-square and ANOVA. Pre-adhered donor hRGCs successfully engrafted in the peripheral retina and demonstrated robust tdTomato-positive cell survival and axon regeneration. RNAseq showed significant upregulation of the axon guidance receptor deleted in colorectal cancer (DCC). Exogenous low- or high-dose netrin treatment did not significantly change the proportion of transplants having any axon regeneration on the host retina compared to PBS-treated controls. However, netrin significantly increased hRGC axon regeneration into the optic nerve head. Pre-adherence of hRGCs from retinal organoids increases survival and axon regeneration after transplant. Netrin is

an effective axon guidance cue to direct regenerated axons to enter the ONH. This strategy may contribute to therapeutic approaches for restoring visual pathways in glaucoma and other optic neuropathies.

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Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.06/K7

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

Support: Research to Prevent Blindness unrestricted gift to the Flaum Eye Institute
Research to Prevent Blindness career development award (MT)
Sigma Xi Grants in Aid of Research G20250315-12502

Title: Neurotrophic signaling and retinal ganglion cell resiliency in photoreceptor degeneration

Authors: *A. ABRAHAM^{1,2}, L. AFRIMA², J. ENDRES², M. TELIAS^{2,3};

¹Pharmacol. and Physiol., Univ. of Rochester Sch. of Med., Rochester, NY; ²Flaum Eye Inst.,

³Del Monte Inst. for Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: During photoreceptor degeneration (PRD), the loss of rods and cones leads to downstream pathophysiological changes in the retina known as remodeling. In late stages, remodeling is characterized by the partial loss of retinal ganglion cells (RGC), the sole output neurons of the retina. Restoring vision requires a functional connection between the retina and the brain, making it essential to understand whether and how RGCs survive in PRD. In analogous pathologies such as glaucoma, the activation of neurotrophins (NT) is sufficient to induce RGC survival. NTs bind to their receptors, tropomyosin receptor kinases (TRK), leading to the activation of downstream survival signals. Here, we hypothesize that NT signaling is necessary for RGCs to survive late-stage PRD. The mouse models used include inherited PRD mice (RD1, Pde6 β ^{RD1/RD1}) and TVRM4 (Rho^{TVRM4}) light induced PRD mice, and wildtype (WT) counterparts. RGC density was assessed by immunolabeling with the RGC specific marker RBPMs. The transcriptional status of all TRKs and NTs was measured in retinal lysates via quantitative real-time PCR. The translational level of TRKs was visualized and quantified via immunohistochemistry in retinal slices or western blot in retinal lysates. In addition, WT and RD1 mice were crossbred with the reporter line TrkB^{tauEGFP}. TrkB signaling was inhibited by intraperitoneal treatment with the selective antagonist ANA-12. We found RGC density in young (P60) RD1 mice to be similar to WT counterparts. However, in old (P300) RD1 mice, RGC density was reduced by ~22%. The expression of TRKs was assessed at the same timepoints. At age P60, TrkB was upregulated in the RGCs of RD1 mice, while TrkA and TrkC expression did not change. By P300, all three TRK subtypes were upregulated in PRD. Strong upregulation of

TrkB in the inner retina was also measured in the TVRM4 mice, only following phototoxic induction of PRD. Interestingly, in P60 RD1 retinas, phosphorylation levels of CREB were significantly downregulated, while phosphorylation of TrkB did not change. Most importantly, acute antagonism of TrkB in young RD1 mice did not affect RGC density but the same manipulation in old RD1 mice accelerated the loss of RGCs by 10%. Our results show that late-stage PRD is accompanied by downstream RGC death. Moreover, we show that PRD triggers TrkB upregulation in RGCs regardless of etiology. Finally, our data suggest that TrkB is necessary for RGC survival, indicating the presence of a NT dependent neuronal survival mechanism in diseases causing blindness due to PRD. These results could be leveraged to devise treatments aimed at improving vision in patients with diseases such as retinitis pigmentosa.

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Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.07/K8

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

Support: SAMSUNG Grant, SRFC-MA2302-05

Title: *Csde1* bridges nuclear and mitochondrial RNA networks to regulate mitochondrial function

Authors: *H. JIN, E. JANG, Y. CHO;

Daegu Gyeongbuk Inst. of Science&Technology., Daegu, Korea, Republic of

Abstract: Neuronal homeostasis requires coordinated gene expression between the nucleus and mitochondria. RNA-binding proteins (RBPs) play essential roles in maintaining RNA stability, translation, and localization. In this study, we demonstrate that CSDE1, a cytoplasmic RBP previously identified as a key factor in axon regeneration, serves as a central mediator of nuclear-mitochondrial communication. By analyzing CSDE1 immunoprecipitation and RNA sequencing analysis (CSDE1 RIP-seq), we found that a group of nuclear-encoded mitochondrial transcripts, particularly those involved in the electron transport chain (ETC), were significantly associated with CSDE1 in mouse dorsal root ganglia (DRG) tissues. We found that CSDE1 was co-localized with mitochondria by immunocytochemistry analysis and detected CSDE1 in mitochondrial fractions by subcellular fractionation analysis. In addition, CSDE1 was co-immunoprecipitated with TOMM20, a co-translational import receptor, in an RNA-independent manner. Upon *Csde1* knockdown, nuclear-derived ETC RNAs and their protein products were reduced from mitochondrial fractions, while mitochondria-encoded RNAs were increased, indicating disrupted normal mitochondrial RNA trafficking and homeostasis. Furthermore, CSDE1 was found to co-localize with mitochondria in axons and interacted with an anterograde motor protein Kinesin-1, implying active transport to axonal injury sites. These findings support

a model in which CSDE1 shuttles nuclear-encoded ETC RNAs to mitochondria, enabling localized protein synthesis critical for sustaining mitochondrial function during neuronal regeneration. This study establishes CSDE1 as a central node of nuclear-mitochondrial communication and highlights its essential role in axonal regeneration.

Disclosures: H. Jin: None. E. Jang: None. Y. Cho: None.

Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.08/K9

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

Support: ALRO – FAPESP Grant: 2023/02615-4
FAPESP Grant: 2024/09568-4

Title: Tempol improves functional and structural outcomes after polyethylene glycol-mediated axonal fusion in rat sciatic nerve injury

Authors: *L. SALAMANCA GUILLÉN¹, K. ROBALLO², L. MELO-THOMAS³, A. S. VIEIRA⁴, L. P. CARTAROZZI⁵, A. L. OLIVEIRA⁶;

¹Univ. Estadual de Campinas, Campinas, Brazil; ²Edward Via Col. of Osteo. Med., Blacksburg, VA; ³Inst. of Anat. and Cell Biol., Marburg, Germany; ⁴IB - DBEF, Univ. Estadual De Campinas, Campinas, Brazil; ⁵Inst. of Biol., ⁶Structural and Functional Biol., Univ. of Campinas - Lab. of Nerve Regeneration, Campinas, Brazil

Abstract: Peripheral nerve transection (neurotmesis) results in immediate and complete interruption of motor and sensory pathways and often necessitates surgical intervention, typically via end-to-end neurorrhaphy. Despite realignment of the stumps, Wallerian degeneration occurs both retrogradely and anterogradely. In the distal stump, axons degrade entirely and are replaced by new sprouts. As this process is slow and incomplete, it frequently leads to muscle atrophy and paralysis, significantly impairing quality of life. An alternative strategy is axonal fusion mediated by polyethylene glycol (PEG), which can rapidly reconnect severed axonal membranes, prevent degeneration, and promote early electrophysiological restoration. Previous studies have shown that the antioxidant methylene blue (MB) improves PEG-fusion outcomes. Here, we investigated whether tempol (4-hydroxy-TEMPO), a membrane-permeable antioxidant with superior redox activity, could further enhance recovery when used in a PEG-fusion protocol. Eight-week-old adult female Lewis rats underwent unilateral sciatic nerve transection and received one of three treatments: end-to-end neurorrhaphy (NRR), MB-PEG-fusion (MB-fusion), or Tempol-PEG-fusion (TMP-fusion). The study was approved by the Institutional Committee for Ethics in Animal Use (CEUA/IB/UNICAMP, protocol #5875-1(A)/2024). Functional recovery was monitored for 8 weeks using CatWalk gait analysis (Peroneal Functional Index, PFI), and compound muscle action potentials (CMAPs) were recorded at baseline, immediately after

fusion, and at 8 weeks post-repair. Histological and immunofluorescence analyses of the sciatic nerve (neurofilament, S100) and spinal cord (synaptophysin, GFAP, Iba-1) were performed to assess axonal structure, Schwann cell activity, synaptic integrity, and glial reactivity. TMP-fusion significantly improved sensorimotor performance (PFI, $p = 0.0232$; step sequence, $p < 0.05$) and preserved CMAP amplitudes ($p < 0.05$) compared to NRR. Increased neurofilament ($p < 0.0001$) and S100 ($p = 0.0004$) expression were observed in both stumps. In the spinal cord, Tempol preserved synaptic coverage ($p < 0.0001$; $p = 0.0003$) and reduced glial reactivity (Iba-1, $p = 0.0035$; GFAP, $p = 0.001$). These results support Tempol as an effective enhancer of PEG-fusion, improving functional, structural, and electrophysiological recovery after neurotmesis.

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Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.09/K10

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

Support: College of Medicine - CoMGRAD to BS
College of Medicine - CoMRAD to AK
Natural Sciences and Engineering Research Council of Canada (NSERC) to AK

Title: Boosting peripheral nerve repair with mesencephalic astrocyte-derived neurotrophic factor (MANF) secreting Schwann cells.

Authors: *B. SIVAKUMAR¹, V. MARTINEZ³, A. KUMAR³, A. KRISHNAN²;

¹Dept. of Anatomy, Physiology, and Pharmacol., ²Anatomy, Physiol. and Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada; ³Dept. of Biochemistry, Microbiology and Immunology, Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Despite the high prevalence of peripheral nerve injuries worldwide, effective pharmacological strategies to promote complete functional nerve regeneration remain elusive. One of the main drawbacks of intrinsic peripheral axon regeneration is the lack of local and sustained availability of growth factors in the regenerating nerves. Therefore, supplementation of growth factors locally and continuously could be a potential therapy for nerve regeneration. MANF is a growth factor that has previously been implied in CNS and PNS repair as well as in the promotion of Schwann cells (SC) dynamics. SCs were previously shown to secrete growth factors like BDNF and NGF to support axon regeneration. In this research, we postulated that nerve resident SCs harnessed to secrete MANF will improve nerve regeneration. We transduced primary SCs with lentiviral vectors carrying a Dox-inducible MANF construct. The expression and secretion of MANF in transduced SCs were validated by western blot and ELISA,

respectively. For neuroprotection and axon regeneration studies, ex-vivo crush injury was performed on a nerve intactly connected to the dorsal root ganglia (DRG) and the preparation was infected with control lentiviruses (L-Dox-EV) or MANF-Lentiviruses (L-Dox-MANF). They were cultured in Matrigel for 5 and 14 days in doxycycline supplemented media. Neuroprotection and axon regeneration in this preparation was quantified after staining the axons with β III tubulin and GAP43, respectively, and using ImageJ Software. Western blot and ELISA assay showed that MANF is significantly expressed and secreted by primary SCs in L-Dox-MANF group compared to L-Dox-EV group. Immunostaining of crushed DRG-nerve preparations 5 days post infection shows significant increase in the number of intact axons, as evident from β III tubulin staining, in the proximal side of the nerve preparation in L-Dox-MANF group compared to L-Dox-EV group, indicating that MANF provides neuroprotection. Immunostaining of crushed DRG-nerve preparations 14 days post infection shows a significant increase in the number of regenerating axons, as evident from GAP43 staining, in the distal end in L-Dox-MANF group compared to L-Dox-EV group, indicating that MANF promotes axon regeneration. Co-immunostaining the nerves with GFAP and MANF confirmed the overexpression of MANF in SCs in the L-Dox-MANF group. Overall, our experiments demonstrate that nerve resident SCs programmed to express and secrete MANF is a potential therapy for peripheral nerve regeneration.

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Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.10/K11

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

Support: University of Missouri Summer Research Fellowship

Title: Effect of intraoperative vagal nerve stimulation on functional recovery in a rat model of iatrogenic RLN transection and repair

Authors: *R. KONDAPANENI¹, A. SCHROEDER², E. MASKROD³, G. EASON⁴, C. DRAGO³, C. SCHELLMAN⁴, S. KINGTON³, G. OTI⁵, K. OSMAN⁶, T. E. LEVER⁶;
¹Univ. of Missouri Sch. of Med., Columbia, MO; ²Dept. of Orthopedic Surgery, ³Col. of Arts and Sci., ⁴Col. of Hlth. Sci., ⁵Dept. of Biomed. Sci., ⁶Dept. of Otolaryngology-Head and Neck Surgery, Univ. of Missouri, Columbia, MO

Abstract: Injury to the recurrent laryngeal nerve (RLN), a branch of the vagus nerve, is a serious complication of anterior neck surgery. Injury to the RLN impairs laryngeal movement and airway protection, leading to dyspnea, dysphonia, and dysphagia—each of which may increase patient morbidity and mortality. Treatments must be developed for patients with RLN injury. This exploratory study investigates the effect of intraoperative vagal nerve stimulation (iVNS)

applied immediately post-RLN transection and repair on functional recovery in Sprague-Dawley rats. Sixteen rats were randomly assigned to iVNS (n=8) or sham-iVNS (control, n=8) groups. Both groups underwent anterior neck incision followed by RLN isolation, transection, and repair within a conduit. The vagus nerve was then isolated and draped over a bipolar electrode. iVNS (40 Hz) or sham-iVNS (0 Hz) was delivered to the vagus nerve for three consecutive 5-minute blocks. Rats were closed and monitored for any health compromise for 7 days post-surgery. Data was collected via translational testing, including videofluoroscopic swallow study, plethysmography, and laryngoscopy. Testing was completed 1-2 weeks pre-operatively, 1-month post-surgery (MPS), and 2MPS. Following the final assessment, rats were euthanized and the larynges/lungs harvested for histology. One rat died due to post-surgical complications (asphyxiation from ingested bedding); all others survived. In all rats, transection of the RLN produced ipsilateral laryngeal paralysis confirmed by intraoperative laryngoscopy. Vagal nerve stimulation was tolerated and evoked swallowing in all iVNS-treated rats, confirmed by teeth/jaw/tongue movement and tongue EMG activity. At the 2MPS timepoint, the iVNS group had significantly greater laryngeal abduction during breathing ($p<0.05$) and improved respiratory parameters (tidal volume, minute ventilation, max inspiratory flow) during maximum challenge as compared to controls. Laryngeal adduction during swallowing remained impaired in both groups at 2MPS, with a large glottal gap increasing aspiration risk. Histology revealed laryngeal denervation atrophy and lung infection consistent with aspiration pneumonia in both groups with only mild improvement in the iVNS group. RLN transection/repair is survivable in adult rats with and without iVNS. Translational testing reveals that iVNS improves breathing but not swallowing-related laryngeal function. Denervation atrophy and aspiration pneumonia are concerns in both groups despite some benefit with iVNS. Further work will include iVNS protocol refinement for improved translational feasibility to human surgical patients.

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Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.11/K12

Topic: D.04. Brain Injury and Trauma

Support: Swiss National Science Foundation (SNSF), grant# 310030_219444

Title: The stimulation of CNS plasticity and repair with CNS-penetrating anti-Nogo-A antibodies

Authors: S. M. JOLY^{1,2}, *V. E. PERNET^{3,2,4,5},

¹Neurol., Dept. of Neurology, Inselspital, Bern Univ. Hospital, Univ. of Bern, Bern, Switzerland, Bern, Switzerland; ²Dept. of BioMedical Res. (DBMR), Univ. of Bern, Bern, Switzerland, Bern,

Switzerland; ³Neurol., Bern Univ. Hospital-Inselspital/University of Bern, Bern, Switzerland; ⁴Regenerative Med. Unit, Univ. Hosp. Ctr. of Quebec, Laval University, Quebec City, Qc, Canada, Quebec, QC, Canada; ⁵Dept. of Mol. Medicine, Fac. of Medicine, Laval University, Quebec City, Qc, Canada, Quebec, QC, Canada

Abstract: Nogo-A is a well-characterized inhibitor of central nervous system (CNS) plasticity. Its neutralization with function-blocking antibodies, such as 11C7 monoclonal antibody, enhances neuronal growth in the injured brain and spinal cord of rodents. However, the efficacy of 11C7 depends on invasive administration methods, across the blood-brain barrier (BBB). Here, our objective was to test two non-invasive strategies to facilitate blood-to-CNS transfer of modified 11C7, across the BBB or across the blood-cerebrospinal fluid barrier (BCSFB). On one hand, a BBB-crossing antibody named 11C7-scFv8D3 was engineered by recombinantly fusing the light chain of 11C7 with the single-chain variable fragment (scFv) of 8D3, an IgG specifically binding mouse TfR1. On the other hand, to determine if folate receptor alpha (FR α) expression in epithelial cells of the choroid plexus can mediate antibody transport across the BCSFB, 11C7 was chemically conjugated to folate (11C7-Fol, 8 folates/antibody). The binding properties of 11C7-scFv8D3 and 11C7-Fol were characterized by biolayer-interferometry (BLI) and ELISA. *In vivo*, antibody concentrations were monitored in plasma, CSF and CNS tissues, 5 min to 24 h after intravenous injection (i.v.) in the tail vein of C57BL/6J mice. Our BLI and ELISA results showed that the fusion of scFv8D3 in 11C7-scFv8D3 and folate conjugation in 11C7-Fol minimally change the affinity for Nogo-A ($KD \leq 1$ pM). After i.v., the plasma concentration of 11C7-scFv8D3 and 11C7-Fol rapidly declined compared with 11C7, suggesting fast receptor-mediated clearance from blood circulation. Surprisingly, the concentrations of 11C7-scFv8D3 and 11C7-Fol were lower than that of 11C7 in the CSF. At 24 h, the level of 11C7-Fol was reduced in the forebrain-midbrain, cerebellum and spinal cord, in comparison with 11C7. However, similar levels of 11C7-Fol and 11C7 were observed when normalized to plasma concentrations. A 2-3-fold increase of 11C7-Fol in the liver suggested that antibody trapping in peripheral organs expressing FR α reduces 11C7-Fol in plasma and CNS penetration. In contrast, 11C7-scFv8D3 was significantly increased in CNS tissues (2-9 fold). In addition, contrary to 11C7-Fol, 11C7-scFv8D3 reached the brain parenchyma in greater amounts than 11C7. In conclusion, our data suggest that targeting TfR1 is an efficient approach to enhance the penetration of Nogo-A-neutralizing antibodies in the CNS of mice. However, folate conjugation does not improve 11C7-Fol uptake in the CNS, presumably due to its capture by peripheral organs expressing FR α .

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Poster

PSTR333: Axon Protection and Regeneration

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR333.12/K13

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

Support: Lonestar Paralysis Foundation Gift
NIH R-01

Title: From rupture to repair: cell-specific roles of calcium, vesicular dynamics, cytoskeletal transport mechanisms, axoplasmic fluid forces in natural and polyethylene glycol-induced sealing of holes in the plasmalemma of axons and oocytes

Authors: *M. L. MENCEL¹, R. SOOD², V. GOKHALE², G. D. BITTNER²;
¹The Univ. of Texas at Austin, Austin, TX; ²Univ. of Texas at Austin, Austin, TX

Abstract: Traumatic damage produces a breach in the plasmalemma triggers calcium influx that mobilizes vesicles that interact to seal the hole in a complex and evolutionary conserved mechanism in eukaryotic cells. We examine similarities and differences in mechanisms by which vesicles **form**, **move** and **interact** to create a barrier at the site of injury in crayfish medial giant axons (MGAs) and *Xenopus* oocytes - two model systems with different strategies for membrane repair. We show that intact MGAs have relatively few cytoplasmic vesicles while oocytes contain many vesicles just interior to the plasmalemma. MGAs use microtubule-based transport and ATP-driven motor proteins (kinesin, dynein) to rapidly move vesicles (60-70mm/d) for long (20-100 μ m) distances. In contrast, oocytes use slower (5-6mm/d) actin-based transport over shorter (5-20 μ m) distances. Vesicle migration in both cells may also utilize bulk cytoplasmic flow—passive cytoplasmic movement driven by osmotic pressure gradients following injury. In MGAs, flow runs parallel to vesicle transport, and hypoosmotic conditions dramatically increase vesicle delivery and accumulation at injury sites. In oocytes, flow is perpendicular to vesicle migration, and osmotic changes have less effect. That is, active transport dominates vesicle trafficking in both cells, but bulk flow can have a synergistic effect, especially in large, elongated cells such as axons. In MGAs, most vesicles (8-15 μ m in diameter) progressively accumulate at the cut end for 10-15min, packing tightly as predicted by the ‘plug’ model of sealing. Rarely (<5% cases), vesicles undergo multiple fusions to form a very large (60-80 μ m) vesicle as predicted by the ‘patch’ model of membrane sealing but still seal the axon progressively rather than a single step. Finally, artificial repair using polyethylene glycol (PEG) bypasses all mechanisms and pathways involved in natural membrane repair. In MGAs, PEG produces rapid collapse and fusion of the cut end within seconds as opposed to minutes in natural sealing. Our data show that basic mechanisms of membrane repair are evolutionarily conserved but natural sealing is influenced by cell-specific factors such as cytoplasmic shape and volume, vesicle numbers and location, and cytoskeletal architecture.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR334.01/K14

Topic: D.05. Spinal Cord Injury and Plasticity

Support: VA RR&D B7332R IP50RX001045
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NIH R01 NS104442
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NIH R01 NS042291
Natural Sciences and Engineering Research Council of Canada RGPIN –
2018 – 06382
Canada Foundation for Innovation

Title: A critical interplay of neural stem cells and rehabilitation to support functional recovery after primate cervical SCI

Authors: *E. S. ROSENZWEIG¹, E. SINOPOULOU³, J. H. BROCK⁴, H. KUMAMARU⁶, E. A. SALEGIO⁷, M. J. CASTLE⁵, J. L. WEBER³, R. WURR⁸, R. MACON⁸, M. W. CHOW³, J. HUIE⁹, N. KYRITSIS¹⁰, L. A. HAVTON¹³, Y. S. NOUT-LOMAS¹⁴, C. J. SPARREY¹⁵, A. R. FERGUSON¹¹, M. S. BEATTIE¹⁶, J. C. BRESNAHAN¹², M. H. TUSZYNSKI²;
²Neurosci., ¹UCSD Dept. of Neurosciences, La Jolla, CA; ⁴Dept Neurosci, ⁵Neurosciences, ³UCSD, La Jolla, CA; ⁶Grad. Sch. of Medicine, Kyushu Univ., Fukuoka, Japan; ⁷ClearPoint Neuro, Solana Beach, CA; ⁸Univ. California, Davis, Davis, CA; ⁹Brain and Spinal Injury Center, Dept. of Neurolog. Surgery, ¹¹Neurolog. Surgery, ¹²Neurosurg., ¹⁰UCSF, San Francisco, CA; ¹³Dept. of Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY; ¹⁴Col. of Vet. Med. and Biomed. Sci., Colorado State Univ., Fort Collins, CO; ¹⁵Simon Fraser Univ., Surrey, BC, Canada; ¹⁶Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA

Abstract: We grafted a new, human embryonic stem cell-derived spinal cord Neural Stem Cell (NSC) line into rhesus monkeys in two spinal cord injury models: right-sided C7 hemisections and right-sided C6 hemi-contusions. Grafts were implanted two weeks after hemisections and 3-4 weeks after hemi-contusions. In hemisected subjects, Human spinal cord NSCs significantly improved functional outcomes on a skilled hand task by 9.2-fold ($P < 0.001$) compared to lesioned controls (object retrieval success $53.4 \pm 19.2\%$ success in grafted monkeys; $5.8 \pm 5.4\%$ in lesioned controls). Hemi-contused subjects exhibited greater anatomical and functional variability, and generally larger lesions, yet still exhibited significant functional improvements: there was a 2.9-fold improvement in grafted animals ($P < 0.001$) with a mean object retrieval success score of $24.6 \pm 11.7\%$ in monkeys with surviving grafts compared to $8.5 \pm 4.7\%$ in monkeys with non-surviving grafts. Notably, effort to engage in rehabilitation correlated with extent of functional recovery *only when a neural substrate was present to support neural plasticity*. In monkeys with complete right-sided hemisections and grafts, rehabilitation effort correlated with functional recovery ($R^2 = 0.77$; $P = 0.059$). In control hemisected monkeys lacking a substrate for structural plasticity, rehabilitation effort did *not* correlate with extent of functional recovery ($R^2 = 0.07$; $P = 0.259$). In monkeys with right-sided contusions and grafts, rehabilitation effort correlated with functional recovery ($R^2 = 0.71$; $P = 0.009$). In monkeys with right-sided contusions that lacked surviving grafts - yet had spared tissue on the lesioned side that could potentially support anatomical plasticity - rehabilitation effort *did* correlate with functional recovery ($R^2 = 0.81$; $P = 0.049$). The extent of correlation of recovery with effort was more significant among contused monkeys with grafts than it was among contused monkeys without grafts, supporting the importance of grafts in providing a mechanism for enhancing functional outcomes. Thus, experience-dependent plasticity interacts with a neural repair strategy to

optimize functional outcomes, suggesting a need to design a dedicated rehabilitation strategy for future clinical trials. This human neural stem cell line exhibits the most extensive efficacy to date of a candidate pro-regenerative therapy for spinal cord injury and merits clinical translation.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR334.02/K15

Topic: D.05. Spinal Cord Injury and Plasticity

Support: VA Merrit Review 1 I01 RX003776-01A2 to PL
VA RR&D Gordon Mansfield Spinal Cord Injury Consortium
I50RX001706-06 to MT

Title: Corticospinal Axonal Regeneration Into and Beyond Cervical Spinal Cord Injury Using a Combination of Neural Stem Cell Grafts and Gene Delivery of BDNF, IGF-1, DCLK2 and Myc and Co-deletion of PTEN/SOCS3

Authors: H. HEURING¹, S. WANG¹, M. MITCHELL¹, A. TORREBLANCA-ZANCA¹, M. J. CASTLE¹, M. H. TUSZYNSKI^{1,2}, *P. P. LU^{1,2};
¹Univ. of California San Diego, La Jolla, CA; ²VA San Diego Healthcare Syst., San Diego, CA

Abstract: Corticospinal tract (CST) axons are critical for control of voluntary movement in humans and their growth must be recruited to restore hand function after SCI. CST axons regenerate into but not beyond spinal cord neural stem cell (NSC) grafts placed into sites of C4 spinal cord injury in mice. To determine whether CST growth could be stimulated to bridge entirely through and beyond NSC grafts, we tested either co-deletion of PTEN/SOCS3, or in separate animals combined overexpression of BDNF, IGF-1, DCLK2 and cMyc. Adult PTEN/SOCS3/tdTomato flox/flox mice underwent C7 injections of retroAAV expressing FLP-dependent Cre, or FLP-dependent mCherry (N=8 group). Three weeks later, we injected AAV-expressing FLP into the motor cortex: this resulted in specific expression of Cre in Forelimb-CST neurons, in turn resulting in deletion of *PTEN/SOCS3* and expression of tdTomato in the PTEN/SOCS3 deleted group or expression of mCherry only in the control group. One week later they received C4 spinal cord bilateral dorsal column lesions to transect the corticospinal projection, followed immediately by implants of NSCs. 6 weeks later, compared to mCherry-expressing control mice, animals with PTEN/SOCS3 deletion exhibited substantially greater regeneration of forelimb CST axons throughout the graft. However, CST axons rarely

regenerated beyond the graft. A separate cohort of PTEN/SOCS3 flox mice (N=8) received additional injections of 3 different AAV9 vectors expressing BDNF and IGF-1, cMyc, and Cre-dependent DCLK2. All vectors were combined in equal concentrations and injected into each of 8 mice one month prior to C4 dorsal column lesions. We did not control for the combination groups because this was an initial experiment to determine whether this combinatorial treatment would promote CST regeneration beyond the lesion; if it did, then we would subsequently test individual genes and smaller sets of combined genes. After 6 weeks, indeed we observed consistent regeneration of CST axons into and beyond the lesion site. For the first time, we observed distant CST axon regeneration through host white matter in addition to host gray matter. CST axons regenerated for a maximal distance of 3 mm into and beyond the lesion. Growth beyond the lesion significantly exceeded the PTEN/SOCS3 knockout group ($P < 0.0001$). A mean of 55.5 ± 11.3 axons regenerated 250 μm beyond the lesion in the multiple gene treatment group compared to 0 ± 0 axons in the PTEN/SOCS3 group ($P < 0.0001$). We conclude that bridging host axon regeneration is feasible through distal host white and gray matter. In ongoing studies we will determine which genes are minimally necessary to support bridging axonal regeneration.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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Program #/Poster #: PSTR334.03/K16

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH R21 NS135479
VA I01 RX003776

Title: Extensive transduction of corticospinal neurons by AAV2retro injection into spinal white matter

Authors: *K. T. NAKASHIMA¹, S. WANG², P. P. LU³, M. J. CASTLE⁴;

¹Neurosciences, Univ. of California San Diego, San Diego, CA; ²Anesthesiol., VA Healthcare System, UCSD, La Jolla, CA; ³UCSD Dept. of Neurosciences, La Jolla, CA; ⁴Neurosciences, UCSD, La Jolla, CA

Abstract: Adult neurons in the central nervous system often fail to regenerate after spinal cord injury (SCI). Regenerative gene therapies could potentially promote corticospinal axon regeneration, restoration of motor circuitry, and functional improvement after SCI, but translational methods for targeted gene delivery to corticospinal neurons are needed. AAV2retro is an engineered variant of the adeno-associated virus 2 (AAV2) capsid that demonstrates greatly enhanced retrograde transduction of projection neurons. When injected into spinal gray matter,

AAV2retro retrogradely transduces neurons in motor cortex that innervate the injected spinal level.

We hypothesized that injection of AAV2retro into the dorsal column white matter immediately rostral of a mouse cervical spinal injury would target transected axons and broadly transduce both forelimb and hindlimb corticospinal neurons. We tested this hypothesis by comparing four groups of mice treated with AAV2retro carrying the tdTomato reporter gene by: 1) injection into the dorsal column white matter rostral of a C4 dorsal column lesion, 2) injection into the intermediate gray matter rostral of a C4 dorsal column lesion, 3) injection into intact dorsal column white matter without SCI, and 4) injection into intact gray matter without SCI. Injection of AAV2retro into dorsal column white matter significantly increased the number of retrogradely labeled corticospinal neurons throughout both forelimb and hindlimb motor cortex relative to gray matter injection. Surprisingly, similarly extensive retrograde transduction of corticospinal neurons was observed after white matter injection in both intact and injured animals, suggesting that AAV2retro can efficiently target axons of passage in uninjured spinal white matter, and that transection by SCI does not make these corticospinal axons more accessible to AAV2retro. We conclude that AAV2retro appears to enter axons of passage in the dorsal white matter of the spinal cord, and that injecting cervical white matter can efficiently target both forelimb and hindlimb corticospinal neurons. This new approach for targeted gene delivery to corticospinal neurons could improve the safety and specificity of regenerative gene therapies for spinal cord injury.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.04/L1

Topic: D.05. Spinal Cord Injury and Plasticity

Support: California Institute of Regenerative Medicine CLIN1-15450
Veterans Administration 1IU1RX005349
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Title: Successful Generation of a GMP Spinal Cord NSC Line for Treatment of Human Spinal Cord Injury

Authors: *T. VOKES¹, A. BLESCH², J. H. BROCK⁴, M. H. TUSZYNSKI³;
²Neurosciences, ³Neurosci., ¹UCSD Dept. of Neurosciences, La Jolla, CA; ⁴Dept Neurosci, UCSD, La Jolla, CA

Abstract: We have developed a scalable, fully GMP-compliant spinal cord neural stem cell (scNSC) product derived from H9 embryonic stem cells (ESCs) for use in human clinical trials targeting spinal cord injury (SCI). These “H9-scNSCs” have previously shown partial restoration

of motor function in rodent and non-human primate SCI models by forming neural relays across lesion sites. Building on prior work in our laboratory optimizing spinal NSC induction using small-molecule patterning (Kumamaru et al., Nat Methods, 2018; Li et al., Ex Neurol, 2024), we adapted the derivation process to meet FDA and scientific standards in a GMP-compliant format. GMP scNSC banks (~3 billion cells per bank) were manufactured at the UC San Diego Advanced Cell Therapy Laboratory (ACTL), with protocols designed to support IND-enabling studies and upcoming Phase 1-2 clinical trials. Phenotypic analysis of these cells in preliminary studies shows increased expression of SOX1 and PAX6, absence of expression of pluripotency markers (OCT4, NANOG), reduced expression of mesodermal genes (Brachyury, TBX6), and expression of HOXC6, indicating spinal cord identity (caudal to C2-3 level). Flow cytometry confirms a heterogeneous NSC population, containing both early NSCs and more differentiated neural progenitor cells (NPCs). Rigorous testing for karyotype, sterility, endotoxin, mycoplasma, and adventitious agents in compliance with FDA standards for human transplantation are in progress. Functionally, H9-scNSCs differentiate into mature, electrically active neurons, as shown by the generation of action potentials and expression of MAP2 and Tau in preliminary studies. We hypothesize that this scNSC population will support spinal cord repair post-transplant by forming neuronal relays and providing a supportive neural microenvironment. This work provides a clinically ready, scalable, and reproducible cell manufacturing platform to enable first-in-human trials for SCI that we propose to begin in 2027. Supported by California Institute of Regenerative Medicine CLIN1-15450 and the Veterans Administration 1IU1RX005349 and B7332R. The authors have no conflicts of interest.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.05/L2

Topic: D.05. Spinal Cord Injury and Plasticity

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NS105478
NS042291
RGPIN 06382

Title: A Clinical Neural Stem Cell Line Extensively Restores Skilled Forelimb Function After Primate Cervical Spinal Cord Injury - Anatomical Findings

Authors: *E. SINOPOULOU¹, E. S. ROSENZWEIG⁴, J. H. BROCK², H. KUMAMARU⁶, E. A. SALEGIO⁷, M. J. CASTLE³, J. L. WEBER⁸, R. WURR⁹, R. MACON⁹, M. W. CHOW⁹, R.

HUIE⁹, N. KYRITSIS⁹, L. HAVTON¹⁰, C. SPARREY¹¹, A. R. FERGUSON¹², M. S. BEATTIE¹⁴, J. C. BRESNAHAN¹³, M. H. TUSZYNSKI⁵;

²Dept Neurosci, ³Neurosciences, ¹UCSD, La Jolla, CA; ⁴Neurosciences, ⁵Neurosci., UCSD Dept. of Neurosciences, La Jolla, CA; ⁶Grad. Sch. of Medicine, Kyushu Univ., Fukuoka, Japan; ⁷ClearPoint Neuro, Solana Beach, CA; ⁸The Scripps Res. Inst., San Diego, CA; ⁹UCD, Davis, CA; ¹⁰Mount Sinai Sch. of Med., NEW YORK, NY; ¹¹Simon Fraser Univ., Surrey, BC, Canada; ¹²Neurolog. Surgery, ¹³Neurosurg., UCSF, San Francisco, CA; ¹⁴Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA

Abstract: We previously demonstrated that human neural stem cells (hNSCs) or multipotent neural progenitor cells (hNPCs) grafted into sites of rodent or rhesus monkey spinal cord injury (SCI) survive, extend axons, form synapses, support host axon regeneration, and improve functional recovery (Lu et al., *Cell* 2012; Lu et al., *Neuron* 2014; Kadoya et al., *Nat Med* 2016; Rosenzweig et al., *Nat Med* 2018). To enable clinical translation we have developed a new, GMP-compatible spinal cord neural stem cell line appropriate for humans: **H9-scNSCs**. This is a human embryonic stem cell-derived Neural Stem Cell line specifically driven to a *spinal cord* identity (Kumamaru et al., *Nat Methods* 2018). We grafted these **H9-scNSC** line into rhesus monkeys in two spinal cord injury models: C7 hemisections and C6 hemi-contusions. Grafts were implanted two weeks after hemisections and 3-4 weeks after hemi-contusions. Detailed functional results are reported in the accompanying poster (Rosenzweig et al.) We demonstrate that H9-scNSC grafts placed into sites of C7 hemisection or C6 unilateral contusion SCI support regeneration of host corticospinal, raphespinal and heavy-chain NF-expressing host axons into the lesion 4 months post-injury. In turn, the grafts extend a mean of $66,200 \pm 25,100$ axons in the hemisection model, and $87,636 \pm 30,136$ axons in the hemicontusion model, into the distal host spinal cord over distances as long as 40 mm. $18 \pm 6\%$ of grafted cells express the neuronal marker NeuN, $51 \pm 6\%$ express the astrocyte marker Sox9, and $9 \pm 2\%$ express the oligodendrocyte marker Olig2. The neuron-to-astrocyte ratio mimics that of normal human spinal cord gray matter. Among cells expressing the neuronal marker NeuN, $71 \pm 3\%$ expressed CamKII, indicating that the majority of neurons adopted an excitatory fate. $15 \pm 4\%$ of NeuN+ cells expressed the inhibitory neuronal marker GABA. **H9-scNSCs** have now been produced under GMP conditions and will undergo clinical translation if ongoing GLP studies demonstrate safety and anatomical efficacy. **ACKNOWLEDGMENTS:** This work was supported by the Veterans Administration (IP50RX001045, B7332R, I01RX002245), NIH (NS104442, NS105478, NS042291), Natural Sciences and Engineering Research Council of Canada (RGPIN 06382), and the Canada Foundation for Innovation.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.06/L3

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NRF RS-2024-00419269
RS-2023-00254156
MOTIE P246800201

Title: Aav9-mediated synaptic gene therapy enhances recovery after spinal cord injury

Authors: *A. SONG¹, Y. NOH², B. CHOI³, J. HYUN⁴;

¹Nanobiomedical Sci., Dankook Univ., Cheonan, Korea, Republic of; ²Dankook Univ., Cheonan-si, Korea, Republic of; ⁴Rehabil. Med., ³Dankook Univ., Cheonan, Korea, Republic of

Abstract: Spinal cord injury (SCI) initiates a cascade of pathological events, including neuronal death, synaptic disintegration, and persistent neuroinflammation, ultimately impairing locomotor recovery. Transcriptomic analyses have identified significant downregulation of critical excitatory synaptic genes—vesicular glutamate transporter 1 (VGLUT1) and leucine-rich glioma-inactivated 1 (LGI1)—following SCI. VGLUT1 is essential for glutamate packaging into synaptic vesicles, while LGI1 regulates synaptic excitability and trans-synaptic stability via interactions with ADAM22/23. Loss of these genes disrupts glutamatergic homeostasis and circuit integrity, exacerbating excitotoxicity and impairing neuroplasticity. AAV serotype 9 (AAV9) is a delivery platform due to its strong neuronal tropism, widespread CNS transduction, and capacity for long-term gene expression in spinal tissue. Given this pathological depletion, we hypothesized that AAV9-mediated gene therapy restoring VGLUT1 and LGI1 expression may promote synaptic repair and functional recovery after SCI. We used AAV9 vectors encoding VGLUT1, LGI1, or both, delivered adjacent to the lesion cavity in a rat moderate contusion SCI model. Animals were monitored for 12 weeks post-injection. In vitro, primary cortical neurons subjected to H₂O₂-induced oxidative damage exhibited increased survival, neurite outgrowth, and synaptic marker expression after AAV9-VGLUT1 or AAV9-LGI1 transduction, confirming neuroprotective effects. In vivo, histological analysis revealed reduced lesion cavity volume and ED1-positive microglia/macrophage infiltration across gene therapy groups, with combined treatment yielding the greatest effect. Behaviorally, treated rats demonstrated significantly enhanced locomotor performance, assessed by the Basso, Beattie, and Bresnahan (BBB) scale and horizontal ladder test. LGI1 therapy conferred slightly greater gains than VGLUT1, yet combination therapy produced the most consistent and sustained improvements. Molecular validation confirmed robust re-expression of VGLUT1 and LGI1, with upregulation of genes involved in excitatory transmission, synaptic remodeling, and neuroimmune modulation. Transcriptomic enrichment identified activation of pathways linked to glutamate release, AMPA receptor trafficking, neuroplasticity, and inflammatory resolution. In conclusion, these findings show that AAV9-VGLUT1 and LGI1 reverses SCI-induced gene depletion, improves synaptic and molecular repair, and enhances motor outcomes. This synaptic gene therapy offers a promising translational strategy for restoring function after SCI.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.07/L4

Topic: D.05. Spinal Cord Injury and Plasticity

Support: FAPESP 2025/10792-9
FAPESP 2013/08028-1

Title: Early beneficial motor effects of neurospheres derived from olfactory epithelium ectomesenchymal stem cells engrafted in rats with moderate spinal cord contusion

Authors: *L. A. HADDAD¹, B. G. M. M. COIMBRA², G. B. SANTOS³, N. DE ARAUJO¹, T. E. P. BARROS-FILHO², R. M. MARCON², A. F. CRISTANTE²;

¹Genet. and Evolutionary Biol., ²Orthopedics, Univ. of São Paulo, Sao Paulo, Brazil;

³Orthopedics, Univ. De São Paulo, Sao Paulo, Brazil

Abstract: Spinal cord injury (SCI) is a major public health concern with devastating clinical and psychological complications. Laminectomy, the gold-standard surgical procedure to decompress the spinal cord canal, has a limited overall impact on patients with SCI. Multiple approaches have been studied to improve clinical outcomes. In previous work, neonate rat forebrain-derived neurospheres (Nsp) in 70% Matrigel® (M7) or M7 alone improved motor function as early as two weeks after moderate thoracic spinal cord contusion (SCC). On the sixth postoperative week, the functional effects of Nsp-M7 were superior to those of M7 or Nsp-M7 that had been treated *in vitro* for 24 hours to initiate neuronal differentiation before surgery. Thus, as presented by us and others, undifferentiated Nsp appear more beneficial than differentiating cells for injury repair. Here, we employed adult rat olfactory epithelium ectomesenchymal stem cells (OSC) to derive Nsp *in vitro* for SCC engraftment in 90% Matrigel® (M9). After nasal septum isolation and culture of dissociated olfactory epithelium, a mixed population of cells was observed *in vitro*. The adherent population was assessed by cytometry. The floating OSC population was harvested and cultured under non adherent conditions for formation of Nsp, which was evaluated for self-renewal and neuronal differentiation. SCC was performed as standardized using the NYU-impactor (NY Univ., 1992; 12.5-mm height) in twelve rats per group, immediately followed by durotomy with or without engraftment of undifferentiated OSC-Nsp in M9 (OSC-Nsp-M9) or M9 only. Functional assessments were conducted for six weeks based on the BBB scale and presence of neurogenic bladder. Spinal cords were collected after rat sacrifice on the sixth postoperative week and analyzed by histology and immunofluorescence assays. OSC-Nsp-M9 was associated with functional motor improvement from as early as seven days after SCI. Animals from both control and M9 groups started improving equally from week 3, when OSC-Nsp-M9 effects were the best among groups (Kruskal-Wallis and Dunn's *post-hoc* tests, $p < 0.05$) through week 5. On the sixth week, OSC-Nsp-M9 and M9 groups did not differ and presented better motor and bladder functions than control. In conclusion, OSC-Nsp-M9 engrafted in rats immediately after SCC associated with motor functional improvement as early as one week after injury. The more easily accessible source of stem cells from adult olfactory epithelium is

amenable to further preclinical research and promising for future translational studies of human autografts. Funding support: FAPESP 2013/08028-1 and 2025/10792-9

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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Program #/Poster #: PSTR334.08/L5

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01DK133195-01A1

Title: Lower urinary tract response to spinal cord epidural stimulation pre- and post-select peripheral neurectomy in spinally injured rats

Authors: *K. M. BEASLEY^{1,2}, D. MEDINA AGUINAGA^{1,2}, B. PEREZ DE CORCHO VAZQUEZ^{1,2}, C. H. HUBSCHER^{1,2};

¹Anatom. Sci. and Neurobio., Univ. of Louisville Sch. of Med., Louisville, KY; ²Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY

Abstract: Spinal cord injury (SCI) often leads to severe impairment of multiple body systems, greatly impacting quality of life. While the urinary bladder is initially areflexic during spinal shock following SCI, reflexive voiding usually develops within 2-12 weeks in humans and 1-2 weeks in the rat. However, voiding post-SCI is often impacted and may display detrusor-sphincter dyssynergia (DSD), which is characterized by uncoordinated bladder and external urethral sphincter (EUS) contractions, causing inefficient emptying and smooth muscle hypertrophy. Likewise, the frequency of bladder contractions may increase and contribute to dangerously high intravesical pressures and storage dysfunction, a condition known as neurogenic detrusor overactivity (NDO). Spinal cord epidural stimulation (scES) is a novel therapy that has been shown to improve lower urinary tract (LUT) function in both humans and pre-clinical experimental models post-SCI. Our group hypothesized that the improvements in LUT function seen with scES result from modulation of the neural networks which project to the bladder or EUS that are located within these sites of stimulation. To gain insight into the neural mechanisms behind scES-induced effects on the LUT, the Hubscher laboratory has developed a model combining thoracolumbar or lumbosacral scES with a neurectomy of either the pelvic, hypogastric, or pudendal nerves in female rats with moderate-severe SCI (215 kdyn) during urethane-anesthetized cystometry-electromyography at 7-, 14-, or 28-days post-injury. Current data suggests that scES is effective for promoting voiding and storage pre- and post-select peripheral nerve transections in moderate-severe T9 contused female rats under voiding cystometry. The data indicates that the improvements in LUT function seen in our current and

previous studies with T9 level SCI is likely driven by multiple peripheral nerves which innervate the urinary bladder rather than a single source of output.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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Program #/Poster #: PSTR334.09/L6

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH Grant NS119475

Title: Transcutaneous spinal cord stimulation to prime step-training and maximize the recovery of spasticity after spinal cord injury

Authors: *T. E. GRAY¹, M.-P. CÔTÉ²;

¹Drexel Univ. Col. of Med. Neurosci. Program, Philadelphia, PA; ²Dept. Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstract: Following spinal cord injury (SCI), people live with profound deficits in locomotion and volitional movement. Improvements to these deficits can be achieved through activity-based therapies. However, following SCI about 75% of patients develop spasticity, a condition involving pathological motor behaviors including heightened muscle tension, involuntary movements, and hyperactive reflexes. These behaviors impede rehabilitation. Transcutaneous spinal cord stimulation (tSCS) is a non-invasive treatment that can diminish spasticity after SCI and contribute to functional recovery through neuroplastic changes. We hypothesize that tSCS can prime step-training further enhancing spasticity reduction. Further, we will determine whether the effect of tSCS is optimal when delivered daily immediately prior to step-training or as a full program prior to initiating step-training. Adult female Sprague-Dawley rats received a severe T9 contusion injury (250 kdyn). Rats were randomly assigned to one of following treatment groups: (1) tSCS, (2) sham stimulation, (3) tSCS immediately followed by step-training each day (acute priming), or (4) tSCS administered for several weeks prior to the onset of step-training (long-term priming). A terminal experiment demonstrated that both tSCS and step-training, whether applied individually or in combination, restored frequency-dependent depression and presynaptic inhibition. tSCS administered prior to step-training led to greater reductions in spasticity compared to tSCS or step-training alone both in the acute priming and long-term priming groups. Our findings indicate that both tSCS and step-training mitigate spasticity symptoms, and administering tSCS immediately before step-training enhances spasticity reduction more effectively than step-training alone.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.10/L7

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Craig H Nielsen Foundation Award #546841
Kentucky Spinal Cord and Head Injury Research Trust #17-5

Title: Effects of chronicity, severity and spinal level on bowel function after contusion in male rats

Authors: ***B. PEREZ DE CORCHO VAZQUEZ**¹, R. F. HOEY⁴, D. MEDINA AGUINAGA², J. FELL¹, C. HUBSCHER³;
²Anatom. Sci. and neurobiology, ³Dept Anatom. Sci. & Neuro, ¹Univ. of Louisville Anatom. Sci. & Neurobio., Louisville, KY; ⁴Anatom. Sci. and Neurobio., MetroHealth Syst., South Euclid, OH

Abstract: The high occurrence of bowel-complications in spinal cord injury (SCI) patients often leads to rehospitalization, affecting morbidity and quality of life of individuals with SCI. In humans, SCI leads to various functional changes that collectively disrupt the ability of the gastrointestinal tract to store and evacuate efficiently. Approximately 75% of the SCI population has symptoms of neurogenic bowel dysfunction which can present as constipation, fecal incontinence, abdominal distension, fecal impaction, among others. The mechanisms behind these deficits, however, are not well understood. In the present study, a clinically relevant rodent T3 or T9 contusion model with graded injury severities (mild, moderate, and severe) was utilized to examine SCI induced bowel dysfunction. In addition, the effect of chronicity in rodents with moderate contusion was studied by performing terminal experiments at different timepoints post-injury (acute, sub-acute, chronic). Outcome measures included both external anal sphincter electromyography (EAS-EMG) and anorectal manometry, which is commonly used in clinical settings to assess colonic dysfunction in individuals with SCI. Significant differences were found in EAS response latency, amplitude and duration of contractile activity in terms of chronicity, severity and location of SCI. Frequency of giant contractions, which are primarily responsible for mass propulsion of material through the colorectum, in contrast was quite variable across individual animals within groups. The findings to date illustrate multiple variables contributing to neurogenic bowel dysfunction following incomplete SCI, all of which are likely to contribute to existing challenges. A complete understanding of the existing complexities surrounding both continence and defecation will be informative for clinical management and provide specific targets for the development of novel therapeutic strategies.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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Program #/Poster #: PSTR334.11/L8

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Morton Cure Paralysis Fund
Bryon Riesch Paralysis Foundation
Craig H. Neilsen Foundation

Title: Restoring Hand Function after Cervical Spinal Cord Injury: A Stimulogenetic Strategy Combining Motor Cortex Stimulation and Neurotrophin-3 Overexpression.

Authors: *A. PAL¹, J. RAJAVONG¹, T. J. CAMPION, III¹, G. KOMA², A. SPENCE², G. M. SMITH¹;

¹Ctr. for Neural Develop. and Repair, ²Bioengineering, Temple Univ., Philadelphia, PA

Abstract: Spinal cord injury (SCI) often leads to persistent upper limb spasticity and motor dysfunction, severely limiting rehabilitation outcomes and quality of life. These deficits are driven in part by maladaptive plasticity and disrupted excitatory-inhibitory balance in spinal circuits. Current therapies offer only partial symptom relief and frequently impair voluntary control. In this study, we tested a combinatorial, stimulogenetic strategy to enhance functional recovery and reduce spasticity after cervical SCI by targeting both motor and sensory pathways. Specifically, we paired repetitive motor cortex stimulation (MCS) with retrograde AAV-mediated overexpression of neurotrophin-3 (NT-3), a neurotrophic factor that supports synaptic plasticity, corticospinal tract (CST) sprouting, and proprioceptive reorganization in spinal motor neurons (MNs) and dorsal root ganglia (DRGs). Adult rats received moderate C5 contusions (200 kDyne) and were randomly assigned to MCS+NT-3, MCS-only, NT-3-only, or sham groups. AAV-retro-NT-3 or AAV-retro-GFP was injected into distal forelimb muscles to transduce MNs and DRGs, and cortical electrodes were implanted for MCS. Functional outcomes were assessed over 10 weeks using H-reflex modulation, a force-based lever-pull task to quantify spastic resistance during both passive and active forelimb flexion/extension, and behavioral assays including skilled reaching, grooming, and locomotion. Histological analysis evaluated CST sprouting, MN survival, and proprioceptive synaptic reorganization. Only the MCS+NT-3 group showed significant restoration of frequency-dependent depression (FDD) of the H-reflex and suppression of abnormal resistance forces—hallmarks of reduced spasticity. Neither MCS nor NT-3 alone was sufficient to normalize these responses. The combined group also exhibited elevated H-reflex thresholds, reduced reflex amplitudes, and sustained improvements in dexterity, distal joint control, and limb kinematics. Histology confirmed enhanced CST sprouting and targeted reorganization of proprioceptive afferents onto MNs, suggesting restored sensorimotor integration. These results demonstrate that pairing MCS with targeted NT-3 gene delivery effectively rebalances spinal circuitry, suppresses upper limb spasticity, and improves

motor outcomes. This dual-modality approach holds strong translational potential for restoring hand function and reducing chronic impairments following cervical SCI.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.12/L9

Topic: D.05. Spinal Cord Injury and Plasticity

Support: DOD award: W81XWH-21-1-0551

Title: Therapeutic effects of spinal stimulation on H-reflex modulation and spasticity reduction in rats with cervical spinal cord injury

Authors: *A. SADEGHI¹, I. MAROSSA², R. L. MURPHY³, R. B. ROBINSON⁴, C. T. MORITZ⁵, S. I. PERLMUTTER⁶, C. P. HOFSTETTER⁷;

¹Department of Neurolog. Surgery, ³Physiol. and Biophysics, ²Univ. of Washington, Seattle, WA; ⁴Univ. of Washington, Edmonds, WA; ⁵Rehabil. Med., ⁶Dept Neurobio. & Biophysics, Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA; ⁷Neurosurg., Dept of Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstract: Traumatic spinal cord injury (tSCI), particularly at the cervical level, often results in permanent sensory and motor deficits, severely impairing upper limb function. Despite advances in interventions and neurorehabilitation therapies, spasticity remains a prevalent complication of the chronic tSCI, often interfering with functional recovery. In this preclinical study, we investigated electrical spinal stimulation as a neuromodulatory treatment to mitigate spasticity and enhance forelimb function. We first conducted terminal electrophysiological studies in urethane-anesthetized rats to determine the effect of paraspinal electrical stimulation at C5 and C6 on spinal reflexes and excitability. H-reflex was recorded and analyzed as an electrophysiological biomarker of spasticity. H-reflex testing was conducted via stimuli applied to the median nerve before, as the baseline, and after electrical stimulation. Spinal stimulation was delivered at 75-80% of movement threshold using either a low frequency (20 or 30 Hz) or a high frequency (100 Hz). Electrical stimulation resulted in immediate and distinct frequency-dependent modulation of spinal reflexes, where low-frequency stimulation increased the H/M ratio by 1.5-fold ($p < 0.001$), while high-frequency stimulation caused a 1.9-fold reduction in the H/M ratio ($p < 0.001$). We then tested the long-term effects of paraspinal stimulation on spasticity. Female Long-Evans rats received a severe hemi-contusion tSCI at the C4 cervical level using an Infinite Horizon Impactor set to 200 kdyn. In the chronic phase of injury, tSCI rats were implanted with spinal electrodes for therapeutic SCS at the cervical level. Additionally, a cuff electrode was placed on the median nerve, and EMG electrodes were implanted in

ipsilesional forelimb muscles to monitor the regulation of abnormal H-reflexes, an electrophysiological biomarker of spasticity. Additionally, we employed a novel elbow rotation robotic device to quantitatively monitor velocity-dependent resistance of the impaired forelimb. We observed a significant increase in peripheral muscle resistance in the injured animals compared to control animals (18.44 ± 3.56 mN vs. 3.56 ± 1.64 mN, respectively; t-test, $p < 0.05$). Preliminary results suggest high-frequency biphasic stimulation can reduce the size of the H-reflex by approximately 37% and suppress spasticity by 33-66% compared to untreated rats. This preclinical study of forelimb spasticity will inform more effective spinal stimulation treatment protocols for spasticity and upper extremity function for people with tSCI.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

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Program #/Poster #: PSTR334.13/L10

Topic: D.05. Spinal Cord Injury and Plasticity

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Title: Gut-brain modulation of taste: Sleeve gastrectomy and fecal microbiome transplant alter taste preferences following spinal cord injury

Authors: J. SNYDER¹, G. M. HOLMES¹, *A. HAJNAL²;

¹Neurosci. and Exptl. Therapeut., ²Penn State Univ. Col. of Med., Hershey, PA

Abstract: Approximately two-thirds of individuals with spinal cord injury (SCI) become overweight or obese. While metabolic surgeries such as sleeve gastrectomy (SG) are widely used to support long-term weight loss and improve glycemic control, it is not well understood how individuals with SCI respond to such interventions compared to those without SCI, particularly in terms of diet-driven behavior and sensory processing. In this study, male Wistar rats received either thoracic (T3) contusion injuries or sham surgeries. After recovery, all rats were fed a high-fat diet (60% kcal from fat) for five to six weeks prior to SG. Taste responses were evaluated using brief-access lick rate tests and 24-hour two-bottle choice (2BC) assays. Current work extends previous findings by exploring the contribution of gut microbial composition through fecal microbiome transplant (FMT), comparing the effects of autologous and heterologous transplants on taste and metabolic outcomes (body composition, metabolic rate, and glycemic control). Prior to diet exposure, SCI rats exhibited reduced licking responses to concentrated sucrose (0.6-1.5 M) and increased responses to sodium chloride (0.01-0.06 M), with no significant differences in 2BC preferences. SG reduced sucrose preference in both SCI and control animals, with SCI rats showing heightened sensitivity to this effect. Additionally, SCI rats that underwent SG displayed elevated cFos activation in the rostral nucleus of the solitary

tract following oral sucrose stimulation compared to non-SCI SG rats. Notably, altered taste responses—reduced sucrose preference, changes in salt and sour sensitivity—were transferrable via FMT from SG-treated donors, supporting a mechanistic role for the gut microbiome in modulating taste behavior following SG. These findings indicate that microbiome shifts, at least in part, contribute to the sensory and metabolic changes induced by SG in SCI conditions, offering new insight into microbial influences on post-surgical outcomes that were not addressed in earlier work.

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Poster

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Title: Polyethylene glycol-600 and photobiomodulation therapy in male Wistar rats following spinal cord injury

Authors: *N. MOJARAD^{1,3}, B. STEWART¹, S. WRIGHT¹, T. DENNISON¹, H. DENBROEDER¹, N. BURNS¹, J. ROSSIGNOL², G. L. DUNBAR, Sr.⁴;
¹Field Neurosciences Inst. Lab. for Restorative Neurol., ²Col. of Med., Central Michigan Univ., Mount Pleasant, MI; ³Program in Neurosci., Central Michigan Univ. Grad. Program In Neurosci., Mount Pleasant, MI; ⁴Psychology/Program in Neurosci., CENTRAL MICHIGAN UNIVERSITY, MOUNT PLEASANT, MI

Abstract: Spinal cord injury (SCI) leads to temporary or permanent neurological damage, causing severe motor, sensory, bladder, and sexual dysfunction. The pathophysiology involves inflammation, oxidative stress, and cell death, damaging vasculature, axons, and membranes, creating a hostile environment for axonal regrowth. Currently, no cure exists, and treatments primarily provide symptomatic relief. Recent studies suggest combinational treatments may be more effective than single therapies by targeting multiple pathological mechanisms simultaneously. Therefore, this study aimed to evaluate the efficacy of two neuroprotective treatments—Polyethylene Glycol (PEG) and Photobiomodulation Therapy (PBMT)—applied individually and in combination to enhance therapeutic outcomes. PEG, a biocompatible synthetic polymer, promotes cell membrane fusion, seals damaged neurons, and reduces glial

scar formation. PBMT, meanwhile, is promising due to its anti-inflammatory effects and tissue repair promotion. For this aim, male Wistar rats underwent a compression injury at the thoracic 9 level. Immediately post-injury, 10 μ L of PEG-600 was applied directly to the lesion in the PEG and combination groups using a Hamilton syringe, while controls received sterile saline. PBMT treatment began 30 minutes post-injury in PBMT and combination groups, applied to the lesion site and eight surrounding points for 15 seconds each, daily for seven weeks. Motor function was assessed by the Basso, Beattie, and Bresnahan (BBB) locomotor scale and horizontal ladder rung walking test before injury, at day three, then weekly for seven weeks. Afterward, rats were euthanized for tissue extraction and immunohistochemical analysis. The BBB test revealed significant group differences ($P=0.009$), with PEG and PBMT groups showing greater motor improvement than the combination and vehicle groups from weeks 3 to 7. This indicates that PEG-600 and PBMT individually promote gross locomotor recovery, whereas their combination did not enhance recovery and performed similarly to controls. The horizontal ladder test showed no significant differences across groups, suggesting fine motor coordination was not restored by treatments. In conclusion, immediate post-injury PEG-600 or PBMT administration enhances gross motor recovery, but their combination lacks additive effects. These results support PEG and PBMT as standalone neuroprotective agents after SCI. Further research should explore mechanisms underlying their effects and optimize combination therapy timing, dosage, or delivery, potentially with adjunctive strategies like stem cell transplantation or neurotrophic factors.

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Poster

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Title: Advancing Human V2a Spinal Interneuron Therapy with GMP-Grade Differentiation and In Vivo Transcriptomics

Authors: *W. KWONG¹, M. DOMINGUEZ¹, A. BANDEL¹, P. HURLEY¹, M. A. LANE², L. ZHOLUDEVA¹;

¹Gladstone Inst., San Francisco, CA; ²Neurobio. & Anat., Drexel Univ., Philadelphia, PA

Abstract: Spinal cord injury (SCI) leads to permanent neurological deficits, and current strategies for enhancing functional recovery rely on spared neural pathways without addressing the underlying tissue loss. Our prior work demonstrated that transplantation of human induced pluripotent stem cell (hiPSC)-derived V2a spinal interneurons (SpINs), identified by VSX2 expression, into a cervical contusion injury in adult rats results in functional synaptic integration with the injured host phrenic circuit and improved diaphragm activity.

To move this therapy toward clinical translation, we applied the previously validated differentiation protocol to three Good Manufacturing Practice (GMP)-grade hiPSC lines and performed immunocytochemistry as well as single-cell RNA sequencing (scRNA-seq) to assess lineage fidelity and differentiation efficiency. One line, designated GMP-1, yielded the highest proportion of VSX2+ neurons at the end of the differentiation protocol (mean = 74.3%, n = 6), compared to other groups. Given the goal of maximizing the proportion of V2a SpINs, GMP-1 was selected for subsequent transplantation experiments.

Ongoing studies will characterize the spatial and molecular features of transplant-host integration, using Visium HD spatial transcriptomic analysis following transplantation of GMP-1-derived SpINs into a rat model of cervical SCI. In prior work, a positive correlation was observed between the number of neurons in the transplant and the degree of host functional recovery. However, the molecular mechanisms underlying integration remain unknown. Spatial transcriptomics enables high-resolution mapping of gene expression within the anatomical context of the graft and injury site, providing the opportunity to identify regional transcriptional differences, characterize interactions at the transplant-host boundary, and determine whether a distinct molecular signature is associated with synaptically integrated neurons, as defined by retrograde, transsynaptic pseudorabies virus (PRV) tracing.

These investigations will inform the cellular and spatial determinants of functional connectivity and support the advancement of GMP-compatible V2a SpINs for regenerative repair in SCI.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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Program #/Poster #: PSTR334.16/L13

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Neilsen Foundation Grant #1175660
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Title: Sacral nerve stimulation for the restoration of bowel function in a rodent model of spinal cord injury

Authors: *N. L. WILKINS¹, W. M. GRILL²;

¹Biomed. Engin., Duke Univ. Pratt Sch. of Engin., Durham, NC; ²Biomed. Engin., Duke Univ., Durham, NC

Abstract: Spinal cord injury (SCI) affects multiple organ systems and has the potential to significantly impact quality of life. Bowel dysfunction, including chronic constipation, slow colonic transit, and incontinence, is seen in 80% of persons living with SCI. Sacral nerve stimulation (SNS) is a minimally invasive, FDA-approved intervention in use for the treatment of bladder and bowel dysfunction in individuals without spinal cord injuries. We are developing a novel paradigm of SNS that uses different temporal patterns to differentiate between promoting continence to treat fecal incontinence and increasing colonic motility to treat chronic constipation in a rodent model of SCI. Nine Sprague Dawley rats (female n=4, male n=5) with thoracic contusion spinal cord injuries underwent acute SNS under urethane anesthesia at four weeks post-injury. SNS was applied with three different temporal patterns (14 Hz continuous stimulation, 30 seconds of stimulation at 18 Hz followed by 80 seconds without stimulation, and 40 seconds of stimulation at 20 Hz followed by 20 seconds without stimulation) for 5 minutes each at 0.8-1.25X motor threshold, both with and without concomitant colorectal distention. During SNS, changes in rectal and colon pressure, at 6, 5, and 4 cm from the anal verge, the number of colon contractions above 5 mmHg, and electromyography of the external anal sphincter (EAS) were recorded to measure changes in colonic motility. Repeated measures ANOVA revealed that there were no significant differences in average colonic pressure between periods with SNS vs. periods without SNS, regardless of stimulation parameters. However, a significant effect of stimulation (without colorectal distention) was detected in the number of colon contractions ($p=0.042$). During periods of SNS, significant differences were also found in activation of the EAS both with ($p=0.0003$) and without ($p=0.004$) colorectal distention, and a pairwise difference was found between 14 Hz continuous stimulation (0.110 ± 0.079 mV) and burst-patterned 20 Hz stimulation (0.098 ± 0.079 mV) ($p=0.023$). These findings illustrate SNS pattern dependent responses in colon activity required to differentiate between SNS for continence vs. increased motility. Although, in the clinical setting stimulation parameters will always need to be personalized to compensate for the heterogeneity in those with SCI, our findings in a rodent model provide the framework for more therapeutic treatment of neurogenic bowel dysfunction in this population.

Disclosures: N.L. Wilkins: None. W.M. Grill: None.

Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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Program #/Poster #: PSTR334.17/L14

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Wings For Life
Paula and Rusty Walter and Walter Oil & Gas Corp Endowment

Title: Human astrocyte containing spinal V2a interneuron organoids mediate enhanced integration and synaptogenesis after transplantation to treat spinal cord injury

Authors: *I. TORRES¹, M. HOGAN², D. DELGADO², J. MEJIA^{3,2}, M. PATEL², R. KRENCIK^{2,3}, P. J. HORNER^{2,3};

¹Neurosci., WEILL CORNELL MEDICINE /Houston Methodist Hosp., Houston, TX; ²Houston Methodist Res. Inst., Houston, TX; ³WEILL CORNELL MEDICINE, New York, NY

Abstract: Spinal cord injury (SCI) pathology interferes with normal circuitry and results in disruption of sensorimotor function. Transplantation of neural progenitor cells has been explored as a therapeutic approach to promote tissue regeneration and reestablishing neuronal connectivity, but functional circuit integration has been experimentally challenging. Given that multiple factors determine SCI pathology and repair, a combinatorial strategy including: 1) a niche specific cell population, such as the V2a excitatory interneurons; 2) a preorganized cell structure, such as neural organoids; and 3) the inclusion of supportive glial cells like astrocytes, may achieve a better outcome than current transplantation approaches. **Here I tested the hypothesis that incorporation of astrocytes into human induced pluripotent stem cell derived spinal organoids will display improved engraftment and functional connectivity compared to neurons alone after transplantation into the lesion site of an experimental SCI model.** To test the ability of astroglia to promote ingrowth in vivo, spinal organoids were injected into the lesion cavity of a C4 hemi-contusion model of SCI at 2 weeks post-injury. Organoids containing astrocytes had increased extension and sprouting of transplant-derived neurites across the lesion border and across multiple segments of into the host gray and white matter. The volume and regional spread of neurite growth induced by astrocyte containing organoids in comparison to the growth induced by spinal cells only is quantified by the application of the stereologic probe fractionator in longitudinal sections of spinal cord. The molecular phenotype of host and graft synaptic connections are also quantified to assess the impact of astroglia on host and graft synaptic integration. Transplant neurites are labeled using the STEM121 antibody and colocalized with Choline acetyl transferase (ChAT) to identify host motoneurons, calcitonin gene related peptide (CGRP) for sensory neurons, vesicular glutamate transporter 2 (VGLUT2) for excitatory connections and gamma-aminobutyric acid (GABA) for inhibitory input. To investigate whether the transplant is making synapses with the host, the pre- and post-synaptic markers synapsin 1 (Syn1) and Homer1 synaptic pairs are imaged by confocal microscopy, respectively. Finally, to assess functional recovery, motor function was assessed using the limb use asymmetry test and grip strength. Preliminary results indicate that astrocyte containing organoids improve the recovery of grip strength. These findings establish the potential of astrocytes to be used as a support cell in transplantation therapies for SCI.

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Poster

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Program #/Poster #: PSTR334.18/L15

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH R01NS099872

Title: The effect of motor-targeted epidural spinal stimulation on spinal cord injury induced neuropathic pain

Authors: *K. D. GREEN¹, C. M. BATISTA², R. L. MURPHY³, R. B. ROBINSON⁴, S. I. PERLMUTTER⁵;

¹Neurobio. and Biophysics, ²Dept. of Physiol. & Biophysics, ³Physiol. and Biophysics, Univ. of Washington, Seattle, WA; ⁴Univ. of Washington, Edmonds, WA; ⁵Dept Neurobio. & Biophysics, Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA

Abstract: Around 50% of people with spinal cord injury (SCI) develop neuropathic pain (SCI-NP) where pain is felt in response to non-noxious or otherwise mild stimuli due to damage to central pain processing circuits. A recent study by Bandres et al. found that acute electrical spinal cord stimulation (SCS) targeted towards recovery of motor function depresses nociceptive transmission in the spinal cord of rats with SCI-NP, suggesting a potential side benefit of motor-targeted SCS. However, it is unclear whether this depression is maintained over longer therapy timelines, which are necessary for clinically relevant motor recovery. Moreover, our lab has found that while injured female rats experience substantial improvement of motor function after SCS, recovery in male rats is less robust and more variable. We hypothesize that this sex difference is due to differences in neuropathic pain levels, known to be different in males and females. Here, we investigated how long-term, closed-loop, epidural SCS affects SCI-NP symptoms in male and female rats with cervical SCI. We assayed three different sensory modalities (touch, heat, cold) to quantify the dimensions of neuropathic pain after both injury and SCS therapy. After injury, we found that rats did not develop mechanical allodynia, but did develop thermal hyperalgesia. Male rats demonstrated more severe hyperalgesia than the female rats. After SCS therapy, male rats' hyperalgesia worsened, whereas it was reduced in females. This finding suggests that there are sex-dependent side-effects of SCS which may be influencing how successful the therapy is at motor rehabilitation.

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Poster

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Program #/Poster #: PSTR334.19/M1

Topic: D.05. Spinal Cord Injury and Plasticity

Support: R01 NS132773

Title: Bioengineered AAVs Enable Targeted Modulation and Real-Time Monitoring of Transgene Activity to Promote Functional Recovery After Spinal Cord Injury

Authors: *M. METCALFE¹, O. STEWARD²;

¹Univ. of Louisville, Louisville, KY; ²Reeve-Irvine Res. Center, Univ. of California Irvine, Irvine, CA

Abstract: Spinal cord injury (SCI) results in devastating, often irreversible loss of function due to the failure of damaged axons to regenerate in the adult central nervous system (CNS). A key barrier to effective therapeutic intervention is the challenge of delivering regenerative molecules with both spatial and temporal precision, while also monitoring their in vivo activity during recovery. To address these challenges, we developed a multifunctional gene therapy platform that integrates bioengineered adeno-associated viruses (AAVs) for cell- and region-specific gene delivery, externally regulated expression and a non-invasive bioluminescence imaging (BLI) to track transgene activity longitudinally in live animals. Using rational capsid design and transcriptional control, we employed retrogradely transported AAVs (AAV-retro) that selectively transduce neurons in the somatosensory cortex projecting to the injured spinal cord. These vectors carry a short hairpin RNA targeting phosphatase and tensin homolog (PTEN), a suppressor of the PI3K/AKT/mTOR pathway. PTEN downregulation enhances intrinsic axonal growth capacity and represents a promising therapeutic strategy in SCI. Our recent studies revealed improved functional outcomes with permanent PTEN deletion via AAV-retro; however, there are late-onset adverse effects, underscoring the need for refined control of gene expression. To address this, we incorporated a doxycycline (Dox)-inducible TetON promoter into AAV-retro/shPTEN, allowing reversible and temporally controlled transgene expression. This control is critical for minimizing side effects and aligning gene expression with optimal windows for neural repair. To monitor transgene activity in real-time, the AAV-retro construct co-expresses a luciferase reporter under the same regulatory elements as the shPTEN cassette. We utilize AkaLuc, a near-infrared luciferase, for deep tissue BLI. In naive mice, intraspinal injection of AAV-retro-shPTEN-AkaLuc followed by Dox administration resulted in robust luminescence in the brain, indicating retrograde transport and successful Dox-dependent transgene activation. Expression was sustained over time and reversible, with no detectable leakiness in the absence of Dox. Together, this work highlights the potential of bioengineered AAVs as precision delivery tools and dynamic biosensors, enabling spatiotemporally controlled gene expression in the injured CNS. This controllable system supports phase-specific therapeutic strategies and provides a versatile platform to modulate neuroplasticity and monitor recovery.

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Poster

PSTR334: Spinal Cord Injury: Therapeutic Strategies: *In Vivo*: Non-Pharmacological

Location: SDCC Halls B-H

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R01AG066653 (RS)
R01CA266004 (RS)
R01AG078702 (RS)
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Title: Normobaric Hyperoxia (N-O₂) Exposure Following Cervical Spinal Cord Injury (SCI) Impacts Recovery

Authors: *G. IGLESIAS MALDONADO^{1,6,7}, K. DEEGAN^{2,6,7}, S. RANA^{1,6,7}, I. GONZALEZ^{1,6,7}, M. MACINTYRE^{1,6,7}, D. FUDGE^{1,6,7}, B. ZIANI³, A. CARBONELL³, R. SUN^{3,8}, D. PLANT^{9,10}, P. K. BOSE^{10,4,5}, D. D. FULLER^{1,6,7};
¹Dept. of Physical Therapy, ²Dept. of Neurosci., ³Dept. of Biochem. & Mol. Biol., ⁴Anesthesiol., ⁵Neurol., Univ. of Florida, Gainesville, FL; ⁶McKnight Brain Inst., Gainesville, FL; ⁷Breathing Res. and Therapeut. Ctr., Gainesville, FL; ⁸Ctr. for Advanced Spatial Biomolecule Res., Gainesville, FL; ⁹Dept. of Physiological Sciences, Col. of Vet. Medicine, Univ. of Florida Gainesville, Florida, Gainesville, FL; ¹⁰Brain Rehabil. Res. Ctr., North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL

Abstract: Spinal cord injury (SCI) causes severe hypoxia in and around the lesion which may exacerbate tissue damage. Hyperbaric O₂ (HBO) treatment during the acute to subacute period can reduce neuronal loss and improve recovery. However, HBO can be challenging to administer. We therefore assessed the impact of a N-O₂ paradigm initiated shortly after cervical SCI. Adult male Sprague Dawley rats received lateral spinal 150Kd contusion at C4. N-O₂ was initiated on the day of SCI and consisted of twice-daily 3-hour exposures to 100% O₂ (1 ATM) for 6-7 days. Control rats received 21% O₂ (1 ATM, balance N₂). The N-O₂ (n=17) and control groups (n=15) had similar post-SCI survival and weight gain. Breathing evaluation using whole-body plethysmography on day 7 post-SCI showed that the maximum inspiratory tidal volume during a powerful chemoreceptor stimulation achieved by inspiring a hypercapnic-hypoxic gas mixture was larger in N-O₂ (1.54±0.32 ml/kg, n=8) vs. control rats (1.16±0.21 ml/kg, n=5, p=0.041). Metabolomic analyses was conducted on blood serum harvested on day 7 post-SCI using a LCMS-VIP HESI-Impact II Bruker liquid chromatography mass spectrometry system. Notably, uric acid (q=0.045) and 2-hydroxyisocaproic acid (q=0.045) were upregulated in the N-O₂ group (n=4) vs. control (n=2). Ongoing studies are using *in vivo* magnetic resonance imaging to examine fractional anisotropy, mean diffusivity, and total edema volume in the cervical cord in both groups. We conclude that a 2x3 hour per day N-O₂ paradigm administered during the acute to sub-acute post-SCI phase does not impact weight gain or survival in rats and may be contributing to improved ability to activate the respiratory neuromuscular system during an acute challenge.

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Poster

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T32HL134621(ABM)
R01HL147554(GSM)

Title: Normobaric hyperoxia increases tissue oxygen pressure in the acutely injured spinal cord

Authors: *K. M. DEEGAN^{1,2,5,6}, P. P. THAKRE^{2,5,6}, S. RANA^{3,5,6}, E. S. BENEVIDES⁴, G. A. IGLESIAS MALDONADO^{3,5,6}, A. B. MARCIANTE^{3,5,6}, E. J. GONZALEZ-ROTHI^{3,5,6}, G. S. MITCHELL^{3,5,6}, D. D. FULLER^{3,5,6};

¹Univ. of Florida, Dept. of Neurosci., Gainesville, FL; ²Dept. of Neurosci., ³Physical Therapy, ⁴Pediatrics, Univ. of Florida, Gainesville, FL; ⁵McKnight Brain Inst., Gainesville, FL; ⁶Breathing Res. and Therapeut. Ctr., Gainesville, FL

Abstract: Traumatic spinal cord injury (SCI) disrupts the spinal cord vasculature causing ischemia and severe tissue hypoxia in and around the lesion area. We used two different rat models of cervical SCI to test the hypothesis that raising the inspired O₂ from 21% to 100% acutely following an SCI accelerates the recovery of spinal PO₂ towards pre-injury values. One group of male Sprague-Dawley (SD) rats received a C4 hemilesion (C4Hx) and were studied during spontaneous breathing under ketamine/xylazine anesthesia (Group 1, n=4). A second group of male SD rats received a C2 hemilesion (C2Hx) and were studied under urethane anesthesia while mechanically ventilated (MV, Group 2, n=5). The partial pressure of O₂ in the spinal cord (sPO₂) was measured before and after SCI using a Unisense OP-50 optode inserted into the ventral mid-cervical (C5) spinal cord (optode tip placed 1.2 mm lateral to spinal midline; 1.7 mm from dorsal surface). In Group 1, the spinal-intact sPO₂ was 29±10 mmHg during room air breathing (21% O₂). After C4Hx, sPO₂ dropped nearly to zero within seconds of injury, and then gradually recovered to a value of 9±2 mmHg after 162±62 seconds. Inspired O₂ was then raised to 100% and sPO₂ rose to 155±71 mmHg, with a variable time course, reaching a stable value after 24±19 min. In Group 2, the spinal-intact sPO₂ was 25±9 mmHg during mechanical ventilation with 21% O₂. sPO₂ immediately decreased post-C2Hx, stabilizing at 7±6 mmHg after 104±76 sec. 100% inspired O₂ increased sPO₂ to 43±11 mmHg after 10±5 min. Thus, normobaric O₂ (100%) mitigates spinal cord tissue hypoxia caudal to an acute SCI. We suggest normobaric O₂ therapy is a viable strategy to raise spinal cord O₂ to attenuate secondary injury and cell death after acute SCI.

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Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR335.01/M4

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH T32 HD101395

Title: Effects of Chronic Spinal Cord Injury on Neuromuscular Junction Transmission

Authors: *M. FARLEY^{1,2}, B. CHEN^{3,1}, M. A. PEREZ^{4,1,5};

¹Northwestern Univ., Chicago, IL; ²Ctr. for Neural plasticity, Arms & Hands, ³Arms Hands Abilitylab, Shirley Ryan AbilityLab, Chicago, IL; ⁴Ctr. for Neural plasticity, Arms & Hands, Shirley Ryan Abilitylab, Chicago, IL; ⁵Edward Hines Junior VA Med. Ctr., Hines, IL

Abstract: Spinal cord injury (SCI) results in a complex array of neurological impairments, including motor and sensory deficits below the level of the lesion. Although the primary damage is localized to the central nervous system, secondary complications involving the peripheral neuromuscular system may contribute to persistent muscle weakness and functional limitations. Particularly changes at the neuromuscular junction (NMJ)—the critical synapse between motoneurons and muscle fibers—could contribute to increased muscle fatigability and weakness. In this study, we used low-frequency (3 Hz) repetitive electrical stimulation of the common peroneal nerve for ten stimuli and recorded compound action potential (CMAPs) from the tibialis anterior (TA) muscle in participants with chronic incomplete SCI (n=8). We found that CMAP amplitude decreased on average by $34.4\% \pm 7.0\%$ between the first and fourth CMAP. This is higher than normative values observed in control subjects. These preliminary findings suggest dysfunction at the NMJ in lower extremity muscles in individuals with chronic incomplete SCI, highlighting the need for further research to determine its prevalence and potential functional consequences.

Disclosures: M. Farley: None. B. Chen: None. M.A. Perez: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR335.02/M5

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH

Title: Propriospinal Contributions to Elbow Flexor and Extensor Muscles in Humans with Spinal Cord Injury

Authors: S. SANGARI¹, *M. A. PEREZ²;

¹Shirley Ryan AbilityLab, Chicago, IL; ²Shirley Ryan Abilitylab, Chicago, IL

Abstract: In addition to direct corticomotoneuronal projections, upper limb motoneurons in humans receive substantial disynaptic corticospinal excitation via the C3-C4 propriospinal system. The propriospinal neurons also integrate extensive peripheral afferent input, enabling parallel modulation of descending motor commands. Clinically, individuals with cervical spinal cord injury (SCI) often demonstrate poorer functional recovery in elbow extensors than in flexors. Although reduced corticospinal drive and enhanced intracortical inhibition to the triceps compared to the biceps brachii has been documented following SCI, the specific role of the propriospinal system in this differential recovery remains unclear. To address this, we used electrophysiological techniques to assess cutaneous suppression of the propriospinal system by stimulating the superficial radial nerve at the ulnar groove during sustained elbow flexion and extension in individuals with and without incomplete cervical SCI. We found greater cutaneous suppression of the propriospinal system in the triceps compared to the biceps in both control and SCI participants. While biceps suppression did not differ between groups, triceps suppression was significantly reduced in SCI participants compared to controls. Furthermore, we confirmed that this difference is not attributable to changes in the magnitude of descending motor drive or afferent input. Our findings suggest that diminished modulation of the propriospinal system by peripheral afferents could further impair descending motor control to the triceps brachii. Collectively, these deficits may underlie the limited functional recovery of the triceps brachii observed after cervical SCI.

Disclosures: S. Sangari: None. M.A. Perez: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR335.03/M6

Topic: D.05. Spinal Cord Injury and Plasticity

Support: National Institute of Neurological Disorders and Stroke
the Veteran Affairs Administration

Title: Combinatorial approaches increasing neuronal activity accelerate recovery after spinal cord injury

Authors: *M. LEE¹, B. CHEN², M. A. PEREZ³;

²Arms Hands Abilitylab, ¹Shirley Ryan AbilityLab, Chicago, IL; ³Ctr. for Neural plasticity, Arms & Hands, Shirley Ryan Abilitylab, Chicago, IL

Abstract: Combinatorial approaches targeting multiple aspects of spinal cord injury (SCI) pathophysiology are needed to maximize functional recovery. We hypothesized that enhancing neuronal activity—by strengthening corticospinal synapses through Hebbian stimulation and increasing neuronal transmission with 4-aminopyridine (4-AP), a potassium blocker—could accelerate locomotor recovery in individuals with chronic SCI. Participants were randomly assigned to receive 10 mg of 4-AP or placebo, where both groups followed with 60 min of Hebbian stimulation targeting corticospinal-motor neuronal synapses supplying leg muscles involved in locomotion and 60 min of standard exercise rehabilitation for 40 sessions over 8-14 weeks. During Hebbian stimulation, 720 paired pulses were delivered to elicit corticospinal action potentials via electrical stimulation of the thoracic spine, ensuring volleys reached the spinal cord 1-2 ms before motor neurons were retrogradely activated through bilateral electrical stimulation of the femoral, common peroneal, and posterior tibial nerves (targeting the quadriceps femoris, tibialis anterior and soleus muscles, respectively). Results showed that participants who received 4-AP exhibited significantly greater improvements in walking speed and endurance, corticospinal excitability, and light touch sensation compared to those who received the placebo. The minimal clinically important difference in walking speed and endurance was achieved after 20 sessions in the 4-AP group, but was not consistently reached in the placebo group. Although walking continued to improve in both groups over the course of 40 sessions, the 4-AP group demonstrated significantly greater progress. Improvement in the 4-AP group was still present approximately 12 months later. These findings suggest that 4-AP represents a strategy to potentiate and accelerate Hebbian stimulation effects on motor recovery in individuals with chronic SCI.

Disclosures: M. Lee: None. B. Chen: None. M.A. Perez: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR335.04/M7

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NICHD Grant 1R03HD114768-01
NINDS
VA
NIDILRR 90SIMS0015

Title: Reticulospinal input to lower limb muscles during locomotion in humans with spinal cord injury

Authors: *D. DE SANTIS¹, M. A. PEREZ²;

²Ctr. for Neural plasticity, Arms & Hands, ¹Shirley Ryan Abilitylab, Chicago, IL

Abstract: The reticulospinal tract (RST) is a major but underinvestigated descending tract that largely contributes to motor output and locomotion in humans. Accumulating evidence suggests that the RST plays a crucial role in the recovery of locomotor function and motor output after SCI. However, little is known about the contribution of the RST to the control of lower limb muscle during locomotion in humans and how this is affected following SCI. Here we evaluated the effect of non-invasive RST stimulation through loud acoustic stimuli (LAS, 115dB) on the onset and amplitude of electromyographic activity of tibialis anterior and quadriceps muscles during locomotion in a group of uninjured individuals and in individuals with chronic incomplete SCI above T10 and weakness in dorsiflexor muscles (motor score ≤ 3). LAS were timed either at the instant of toe-off or heel strike of the weaker leg (right leg in uninjured controls) detected through custom in-sole footswitches. EMG activity was recorded from the Rectus Femoris (RF), Tibialis Anterior (TA), Biceps Femoris, and Soleus muscles bilaterally. The participants were asked to walk at 2 different speeds: (1) a self-paced comfortable speed, and (2) a pre-selected slow speed (0.4 miles/h) if able. The slow speed was selected to provide a lower bound for comparison with individuals with severely impaired gait and to be able to account for speed-related changes in muscle activity and increased postural and dynamic challenges encountered during slow walking (Den Otter et al., 2004). All test conditions were randomized. We found that LAS elicited well defined early-onset responses (60-70ms) in uninjured controls, consistent with a previous report (Nieuwenhuijzen et al., 2000). These responses were either inhibitory when elicited during early swing (toe-off) followed by facilitation around 100ms post LAS, or facilitatory when elicited during early stance (heel-strike). The average response in the TA was greater at lower speed and during toe-off stimulation. The RF showed little modulation, mostly evident when LAS was delivered at heel-strike. However, in individuals with SCI facilitatory responses were greatly depressed and early-onset inhibitory responses absent. Our results suggest that reticulospinal output during locomotion may be reduced after SCI, which could possibly contribute to locomotor deficits.

Disclosures: D. De Santis: None. M.A. Perez: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR335.05/M8

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH

Title: Clonus-associated differences in cutaneous reflex among individuals with spinal cord injury

Authors: *E. CURUK¹, M. A. PEREZ², C. HECKMAN³;

¹Shirley Ryan AbilityLab, Chicago, IL; ²Ctr. for Neural plasticity, Arms & Hands, Shirley Ryan Abilitylab, Chicago, IL; ³Dept. of Neurosci., Northwestern Univ., Oak Park, IL

Abstract: Ankle clonus is an involuntary, rhythmic muscle contraction often observed in humans with spinal cord injury (SCI). While clonus is associated with spasticity syndrome, its underlying neural mechanisms are not fully understood. Reduced inhibitory control and changes in motoneuron persistent inward currents may lead to the prolonged involuntary muscle activity observed in clonus. This study aims to investigate the presence of prolonged clonus and its relationship to the cutaneous reflex (the long-lasting reflex; LLR). To achieve this, we examined ankle clonus using clinical tests, including the leg drop and manual ankle stretch tests. The LLR was triggered by electrically stimulating the medial plantar nerve, with responses recorded from the tibialis anterior (TA) and soleus (SOL) muscles. In addition, we measured the SOL H-reflex and maximal M-wave (M-max) by stimulating the posterior tibial nerve at the popliteal fossa. Our results showed strong reliability for the clonus tests (ICC=0.76), excellent consistency for the manual stretch test across different days (ICC=0.99), and moderate reliability for the drop test (ICC=0.69). We found that the LLR duration in the TA muscle was longer in individuals with SCI with sustained clonus (1.51 ± 0.86 s) compared to those without clonus (0.31 ± 0.13 s). Similarly, the SOL muscle LLR was also prolonged in those with sustained clonus (0.27 ± 0.20 s) compared to those without clonus (0.08 ± 0.04 s). Furthermore, the H/M ratio in the SOL muscle was higher in individuals with SCI with sustained clonus (0.56 ± 0.20) than in those without clonus (0.21 ± 0.23). These findings indicate that changes in the neuromodulation of motoneuron excitability play a role in the development of prolonged ankle clonus in people with SCI.

Disclosures: E. Curuk: None. M.A. Perez: None. C. Heckman: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR335.06/M9

Topic: D.05. Spinal Cord Injury and Plasticity

Support: VA
NINDS
NICHD

Title: Biomarker for recovery following severe spinal cord injury

Authors: *T. T. WU¹, D. DE SANTIS², M. A. PEREZ^{3,4,5};

¹Univ. of Illinois at Urbana-Champaign, Champaign, IL; ²Arms and Hands lab, ³Ctr. for Neural plasticity, Arms & Hands, Shirley Ryan Abilitylab, Chicago, IL; ⁴Edward Hines Jr. VA Hosp., Hines, IL; ⁵Physical Med. and Rehabil., Northwestern Univ., Chicago, IL

Abstract: A motor-complete spinal cord injury (SCI) results in the complete loss of voluntary motor control below the level of injury. Only a small proportion of individuals experience partial motor recovery during inpatient rehabilitation. Both animal and clinical studies suggest the existence of a critical time window immediately following SCI during which neuroplasticity potential is heightened. However, there is currently no biomarker to identify, early after injury, which individuals have the potential for recovery. We hypothesize that among individuals with motor-complete SCI, those presenting with more severe spasticity at admission will demonstrate greater neurological and functional recovery during inpatient rehabilitation compared to those with less severe spasticity. This ongoing study enrolls individuals classified as having T10 or above injuries, categorized as AIS A or B, who are admitted to an inpatient rehabilitation facility. Evaluations are conducted at two time points: within 2-3 weeks of admission, and again 2-3 weeks prior to discharge. The primary study endpoints are: Spasticity, measured by the Modified Ashworth Scale, kinematic analysis via the Pendulum Test, and the Spinal Cord Assessment Tool for Spastic Reflexes (SCATS); Motor and sensory recovery, assessed through the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI); Quality of life, evaluated using the Spinal Cord Injury-Quality of Life (SCI-QOL) survey. Findings from our site will be presented at the upcoming Society for Neuroscience (SfN) meeting.

Disclosures: T.T. Wu: None. D. De Santis: None. M.A. Perez: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR335.07/M10

Topic: D.05. Spinal Cord Injury and Plasticity

Support: R35NS122336

Title: Startle-induced modulation of reciprocal inhibition following incomplete spinal cord injury: evidence for reticulospinal interactions

Authors: *B. CHEN^{1,2,4}, B. A. DEFOREST¹, M. A. PEREZ^{1,2,4,3},

¹Shirley Ryan AbilityLab, Chicago, IL; ²Physical Med. & Rehabil., ³Dept. of Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; ⁴Hines VA hospital, Chicago, IL

Abstract: Spinal cord injury (SCI) disrupts inhibitory circuits, yet how descending pathways modulate inhibition between antagonist muscles remains unclear. We tested whether reticulospinal activation via an auditory startle (115 dB) differentially alters reciprocal inhibition in ankle dorsiflexor (tibialis anterior, TA) and plantarflexor (soleus, SOL) muscles during submaximal contractions (~10% MVC) in 16 individuals with incomplete SCI and 16 uninjured controls. Additionally, we assessed motoneuronal excitability using F-waves in both muscles under the same conditions. In healthy participants, the startle stimulus significantly reduced

reciprocal inhibition in TA but not in SOL. In contrast, individuals with SCI exhibited startle-induced reduction of reciprocal inhibition in both TA and SOL. F-wave analysis revealed no significant effect of the startle stimulus on motoneuronal excitability in either group. Notably, greater startle-induced disinhibition of SOL was associated with lower SOL maximal voluntary contraction in the SCI group. These results provide the first human evidence that reticulospinal pathways suppress reciprocal inhibition, and suggest that post-injury reorganization of descending pathways enhances disinhibition of ankle plantarflexors, likely due to reduced descending drive. Our results highlight the importance of reticulospinal circuits in shaping spinal inhibitory control following SCI and may inform future interventions targeting spasticity and motor recovery.

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Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

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Program #/Poster #: PSTR335.08/M11

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NSF Grant 1748986

Title: Alterations in Maternal Care toward Spinal-Transected Offspring

Authors: *A. C. MARTES, M. R. BRUMLEY;
Idaho State Univ., Pocatello, ID

Abstract: Effects of neonatal neural injury are well established in developmental research. Rats are commonly used in such models; however, the role of maternal care has received limited attention. Maternal behavior significantly influences offspring development, affecting outcomes such as reflexes and social behavior (Rincón-Cortés & Sullivan, 2016; Kauer et al., 2016), and is shaped by external cues. This study investigated whether maternal care is altered in response to spinal cord injury in offspring by comparing maternal behavior directed toward pups with a spinal cord transection versus sham-operated controls. Subjects were 20 adult female Sprague Dawley rats and their litters. On postnatal day (P)1, pups received either a complete low-thoracic spinal cord transection or sham surgery. Maternal behaviors (e.g., licking/grooming, anogenital licking, nursing postures, off-nest time, maltreatment) were continuously recorded for 12 hours on P4, P8, and P12. Four 20-minute video segments per day were analyzed, and durations of each behavior were scored. Linear mixed models were used to assess within- and between-subject effects. No maltreatment behaviors were exhibited and thus were excluded from analyses. No significant effects of surgery condition or age were observed for passive nursing, arched-back nursing, nest building, on-nest, or off-nest behavior. A significant age effect was found for blanket nursing, $p = 0.002$, $F(1, 236) = 10.229$, with increased duration on P8 and P12 relative to P4. Anogenital licking (AGL) showed significant main effects of surgery condition (p

= 0.013, $F(1, 236) = 6.328$) and age ($p = 0.014$, $F(1, 236) = 6.161$); dams engaged in shorter durations of AGL with spinal-transected pups, and AGL across both surgical conditions peaked at P8. A main effect of surgery condition was observed for general licking/grooming, $p = 0.05$, $F(1, 236) = 3.864$, and an age effect was seen for pup retrieval, $p = 0.003$, $F(1, 236) = 9.334$, with shorter retrieval durations at P12. These findings indicate that maternal behavior is modulated by offspring neural injury. Given the developmental significance of behaviors such as AGL toward offspring (Kauer et al., 2016), maternal care should be considered a potential moderating factor in studies of neonatal injury and recovery. Future research will explore experimental manipulation of maternal behavior and its relationship to offspring recovery outcomes.

Disclosures: A.C. Martes: None. M.R. Brumley: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR335.09/M12

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NSF Grant 1748986

Title: The Effects of Neonatal Spinal Cord Injury and Treadmill Training on Sensorimotor Reflexes

Authors: *L. D. HERRERA¹, A. L. BOZEMAN², A. MARTES¹, T. CAMPBELL³, N. COLLINS⁴, T. L. ROTH⁴, M. R. BRUMLEY¹;

¹Idaho State Univ., Pocatello, ID; ²Emory Univ. Sch. of Med., Atlanta, GA; ³ChristianaCare Gene Editing Inst., Newark, DE; ⁴Univ. of Delaware, Newark, DE

Abstract: After a spinal cord injury (SCI), motor function is often impaired. However, the developing spinal cord retains neuroplasticity potential due to the infant lesion effect, offering a critical window for recovery. Locomotor training has been shown to enhance neuroplasticity and promote functional reorganization through movement-related sensory input. Both age and training are important considerations for spinal adaptability and recovery. The purpose of this study was to examine the effects of neonatal spinal cord injury (SCI) and early treadmill training on sensorimotor reflexes in rats. Eighty rats were divided into eight groups: 2 sexes (male or female) x 2 surgical conditions (spinal-transected or sham) x 2 training conditions (trained or untrained), with 10 subjects per group. On postnatal day 1 (P1), rats underwent either a complete low-thoracic (T8-T10) spinal cord transection or sham surgery. Half of the rats received treadmill training from P10 to P17, consisting of three 10-minute sessions per day. On P18, all rats were tested for surface righting, hindlimb placing, and hindlimb crossed-extensor reflexes. Reflex latency and response presence were recorded. The results revealed a significant difference between surgical conditions across all sensorimotor reflexes. Spinal-transected rats showed

significantly delayed righting reflexes and exhibited more absent hindlimb placing and crossed-extensor reflex responses compared to sham rats. However, some spinal-transected rats did respond to sensory input, indicating that spinal processing was retained despite being isolated from the brain. Notably, treadmill training did not improve sensorimotor reflex performance. These findings highlight the importance of developmental timing in recovery and suggest that further optimization of locomotor training schedules may be necessary to maximize recovery. This study advances our understanding of how early SCI affects sensorimotor function and recovery in the developing spinal cord.

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Poster

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Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS111234
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Title: Modulation of intraspinal local field potentials by sensory afferent feedback and spinal cord injury

Authors: *G. N. MORENO ROMERO^{1,2}, M. F. BANDRES^{1,2}, J. G. MCPHERSON^{3,1,2};
¹Biomed. Engin., Washington Univ. in St. Louis, St Louis, MO; ²Physical Therapy, Washington Univ. Sch. of Med., St Louis, MO; ³Anesthesiology, Pain Center, Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Local field potentials (LFPs) have long been used to characterize the structure and function of supraspinal neural networks across temporal, frequency, and spatial domains. However, investigations of LFPs in spinal networks remain considerably more limited. The goal of this work is to define if and how natural sensory feedback modulates intraspinal LFPs across structural and functional regions of the lumbar gray matter *in vivo*. We then ask whether these spatiotemporal features of intraspinal LFPs are altered below a chronic spinal cord injury (SCI) and by the presence of SCI-related neuropathic pain (SCI-NP). All experiments were approved by the WUSTL IACUC. The study utilized adult male Sprague-Dawley rats including 5 neurologically intact rats, 5 rats with chronic SCI (T8 contusion) but no behavioral signs of SCI-NP, and 5 rats with SCI-NP. Electrophysiological recordings were performed 6-8 weeks post-injury under urethane for anesthesia. Microelectrode arrays were implanted into the spinal grey matter at the level of the L5 dorsal root entry zone, enabling extracellular neural recordings throughout the dorsoventral extent of the L5 spinal segment. Broad-band LFP signals were band-

limited to frequency ranges commonly defined for supraspinal networks: theta, alpha, beta, low gamma, high gamma, and high frequency oscillation bands 1 and 2 (HFO1, HFO2). During periods of spontaneous neural activity (no induced sensory feedback), power in the low gamma range (30-70 Hz) was greater than that in the high gamma range (70-200 Hz) across sensory dominant, sensorimotor integrative, and motor dominant regions of the gray matter and regardless of whether the spinal cord was neurologically intact or chronically injured. Interestingly, however, this trend was reversed during periods of nociceptive transmission, with high gamma power meeting or exceeding that of low gamma across regions and cohorts. We also observed in all cohorts increased power in the HFO1 (200-500 Hz) and HFO2 (500 to 1,000 Hz) ranges during nociceptive transmission relative to periods of spontaneous transmission. Future work will be required to determine the potential functional implications of these findings and whether intraspinal LFPs can be modulated independently of ongoing spiking activity.

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Poster

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Program #/Poster #: PSTR335.11/M14

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Neilson Grant # 1000927

Title: Administration of bumetanide after spinal cord injury mutes the effect of nociceptive stimulation, reducing plastic potential and pain-induced tissue loss

Authors: *P. CHANDRAMOHAN¹, K. E. HUDSON³, T. JOHNSTON⁴, J. W. GRAU²;
¹Psychological & Brain Sci., ²Texas A&M Univ., College Station, TX; ³Vanderbilt Univ. Med. Ctr., Nashville, TN; ⁴McGovern Med. Sch. at UTHealth Houston, Houston, TX

Abstract: Prior work has shown pain (nociceptive stimulation) caudal to a spinal cord injury (SCI) can engage spinal circuits that contribute to the development of neuropathic pain and impact long-term recovery. In rats that have received a complete thoracic transection, animals given noxious electrical stimulation (shock) to one hind leg whenever the leg is extended learn to maintain a flexion response that reduces net shock exposure. When stimulation is given independent of leg position (uncontrollable), animals fail to learn. Further, uncontrollable stimulation induces a state of neural over-excitation that enhances reactivity to non-noxious mechanical stimulation, an allodynic-like effect indicative of neuropathic pain. Uncontrollable stimulation also impairs long-term recovery in rats that have received a contusion injury. Research suggests that SCI may amplify the consequences of nociceptive stimulation by inducing a biochemical modification (the down-regulation of the co-transporter KCC2) that reduces GABAergic inhibition. Supporting this, we have shown that pharmacological treatments (e.g., administration of bumetanide) that restore GABA-dependent inhibition block the

development of neuropathic pain after SCI. Here we examine whether this drug treatment regulates other consequences of nociceptive stimulation. Male rats that had received an upper thoracic (T2) transection were given bumetanide (i.t.) and tested in our learning paradigm. Restoring GABAergic inhibition blocked learning. Other animals were given bumetanide followed by uncontrollable stimulation. The capacity to learn was assessed the next day. In vehicle treated animals, uncontrollable shock induced a learning impairment. This effect too was blocked by bumetanide. Finally, we examined whether bumetanide would attenuate the adverse effect of nociceptive stimulation on behavioral function and tissue loss in rats that had received a lower thoracic (T12) contusion injury 24 hrs earlier. In vehicle-treated rats, brief nociceptive stimulation to the tail induced an acute disruption in locomotor performance and increased hemorrhage at the site of injury. Pretreatment with bumetanide blocked these effects. The results are consistent with prior work showing that SCI can reduce GABA-dependent inhibition and that this depolarizing shift enables plasticity, which fosters both adaptive learning and the development of maladaptive plasticity. The latter appears to contribute to tissue loss after a contusion injury. Importantly, this adverse effect is blocked by a drug treatment known to restore GABA inhibition.

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Poster

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Topic: D.05. Spinal Cord Injury and Plasticity

Support: FAPESP Ph.D Scholarship: 2024/09098-8
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FAPESP Grant: 2022/06609-6

Title: Motoneuron in Dire Straits: GLT1 upregulation following spinal ventral root axotomy improves neuronal survival by reducing glial reactions and muscle atrophy

Authors: *K. SILVA MULLER, A. L. OLIVEIRA, L. P. CARTAROZZI;
Univ. of Campinas, Campinas, Brazil

Abstract: Ventral root crush (VRC) is a severe form of nerve injury that leads to the loss of up to 50% of spinal motoneurons within the first month, also triggering widespread morphofunctional changes in the spinal cord parenchyma. As such, glial reactivity and synaptic remodeling may worsen the overall outcome, what is connected with the anterograde changes along the neuromuscular axis, resulting in substantial muscle atrophy and motor deficits. Therefore, novel strategies to protect spinal motoneurons are highly relevant for translation, especially when delivered systemically. Sulbactam, a β -lactam compound with demonstrated

neuroprotective effects in cerebral ischemia, partly via modulation of glial responses through glutamate transporter-1 (GLT1) upregulation, emerges as a promising bioactive molecule. Herein, we investigated the progression of motoneuron degeneration, reactive gliosis, and muscle atrophy following L4-L6 VRC in female C57BL/6JUnib mice, and compared with Sulbactam (10 mg/Kg, i.p.) treatment (n = 6/group; CEUA-UNICAMP: 6521-1/2024). Spatiotemporal changes in microglia activation (Iba-1), astrogliosis (GFAP), motoneuron survival (Nissl), and tibialis cranialis muscle integrity (H&E) were assessed at 1, 3, 5, 7, 14, and 28 days post-injury (dpi). Sulbactam was administered for 3 days post-lesion, and the outcomes were assessed at 28 dpi. VRC induced progressive motoneuron loss, with 20% motoneuron death by 7 dpi, and up to 51% by 28 dpi. Microglial activation increased sharply by 3 dpi (3.7-fold), and 7 dpi (4.0-fold), and gradually declined by 28 dpi (2.1-fold). Astrogliosis peaked at 7 dpi (3.9-fold), and kept elevated up to 28 dpi (2.6-fold). Muscle fiber area progressively decreased, with significant 66% atrophy at 28 dpi ($p<0.001$). Importantly, sulbactam treatment significantly attenuated microglial reactivity (1.5-fold, $p<0.001$) and astrogliosis (1.6-fold, $p<0.001$), rescued 70% of the axotomized motoneurons ($p<0.001$) and decreased muscle atrophy (36%, $p<0.001$) at 28 dpi. Overall, the present results suggest that early sulbactam treatment effectively rescues motoneurons and modulates glial responses. These findings indicate the potential for clinical translation following proximal lesions, such as brachial plexus injury.

Disclosures: K. Silva Muller: None. A.L. Oliveira: None. L.P. Cartarozzi: None.

Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR335.13/M16

Topic: D.05. Spinal Cord Injury and Plasticity

Support: A Grant-in-Aid for Specially Promoted Research from MEXT to MY (KAKENHI no. 20H05628)

Title: A Synthetic Synapse Organizer Rescues Hand Dexterity and Induces Corticospinal Plasticity in Spinal Cord Injured Macaques

Authors: *S. PEYDA¹, R. YAMAGUCHI^{1,2}, S. UENO², K. ISA¹, K. SUZUKI^{4,5}, V. CHANG⁴, H. SASAKURA⁶, K. M. MATSUDA⁵, K. KOBAYASHI⁷, K. TAKEUCHI⁶, H. ONOE^{1,3,8}, R. ARICESCU⁴, M. YUZAKI⁵, T. ISA^{1,2,3,7};

¹Dept. of Neuroscience, Div. of Neurobio. and Physiol., Kyoto University, Grad. Sch. of Med., Kyoto, Japan; ²Inst. for the Advanced Study of Human Biol. (WPI-ASHBi), ³Human Brain Res. Ctr., Kyoto Univ., Kyoto, Japan; ⁴MRC Lab. of Mol. Biol., Cambridge, United Kingdom; ⁵Dept. of Neurophysiol., Keio University, Sch. of Med., Tokyo, Japan; ⁶Intl. Ctr. for Brain Sci., Fujita Hlth. Univ., Toyoake, Japan; ⁷Natl. Inst. for Physiological Sci., Okazaki, Japan; ⁸Dept. of Pharmaceut. Sci., Kobe Gakuin Univ., Kobe, Japan

Abstract: Humans and certain non-human primates have evolved extraordinary hand dexterity. Dexterous hand movements are controlled by signals from the brain's primary motor cortex (M1) to motor neurons in the cervical spinal cord, transmitted via the corticospinal tract (CST). The CST circuits are shaped and maintained by synapse organizing proteins, but their integrity is disrupted following a spinal cord injury (SCI). SCI affecting cervical segments leads to impaired or lost hand function. Functional recovery is challenging, but alluringly, CPTX—a novel synthetic synapse organizing protein connecting pre-synaptic nerve terminals and post-synaptic excitatory AMPA-type glutamate receptors—has been shown to restore walking ability in SCI mice by re-establishing lost communication between central neurons (*Suzuki et al., Science 2020*). Building on this, we hypothesized CPTX might recover hand function in a non-human primate SCI model.

To this end, we first trained macaques in a reach-and-grasp task: picking up small potato pieces from a narrow slit using only the index finger and thumb. Next, hand function was deprived by sub-hemisection targeting the cervical CST at the C6/C7 level. Seven days after injury, monkeys randomly received bilateral injections of either CPTX or buffer solution (vehicle) at locations surrounding the injury. Surgeons and experimenters remained blinded for 120 days while dexterity was evaluated using marker-less pose estimation via a deep-learning network (*Mathis et al., Nature Neuroscience 2018*). Unblinding revealed that CPTX-treated monkeys recovered the dexterous hand function significantly earlier than vehicle-treated monkeys, and exhibited a higher quality of recovery as their hand function resembled pre-lesional movements. Furthermore, viral vectors injected bilaterally into the M1 of CPTX-treated monkeys induced massive plasticity of the corticospinal tract at both subcortical and upper cervical segments. These findings suggest that CPTX may leverage the central nervous system's neuronal reservoir to rescue lost dexterity and accelerate hand function recovery in spinal cord injury patients to reduce their suffering and time spent in rehabilitation.

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Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

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Topic: D.05. Spinal Cord Injury and Plasticity

Support: California Institute for Regenerative Medicine DISC2-14180 (LVZ)
National Institutes of Health grant R01 NS104291 (MAL)
Roddenberry Foundation (DS)

Title: The Effects of Optogenetic Stimulation on Human Induced Pluripotent Stem Cell-Derived V2a Spinal Interneurons

Authors: *M. DOMINGUEZ¹, A. BANDEL¹, P. HURLEY¹, W. KWONG¹, Y. CHANG², J. T. PAZ³, M. A. LANE⁴, D. J. BOURBEAU⁵, D. SRIVASTAVA¹, L. ZHOLUDEVA¹;

¹Gladstone Inst., San Francisco, CA; ²UCSF, San Francisco, CA; ³Neurol., Gladstone Inst. and UCSF, San Francisco, CA; ⁴Neurobio. & Anat., Drexel Univ., Philadelphia, PA; ⁵Cleveland FES Ctr., Cleveland, OH

Abstract: Spinal cord injury (SCI) results in extensive damage to neural networks, causing severe and permanent functional deficits. The neuroplastic potential of the injured spinal cord, mediated in part by spinal interneurons (SpINs) can contribute to some limited recovery, but deficits persist without tissue repair. Cell transplantation, specifically the transplantation of excitatory “V2a” SpINs, can enhance spinal repair through restored tissue integrity and introduction of neural building blocks capable of synaptic integration with damaged host networks. However, without a consistent means of driving integration, transplanted SpINs show variable donor to host connectivity. We hypothesize that neural stimulation can be used to promote neurite outgrowth and more consistent synaptic integration and connectivity. Using an optogenetic human induced pluripotent stem cell (hiPSC) line differentiated into V2a SpINs, we tested the effects of light-induced stimulation on neurotrophin production (using ELISA to measure BDNF synthesis), neurite growth (using live-cell imaging) and neuronal activity (using multielectrode array and patch clamp) in vitro. We cultured V2a SpINs for 5 weeks, optogenetically stimulating them 3 times per week (2ms pulse width, 20Hz, 1 hour total). Blue light stimulation increased BDNF synthesis, neurite outgrowth, and increased overall neuronal activity compared to unstimulated and wildtype controls. Future studies will assess whether optogenetic stimulation can enhance connectivity of transplanted SpINs in an animal model of SCI.

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Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR335.15/N2

Topic: D.05. Spinal Cord Injury and Plasticity

Support: R01NS079702
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Yant Family Spinal Cord Regeneration Fund

Title: Promoting plasticity of forelimb motor circuitry in chronic cervical contusion spinal cord injury

Authors: *K. CROWTHER¹, A. C. LEPORE¹, M. LYTTLE², S. LI³;

¹Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA; ²Thomas Jefferson Univ., Philadelphia, PA; ³Shriners Hosp. Pediatric Res. Center, MERB-655, Temple Univ., Philadelphia, PA

Abstract: Spinal cord injury (SCI) globally affects approximately fifteen million individuals, often causing permanent loss of function. The top recovery priority for individuals impacted by high-level SCI is arm/hand function. While the vast majority of pre-clinical SCI research has focused on therapeutically targeting early phases post-injury, the reality is that most patients have been injured for years. It is therefore imperative to develop strategies to circumvent endogenous factors inhibiting circuit reconnection in the chronic phase of SCI, which is a highly challenging problem. We have previously shown that phosphatase and tensin homolog (PTEN) inhibition can successfully promote regeneration of respiratory neural circuitry and recovery of diaphragm function in multiple models of chronic cervical SCI. In this study, we sought to utilize PTEN inhibition in an effort to induce regeneration of highly-important forelimb motor circuitry. We performed a cervical level-5 (C5) hemicontusion SCI, and we then systemically delivered either PTEN antagonistic peptide (PAP) or vehicle-only to SCI rats starting in the chronic phase of injury. We employed a battery of behavioral analyses and found that PAP promoted significant and persistent recovery of forelimb motor function. Additionally, we traced a number of important motor circuits using pseudorabies virus (PRV) and biotinylated dextran amine (BDA) for retrograde trans-synaptic labeling and anterograde labeling, respectively. We are examining whether PAP-based PTEN modulation promotes regrowth of axon populations involved in forelimb motor control and, importantly, are exploring which forms of circuit plasticity are capable of driving functional recovery in chronic cervical SCI.

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Poster

PSTR335: Spinal Cord Injury: Plasticity and Recovery II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR335.16/N3

Topic: D.05. Spinal Cord Injury and Plasticity

Support: WFL-US-06/22
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Title: Combination of ventral spinal stimulation with spinally patterned pre-networked V2a excitatory interneurons and astrocytes enhances integration and survival of spinal tissue grafts after SCI.

Authors: *M. HOGAN¹, I. TORRES³, C. STIGLIANO¹, X. TANG⁴, M. PATEL¹, S. LAVEKAR², R. KRENCIK², P. J. HORNER²;

²Neurosurg., ¹Houston Methodist Res. Inst., Houston, TX; ³Neurosci., Weill Cornell Med. Col., New York, NY; ⁴Dept of Neurosurg, Baylor Coll of Med., Houston, TX

Abstract: After spinal cord injury (SCI), integral cells are lost or disconnected, and recovery via plasticity of spared circuitry has a functional ceiling. Cell replacement therapy is an enticing target for restoring function following SCI. Advances in neural graft technology have led to cell survival within the injured spinal cord and extensive growth, but evidence of functional host and graft connectivity remains limited. We addressed barriers by pre-networking niche appropriate neural cell types and providing guidance and integration signals via targeted electrical stimulation to motor associated circuitry of the impaired forelimb. **Pre-networking niche appropriate neural cell types** We hypothesize V2a excitatory interneurons play a role in establishing relays between damaged circuits to restore motor function after SCI. We optimized culture systems to enrich for V2a excitatory interneurons in a rapidly maturing neural hIPS derived cell line. Inclusion of 20-30% astrocytes can induce networked activity within two weeks of Neurogenin1 induction after ventral and spinal patterning (Retinoic Acid, Purmorphamine, DAPT, BDNF, GDNF and Ascorbic Acid). Cell combinations can be formed into precise sizes of organoids or embedded in alginate ribbons for injection into the spinal cord. Injection methods were optimized for consistent cell number delivery of pre-networked grafts. Ventral spinal neurons (Chx10+, ~72% and ChAT+, ~13%) persist within pre-networked grafts at 10 weeks post engraftment. We found that pre-networked grafts retained higher survival, structural organization and outgrowth compared to suspension grafts of the same cell populations. **Guidance and integration signals via targeted electrical stimulation** Targeted electrical stimulation of the ventral spinal cord may provide integration cues to enhance directed connectivity of spinal grafts. We developed a wireless stimulator for ventral spinal stimulation (VSS) that restores skilled reaching after cervical SCI in a stimulation independent manner. VSS activates unique circuitry compared to clinically traditional dorsal stimulation and dorso-ventral transcriptional activity patterns within cells in the spinal cord. Outgrowth assays indicate ventral electric fields can induce a dorso-ventral and rostro-caudal deflection in axon trajectory from a neural stem cell graft in an injured spinal cord implying electrical currents may be leveraged to target neural graft growth. This research was funded in part by Paula and Rusty Walter and Walter Oil & Gas Corp Endowment and Wings for Life.

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Poster

PSTR336: Descending Pain Modulation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.01/N4

Topic: E.01. Somatosensation – Pain and Itch

Title: The electrophysiological and behavioral evidence of the anti-nociceptive effect of high-intensity electrical brain stimulation in a conscious state: an exploratory study

Authors: *J. TREJO, D. IBARRA, Z. WANG, Y. B. PENG;
Univ. of Texas, Arlington, Arlington, TX

Abstract: The implementation of electroconvulsive therapy (ECT) has long been utilized for many decades since Ugo Cerletti and Lucio Bini's 1938 development. More specifically, the shock stimulation of ECT has been mostly well known to be used with major depressive patients who have revealed resistance to other modes of treatment and has also been used in patients with chronic pain. The local field potential (LFP) is a powerful electrophysiological tool that records the summation of electrical activity of the brain around the tip of an electrode. The LFP can be subdivided into five frequency bands: delta (0.1 - 3 Hz), theta (3 - 7 Hz), alpha (7 - 12 Hz), beta (12 - 30 Hz), and gamma (30 - 100 Hz), and their power is used to reflect the change of specific brain activities. In the present study, to explore how ECT is effective in alleviating formalin-induced nociception, we recorded continuous LFP in four distinct regions of the male adult Sprague-Dawley rat brain in a conscious state: anterior cingulate cortex (ACC), bilateral central amygdala (L-CeA, R-CeA), and the ventral tegmental area (VTA). The hypothesis is that ECT will demonstrate anti-nociceptive and analgesic properties by suppressing the LFP power and by reducing the formalin-pain behavioral response. In a freely-moving state, the LFP was recorded simultaneously with the behavioral pain test score in two conditions: formalin-only (control) and formalin-ECT (experimental). In the formalin-only group after a 10 min baseline recording, under brief isoflurane anesthesia, 3% 50µl formalin was injected into the left hind paw. The animal's LFP was recorded while simultaneously scoring the behavioral activity for 60 min. In the formalin-ECT group, similarly, the rat was given formalin injection followed by 3 ECT stimulations at 50 pulses/s, 0.7 ms, 50 mA for 2 s, with interstimulus interval of 10 s. The same LFP and behavioral activity was recorded for 60 min. The power spectrum analysis revealed a trend of an inhibitory LFP effect post-ECT administration. In the behavioral testing, results demonstrated significance at the 40 min point, with the formalin-group showing a lower behavioral pain score. The hypothesis is partially supported by the LFP data, but not currently by the behavioral data.

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Poster

PSTR336: Descending Pain Modulation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR336.02/N5

Topic: E.01. Somatosensation – Pain and Itch

Support: Japan Pain Foundation
JSPS KAKENHI Grant 22KJ2540, 24KK0179, 25K02963
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Title: A randomized controlled clinical trial of home-based telemedicine for patients with chronic musculoskeletal pain

Authors: *K. HAYASHI¹, K. MIKI²;

¹Kyoto Univ., Kyoto, Japan; ²Fac. of Hlth. Sci., Osaka Yukioka Col. of Hlth. Sci., Ibaraki, Japan

Abstract: Non-pharmacologic self-management strategies including education and exercise are recommended in treatment for patient with chronic pain. Telemedicine is a practice of medicine using electronic communication, information technology, or other means between a physician in one location and a patient in another location. The present randomized controlled clinical trial aimed to compare the effects of home-based telemedicine with wearable device and usual care, compared with usual care, on pain-related outcomes in patients with chronic musculoskeletal pain. This trial tested the hypothesis that home-based telemedicine with a wearable device has an additional effect on pain-related outcomes, compared with usual care. The patients with chronic musculoskeletal pain were randomly allocated into usual care group or telemedicine group. All patients in both groups received usual care following recommendations from clinical practice guidelines. The in person face-to-face treatment by orthopedics and physical therapy in clinic was performed once a month for 6 months. Telemedicine group received telemedicine with wearable devices and usual care. Telemedicine was performed 20 minutes per session, every week, for 6 months, by the referring physical therapist. The patients used a wrist-worn wearable device, Fitbit Inspire HR, all day and all night. A primary outcome measure was a pain-Numeric Rating Scale (NRS). Secondary outcome measures were Pain Catastrophizing Scale (PCS), Hospital Anxiety and Depression Scale (HADS), Pain Disability Assessment Scale (PDAS), and EuroQol-5 Dimensions-3 level (EQ-5D-3L). Seventy-one participants were analyzed. The pain-NRS score was significantly decreased at 6 months, but not early periods in telemedicine group compared with baseline. Pain-NRS score at 6 months in telemedicine group was significantly lower than usual care group. Telemedicine group had a superior effect at 6 months on PCS, HADS, PDAS, and EQ-5D-3L, compared with usual care group. The present randomized controlled clinical trial firstly showed home-based telemedicine with wearable devices and usual care has a modest effect on pain-related outcomes than those usual care in patients with chronic musculoskeletal pain. The home-based telemedicine with a wearable device could have an additional therapeutic effect on symptoms in patients with chronic musculoskeletal pain. The telemedicine or wearable devices lead to provide better knowledge and concrete feedback on physical activity, motivate behavior change, facilitate daily exercise at home, reinforce wellness group activities and goals, and close at a distance between physicians and patients.

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Poster

PSTR336: Descending Pain Modulation

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Program #/Poster #: PSTR336.03/N6

Topic: E.01. Somatosensation – Pain and Itch

Support: JSPS KAKENHI Grant Numbers (22KJ2540, 24KK0179, and 25K02963)
Casio science promotion foundation
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Title: Endogenous pain modulation and pain catastrophizing are associated with delayed onset muscle soreness severity

Authors: *K. KASHIMA¹, N. OOKA¹, Y. FUKUSHIMA¹, G. BERARDI², A. JANOWSKI², Y. NIWA³, T. HIRONO¹, Y. SHIRO⁴, T. YAMAGUCHI¹, S. YAMADA¹, K. HAYASHI¹;
¹Kyoto Univ., Kyoto, Japan; ²Univ. of Iowa, Iowa City, IA; ³Kyoto Univ. Hosp., Kyoto, Japan;
⁴Nagoya Gakuin Univ., Nagoya, Japan

Abstract: A single bout of eccentric exercise induces muscle damage characterized by the development of muscle pain and swelling. The experience of pain is multifactorial and can be due to nociceptive stimuli as well as neurophysiological and psychosocial factors. Endogenous pain modulation is commonly assessed by temporal summation (TS) and conditioned pain modulation (CPM). Pain catastrophizing is a negative cognitive and affective response to pain. Heightened TS, deficient CPM, or high pain catastrophizing predicts the development of persistent pain and the efficacy of analgesics. The present study aimed to investigate whether neurophysiological and psychosocial factors predict delayed onset muscle soreness (DOMS) severity. Twenty-one healthy participants performed eccentric knee extensor contractions to induce DOMS. Pressure pain thresholds (PPT) on the ipsilateral quadriceps muscle were measured by using a pressure algometer before and 48 hours after the eccentric contractions. TS and CPM were evaluated before the eccentric contractions. For TS measurement, the pressure stimuli were applied to the ipsilateral quadriceps muscle using a pressure algometer. Participants were instructed to continuously rate their pain for each stimulus on a visual analogue scale (VAS). Greater increases in reported pain from the first to the tenth stimuli indicate greater ascending facilitation. For CPM measurement, the test pain was the PPT on the ipsilateral quadriceps muscle. The conditioning stimulus was a clamp that pinched the earlobe. CPM was measured as the change in PPT at the ipsilateral quadriceps, with greater values indicating greater descending pain inhibition. Pain catastrophizing was measured using the Pain Catastrophizing Scale (PCS), with higher scores indicating greater catastrophizing. Our paradigm led to a successful induction of TS and CPM. Participants reported a mean increase of 0.51 ± 1.97 points in VAS scores with the TS paradigm. Likewise, participants experienced 16.54 ± 17.87 % increase in PPTs with the CPM protocol. The eccentric exercise protocol for inducing DOMS decreased PPT on the quadriceps muscle (29.23 ± 11.16 N), compared to pre-exercise (35.12 ± 11.72 N). Lower CPM efficiency or elevated PCS scores are correlated with a significant decrease in PPTs from baseline to 48 hours ($p < 0.05$). TS was not correlated with the decrease in PPTs from baseline to 48 hours. The present study suggests endogenous pain modulation and pain catastrophizing have the potential to predict DOMS severity. Baseline

assessments of CPM and pain catastrophizing are potentially markers to help determine the extent of an individual's response to acute pain.

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Poster

PSTR336: Descending Pain Modulation

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.04/N7

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH NIDCR Intramural Research Program

Title: A new pontine center in descending pain control

Authors: *T. Li^{1,2,3,4},

¹Brown-NIH Grad. Partnership Program, Bethesda, MD; ²Natl. Inst. of Dent. and Craniofacial Res., Bethesda, MD; ³Natl. Ctr. for Complementary and Integrative Hlth., Bethesda, MD;

⁴Brown Univ., Providence, MD

Abstract: Pain sensation changes according to expectation, context, and mood, illustrating how top-down circuits affect somatosensory processing. Here, we used an intersectional strategy to identify anatomical and molecular-spatial features of supraspinal descending neurons activated by distinct noxious stimulation. This approach captured known descending pain pathways as well as spinal projecting neurons that are anatomically mapped to Barrington's nucleus in the dorsal pontine tegmentum. We determined that this population of neurons expresses corticotropin-releasing hormone in Barrington's nucleus (Bar^{Crh}) and exhibits time-locked firing in response to noxious stimulation. Chemogenetic activation of Bar^{Crh} neurons attenuated nocifensive responses as well as tactile neuropathic pain, while silencing these neurons resulted in thermal hyperalgesia and mechanical allodynia. Mechanistically, we demonstrated that pain-related input from the ventrolateral periaqueductal gray recruits Bar^{Crh} neurons, reduces ascending nociceptive transmission, and preferentially activates spinal dynorphin neurons to mediate analgesia. Our data expose a pontine inhibitory descending pathway that powerfully controls nocifensive sensory input to the brain.

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Poster

PSTR336: Descending Pain Modulation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.05/N8

Topic: E.01. Somatosensation – Pain and Itch

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2022R1A2C3009749; RS-2023-00302281)

Title: Sleep promotes neuropathic pain via Homer1a-mediated dendritic spine dysgenesis in the ventrolateral periaqueductal gray

Authors: *A. HO¹, M. PARK², T. KIM¹;

¹Biomed. Sci. and Engin., ²Biomed. science and engineering, Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of

Abstract: Neuropathic pain (NP), a chronic condition arising from nervous system pathology, is frequently comorbid with sleep disorders, exhibiting bidirectional influences. The immediate early gene Homer1a, a known regulator of synaptic homeostasis, is upregulated in both sleep deprivation and NP. However, the causal role of sleep loss-induced Homer1a in NP chronification remains elusive. We hypothesized that sleep disruption drives chronic NP by inducing Homer1a-mediated dendritic spine destabilization, impairing endogenous pain inhibitory circuits. To investigate this, adult male C57BL/6J mice underwent 6h, 12h, or 5 days of total sleep deprivation. Mechanical and thermal hypersensitivity were assessed using Von Frey and Hargreaves tests, respectively. Homer1a mRNA expression in the ventrolateral periaqueduct grey (vLPAG) was quantified via qRT-PCR. To evaluate vLPAG-rostral ventral medulla (RVM) projection neuron dendritic spine dynamics, AAV1-DIO-CHR2-EGFP-WPRE and AAVrg-hSyn-Cre-WPRE-hGH were stereotactically injected into the vLPAG and RVM, respectively. Confocal microscopy and Imaris software were used for dendritic spine analysis three months post-injection. The result indicates that sleep deprivation induced thermal and mechanical hypersensitivity, correlating with elevated Homer1a mRNA in the vLPAG. Morphological analysis revealed that 24h of sleep loss significantly reduced overall dendritic spine density and shifted spine morphology towards immature forms, indicative of impaired synaptic maturation. A similar pattern of spine remodelling was observed following sciatic nerve crush injury, suggesting a convergence of sleep deprivation and neuropathic pain on vLPAG-RVM circuit structural plasticity. Our findings suggest that sleep disturbance contributes to NP development or maintenance by impairing descending pain inhibition via Homer1a-mediated synaptic alterations in the vLPAG.

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Poster

PSTR336: Descending Pain Modulation

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Topic: E.01. Somatosensation – Pain and Itch

Support: NASU grant 0124U001556
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FCT, I.P. UIDB/04293/2020

Title: Descending control of nociceptive processing in spinal lamina X

Authors: I. BLASHCHAK¹, S. V. ROMANENKO¹, O. HALAIDYCH¹, K. KOROID¹, V. KROTOV¹, A. NECHAI², N. V. VOITENKO^{3,2}, B. V. SAFRONOV⁴, Y. M. USACHEV⁵, *P. V. BELAN^{6,2};

¹Bogomoletz Inst. of Physiol., Kyiv, Ukraine; ²Kyiv Academic Univ., Kyiv, Ukraine; ³Dobrobut Acad. Med. Sch., Kyiv, Ukraine; ⁴Neuronal Networks, IBMC 503828360, Porto, Portugal; ⁵Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA; ⁶Bogomoletz Institute of Physiol., Kyiv, Ukraine

Abstract: OFF cells in the rostral ventromedial medulla (RVM) control nociceptive transmission in the spinal cord. Their axons descending in the dorsolateral funiculus (DLF) project to several spinal areas, including lamina X. Unfortunately, little is known about how the activity of RVM OFF cells affects the primary afferent (PA) input to and local network in lamina X. To address these issues, we used an *ex-vivo* spinal cord preparation and recorded whole-cell responses of lumbar lamina X neurons to electrical PA and DLF stimulations. We have found that the DLF stimulation with the pattern resembling firing in the RVM OFF cells produced prolonged dorsal root potentials, almost complete presynaptic inhibition of PA inputs and effect on excitatory and inhibitory network activity in lamina X. Furthermore, the DLF stimulation regulates the output of lamina X neurons through presynaptic action on terminals of PAs and local interneurons. PA inputs and network-driven excitatory activity are gradually and differentially modulated by the frequency and duration of single DLF discharges in a time window of 10-50 s. Thus, intrinsic OFF cell discharges are likely to set responsiveness of lamina X neurons to PA and local inputs, thus regulating nociception. Strong and prolonged activation of descending pathways first results in an almost complete block of PA input to lamina X neurons and then in a gradual decrease of this block due likely to depletion of vesicles in the presynaptic terminals of descending fibers. Thus, the potency of descending control is a trade-off between the release, diffusion and reuptake of neuromodulators and recovery of pool of secretory vesicles. An application of noradrenaline evoked, in a fraction of lamina X neurons, effects similar to those induced by the DLF stimulation and could occlude them, therefore, implying that the adrenergic system plays an important role in the RVM-mediated descending control. We conclude that activity of descending pathways results in a gradual accumulation of released neuromodulators in the extracellular milieu of lamina X that produces slow presynaptic depolarization leading to the powerful control of PAs and local network.

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Poster

PSTR336: Descending Pain Modulation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.07/N10

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS111234
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Title: Paradoxical antinociception observed in vivo after $\alpha 2$ adrenergic receptor blockade

Authors: *J. LUCAS-ROMERO¹, M. F. BANDRES², J. G. MCPHERSON³;

¹Washington Univ. in St. Louis, Saint Louis, MO; ²Biomed. Engin., Washington Univ. In St. Louis, St Louis, MO; ³Physical Therapy, Anesthesiology, Biomed. Engin., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: The descending noradrenergic system has been described as a key element in the modulation of nociception at the spinal cord level. Noradrenaline exerts its actions through a variety of receptors expressed among different populations of spinal cord neurons and primary afferents. Its antinociceptive effects are primarily mediated by the $\alpha 2$ receptor, a $G_{i/o}$ protein-coupled receptor that inhibits neurotransmitter release from primary afferents and hyperpolarizes local spinal neurons, thereby contributing to pain modulation in the central nervous system. In this study, in vivo recordings from the spinal cords of anesthetized rats were obtained using microelectrode arrays, allowing the measurement of spinal neuronal population responses to noxious peripheral stimuli and the evaluation of pharmacological manipulations of the descending noradrenergic system. To this end, we administered RX821002, a selective $\alpha 2$ receptor antagonist, both intraperitoneally and intrathecally, to assess its effects on nociceptive transmission.

Interestingly, $\alpha 2$ receptor blockade induced a paradoxical antinociceptive effect under several experimental conditions, evident at both the single-neuron and population levels. This effect was found to be nociceptive-specific, reversible, and could also be observed when a non-specific noradrenergic blockade was performed using phentolamine. Furthermore, the depression of neural response after noxious stimulation was not limited to sensory-dominant regions of the spinal cord; it was also observed at every spinal cord level, including the motor regions at the ventral horn.

Noradrenaline release at the spinal cord is subject to regulation by a variety of spinal and supraspinal mechanisms. The descending noradrenergic fibers express $\alpha 2$ autoreceptors, which modulate noradrenergic release. We hypothesized that the blockade produced by RX821002 released the descending fibers from this feedback control mechanism, thereby effectively increasing the noradrenergic drive in the spinal cord. This increased drive would explain the antinociceptive effects observed, although further research will be needed to clarify the physiological relevance and relative importance of this mechanism.

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Poster

PSTR336: Descending Pain Modulation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.08/N11

Topic: E.01. Somatosensation – Pain and Itch

Title: Predicting Placebo-Induced Analgesia Based on Personality Traits

Authors: *Z. WEI¹, B. PETRE², T. D. WAGER³;

¹Psychological and Brain Sci., Dartmouth Col., HANOVER, NH; ³Psychological and Brain Sci.,

²Dartmouth Col., Hanover, NH

Abstract: Despite previous studies revealing associations between placebo effects and personality traits (Kern et al., 2020), brain activity patterns, as well as certain behavioral indicators (Botvinik-Nezer et al., 2024), however, recent meta-analytic findings have questioned the robustness and consistency of these associations (Kang et al., 2023). In this large-scale, pre-registered fMRI study (N = 400), we investigated whether placebo analgesic treatment modulates the expression of multivariate fMRI signatures associated with pain (thermal-selective and mechanical-selective) and multimodal negative affect (Ceko et al. 2022), and whether these effects can be predicted by individual differences in personality traits. Placebo treatment caused robust analgesia in conditioned thermal pain that generalized to unconditioned mechanical pain. In the brain, the placebo significantly reduced expression of Ceko et al.'s (2022) general negative affect signature in response to thermal pain ($\beta = -0.011$, $SE = 0.005$, $t(235.65) = -2.32$, $p = 0.021$, Cohen's $d = -0.11$). Placebo-induced reductions in negative affective signature responses to mechanical pain were marginally significant ($\beta = 0.010$, $SE = 0.005$, $t(452.55) = 1.89$, $p = 0.059$, Cohen's $d = 0.09$). Placebo significantly reduced responses in both the thermal pain-selective model response to heat ($\beta = 0.018$, $SE = 0.005$, $t(456.33) = 3.38$, $p = 0.001$, Cohen's $d = 0.16$) and mechanical pain-selective model to mechanical stimuli ($\beta = -0.008$, $SE = 0.003$, $t(240.65) = -2.61$, $p = 0.010$, Cohen's $d = -0.12$). Predictive analyses showed that higher scores in Emotion Regulation (ER) were associated with stronger neural placebo effects, as evidenced in both the general negative affect model for the mechanical pain condition ($\beta = 0.055$, $p = 0.027$, $d = 0.226$) and in the mechanical pain model ($\beta = 0.035$, $p = 0.0095$, $d = 0.265$). Higher scores on Positive Affect (PANAS-positive) were also associated with stronger placebo effects in the mechanical pain model ($\beta = 0.010$, $p = 0.047$, $d = 0.202$). In sum, individuals with better emotion regulation abilities and those more likely to experience positive emotions demonstrate greater neural placebo analgesic effects.

Disclosures: Z. Wei: None. B. Petre: None. T.D. Wager: None.

Poster

PSTR336: Descending Pain Modulation

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Program #/Poster #: PSTR336.09/N12

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS122830
NIH Grant T32GM075770

Title: Glycine Receptor $\alpha 3$ Subunit Contributes to Resolution of Postoperative Pain

Authors: *Y. WANG¹, T. DEAKIN², R. E. RHOADES¹, P. TANG³, Y. XU⁴;

²Anesthesiol., ³Anesthesiol. and Perioperative Med., ¹Univ. of Pittsburgh, Pittsburgh, PA;

⁴Anesthesiol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA

Abstract: Postoperative (PO) pain is a common clinical complication that typically resolves within 7-10 days. However, 3-10% of patients experience chronification of pain lasting >3 months or even permanently, significantly impairing quality of life for these patients. The mechanisms underlying PO pain chronification remain poorly understood. Glycine receptor $\alpha 3$ (GlyR $\alpha 3$), a subunit of the inhibitory glycine receptor family, plays a critical role in pain processing and recovery. We hypothesized that GlyR $\alpha 3$ contributes to the resolution of PO pain, and that loss of GlyR $\alpha 3$ delays recovery from PO pain states. We assessed pain behavior in 32 female mice—16 wild type (WT) C57BL/6J and 16 global GlyR $\alpha 3$ knockout (KO) mice. Mice were further divided into two cohorts for modeling acute and chronic PO pain. Acute pain was induced by a plantar incision on the left hind paw, while chronic pain was modeled by skin/muscle incision and retraction (SMIR) surgery on the left thigh. Pain behaviors were evaluated using von Frey, Hargreaves and thermal gradient tests (TGT; self-reported thermal preference). In the acute model, both WT and KO mice developed robust mechanical and thermal hypersensitivity following plantar incision, quantifiable by von Frey and Hargreaves tests, with no significant genotype differences. However, TGT revealed a distinct phenotype: KO mice displayed a persistent shift toward colder temperature preference, lasting over 37 days post-surgery, whereas WT mice recovered to their baseline thermal preference on PO days 7-9. This suggests that TGT captures subtle affective or sensory alterations that are missed in the traditional pain assessments based on supraspinal reflexes. In the chronic SMIR model, von Frey testing showed prolonged mechanical hypersensitivity in KO mice, with recovery delayed to PO Day 24 and not resolved until PO Day 41. In contrast, WT mice began to recover by Day 14 and fully resolved by Day 30. The recovery delay in KO mice highlights a significant role for GlyR $\alpha 3$ in modulating the chronification of mechanical hypersensitivity. Neither genotype exhibited thermal hypersensitivity in this model based on Hargreaves testing, further emphasizing the specific contribution of GlyR $\alpha 3$ to the mechanical dimension of chronic PO pain. These findings demonstrate that GlyR $\alpha 3$ facilitates resolution of both acute and chronic pain states, particularly through recovery of mechanical sensitivity and thermal preference. Loss of GlyR $\alpha 3$ delays behavioral recovery and may disrupt homeostatic inhibition required for pain resolution. These results suggest GlyR $\alpha 3$ as a potential molecular target for therapies aimed at preventing the chronification of PO pain.

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Poster

PSTR336: Descending Pain Modulation

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Program #/Poster #: PSTR336.10/N13

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS130882

Title: Delta opioid receptors reverse pituitary adenylate cyclase-activating peptide (PACAP) stimulation of GABA release in the vIPAG during opioid-induced hyperalgesia

Authors: *S. L. INGRAM¹, L. C. PATTI¹, C. M. DE ANDA GAMBOA², M. C. BELLINGHAM³, A. A. PRADHAN⁴;

¹Univ. of Colorado Anschutz Med. Campus, Aurora, CO; ²Univ. of Colorado - Anschutz, Aurora, CO; ³Sch. of Biomed. Sci., Univ. of Queensland, Brisbane, Australia; ⁴Ctr. for Clin. Pharmacol., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Opioid induced hyperalgesia (OIH) is prevalent chronic pain condition with few treatment options. Pituitary adenylate cyclase-activating peptide (PACAP) and its receptor, PAC1 are upregulated in OIH in the ventrolateral periaqueductal gray (vIPAG). Delta opioid receptors (DORs) in the vIPAG are upregulated in chronic pain states. Given that these neurotransmitter systems have opposing effects on adenylyl cyclase activity, we hypothesized that the effects of PACAP on vIPAG neurons would be reversed with DOR agonists. Whole-cell patch-clamp experiments examined changes to neuronal excitability and GABAergic spontaneous and evoked synaptic transmission in C57/B6 mice treated with saline injections or morphine (20 mg/kg 2X daily for 3 days, 40 mg/kg twice on day 4) to induce OIH. Brain slices were taken on Day 5 for electrophysiological recordings. In current-clamp experiments in naïve mice, PACAP (1 - 5 nM) hyperpolarized neurons but had minimal effects on excitability in the presence of GABA_A and GABA_B antagonists, gabazine (10 μ M) and CGP55845 (10 μ M), respectively. There was a notable increase in excitability in phasic firing vIPAG neurons suggesting that there is a selective activation of a subpopulation of vIPAG neurons. In voltage-clamp experiments, PACAP potentiated both spontaneous release of GABA and evoked GABAergic inhibitory postsynaptic currents (IPSCs). The effect of PACAP on evoked IPSCs is concentration-dependent with an EC₅₀ of 2 nM (95% CI 0.83 - 3.3 nM) and reversed with the PAC1 inhibitor, M65 (100 nM). PACAP-mediated potentiation of evoked IPSCs is greater in OIH-treated mice ($142 \pm 9\%$ compared to saline-treated mice $94 \pm 7\%$, $t(23) = 3.6$, $p=0.001$). The DOR antagonist, deltorphin (1 μ M), had nonsignificant effects in both saline and morphine-treated rats alone. However, deltorphin reversed the effects of PACAP in both treatment groups with a trend toward greater inhibition in OIH-treated mice. These data indicate that PACAP signaling is enhanced after development of OIH and that DOR agonists and PAC1 antagonists may be effective analgesics for OIH through actions in the vIPAG. The role of adenylyl cyclase and protein kinase A in the PACAP potentiation and DOR inhibition are currently being tested to better understand how PACAP induces OIH through increasing GABA release in the vIPAG.

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Poster

PSTR336: Descending Pain Modulation

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Title: Top-down control of the descending pain modulatory system drives placebo analgesia

Authors: *J. CHANG-WEINBERG^{1,2}, G. LIVRIZZI^{1,2}, J. LIAO¹, D. A. JOHNSON¹, S. T. LUBEJKO^{1,3}, C. DONG⁴, L. TIAN^{5,4}, M. R. BANGHART¹;

¹Dept. of Neurobiology, Sch. of Biol. Sci., ²Biol. Sci. Grad. Program, ³Neurosciences Grad. Program, Univ. of California San Diego, La Jolla, CA; ⁴Dept. of Biochem. and Mol. Medicine, Sch. of Med., Univ. of California Davis, Davis, CA; ⁵Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: In placebo analgesia, prior experience and expectations lead to pain suppression upon administration of an inert substance, however, detailed characterization of its analgesic effects is lacking. To more thoroughly examine placebo-induced analgesia, we reverse-translated a conditioned placebo protocol from humans to mice using systemically administered 10mg/kg morphine. Surprisingly, the placebo effect suppressed both nociception and unconditioned emotional-motivational pain-related behavior in C57Bl/6J mice aged 8-60 weeks. Further, we demonstrate that placebo can be conditioned for both thermal and mechanical nociception using the hotplate and pinprick assays, and the analgesic effect generalizes across pain modalities. Systemic administration of the opioid-receptor antagonist, naloxone at a dose of 3mg/kg, prevents placebo analgesia in both thermal and mechanical conditioned placebo, suggesting convergence of multiple pain modalities in the descending pain modulatory system. Using chemogenetic approaches, we observed that descending pain modulatory neurons in the periaqueductal gray (PAG) are critical for both morphine and placebo antinociception. The placebo effect depends on input to the PAG from the medial prefrontal and anterior cingulate cortices, but not anterior insular cortex. Conditioning enhances noxious stimulus-evoked endogenous opioid release in the PAG to produce analgesia consistently in male, but not female mice. Our results suggest that cortical control of the descending pain modulatory system (DPMS) is gated by rapid endogenous opioid signaling in the PAG during placebo trials. This study

bridges clinical and preclinical research, establishing a central role for the DPMS in placebo analgesia.

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Poster

PSTR336: Descending Pain Modulation

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.12/N15

Topic: E.01. Somatosensation – Pain and Itch

Title: A spatially-resolved multi-modal map of the mouse medulla

Authors: *L. YANG¹, F. LIU², M. KAWATANI², Y. ZHANG², H. HAHM², T. OKUDA³, M. HEITMEIER², V. K. SAMINENI³;

¹Washington Univ. in St. Louis Neurosci. PhD Program, St. Louis, MO; ²Washington Univ. in St. Louis, St. Louis, MO; ³Washington Univ. in St. Louis, St Louis, MO

Abstract: The mouse medulla is a crucial brainstem region for regulating essential bodily functions and connecting various brain regions and the spinal cord. Early neuroanatomy and physiology studies have shown that the medulla consists of several sub-nuclei containing neuronal cells with distinct morphologies and involved in various aspects of basic and vital functions, but the molecular and neuroanatomical organization of the heterogenous medulla neuronal populations remains elusive. To generate a high resolution, multi-modal map of the mouse medulla neurons, we combined Projection-TAGs, a high-throughput neuroanatomical tool we recently introduced, with spatial transcriptomics, allowing us to examine the spatial organization, molecular profiles, and projection patterns of medulla neurons at single-cell resolution covering ascending brain and descending spinal targets. This multi-modal spatial map provides rich insights into the molecular and neuroanatomical architecture of the mouse medulla and a foundation for functional investigations of neural circuits underlying diverse medulla functions in health and dysfunction in diseases.

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Poster

PSTR336: Descending Pain Modulation

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR336.13/N16

Topic: E.01. Somatosensation – Pain and Itch

Support: NSFC 82130032
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Title: Descending projection neurons in the primary sensorimotor cortex regulate neuropathic pain and locomotion in mice

Authors: *G. WANG¹, X. HOU³, Z. ZHOU⁴, Y.-Q. ZHANG²;

¹Inst. of Brain Sci., ²Fudan Univ., Shanghai, China; ³Inst. of Brain Science, Fudan Univ., Shanghai, China; ⁴Fudan university, Shanghai, China

Abstract: Objective Cortical descending modulation of sensory processing in the spinal cord has been revealed, the role of cortico-brainstem projections in sensory and motor regulation remains poorly investigated. **Results** (1) We identified two previously unappreciated subpopulations in the primary sensorimotor cortex (SM1), which are anatomically and electrophysiologically distinct but functionally similar, projecting to the periaqueductal gray (PAG) and rostroventral medulla (RVM), respectively. (2) Postsynaptic PAG glutamatergic neurons then project to the noradrenergic (NA) neurons in the locus coeruleus (LC) and 5-HT neurons in the RVM to drive spinal NA and 5-HT anti-nociception. (3) RVM enkephalinergic neurons that receive SM1 projections directly drive spinal opioid analgesia. (4) Silencing these two SM1 subpopulations impair motor behaviors and motor-induced analgesia, but does not affect behavioral responses to cutaneous sensory stimuli. **Conclusion** Our findings indicate that these brainstem-projecting SM1 subpopulations are crucial for cortical descending pain modulation, which could be exploited to optimize pain relief approaches.

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Poster

PSTR336: Descending Pain Modulation

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Topic: E.01. Somatosensation – Pain and Itch

Support: National Natural Science Foundation of China (NSFC) grants (82330036)
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Title: A sensory-motor-sensory circuit underlies antinociception ignited by primary motor cortex

Authors: *F. WANG;
Fourth Military Med. Univ., Xi'An, China

Abstract: Sensory-motor integration is crucial in the processing of chronic pain. Primary motor cortex (M1) is emerging as a promising target for chronic pain treatment. However, it remains elusive how nociceptive sensory inputs influence M1 activity, and how rectifying M1 defects in

turn regulates pain processing at cellular and network level. We show that injury/inflammation leads to hypoactivity of M1^{Glu} pyramidal neurons by excitation-inhibition imbalance between primary somatosensory cortex (S1)-M1. The impaired M1 output further weakens inputs to excitatory parvalbumin neurons of lateral hypothalamus (LH^{PV}) and impairs descending inhibitory system, and hence exacerbating spinal nociceptive sensitivity. When rectifying M1 defects with rTMS, the imbalance of S1-M1 microcircuitry can be effectively reversed, which aids to restore the ability of M1 to trigger descending inhibitory system, thereby alleviating nociceptive hypersensitivity. Thus, a sensory-motor-sensory loop is identified for pain-related interactions between sensory and motor system and can be potentially exploited toward treating chronic pain.

Disclosures: F. Wang: None.

Poster

PSTR336: Descending Pain Modulation

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Topic: E.01. Somatosensation – Pain and Itch

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Title: Craniofacial pain unmasks a glycinergic descending circuit controlling bodily morphine-resistant mechanical allodynia

Authors: D. DONG, J. HUO, G. YIN, *L. CHENG;
Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Bodily mechanical allodynia (MA) pathways are pre-existing but are normally gated in the spinal dorsal horn (SDH). A critical gap persists in understanding how craniofacial pain engages descending circuits to unmask systemic MA. Here, we developed a mouse model of intense craniofacial pain-induced bilateral hindpaw MA (CFH) to study descending control of MA. It is unexpected that CFH model-induced MA is resistant to systemic morphine treatment. Using CFH model, we identified a distinct glycinergic population (Glyt2⁺) from the rostral ventromedial medulla (RVM) to the SDH (RVM^{Glyt2+}→SDH) that controls the latent morphine-resistant MA. We show that the RVM^{Glyt2+}→SDH neurons receive direct axon projections from TG neurons as well as neurons from multiple sensory/emotional/stress-related brain regions. We demonstrate that craniofacial pain activates TG neurons and recruits sensory/emotional/stress-related brain regions to drive hyperactivity in RVM^{Glyt2+}→SDH neurons, which in turn unmasked the normally gated morphine-resistant bodily MA. Silencing or ablating these neurons

abolished morphine-resistant MA in the CFH model, whereas their chemogenetic activation unmasked the latent morphine-resistant MA pathways in naïve mice. Mechanistically, RVM^{Glyt2+} neurons unmask morphine-resistant MA pathways through directly exciting SDH NR3A-expression somatostatin (SOM) positive neurons via the excitatory “Glycine-GluN3A” synapse. These findings reveal a previously unrecognized descending circuit (TG→RVM^{Glyt2+}→SDH^{SOM+/-GluN3A+}) that selectively governs opioid-resistant forms of tactile pain, offering new avenues for targeted therapies.

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Poster

PSTR337: Pain Mechanisms and Targets

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR337.01/O3

Topic: E.01. Somatosensation – Pain and Itch

Support: National Institutes of Health grants GM130814 (J.Y.)

Title: Antinociceptive effect of nimodipine in paclitaxel-induced neuropathic pain

Authors: *Y. LU¹, G. CHEN², J. YAN², S. ABDI¹;

¹Dept. of Pain Med., ²Dept. of Anesthesiol. & PeriOperative Med., The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Neuropathic pain, resulting from nerve injury or chemotherapeutic agents like paclitaxel, presents a major clinical challenge due to limited treatment options and poor efficacy of current analgesics. Calcium channel blockers such as nimodipine, traditionally used for cerebral vasospasm, have recently shown potential for modulating pain pathways. However, their effectiveness in preclinical models of neuropathic pain remains to be fully elucidated. This study investigated the antinociceptive effects of nimodipine in mouse models of paclitaxel-induced neuropathic pain, PINP (paclitaxel, 2 mg/kg, i.p., one injection every other day for total of four injections). Once mechanical hypersensitivity (mechanical allodynia) was established, mice were divided into 3 groups (Group1: control group, treated with saline i.p.; Group 2: treated with nimodipine at doses of 2 mg/kg i.p.; and Group3: treated with nimodipine of 20 mg/kg i.p.). Mechanical nociceptive thresholds were assessed using Von Frey filaments at various time points post-treatment. In PINP mice, 2 mg/kg nimodipine produced a partial antinociceptive effect. In contrast, 20 mg/kg nimodipine significantly increased mechanical thresholds, demonstrating robust and sustained antinociceptive effects. The onset of effect of nimodipine was evident at 30 minutes post injection and lasted up 4 hours in both treatment groups. On the contrary, there was no change in the mechanical threshold of the control group. These findings suggest that nimodipine exhibits antinociceptive properties in paclitaxel-induced neuropathic pain. However, further studies using L-type calcium channel blockers as potential therapeutics for PINP needed to be done.

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Poster

PSTR337: Pain Mechanisms and Targets

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR337.02/O4

Topic: E.01. Somatosensation – Pain and Itch

Title: The analgesic effect of hepatocyte growth factor on transient receptor potential vanilloid 1-mediated pain

Authors: *Y. HAN;
Hanyang Univ., Seoul, Korea, Republic of

Abstract: Hepatocyte growth factor (HGF) is known for its important roles in tissue recovery processes and anti-inflammatory properties; however, its role in pain modulation remains poorly understood. This study investigates the effects of HGF in pain modulation and its interaction with the pain receptor transient receptor potential vanilloid 1 (TRPV1), providing insights into the intricate interplay between HGF and pain regulation. In the study of pain behavior using adult male C57BL/6 wild-type mice, we found that both recombinant HGF and its natural variant NK1 significantly reduced TRPV1-associated pain behaviors, particularly in thermal and capsaicin-induced pain, without affecting mechanical sensitivity. In dorsal root ganglion (DRG) neurons, HGF inhibited capsaicin-induced calcium influx, which was reversed by c-Met receptor inhibitor, suggesting a specific analgesic mechanism targeting the HGF/c-Met-TRPV1 axis. Further studies revealed that the activation of Akt signaling downstream within the HGF/c-Met pathway contributes to TRPV1 inhibition. Notably, in a Complete Freund's Adjuvant (CFA)-induced inflammatory pain model, intraplantar administration of HGF led to a reduction of mechanical hyperalgesia, while TRPV1 knockout mice failed to develop CFA-induced hyperalgesia. Collectively, these findings propose a novel role of HGF as an endogenous modulator of nociception via the c-Met-Akt-TRPV1 axis, suggesting a potential therapeutic target for TRPV1-related pain conditions.

Disclosures: Y. Han: None.

Poster

PSTR337: Pain Mechanisms and Targets

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR337.03/O5

Topic: E.01. Somatosensation – Pain and Itch

Support: NS128543

Title: Targeting the nociceptor endocytotic AP2 complex attenuated osteoarthritic pain behavior and prevented bone erosion

Authors: *A. COOPER¹, A. BHATTACHARJEE²;

¹Neurosci., State Univ. of New York, Univ. at Buffalo, Buffalo, NY; ²Pharmacol. and Toxicology, SUNY-Buffalo, Buffalo, NY

Abstract: Osteoarthritis (OA) is a degenerative joint condition that leads to chronic pain and the need for pain relief. Our previous work has indicated that inhibition of the nociceptor endocytotic adaptor protein complex 2 (AP2) inhibited neuronal hyperexcitability and inflammatory pain behavior. Here we demonstrate that OA pain behavior is reduced after genetic and local pharmacological inhibition of the nociceptor AP2 complex. Monoiodoacetate (MIA) was used to induce knee joint OA and pain behaviors were assessed by dynamic weight bearing (DWB) and von Frey mechanical sensitivity. *In vivo* AP2A2 genetic knockdown attenuated established OA pain-like behaviors in mice. Similarly, a lipidated AP2 complex decoy peptide achieved reduction in pain behavior in Sprague-Dawley rats. Specifically, a single intra-articular injection of either the AP2 inhibitor peptide or a scrambled peptide control was administered to the arthritic knee after verification of pain behavior. The AP2 inhibitor peptide demonstrated weeks of reduced pain behavior after a one-time injection. Upon completion of behavioral testing, MIA injected knee joints as well as the contralateral healthy knee joints were collected for analysis of disease progression. First, arthritis was validated using histological analyses of cartilage. Microcomputed tomography (micro-CT) analysis was used to evaluate the integrity of the joints. Arthritic knees that received the AP2 inhibitor peptide exhibited a preservation of bone volume in MIA-injected knees, which was comparable to that observed in healthy control knees. Conversely, the scrambled peptide-treated group exhibited the typical subchondral bone loss characteristic of this OA model. These findings suggest that inhibition of AP2 may alleviate OA pain and mitigate bone loss.

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Poster

PSTR337: Pain Mechanisms and Targets

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Program #/Poster #: PSTR337.04/O6

Topic: E.01. Somatosensation – Pain and Itch

Support: KSN2511011
RS-2023-00262398

Title: Trpv1 antagonism mediates the analgesic effects of mechanical acupoint stimulation in a mouse model of carrageenan-induced inflammatory pain

Authors: *S.-Y. KANG¹, S. BANG²;
¹KIOM, Daejeon, Korea, Republic of; ²KIOM, DAEJEON, Korea, Republic of

Abstract: We recently developed a mechanical acupuncture instrument (MAI) that applies mechanical stimulation to acupuncture points in effectively treating hypertension and addiction in animal models. However, its analgesic effect on inflammatory pain remains unclear. Here, we aimed to determine the optimal duration of MAI treatment at any given acupuncture point to improve analgesic effects. We also investigated whether MAI treatment or transient receptor potential vanilloid 1 (TRPV1) inhibition exhibit analgesic effects and influence TRPV1 expression and glial cells in the spinal cord in mice. The capsaicin test was used to identify the most effective acupoints and optimal treatment times for MAI. Additionally, we induced inflammatory pain in mice by administering a 2% carrageenan via intraplantar injection. To assess the analgesic effects of MAI treatment and TRPV1 inhibition, we evaluated pain-related behavior using von Frey filaments and a thermal stimulator applied to the hind paw. Here, we observed that MAI treatment for 60 s at ST36 showed optimal analgesic effects, including inflammatory pain-related behaviors. To investigate the involvement of TRPV1, we intrathecally administered capsazepine, which reduced carrageenan-induced mechanical allodynia and thermal hyperalgesia. Additionally, MAI treatment and capsazepine administration effectively suppressed the carrageenan-induced upregulation of TRPV1 and glial cells in the spinal cord. Our findings show that MAI application at ST36 effectively alleviates inflammatory pain behaviors and suppresses spinal TRPV1 activation. These results strongly support MAI as a valuable clinical therapy to manage inflammatory pain.

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Poster

PSTR337: Pain Mechanisms and Targets

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Topic: E.01. Somatosensation – Pain and Itch

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Title: Tlr4 activation in dorsal root ganglia neurons increases trpv1 activity through pka signaling

Authors: *J. B. LEMES¹, K. FRANCO MALANGE³, A. PANICHKINA¹, J. NAVIA PELAEZ⁴, S. CHOI², G. GONCALVES DOS SANTOS⁵, S. DOCHNAL⁶, M. CORR^{7,1}, Y.

MILLER⁸, T. L. YAKSH⁹;

¹UCSD, San Diego, CA; ²UCSD, La Jolla, CA, ; ³Anesthesiol., Univ. California San Diego, San Diego, CA; ⁴St. Louis Univ., Saint Louis, MO, ; ⁵Dept. of Anesthesiol., Univ. of California San Diego, Hillcrest, CA; ⁶Univ. of California San Diego, La Jolla, CA; ⁷La Jolla, CA, ; ⁸UC San Diego, La Jolla, CA; ⁹UCSD Anesthesia Lab. 0818, La Jolla, CA

Abstract: Nociceptive processing is multifactorial and involves several membrane receptors and their interaction. It was recently shown that TRPV1 activity is modulated by TLR4 activation, and this interaction plays a key role in initiating and maintaining pain states. However, the mechanism of the potentiated TRPV1 response due to TLR4 agonism in sensory neurons is unknown, although intracellular kinase activity is a strong candidate. To address that issue, dorsal root ganglia cell cultures from WT and *Tlr4*^{-/-} mice were prepared for assessing intracellular calcium transients, treated with lipopolysaccharide (LPS: TLR4 agonist) for 30 minutes, and acute capsaicin stimuli. TLR4 activation by LPS was assessed by blocking with LPS-RS (TLR4 antagonist). Flow cytometry was used to quantify TRPV1 protein expression at the neuron surface in suspended DRG cells with or without LPS treatment. In addition, behavioral nociception response was evaluated in male mice after TRPV1 activation via intraplantar injection of capsaicin. The role of kinase proteins was assessed using specific inhibitors (PKAi: KT5720 or PKCi: Chelerythrine chloride) applied to WT-DRG neurons or intrathecally prior to LPS. Our results showed that LPS incubation in DRG neurons enhances calcium influx after TRPV1 activation (capsaicin stimuli) via TLR4 receptors, and the blocking of PKA but not PKC reversed this augmentation caused by LPS. To verify the *in vivo* effects of this signaling identified *ex vivo* on pain behavior, mice received intrathecal LPS or PKAi + LPS, and the mechanical threshold of hind paws was measured by von Frey filament. Animals treated intrathecally with LPS presented higher spontaneous nociceptive response after intraplantar capsaicin and mechanical allodynia and this effect was prevented by the intrathecal PKA inhibitor. Together, these results display the relevance of TLR4 in modulating the excitability of nociceptors by regulating the response to TRPV1 activator, a key player in pain transmission, through PKA signaling.

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Poster

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Title: Expression of M1 macrophages markers in the dorsal root ganglion of male and female mice following induction of hyperalgesic priming

Authors: *N. OOKA¹, Y. FUKUSHIMA¹, K. KASHIMA¹, A. F. SMITH², K. A. SLUKA², R. NAKAHARA³, M. NAGAI-TANIMA¹, A. ITO¹, S. YAMADA¹, K. HAYASHI¹;
¹Kyoto Univ., Kyoto, Japan; ²Univ. of Iowa, Iowa, IA; ³Univ. of Washington, Seattle, WA

Abstract: Chronic pain is one of the most prevalent diseases worldwide. Women are disproportionately affected by chronic pain compared to men. Hyperalgesic priming, a form of neuroplasticity triggered by inflammatory mediators or injury, is considered a pivotal mechanism underlying the transition to chronic pain. Sex differences in the hyperalgesic priming model are contradictory depending on the priming stimulus. Dorsal root ganglion (DRG) sensory neurons play a key role in the transition from acute to chronic pain. Further, macrophages interact with DRG neurons, can release excitatory cytokines to sensitize sensory neurons, and enhance neuropathic pain. We hypothesized that an increase in inflammatory macrophages in the DRG (M1 phenotype) would contribute to hyperalgesic priming in male and female mice. Male and female 8-week-old C57BL/6J mice were randomly assigned to a hyperalgesic priming group or a pain-free control group. The hyperalgesic priming model was induced by an intraplantar injection of IL-6 (0.1ng, 25µl), followed by PGE2 (i.pl., 100ng, 25µl) at the same site 7 days later. Control mice received two intraplantar injections of normal saline, 7 days apart. Pain behavior was measured by determining the paw withdrawal threshold (PWT) to mechanical stimuli using an electronic von Frey anesthesiometer before and after injection. Macrophage phenotype was assessed using quantitative polymerase chain reaction (qPCR). The ipsilateral L4-L6 DRG were collected and pooled 1 day after injection of PGE2 and processed with qPCR analysis for F4/80 and CD11b as M1 macrophage markers. The ipsilateral, but not contralateral, PWT decreased 1 day after PGE2 injection, and remained decreased at least 3 days, in both male and female mice compared to controls. There were no sex differences in the peak and recovery of the pain behaviors. No changes in F4/80 or CD11b mRNA were observed between groups in the ipsilateral L4-L6 DRG in either male or female. The hyperalgesic priming model produced increased pain behaviors equally in male and female mice in the present study. There was no increase in expression of M1 mRNA markers in macrophages following the induction of the IL-6 induced hyperalgesic priming model. Future studies will examine for macrophage content and protein expression of M1 and M2 markers.

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Poster

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Title: Synovial mast cell expressed MrgprB2 mediates arthritis pain

Authors: *L. QU¹, L. YANG¹, C. LIU², W. LIU²;

¹Clin. Res. Ctr., ²Anesthesiol., The Second Affiliated Hospital, Hengyang Med. School, Univ. of South China, Hengyang, China

Abstract: The management of joint pain in rheumatoid arthritis (RA) represents a huge challenge for clinicians, in part because of limited efficacy and serious side effects of current treatments. Thus, novel effective mechanism-targeted treatments are urgently needed for RA pain. Mast cells (MCs) present in the synovium have been implicated in RA pathogenesis. Mas-related G-protein-coupled receptor B2 (MrgprB2), the orthologue of human MRGPRX2, is exclusively expressed in connective tissue MCs and activated by various basic secretagogues. Yet, it is unclear whether MrgprB2/X2 contributes to the development of RA pain and disease progression. Here, we show that MrgprB2 is expressed in synovial MCs. Furthermore, MrgprB2 expressing MCs formed close contacts with joint sensory fibers in the synovium. MrgprB2/X2 mRNA expression level was upregulated in synovial tissues of mice with collagen II-induced arthritis (CIA) and RA patients. Genetic deletion and pharmacological blockade of MrgprB2 significantly attenuated arthritis pain and joint swelling in the CIA model. Similar effects were also observed in mice in which MrgprB2 expressing MCs were genetically ablated. In vivo DRG electrophysiological recordings revealed that genetic deletion of MrgprB2 reduced hyperactivity of joint sensory afferents in the CIA. In naïve mice, chemical or chemogenetic activation of synovial MrgprB2/X2 is sufficient to trigger behavioral signs of joint hypernociception and inflammation. In vivo DRG imaging assay showed that MrgprB2 activation of synovial MCs activated peripheral terminals of joint nociceptors. These findings suggest that MrgprB2/X2 mediates the development of arthritis pain via the activation of synovial MCs, and thus define MrgprB2/X2 as a new potential therapeutic target for treating RA pain.

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Poster

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Topic: E.01. Somatosensation – Pain and Itch

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Title: Mast cells expressing VEGF are adjacent to visceral afferents that drive pelvic tactile allodynia in a mouse model of endometriosis

Authors: S. ACHARYA¹, M. V. TATAPUDI¹, H. CLARK¹, N. DONNELLAN³, B. K. TAYLOR¹, *K. ROMAN^{2,1};

¹Dept. of Anesthesiol. and Perioperative Med., ²Univ. of Pittsburgh, Pittsburgh, PA; ³Dept. of Obstetrics, Gynecology and Reproductive Sci., UPMC Magee-Womens Hosp., Pittsburgh, PA

Abstract: Endometriosis (EM) impacts approximately 9 million women in the United States. EM lesions are heavily innervated, infiltrated with mast cells (MCs), and have increased levels of vascular endothelial growth factor (VEGF) from an unclear source. We used a non-invasive mouse model of EM to test the hypothesis that an interaction between MCs that express VEGF and visceral afferents contributes to EM-associated pelvic pain. Briefly, female C57BL/6J donor mice (6 weeks old) received an injection of estradiol benzoate (10ug), and 4 days later, each uterine horn was excised and placed in Hank's Balanced Salt Solution (HBSS) and minced. Recipient mice received an intraperitoneal (i.p.) injection of HBSS (500ul; 'Shams') or HBSS+donor mice minced uterine horn (500ul; "EM mice"). To assess the development of pelvic tactile allodynia, von Frey (vF) filaments were applied to the suprapubic region before (baseline) and 28 days after tissue injection. Our first experiment revealed that VEGF-expressing MCs are adjacent to CGRP+ afferents in the uterine tissue of EM mice but not Sham controls. Next, we suprapubically applied von Frey filaments to assess mechanical hypersensitivity after ablation of TRPV1-expressing sensory neurons with intrathecal (i.t.) capsaicin (CAP; 1ug) or resiniferatoxin (RTX; 25ng). In Shams, neither vehicle, CAP, nor RTX changed mechanical thresholds. In EM mice, CAP, or RTX, but not saline, reversed hypersensitivity (p<0.05). Follow-up studies demonstrated that intrauterine VEGF dose-dependently induced pelvic tactile allodynia in non-EM mice; thus, we investigated the effect of afferent ablation in this model. RTX but not vehicle reversed VEGF-induced hypersensitivity (p<0.05). Altogether, we concluded that 1) MCs expressing VEGF are in close proximity to peptidergic afferents in EM, and 2) TRPV1-expressing primary afferent neurons drive EM-associated and VEGF-induced pelvic tactile allodynia. This study suggests targeting neuroimmune interactions between MCs expressing VEGF and TRPV1-positive afferents is a potential therapeutic method to alleviate chronic pelvic pain in endometriosis.

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Poster

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Title: Hiv-1 gp120 derived circulating extracellular vesicle, an unknown chronic algogen for hiv-associated pain

Authors: *S. YUAN^{1,2}, J. LIEW², J. BEI³, A. PAL², K. ARAYA⁶, V. TAT⁴, I. J. GAMEZ², Q. CHANG³, C. HAINES², B. GONG⁵;
²Neurobio., ³Pathology, ⁴Dept. of Pharmacol. and Toxicology, ¹Univ. of Texas Med. Br., Galveston, TX; ⁵Univ. of Texas Med. Br., Pathology, TX; ⁶Dept. of Pharmacol. and Toxicology, The Univ. of Texas Med. Br., Galveston, TX

Abstract: HIV associated chronic pain (HIV-PAIN) is one of the critical comorbidities of HIV infection and remains 30-60% prevalent in HIV infected patients despite viral suppression achieved by antiretroviral therapy (ART). Current work aims to understand the mechanism why the chronification of HIV-PAIN maintains and cannot be dramatically decreased by ART. We proposed that small extracellular vesicle (sEV) play an important role in maintaining chronicity of HIV-PAIN. We isolated circulating gp120 derived sEV(gp120-sEV) from gp120 induced pain mice (gp120-PAIN) which was created to replicate HIV-PAIN in clinical. gp120-sEV was proven to be absent of gp120 by western blot. Following intrathecal transfusion of gp120-sEV, naïve recipient mice exhibited an extensive pain phenotype, including mechanical allodynia, thermal hyperalgesia, and cold allodynia, tested by novel dry ice vapor cold test we invented. RNA-sequence analysis suggests that gp120-sEV induced alternative expression of genes pertinent to nociception and neuroinflammation. These findings provide direct evidence that circulating gp120-sEV functions as endogenous chronic algogens, which is exempt being a target of ART and whose algogenic efficacy was even stronger and longer than gp120 itself. The endogenous gp120-sEV enhances the gp120-initiated pain and critically extends the pain chronification. Thus, we build up a paired algogens testing system. including test gp120 in donor mice and gp120-sEV in recipient mice, to distinguish the initiating (exogenous, gp120) algogen and chronic (endogenous, gp120-sEV) algogen. This paired algogens concept provides a new mechanistic insight into the pathogenesis of chronification of HIV-PAIN, allowing develop new analgesic target for HIV-PAIN by targeting algogenic gp120-sEV.

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Poster

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Title: Brain-derived neurotrophic factor in dorsal root ganglion and spinal dorsal horn in a mouse model of osteoarthritis

Authors: *Y. FUKUSHIMA¹, N. OOKA¹, K. KASHIMA¹, J. B. LESNAK², A. N. PLUMB², R. NAKAHARA³, M. NAGAI-TANIMA¹, A. ITO¹, S. YAMADA¹, K. HAYASHI¹;

¹Kyoto-University, Kyoto, Japan; ²Univ. of Texas at Dallas, Dallas, TX; ³Univ. of Washington, Seattle, WA

Abstract: Osteoarthritic pain is classically considered a nociceptive pain condition arising from abnormal loading of a damaged joint. Meanwhile, the existence of a neuropathic component in osteoarthritic pain has been proposed in human and rodent studies. Brain-derived neurotrophic factor (BDNF) in dorsal root ganglion (DRG) or spinal dorsal horn likely contributes to the sensitization of neurons, and produces pain in a variety of pain models. However, their contributions in the monoiodoacetate-induced osteoarthritis model are unclear. The purpose of this study was to compare the expression of BDNF in DRG and spinal dorsal horn in an animal model of monoiodoacetate-induced osteoarthritis. C57BL/6J mice, aged 8 weeks, were used in this study. The mice were randomly assigned to the osteoarthritis group or pain-free control group. The osteoarthritis model was induced by an intra-articular injection into the left knee joint of 10 µl monoiodoacetate in normal 0.9% saline under anesthesia with isoflurane. The control mice received an intra-articular injection of normal 0.9% saline. Behavioral assessment was performed by measuring the mechanical paw withdrawal threshold bilaterally using an electronic von Frey anesthesiometer. The lower paw withdrawal thresholds indicate greater sensitivity. BDNF mRNA expression was measured in ipsilateral L3-5 DRG and spinal dorsal horn isolated from the osteoarthritis group and pain-free control 5 days post injection using quantitative real-time PCR. The ipsilateral paw withdrawal threshold was significantly decreased at 5 days after intra-articular injection in the osteoarthritis group, compared to before the injection or pain-free control group ($p < 0.05$). The decreased threshold was maintained for at least 4 weeks after the injection. There was no difference both between and within the groups in the contralateral paw. BDNF mRNA expression was significantly increased in ipsilateral DRG in the osteoarthritis model at 5 days after intra-articular injection, compared to controls ($p < 0.05$). There was a trend towards significance of BDNF mRNA expression in ipsilateral spinal dorsal horn. BDNF mRNA expression in DRG was increased in the osteoarthritis model. BDNF in the primary or secondary sensory neurons could contribute to sensitization and pain hypersensitivity.

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Poster

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Title: Growth Hormone Regulates Distinct STAT-Mediated Neuroimmune Mechanisms in Neonatal Inflammatory Pain

Authors: *A. O. FADAKA¹, O. ECKSTEIN¹, G. M. TRIPATHI¹, M. C. HOFMANN¹, R. C. HUDGINS², M. P. JANKOWSKI¹;

¹Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; ²Anesthesia, Cincinnati Children's Res. Fndn., Cincinnati, OH

Abstract: Neonatal inflammatory pain can lead to long-term alterations in pain sensitivity, yet the underlying mechanisms remain poorly understood. Our previous work demonstrated that growth hormone (GH) regulates inflammatory pain in neonates through neuronal STAT1 expression, rather than Serum Response Factor (SRF), distinguishing inflammatory muscle injury from incision-related hypersensitivity. Specifically, GH-dependent STAT1 signaling regulated neuronal sensitization and acute pain-like behaviors via peripheral CCL2 release, driving macrophage infiltration into injured tissue. While GH was also found to influence the chemokine CXCL10 and mast cell recruitment, the underlying mechanism remained unclear. New analysis of our bulk RNA-Seq data from dorsal root ganglia (DRGs) indicated that GH also modulated neuronal STAT2 expression. Therefore, we investigated whether GH regulated mast cell infiltration through STAT2-mediated control of CXCL10. We used nerve targeted siRNA mediated knockdown of neuronal STAT2 or CXCL10 in neonatal mice prior to inducing muscle inflammation with 1% carrageenan. Knockdown of sensory neuron STAT2 significantly reduced pain-like behaviors, including spontaneous paw guarding and mechanical hypersensitivity at one day post-injury. Pain-like behaviors typically persisted through day 3 in control inflamed mice but were also markedly attenuated following neuronal CXCL10 knockdown. Furthermore, neuronal STAT2 knockdown led to a marked decrease in CXCL10 expression in the DRGs and attenuated mast cell accumulation in the injury site. These findings uncover distinct, STAT-dependent immune mechanisms by which GH modulates neonatal inflammatory pain, highlighting GH-STAT1-CCL2 and GH-STAT2-CXCL10 as parallel but specific regulators of macrophage and mast cell responses, respectively. This work provides new insight into neonatal

neuroimmune interactions and suggests potential therapeutic targets for managing early-life inflammatory pain.

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Poster

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Title: Role of NaV1.7 in joint inflammation and nerve sprouting in a rheumatoid arthritis model

Authors: *K. FRANCO MALANGE¹, G. GONCALVES DOS SANTOS³, J. VAZQUEZ MORA⁶, Y. DU⁴, J. B. LEMES⁷, S. DOCHNAL⁸, C. MORADO URBINA⁵, J. JIMENEZ-ANDRADE⁹, M. CORR², K. B. DORE¹⁰, T. L. YAKSH¹¹;

¹Univ. California San Diego, San Diego, CA; ²Univ. California San Diego, La Jolla, CA; ³Dept. of Anesthesiol., Univ. of California San Diego, Hillcrest, CA; ⁴Univ. of California San Diego, La Jolla, CA; ⁵Anesthesiol., Univ. of California San Diego, San Diego, CA; ⁶Karolinska Inst., stockholm, Sweden; ⁷Anesthesiol., ⁸UCSD, San Diego, CA; ⁹Univ. Autónoma De Tamaulipas, Reynosa, Mexico; ¹⁰Neurosciences, UCSD Dept. of Neurosciences, La Jolla, CA; ¹¹UCSD Anesthesia Lab. 0818, La Jolla, CA

Abstract: Disability arising from rheumatoid arthritis (RA) has a profound impact upon quality of life in over 50 million Americans. Current therapeutics represent major advances in management of clinical signs but often do not resolve the associated pain, suggesting mechanisms that support the pain phenotype beyond simple inflammation-induced terminal sensitization. Our work with the K/BxN serum-transfer murine model has demonstrated a parallel profile that mimics the characteristics of RA, resulting in a long-lasting but reversible articular inflammation, a robust post-inflammatory pain that persists long after resolution of the inflammatory phenotype, and as discussed below, nociceptor joint sprouting and analgesic pharmacology, all mimicking a polyneuropathic phenotype. Our results showed that K/BxN mice display an allodynic and inflammatory phenotype 7 days after the arthritic serum transfer. To assess the characteristics of the changes in excitability induced by the K/BxN serum transfer, DRG neurons harvested from K/BxN mice were examined in culture for membrane patch clamping. We found that such hyperexcitable membrane states in the K/BxN mouse are maintained by enhanced sodium currents which are blocked by the selective NaV 1.7 antagonist Prototoxin-II (PTx-II). These findings are aligned with behavioral studies that have shown that

K/BxN mice treated with intrathecal PTx-II show a transient reversal of allodynic thresholds at the inflammatory phase. Altogether, such initial findings support investigating the effects of such a therapeutic target in the K/BxN model. Our previous work showed that epigenetically repression of NaV 1.7 transcription using intrathecally delivered Zinc-Finger proteins packed in Adeno Associated Viral vectors (AAV-ZF-NaV1.7) produces long lasting but nonpermanent gene repression of NaV1.7 in primary afferents and results in a robust analgesic profile in a variety of pain conditions. Here, the intrathecal AAV-ZF-NaV1.7 reversed the tactile algesic effect on both skin and joint dermatomes affected in the K/BxN model. Unexpectedly, NaV 1.7 repression resulted in a significant reduction in joint inflammation (arthritis score) and more importantly, the knock-down of NaV 1.7 robustly reduced the joint nerve sprouting of both polymodal-C-nociceptors and post ganglionic sympathetic fibers that maintain the chronic phenotype of K/BxN mice. The results suggest that blockade or repression of NaV 1.7 will mitigate the arthritic pain phenotype through modulating the afferent nociceptor traffic otherwise mediated by NaV1.7, and ongoing block of nociceptor signaling has a disease-modifying effect upon the RA phenotype.

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Poster

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Title: Ectopic sensory nerve innervation mediated by PAR2 drives the development of chronic pain induced by bone cancer or tendon injury

Authors: H. YAO, *Z. ZHU, X.-J. SONG;
Dept. of Med. Neuroscience, SUSTech Ctr. for Pain Med. and Med. Sch., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Cancer or severe injury in peripheral tissue leads to intractable and persistent pain, while effective treatments for pain relief remain limited. There is evidence suggesting that ectopic sensory nerve innervation in tumor or injured tissue exacerbates chronic pain development. However, the underlying cellular and molecular mechanisms remain elusive. In this study, we used tumor cell implantation (TCI) model in tibia to produce cancer pain and Achilles tendon partial transection injury (TTI) model to produce tendon injury-induced pain.

Combining transgenic nociceptor reporter (Trpv1^{Cre}; Ai9) mice and immunofluorescence, we characterized ectopic sensory nerve innervation in cancer-invaded bone tissue and injured tendon tissue. We also used western blot and immunofluorescence to analyze the alterations of the signaling molecules related with sensory nerve remodeling. Our results showed that both TCI and TTI increased the abundance of nociceptor innervation in cancer-invaded bone tissue and injured tendon tissue. We identified that the proteinase-activated receptor 2 (PAR2) was up-regulated in both soma of nociceptors in dorsal root ganglion (DRG) and their peripheral tissue-innervated nociceptive terminals following TCI or TTI. Increased PAR2 enhanced the plasticity of DRG nociceptors by activating cAMP/PKA signaling and promoting phosphorylation of PKC, CaMKII, and CREB. Pharmacological or genetic perturbation of PAR2 in DRG nociceptors decreased the ectopic sensory nerve innervation and ameliorated bone cancer pain and tendon injury-induced pain. Besides, systemic treatment of vitamin B complex (VBC) comprising thiamine (B1), pyridoxine (B6), and cyanocobalamin (B12) produced effective analgesia on TCI- or TTI-induced pain and suppressed ectopic sensory nerve innervation by blocking PAR2 activation. Our study demonstrates a critical role of PAR2 in the ectopic sensory nerve innervation during the development of chronic pain induced by bone cancer or tendon injury. Our data also suggest that targeting PAR2, for instance with VBC, is a promising therapeutic strategy for pain management in cancer pain and tendon injury-induced pain.

Disclosures: H. Yao: None. Z. Zhu: None. X. Song: None.

Poster

PSTR337: Pain Mechanisms and Targets

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR337.14/O16

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R35GM119522

Title: Elevated oxidative stress impairs inflammation resolution and prolongs postoperative pain in a rodent surgical incision model

Authors: *R. HELL¹, C. GOODNOUGH², E. GROSS²;

¹Dept. of Anesthesiology, Perioperative and Pain Med., Stanford Univ., Stanford, CA; ²Dept. of Anesthesiology, Perioperative and Pain Med., Stanford Univ., Palo Alto, CA

Abstract: Postoperative pain remains a major clinical challenge. A surgical incision triggers the generation of reactive aldehydes, that are cytotoxic byproducts of lipid peroxidation, that are metabolized by aldehyde dehydrogenase 2 (ALDH2). Deficient ALDH2 activity, which is prevalent in 8% of the world population that carry an ALDH2*2 genetic variant, leads to aldehyde accumulation and increased oxidative stress. This imbalance may impair the resolution of inflammation and prolong pain. We investigated whether ALDH2 dysfunction exacerbates postoperative inflammatory pain by disrupting macrophage-mediated immune regulation. Using

a mouse model carrying the ALDH2*2 (E487K) variant associated with reduced ALDH2 activity, we performed hind paw incisions and evaluated mechanical hypersensitivity over five days. Proinflammatory cytokines (IL-1 β and IL-6) were quantified in skin samples at multiple time points, and histological analysis was conducted 72 hours post-incision. To investigate cellular mechanisms, bone marrow-derived macrophages from ALDH2*2 and wild-type mice were assessed *in vitro* for their migratory ability and capacity for efferocytosis. Mice with impaired aldehyde clearance exhibited prolonged mechanical hypersensitivity and elevated levels of IL-1 β and IL-6 in the early postoperative period. Histological analysis revealed greater neutrophil accumulation and reduced macrophage density at the wound site in the ALDH2*2 mice relative to wild type ALDH2 mice. *In vitro*, ALDH2*2 macrophages demonstrated impaired migration and decreased ability to engulf dying cells, suggesting that dysfunctional macrophage behavior contributes to sustained neutrophilic inflammation. This failure to resolve inflammation may underlie the prolonged mechanical hypersensitivity seen for the ALDH2*2 variant when subjected to a surgical incision. These findings establish a mechanistic link between oxidative stress and postoperative pain, mediated by impaired macrophage function. Individuals with reduced ALDH2 enzyme activity may be at greater risk for prolonged postoperative inflammation and pain. Enhancing aldehyde metabolism or restoring macrophage resolution capacity may represent promising therapeutic strategies to improve recovery outcomes for patients requiring surgery.

Disclosures: R. Hell: None. C. Goodnough: None. E. Gross: None.

Poster

PSTR337: Pain Mechanisms and Targets

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR337.15/P1

Topic: E.01. Somatosensation – Pain and Itch

Support: NIDCR grant DE022129

Title: Temporomandibular disorder- neurexin 3 role in myofacial pain

Authors: *P. KRAMER¹, M. UMORIN¹, L. NGUYEN²;

¹Texas A&M Univ. Sch. of Dent., Dallas, TX; ²Texas A&M Univ. Col. of Dent., Dallas, TX

Abstract: Background: Temporomandibular disorders often involve myofascial pain. Animal models for myofascial pain include placing a ligature around the masseter tendon of a rat. Previously, we reported that expression of neurexin 3, a cell adhesion molecule that has an important role in GABA release, is decreased in the central amygdala (CeA) after inducing a pain response. Therefore, we hypothesized that tendon ligature pain in male rats is due to inhibition of neurexin 3 expression in the CeA. Methods: To test this hypothesis, we used a combination of gene knockdown, c-fos staining and pain behavior experiments. Behavioral tests included von Frey testing of the orofacial region and spontaneous measures pain as quantitated

by meal duration. Results: The results indicated that ligature of the tendon and knockdown of neurexin 3 expression in the CeA significantly increased c-fos positive neurons within the trigeminal nucleus caudalis, the trigeminal ganglia, the lateral parabrachial and the CeA. This increase in c-fos staining was present when behavioral pain increased. Conclusions: The decrease in neurexin 3 in the CeA increased the activity of neurons within the orofacial pain pathway after ligature of the masseter tendon. This increased pain response is potentially due to decreased GABA release, attenuating the inhibition of ascending pain signals.

Disclosures: P. Kramer: None. M. Umorin: None. L. Nguyen: None.

Poster

PSTR337: Pain Mechanisms and Targets

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Program #/Poster #: PSTR337.16/P2

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH AT008742

Title: Interactions among ketogenic diet, inflammatory pain, and estrous stage in female mice

Authors: *D. N. RUSKIN¹, S. A. MASINO²;

¹Trinity Col., Hartford, CT; ²Trinity Col., HARTFORD, CT

Abstract: Chronic pain is often driven by inflammation, highlighting the importance of anti-inflammatory strategies. Research suggests that the ketogenic diet (KD) is anti-inflammatory. Our previous work in male mice found that KD treatment significantly reduced tactile allodynia due to inflammatory pain. Here, to investigate sex differences, the effects of KD on tactile allodynia due to inflammatory pain were investigated in female mice. Estrous stage was noted to account for the potential influence of sex hormones. Female mice were maintained on a control diet or KD before receiving intraplantar injection of complete Freund's adjuvant (CFA) to induce inflammation. Tactile sensitivity was assessed using von Frey, spontaneous behavior was evaluated via marble burying, and inflammation was assessed with paw swelling. CFA injection produced significant swelling, increasing from 4h to 2d, with KD reducing the swelling at both time points. KD treatment reduced overall tactile sensitivity but that significant tactile allodynia was only present at 4h. Tactile allodynia showed complete recovery at 2d, and there was no effect of KD apart from the net decrease in sensitivity noted in uninjected paws. The effect of estrous stage was limited to KD-fed mice: reduced allodynia was found in KD-fed mice in proestrus. KD increased marble burying overall, and this spontaneous behavior was not affected significantly by CFA. We found that female mice recovered from CFA-related allodynia faster than males, and, unlike males, the trajectory of that recovery was unchanged by KD. Taken together, there are mixed effects of KD on CFA-induced behavioral and physiological changes in male and female mice and the mechanisms remain poorly understood.

Disclosures: D.N. Ruskin: None. S.A. Masino: None.

Poster

PSTR337: Pain Mechanisms and Targets

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Program #/Poster #: PSTR337.17/P3

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH NIDCR R15 DE025970
NIH NIDCR F31 DE031959
TWU Small Grant
TWU Center for Student Research Small Grant

Title: Estrogen Modulates Macrophage Cytokine Release In Vitro and Correlates with Immune-Glial Changes in the Trigeminal Ganglia During Orofacial Inflammation

Authors: *A. BASNET¹, T. M. HICKMAN², L. K. HANSON², S. SINHA¹, D. L. AVERITT²;
²Biol., ¹Texas Woman's Univ., Denton, TX

Abstract: Orofacial pain is 2-4x more common among women than men and the gonadal hormone estrogen has been evidenced to play a role in sex differences. Trigeminal sensory neurons innervate the orofacial region, relaying pain signals to the central nervous system. Orofacial inflammation has been linked to the infiltration of immune cells, including macrophages, into the trigeminal ganglia at the sensory neuron cell bodies; however, their role in trigeminal pain remains unclear. We quantified cytokines present in the supernatant of IC-21 murine macrophages (intermediate monocyte-macrophage stage of development, AATC) and J774A.1 murine macrophages (mature macrophage phenotype; AATC) following treatment with 50 nM 17 β -estradiol (E2) or vehicle as a negative control. The release of cytokines and chemokines was quantified using a Proteome Profiler Cytokine Array Kit (R&D Systems). We found that several key proinflammatory cytokines were increased by E2 in both macrophage cell lines, notably the cytokine interleukin-6 (IL-6) and the chemokine C-X3-C motif ligand 1 (CX3CL1; also known as fractalkine). This suggests that estrogen could alter the release of proinflammatory mediators from macrophages and crosstalk with sensory neurons and other immune cells within the cell bodies. We hypothesized that the secretion of mediators by macrophages influences the number, timing, and activation state of other immune cell types, and nonneuronal cells, including satellite glial cells in trigeminal ganglia. Estrogen polarization of macrophages at the trigeminal ganglia may amplify orofacial pain signaling at trigeminal sensory neurons to a greater degree in females than males. To test this *in vivo*, we injected the Complete Freund's Adjuvant (CFA) into the right masseter muscle of adult female and male rats as a rat model of inflammatory orofacial pain. Immune cells were isolated using enzymatic methods from bilateral trigeminal ganglia on days 1-14 post-injection and analyzed by flow cytometry. The total CD45⁺ leukocyte population increased after CFA injection on days 3, 5, and 7, with myeloid cells (CD11b⁺) predominating over lymphocytes (CD3⁺) across all three time points compared to sham. In parallel, we are currently performing immunohistochemistry at the same time points to assess satellite glial cells using glutamine synthetase and GFAP markers.

Ultimately, these data will contribute knowledge of immune cell infiltration at the trigeminal ganglia of male and female rats, serving as a basis for future hypotheses on sex differences in the neuroimmune interaction in sensory neurons.

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Poster

PSTR337: Pain Mechanisms and Targets

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Topic: E.01. Somatosensation – Pain and Itch

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NIH NIDCR F31 DE031959
TWU Small Grant
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Title: Sex differences in P2RX3 expression in the TRPV1 population of trigeminal sensory neurons in a rat model of stress exacerbated orofacial pain

Authors: *B. ISLAM¹, D. CANTU¹, A. MENDOZA¹, O. DARWISH², D. L. AVERITT¹;
¹Biol., ²Mathematics and Computer Sci., Texas Woman's Univ., Denton, TX

Abstract: Psychological stress contributes to and amplifies orofacial pain, which is more prevalent in women. Recently, we found that exposure to sub-chronic stress exacerbates inflammatory orofacial pain to a greater degree in females than males. We performed transcriptomic analysis of the rat trigeminal ganglia (TG) to identify sex-specific differentially expressed genes (DEGs) to gain insight into neuroplasticity occurring in the sensory ganglia during stress-exacerbated orofacial pain. Adult male and female Sprague-Dawley rats (250-350g) received an injection of complete Freund's adjuvant (CFA) or saline into the right vibrissal pad. All rats were subjected to the forced swim test (FST, sub-chronic stress) or sham paradigm for 3 days. Following day 3 of FST, ipsilateral TG were extracted and flash-frozen in liquid nitrogen. Next-generation RNA sequencing was employed to examine the TG transcriptomic profile of rats exposed to FST (Saline+FST) or CFA and FST (CFA+FST) compared to negative controls (Saline+Sham). We identified numerous pain-related sex-specific DEGs following FST. Genes related to the extracellular matrix and GABA neurotransmission, such as collagen type III alpha 1 chain (*Col3a1*) and solute carrier family 13 member 3 (*Slc13a3*), were found to be significantly upregulated in males. The genes encoding transmembrane protein 45b (*Tmem45b*) and P2X purinergic receptor 3 (*P2rx3*) were found to be significantly upregulated in stressed females compared to males. We then performed quantitative polymerase chain reaction (qPCR) for the *Tmem45b* and *P2rx3* genes. Our data shows greater upregulation of *P2rx3* and *Tmem45b*

genes in stressed females compared to males. We next performed immunohistochemistry (IHC) to observe P2RX3 protein expression within a subpopulation of nociceptive sensory neurons that express transient receptor potential vanilloid 1 (TRPV1) ion channels. While similar P2RX3 expression was observed across the male and female TG, we report a significantly greater expression of P2RX3 in the TRPV1 positive sensory neurons in stressed females compared to males. To gain insight into the effect of gonadal hormones on P2RX3 expression, we performed IHC for P2RX3 expression in TG collected from each stage of the estrous cycle, ovariectomized females, and naïve males. Our data indicate higher expression of P2RX3 in female rats during estrus. Together, these data provide evidence that stress alters P2RX3 expression in the trigeminal sensory ganglia, which is highly sexually dimorphic under pathological conditions related to stress and under the modulation of gonadal hormones.

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Poster

PSTR337: Pain Mechanisms and Targets

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Program #/Poster #: PSTR337.19/P5

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS116759

Title: Sex-specific metabolic differences drive post-surgical pain

Authors: ***M. HAQUE**¹, **P. KUPPUSAMY**², **O. K. MELEMEDJIAN**³;

¹Univ. Maryland, Baltimore, Baltimore, MD; ²Univ. of Maryland, Baltimore, MD; ³Dept. of Neural & Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD

Abstract: Pain is a complex, multifactorial experience that differs significantly between biological sexes. Females typically experience greater post-surgical pain compared to males. However, the biological mechanisms driving these sex differences remain poorly understood, particularly regarding how sex-specific metabolic regulation influences pain development. Our previous work demonstrated that mitochondrial pyruvate oxidation is disrupted in various pain conditions, causing a metabolic shift toward glycolytic extrusion of metabolites that activate nociceptive pathways. Importantly, pain resolution requires compensatory upregulation of glutamine oxidation rather than simple reversal of these metabolic changes. Based on this foundation, we investigated these mechanisms using a hyperalgesic priming mouse model with intraplantar incision as the initial insult, followed by prostaglandin E₂ (PGE₂) to model pain chronification. Our findings revealed distinct sex-dependent pain patterns that mirror clinical observations. In acute surgical pain, males developed peak pain intensity faster, while females showed slower onset but developed more severe pain that lasted longer. In chronic post-surgical pain, females experienced more severe chronic pain compared to males. We tested several

metabolic interventions in both acute and chronic post-incisional pain. Oxamate, a lactate dehydrogenase inhibitor that blocks lactate production, showed greater anti-allodynic effect in males than females. In contrast, dichloroacetate (DCA), which increases mitochondrial pyruvate oxidation by inhibiting pyruvate dehydrogenase kinase, did not show sex dimorphism. Similarly, treatment with dimethyl α -ketoglutarate, a cell-permeable form of alpha-ketoglutarate, also showed no sex differences. These findings reveal that metabolic interventions vary by sex. Lactate-mediated mechanisms show clear sexual dimorphism, while mitochondrial pyruvate oxidation and alternative metabolic pathways do not. Importantly, our results mirror clinical observations where females experience worse post-surgical pain outcomes, potentially validating the translational relevance of targeting specific metabolic pathways for sex-informed pain therapeutics.

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Poster

PSTR337: Pain Mechanisms and Targets

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Program #/Poster #: PSTR337.20/P6

Topic: E.01. Somatosensation – Pain and Itch

Support: NS045594
NS135157

Title: Neuropathic Spontaneous pain is intensified by brief stress exposure in a sex-dependent manner in mice

Authors: *L. UNDERWOOD¹, D. DENARDIN LÜCKEMEYER¹, W. XIE³, J. A. STRONG², S. LACKEY⁴, J.-M. ZHANG⁵;

¹Anesthesiol., Univ. of Cincinnati, Cincinnati, OH; ²Univ. of Cincinnati, Cincinnati, OH, ;

³Anesthesiol., Univ. Cincinnati Coll Med., Cincinnati, OH; ⁴Cincinnati, OH, ; ⁵Col. of Med., Cincinnati, OH.

Abstract: Recent research demonstrates a connection between neuropathic spontaneous pain, characterized by episodic shooting or stabbing sensations, and vascular response-evoked clustered firing of injured sensory neurons. Given that the “fight-or-flight” stress response typically causes vasoconstriction and elevated blood pressure through sympathetic nervous system activation, we are interested in investigating whether acute stress-induced cardiovascular responses influence neuropathic spontaneous pain. Behavioral signs of spontaneous pain (e.g., paw shaking, flinching, licking) was videorecorded for 30 min in mice of both sex with spared nerve injury (SNI) on postoperative day 21-28, followed by 10 min of restraint in a plastic cylinder. Video recordings were repeated for another 30 min after stress exposure. Results showed that acute stress significantly increased spontaneous pain scores, indicated by a higher number of shakes or flinches of the ipsilateral hind paw. This increase in pain score was

observed only in male mice possibly due to higher baseline pain scores in female mice. Acute stress also increased number of licks, but only in male mice. When the α -blocker phentolamine was administered intraperitoneally (i.p.) immediately before restraint, acute stress failed to enhance spontaneous pain. Interestingly, in naive mice without nerve injury, acute stress increased the number of licks in both sexes suggesting that licking may reflect stress response rather than pain. It is well documented that acute stress can lead to a short-term reduction in pain sensitivity under physiological conditions. These findings demonstrate that, under pathological conditions, acute stress can enhance spontaneous pain.

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Poster

PSTR338: Olfaction: Behavior, Perception, and Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR338.01/P7

Topic: E.03. The Chemical Senses

Support: NIH DC019124

Title: Contextually driven communication between hippocampus and the olfactory system

Authors: *N. AGRAWAL¹, O. D. ESCANILLA², D. E. SCOTT³, L. M. KAY⁷, D. M. SMITH⁴, T. A. CLELAND⁵, C. LINSTER⁶;

¹Neurobio. and Behavior, ²Neurobio. and behavior, ⁴Dept. of Psychology, ⁵Psychology, ³Cornell Univ., Ithaca, NY; ⁶Cornell Univ., Freeville, NY; ⁷Dept. of Psychology, Univ. of Chicago, Chicago, IL

Abstract: Odor meanings are often shaped by the context in which they are encountered, allowing a neutral odor to acquire positive or negative valence depending on the context. Previous work has shown connections between hippocampus and olfactory cortex to be important for spatial and temporal context dependent odor perception. Selective lesioning of connections between the ventral Hippocampus (vHC) and Anterior Olfactory Nucleus (AON) led to decreased contextual odor memory formation and retrieval. We hypothesized that functional neural connectivity between these 2 areas would be stronger when rats perform tasks in which spatial context is needed to obtain food from an odorized dish. In this study, we recorded Local Field Potentials (LFPs) from vHC and AON in male rats (n=4) performing a contextually cued olfactory decision task. The task utilized a three-chamber box, with two distinct contexts (a black and a white chamber) and a central, neutrally-colored waiting area. In each context chamber, rats chose between two differently scented odor dishes, one of which contained a food reward. For the context-dependent task, the rewarded odor was determined by the chamber's color, with opposite reward contingencies in each context. In a control context-irrelevant task, the food reward was always in the same odor dish, irrespective of the chamber's color. We truncated the

neural data around behavior events of interest - door opening of the context chamber and rat digging in the odorized dish. We found that the power of both AON($f(1, 36) = 9.250$; $p < 0.001$) and vHC ($F(1, 36) = 8.8082$; $p = 0.007$) was higher in the context relevant task than context irrelevant task. This suggested that activity in both these areas was higher when the information needed to be integrated. Coherence between AON and vHC increased notably right before the rat made its decision ($F_{\text{predig}}(1, 15) = 9.124$; $p = 0.009$), with AON leading vHC in phase. This increase was not seen in the context irrelevant task. Further, coherence was highest in the beta frequency band (12-30Hz) ($F(3, 35) = 10.504$; $p < 0.001$), which is consistent with literature of beta frequency being important in associative memory tasks. Correlation of beta-band coherence and the time it took for the rat to dig to find the reward showed a significant negative correlation for context-relevant task ($R = -0.273$ $p = 0.001$) and not for context irrelevant task ($R = -0.131$ $P = 0.100$). This suggests that the higher coherence between AON and vHC, the faster the decision process can be achieved. This is a novel study that highlights the importance of inter-areal communication in the brain for a task that requires integration of different sensory modalities and valences.

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Poster

PSTR338: Olfaction: Behavior, Perception, and Neurophysiology

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR338.02/P8

Topic: E.03. The Chemical Senses

Support: NIH/NIDCD R01 DC014701
NIH/NIDCD R01 DC019124

Title: Whole-brain neural representations of innately fear-inducing odor responses in mice

Authors: *Y. CHEN, M. EINHORN, C. YANG, T. A. CLELAND;
Psychology, Cornell Univ., Ithaca, NY

Abstract: Neural representations of external events or internal states are complex and diverse in their implications, potentially incorporating, for example, physical sensory information, the influences of prior learning, contextual factors, expected values and contingencies, and the construction of appropriate motor responses. Brain activity underlying the multiple aspects of these rich representations, however, is widely distributed, which has hindered efforts to measure and study the broad construction of neural representations at this scale. Recent advances in brain clearing techniques and light sheet fluorescence microscopy have made it possible to visualize neural activation patterns across entire mouse brains through the measurement of immediate early gene (IEG) expression. To investigate how odor representations are modulated by the odor's meaning or implications, we studied the responses of mice to the presentation of 2,4,5-

trimethylthiazoline (TMT), an odorant known to evoke innate fear-related behaviors in mice. Specifically, we exposed FosTRAP-Ai14 mice to TMT, to one of two neutral valence odors, or to no odorant while they were under the influence of 4-hydroxytamoxifen (4OHT). Administration of 4OHT gates the permanent expression of a tdTomato fluorophore in Fos-expressing neurons, thereby recording the odor-induced Fos response across the brain. Using Annolid behavior analysis software, we tracked the mice and analyzed their behavior through three metrics: motion index (mobility/freezing), place preference (time spent near the odor vs. far away), and velocity (used to assess immobility and escape-like darting responses). We observed that mice exposed to TMT spent significantly less time on the odor-proximal side of the enclosure and exhibited increased periods of immobility compared to mice exposed to the control odors or no odor. Brains then were extracted whole, cleared using aqueous methods, and imaged with a light sheet microscope. We registered the brains to the Allen Institute CCFv3 using an ANTs-based method, identified and counted Fos-expressing neurons, and applied both the Allen and Paxinos/Franklin atlases to the registered image in order to assign each Fos-expressing neuron to an atlas-defined brain region and compare Fos expression profiles across experimental groups. We also immunohistochemically labeled odor-gated Fos expression profiles in whole brains to compare the two labeling methods and assess the plausibility of within-subjects comparisons.

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Poster

PSTR338: Olfaction: Behavior, Perception, and Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR338.03/P9

Topic: E.03. The Chemical Senses

Support: Intramural Research Program at the National Institute on Drug Abuse ZIA DA000642

Title: Exploring lateralized processing in the human olfactory system

Authors: *C. U. RAITHEL, J. WORTHY, R. SULLIVAN, T. KAHNT;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The human olfactory system receives sensory information from two anatomically distinct nostrils. Existing evidence on how these separate streams of information are processed in the brain and used for behavior is inconclusive. Furthermore, nasal cycling is often ignored in the literature or discussed purely as an afterthought. In this study, we ask how the olfactory system encodes sensory inputs from the left and right nostril, and how both behavior and neural representations are influenced by nasal cycle. For this purpose, we developed a novel olfactory perceptual decision-making task in which we deliver binary odor mixtures to the left or right nostril of participants and ask them to indicate which of the two odor components is dominant.

We simultaneously record brain activity using fMRI and measure nasal airflow in each nostril separately to account for differences in neural representations as a function of the nasal cycle. Initial pilot data suggest that participants can successfully discriminate the odor mixtures delivered unilaterally, regardless of which nostril the odorant is presented to. However, task performance accuracy is significantly influenced by nasal cycle, raising the intriguing question of whether these behavioral results are reflected in corresponding (e.g., lateralized) neural representations. Analysis of neural data will elucidate how the olfactory system encodes sensory information from two separate input streams, and whether this coding is impacted by differential airflow to the two nostrils.

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Poster

PSTR338: Olfaction: Behavior, Perception, and Neurophysiology

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Program #/Poster #: PSTR338.04/P10

Topic: E.03. The Chemical Senses

Support: NIH
Wu Thai Neurosciences Institute at Stanford University

Title: Early life experiences shape begging behavior in social poison frog tadpoles

Authors: *N. AKBARI¹, A. S. RAO², L. A. O'CONNELL¹;
¹Biol., Stanford Univ., Stanford, CA; ²Stanford Univ., Palo Alto, CA

Abstract: The ability of infants to distinguish caregivers from non-caregivers is a fundamental survival behavior early in life. In many species, olfactory cues play a key role in mediating these critical social interactions. By enabling context-specific responses, such as approaching caregivers or avoiding potential threats, the proper processing of olfactory information ensures that offspring receive care and avoid predation. However, the neural mechanisms underlying this process remain poorly understood. *Ranitomeya imitator* tadpoles offer a powerful model for studying the neural basis of social recognition. These tadpoles exhibit complex social behaviors, including caregiver recognition and energetically costly motor displays such as begging to signal their needs. In this species, olfactory cues are both necessary and sufficient for identifying caregivers. We demonstrate that tadpoles reared in isolation (N = 13) or with conspecifics (N = 10) reliably beg more toward a female conspecific than toward a female of a closely related predatory species, *Ranitomeya variabilis*, which is known to deposit predatory tadpoles into *R. imitator* pools. However, when *R. imitator* tadpoles are raised for four weeks in tanks exposed to olfactory cues from *R. variabilis* tadpoles (N = 10), they no longer show a preference for the conspecific female. Our findings show that exposure to social odors facilitates begging preference in *R. imitator* tadpoles suggesting that caregiver identity is learned through exposure to olfactory cues. To understand the mechanism by which social conditions govern begging

preferences, we are investigating gene expression differences in the olfactory bulb of these tadpoles. Since *X. laevis* tadpoles conditioned with kin and non-kin odorants have a different ratio of dopamine to GABA neurons, we expect to see a change in the distribution of excitatory and inhibitory neurons in different socially conditioned tadpoles. This work explores how social behavior in infants develops, focusing on both behavior and gene expression patterns in the brain. Our findings will help us better understand how the brain supports these important behaviors early in life.

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Poster

PSTR338: Olfaction: Behavior, Perception, and Neurophysiology

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Program #/Poster #: PSTR338.05/P11

Topic: E.03. The Chemical Senses

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

Title: Abnormal olfactory behavior in forebrain-specific *Ccn2* knockout mice

Authors: *T. YANG¹, L.-J. LEE², H.-C. CHANG²;

¹Anat. and Cell Biol., Natl. Taiwan Univ. Col. of Med., TAIPEI, Taiwan; ²Anat. and Cell Biol., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstract: Cellular communication network factor 2 (CCN2) is a matricellular protein that plays an important role in the connective tissues. CCN2 is also expressed in the nervous system, particularly in olfaction-related brain regions; however, its role is still unclear. To explore the function of CCN2 in the brain, we generated forebrain-specific *Ccn2* knockout (Fb*Ccn2* KO) mice. In this study, we examined their olfactory behaviors. Mice lacking *Ccn2* in the forebrain exhibited normal olfactory discrimination. However, homozygous Fb*Ccn2* KO mice spent a longer time sniffing bedding collected from unfamiliar mice of the opposite sex compared to the same sex and control groups. After exposure to the bedding, we examined c-FOS expression in the olfactory bulb and various olfaction-related brain regions. Our results showed that in Fb*Ccn2* KO mice, olfactory cues in heterosexual bedding elicited stronger signals in some main olfactory bulb- and accessory olfactory bulb-related regions compared with controls. Changes in gene expression in the olfactory bulb of Fb*Ccn2* KO mice suggested altered functions. Olfactory dysfunction is associated with psychiatric disorders and neurodegenerative diseases; our Fb*Ccn2* KO mice might be useful in elaborating the relationship between olfaction and neuropsychiatric illnesses, as well as informing therapeutic strategies for these conditions.

Disclosures: T. Yang: None. L. Lee: None. H. Chang: None.

Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR338.06/P12

Topic: E.03. The Chemical Senses

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Title: Pallium-encoded valence-specific chemosensory amplification of eye-body coordination in larval zebrafish

Authors: ***K. SY**^{1,2}, **D. C. CHAN**², **J.-J. ZHANG**², **C. FENG**², **K.-Y. WONG**³, **Y. MU**⁴, **Y. HU**⁵, **H. KO**²;

¹Univ. of Göttingen, Göttingen, Germany; ²The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong; ³The Univ. of Hong Kong, Hong Kong, Hong Kong; ⁴Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China; ⁵The Hong Kong Univ. of Sci. and Technol., Kowloon, Hong Kong

Abstract: Coordinated eye-body movements are essential for adaptive behavior, yet little is known about how multisensory input, particularly chemosensory cues, shapes this coordination. Using our enhanced Fish-on-Chips optofluidic platform, we uncovered complex dynamics in how larval zebrafish coordinate saccadic eye movements with tail flips. Under baseline conditions, spontaneous tail flips dynamically align with saccades in frequency and direction for coordinated turns. Chemosensory valence further modulates this coordination: death-associated cues intensify both the strength and frequency of coupled saccade-tail flips during turns, whereas food-related cues promote forward gliding without altering saccade coupling. Concurrent brain-wide neuronal imaging reveals that the zebrafish pallium represents the transformation of aversive valence into enhanced saccade-tail coordination, with stronger coupling associated with higher pallium activation. These findings uncover the neural basis by which chemosensory inputs of different valences distinctly regulate eye-body coordination to drive adaptive locomotion in a developing vertebrate, offering insights into principles of sensory-motor integration.

Disclosures: **K. Sy:** A. Employment/Salary (full or part-time): The Chinese University of Hong Kong, University of Göttingen. **D.C. Chan:** A. Employment/Salary (full or part-time): The Chinese University of Hong Kong. **J. Zhang:** A. Employment/Salary (full or part-time): The Chinese University of Hong Kong. **C. Feng:** None. **K. Wong:** A. Employment/Salary (full or part-time): The University of Hong Kong. **Y. Mu:** A. Employment/Salary (full or part-time):

Institute of Neuroscience, Chinese Academy of Sciences. **Y. Hu:** A. Employment/Salary (full or part-time): The Hong Kong University of Science and Technology. **H. Ko:** A. Employment/Salary (full or part-time): The Chinese University of Hong Kong.

Poster

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Topic: E.03. The Chemical Senses

Support: NIH Grant F31DC021353
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Title: Changes in the olfactory bulb TAAR4-related circuits evoked by critical period exposure to phenethylamine persist following recovery from olfactory sensory neuron ablation

Authors: ***J. D. GREGORY**¹, C. E. CHEETHAM²;
¹Neurobio., ²Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Critical periods are essential for proper development of mammalian sensory circuits. In the mouse olfactory system there is an early postnatal critical period during which errors in glomerular map formation can be corrected. Furthermore, critical period exposure can alter the valence of innately recognized odorants. Mice exposed during the olfactory critical period to the innately aversive odorant phenethylamine (PEA), detected by olfactory sensory neurons (OSNs) expressing the trace amine-associated receptor 4 (TAAR4, T4), do not demonstrate stereotypical innately avoidant behaviors, but rather are attracted to PEA. This change in behavior is accompanied by an increase in the number of TAAR4 glomeruli per olfactory bulb (OB). Here, we seek to address two key questions: how neonatal PEA exposure alters the odor responsiveness of OB output neurons, and whether the changes in valence toward PEA persists following complete ablation and subsequent repopulation of OSNs. To address the first question, we injected mice with AAV5-syn-GCaMP6s to record PEA-evoked calcium responses in mitral and tufted cells using in vivo 2-photon microscopy. In juvenile PEA-exposed mice, we found both a significant increase in preference toward PEA and a reduction in mitral and tufted cell responses to PEA. To address the second question, PEA-exposed T4-RFP^{+/+} mice underwent either repeated odor preference testing or chronic in vivo 2-photon imaging of the T4 glomeruli to address whether the behavioral and/or structural alterations induced by critical period PEA exposure persist after OSN ablation. Repeated odor preference testing revealed that six weeks after MMZ treatment, when OSNs have repopulated and reinnervated the OB, preference for PEA was retained in mice that had undergone critical period PEA exposure. Together, these data suggest that altered odor preference as a result of critical period PEA exposure can be explained by a combination of structural changes seen in the glomeruli and a reduction in PEA-evoked responses in output neurons. Furthermore, the altered odor preference is maintained following

complete OSN ablation and subsequent regeneration. This highlights the complexity of the interaction between critical period experiences and the formation of innate behaviors.

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Poster

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Program #/Poster #: PSTR338.08/Q2

Topic: E.03. The Chemical Senses

Support: BPRI Grant GR0036649

Title: Functional Analysis of the Alpha and Beta Lobes in *Schistocerca Americana*

Authors: *J. KELLEY¹, B. RAMAN²;

¹Washington Univ. In St. Louis, St. Louis, MO; ²Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Olfaction is an important sensory modality that drives a wide repertoire of appetitive, navigational, and courtship behaviors in insects. How odorants elicit responses in different neural circuits, which are then mapped onto behavioral decisions, is a key question in the field. Much research has focused on how olfactory information is processed in early neural centers such as the antennal lobe (AL) and the mushroom body (MB). However, how neural activity in the early olfactory pathway is reformatted to support different behavioral outcomes is poorly understood. This is the question we sought to answer. We focused primarily on characterizing the neural responses in the alpha and beta lobes of the mushroom body in *Schistocerca americana*. Odorant molecules are detected by ~100,000 olfactory receptor neurons (ORNs) on each locust antenna. This information is relayed to ~800 projection neurons (PNs) in the locust antennal lobe (AL). Information about odor identity and intensity is represented by patterns of spiking activity that are distributed across an ensemble of PNs in the AL. This activity is then relayed to downstream processing centers such as the MB and lateral horn. In the mushroom body, PNs synapse onto Kenyon cells which then transmit information to mushroom body output neurons (MBONs) in the alpha and beta lobes. Recent studies suggest that MBONs have a high degree of correlation with olfactory behavioral outputs. Research in cockroaches has linked feeding behaviors to beta-lobe activity (Arican et al., 2023), and studies in honeybees (Strube-Bloss et al., 2011) and locusts (Cassenaer, Laurent, 2007) have demonstrated that beta-lobe MBON activity is plastic. Building on these results, we sought to examine the link between locust MBON activity and their behavioral relevance. Consistent with prior results, we found that beta-lobe MBONs are promiscuous in their response to odor stimulation. Beta-lobe MBONs were activated during the odor presentation (ON responses) and after termination (OFF responses). Our initial results indicate that the ON responses in beta-lobe MBONs are less discriminative than the OFF responses. We believe that this selectivity of OFF responses is important for driving locust

behaviors such as odor plume tracking. We are currently testing this hypothesis using electrical stimulation of each lobe. Alpha-lobe MBONs were more selective in their responses, responding to only a few odorants in the odor panel. While the behavioral role of activity in this olfactory circuit is currently unclear, we are actively combining electrophysiology studies with electrical stimulation of each lobe to determine how MBON activity drives motor outputs.

Disclosures: J. Kelley: None. B. Raman: None.

Poster

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Topic: E.03. The Chemical Senses

Support: NIH 1R01DC020892-01
NSF 2014217

Title: Behavioral and neurobiological evidence for a role of active sensing in appetitive learning in the honey bee *Apis mellifera*

Authors: *H. LEI¹, B. H. SMITH²;
²Schl Life Sci., ¹Arizona State Univ., Tempe, AZ

Abstract: Animals are frequently seen to align their sensory organs with dynamic stimuli in the environment to maximize information input, a behavior described as active sensing such as sniffing in dogs. Recent findings indicate that insects modify their antenna movement based on the orientation and/or saliency of incoming olfactory signals, suggesting a strong correlation between antenna movement and active sensing. Whereas a modified antenna movement can alter sensory responses to odors, it's largely unknown how it may affect olfactory learning. Here, we have taken mechanistic approaches to address this question. First, honey bee foragers were subjected to a differential training protocol where one odor (CS+) was rewarded with sugar water (US) and the other odor (CS-) was punished with salt water, and later tested with CS+ and CS- under a series of concentrations. Second, bee's antennae were either fixed or not fixed during the training protocol and the training results were assessed with proboscis extension reflex (PER). Third, electrophysiological recordings were performed from the antennae and the primary olfactory center, i.e. antennal lobes, while the bees were differentially conditioned under fixed or free antenna treatments. We have found that (1) antennae respond to odors more consistently when they are free to move; (2) whether the antennae are free or fixed, bees learn the CS-US association equally well; (3) however, the neuronal responses in the antennal lobe show more distinction between CS+ and CS- when the antennae are free than are fixed; (4) the neuronal responses correlate better with the learning performance measured by electromyograms underlying PER than by the binary scores of PER. In summary, our results support the notion that active sensing affects associative learning.

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Poster

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Program #/Poster #: PSTR338.10/Q4

Topic: E.03. The Chemical Senses

Support: NIDCD R00 DC019505

Title: In vivo dynamics of dopaminergic circuits in the mouse olfactory bulb

Authors: *P. AMBROSI, E. MOSS;

Dept. of Anesthesiol. and Perioperative Med., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Sensory perception and the encoding of sensory information are shaped by internal states and task demands. Variable stimulus encoding is already evident in primary sensory regions like the olfactory bulb, but the integration of intrinsic and extrinsic signals by sensory circuits is poorly understood. We are using the mouse olfactory system to study how non-sensory cues regulate information processing in sensory circuits. We hypothesize that local dopamine neurons (DANs) in the olfactory bulb (OB) mediate task-dependent changes in odor encoding by mitral and tufted cells (MTCs), the principal cells of the OB. Odor-evoked MTC activity becomes sparser as task demands transition from coarse to fine odor discrimination, but the mechanisms underlying this flexible encoding are unknown. OB DANs are well-positioned to mediate task-dependent changes in olfactory perception because (1) unlike most OB interneurons, DANs can link up to 50 glomeruli, enabling interglomerular communication, (2) OB DANs release dopamine and GABA, modulating MTC activity via D1, D2 and GABAA receptors, and (3) OB DANs receive cholinergic inputs from the basal forebrain, a major source of top-down regulation in the brain. Using two-photon imaging of the OB through a chronic cranial window, we are quantifying the spatial-temporal dynamics of OB DAN activity and DA release in the OB as mice perform an odor discrimination task. Preliminary results confirm that head-fixed mice learn to rapidly transition between coarse and fine odor discrimination in a two-alternative forced choice (2AFC) task. Simultaneously, using a high field number resonance scanning two-photon microscope, we can image the entire dorsal surface of both OBs in awake behaving mice with high spatial-temporal resolution. Since OB DANs are characterized by tyrosine hydroxylase (TH) expression, we have targeted the expression of genetically encoded calcium and voltage sensors (jGCaMP8f and JEDI-2P, respectively) to OB DANs via AAV injections in the OB of TH-Cre mice. Preliminary results suggest that OB DAN activity is heterogeneous across glomeruli and odor-dependent. On-going experiments are comparing two genetically encoded dopamine sensors, GRAB-DA and dLight, in the OB. Our results will help us better understand the neuromodulation of olfaction and the neural circuits behind flexible stimulus encoding.

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Poster

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Topic: E.03. The Chemical Senses

Support: NIH R01DC022320
NIH U19 NS112953

Title: Neural dynamics underlying extraction of behaviorally relevant information in the olfactory bulb

Authors: *S. M. TETRICK¹, M. KARADAS², S. CEBALLO², E. KOULAKOVA³, D. RINBERG⁴;

¹Neurosci. Inst., New York Univ., New York, NY; ²Neurosci. Inst., NYU Langone Hlth., New York, NY; ³NYU Neurosci. Inst., Huntington, NY; ⁴Neurosci. Inst., NYU Neurosci. Inst., New York, NY

Abstract: Olfaction plays a critical role in guiding behavior across species, yet the contributions of local olfactory bulb (OB) processing and higher-order cortical feedback to behaviorally relevant feature extraction remain incompletely understood. In mammals, odor inhalation evokes complex spatiotemporal activity patterns across glomeruli in the OB, driving mitral (MCs) and tufted cells (TCs) that relay information to downstream brain regions. This project investigates how odor representations are transformed from glomeruli to MCs and TCs in awake, behaving animals, and how these transformations contribute to sensory-guided decision-making. We employ a go/no-go behavioral paradigm in conjunction with one- and two-photon calcium imaging to monitor MC/TC activity linked to a single, optogenetically tagged glomerulus. We selectively activate a channelrhodopsin-2 expressing glomerulus (M72S50 mouse line) during a stimulation detection task, allowing us to isolate behaviorally relevant signals to a single glomerular channel while also introducing background odors to assess perceptual discrimination. We first assess the behavioral relevance of single glomerular activation by varying stimulation timing and sensory context. Second, we identify functionally connected MCs and TCs and characterize the response features that encode task-relevant information. We will investigate whether MC/TC activity associated with the optogenetically-stimulated glomerulus is modulated by behavioral context. Specifically, we will compare stimulation-evoked MC/TC activity at defined phases of the sniff cycle between two conditions: passive odor presentation in naive animals and active stimulation detection in animals trained on our behavioral task in the presence of background odors.

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Poster

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Program #/Poster #: PSTR338.12/Q6

Topic: E.03. The Chemical Senses

Title: Relevant features of the odor code for generalization in the mouse olfactory bulb

Authors: *S. KARIMIMEHR¹, S. CEBALLO², M. KARADAS², D. RINBERG³;
²Neurosci. Inst., ¹NYU Langone Hlth., New York, NY; ³Neurosci. Inst., NYU Neurosci. Inst., New York, NY

Abstract: Odor stimuli evoke rich spatiotemporal patterns of neural activity in the olfactory bulb, particularly within the glomeruli and downstream mitral/tufted (MT) cells. Yet, it remains unclear which features of this neural code are behaviorally relevant, especially in the context of generalization. In this study, we combined behavioral and imaging approaches to identify which aspects of odor-evoked activity predict perceptual generalization in mice. Using a two-alternative forced-choice (2AFC) task, mice were trained to discriminate a specific ternary odor mixture ("Target") from a variety of non-target mixtures composed of the same three odorants in different ratios. We then assessed generalization in three domains: **within-class generalization** (mixtures near the target), **between-class generalization** (odor mixtures with qualitatively different component ratios), and **generalization across concentrations** of the same mixtures. To uncover the neural correlates underlying these perceptual behaviors, we performed two-photon calcium imaging in mice expressing the fast calcium indicator GCaMP8s/8m/6f in postsynaptic glomeruli and MT cells. We found that the **temporal sequence of activation**—particularly within the first 200 ms of odor presentation—strongly predicted behavioral responses across all generalization domains. Neural activity outside this early temporal window was less informative, suggesting that the brain prioritizes early-arriving information for rapid and flexible odor decisions. Together, these findings identify **temporal features** of the olfactory code as key determinants of generalization in the mouse olfactory bulb. Our work provides new insight into how dynamic neural representations support both fine discrimination and broad generalization across odor space.

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Poster

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Topic: E.03. The Chemical Senses

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Title: Alignment of drifting olfactory representations in the piriform cortex permits linear decoding

Authors: *E. C. BAXTER¹, A. J. FINK², J. E. FITZGERALD²;

¹Engin. Sci. and Applied Mathematics, ²Neurobio., Northwestern Univ., Evanston, IL

Abstract: In the olfactory (piriform) cortex, patterns of spiking activity evoked by individual odorants permit odor identification. However, these population responses gradually evolve on daily to weekly timescales, a phenomenon termed ‘representational drift’. A natural question related to perceptual constancy in the olfactory system thus ensues: what properties of odorant representations in piriform cortex, if any, are invariant under representational drift? Previous work (Schoonover et al., 2021) has shown that the classification accuracy of a linear support vector machine trained on population responses and odorant identities from trials recorded on a single day reaches chance levels when tested on population responses and odorant identities from trials recorded one month later. Efforts to identify drift-invariant geometry in these experimental data were not successful. Here, we reinvestigated these data and uncovered some drift-invariant structure. In particular, we found day-specific orthogonal transformations that, when applied to neural space, are sufficient to maintain greater-than-chance classification accuracy across all test days using the same classifier. Notably, these orthogonal transformations are computed using only population responses, not odorant identities. Since these orthogonal transformations explain away enough drift to successfully decode odorant identities from population responses in piriform cortex, this finding suggests that drift in piriform cortex may manifest in orthogonal transformations on neural space. This geometry extends the Schoonover et al. finding that accurate linear decoding of odor identity is successful on each day, as the day-dependent orthogonal transformation of the activity data can be paired with a day-dependent linear decoder that accounts for the transformation. If drift does manifest in orthogonal transformations, then a skew-symmetric plasticity rule (e.g. spike-time-dependent plasticity) may provide a natural candidate model to describe the underlying mechanism of drift in piriform cortex. Overall, in capturing drift-invariant structure in the population responses of piriform cortex, this line of investigation is a critical step forward in understanding perceptual constancy in the olfactory system.

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Title: Multivariate signatures of predictive coding in the human olfactory system

Authors: *S. H. LYONS, J. A. GOTTFRIED;
Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Predictive coding is a potentially unifying theory of perception, which states that the brain aims to predict incoming sensory stimuli and minimize prediction error. While this theory has received ample attention in domains like vision and audition, olfactory predictive coding has remained relatively understudied. To fill this gap, we recruited participants (N = 25) to complete a probabilistic odor prediction task under ultra-high-field 7-Tesla functional MRI, allowing us to explore patterns of brain activity at high resolution. During this task, participants learned shape-odor relationships, in which a given shape (a circle or square) predictively cued an upcoming odor (pine or banana). We also controlled for physiological parameters by measuring participants' sniffing, respiration, and heart rate. Participants were successfully able to predict the odors and minimize prediction error over time. Additionally, we fit several competing models to participants' behavior, and using a model comparison procedure, we confirmed that a predictive-coding style model best explained the behavioral data. Consistent with these behavioral results, we discovered multivariate patterns of brain activity associated with olfactory predictions and prediction errors. Moreover, these patterns were modulated by odor uncertainty, as hypothesized by predictive coding. Overall, these results provide novel support for predictive coding in the human olfactory system.

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Poster

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Topic: E.03. The Chemical Senses

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Title: Synaptic inhibition in the olfactory bulb modulates threat perception in mice

Authors: S. PANDEY¹, S. THORAT¹, S. DAS¹, L. RANJAN¹, D. RAWAT¹, K. THOMAS¹,
*N. ABRAHAM²;

¹Dept. of Biol., ²Indian Inst. of Sci. Educ. and Res. - Pune, Pune, India

Abstract: Innate responses to threat stimuli are genetically predetermined, for example, the fear reaction shown by rodents toward predator scent. TMT (2,3,5-Trimethyl-3-thiazoline) is a volatile sulphur-containing single molecule kairomone derived from fox feces, and has been demonstrated to function as a predatory cue to rodents. Exposure to this odor leads to avoidance and freezing responses, indicative of fear. The amygdala plays a crucial role in assigning emotional valence to incoming sensory information, thereby enabling instinctive behaviors such as active exploration, evading predator scents, or freezing. Olfactory information is transmitted to various brain regions, including the amygdala via projection neurons of the olfactory bulb. The inhibitory interneuronal population modulates the spiking activity of excitatory projection neurons (Mitral/Tufted cell) and helps refine the odor percept. Although the Amygdala and Periaqueductal Gray (PAG) have been demonstrated to be crucial in eliciting defensive behaviors and mediating transitions between fight, flight, and freezing responses, the role of the pre-cortical circuitry remains largely unexplored. Here, we explain mice' behavioral states in response to TMT using LabGym, and Bonsai. Further, we recorded calcium signals from GAD-65 expressing interneurons while animals exhibited these different states. Mobile and immobile behavioral states had shown differential recruitment of these inhibitory interneurons. The optogenetic modulation of these neurons confirmed the role of synaptic inhibition in modulating threat perception.

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Peduciba posgrados x3
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Title: Early Sensory Disconnection in the Olfactory System During Sleep

Authors: *M. L. CAVELLI¹, D. SERANTES², D. GALLO², M. GARCIA², J. GONZALEZ³, P. TORTEROLO²;

¹Physiol., ²Facultad de Medicina. UdelaR, Montevideo, Uruguay; ³Dept. of Psychiatry, New York Univ. Grossman Sch. of Medicine, New York, NY, USA, New York, NY

Abstract: As we fall asleep each night, we partially disconnect from our surroundings. This sensorimotor disconnection is characterized by a reduced ability to respond to external stimuli,

reflected in an increased arousal threshold. Despite the risks associated with this diminished reactivity, sleep is a biological necessity, suggesting that this disconnection serves essential functions. The neural mechanisms underlying this phenomenon remain poorly understood. Most theories propose that the thalamus and cortex are central in modulating sensory transmission during sleep. However, recent evidence suggests that sensory disconnection may originate in more peripheral structures, before reaching these higher-order brain areas. The olfactory system, composed of the olfactory bulb and associated cortices, lies just one or two synapses away from the external environment. In the nasal cavity, sensory cells detect chemical and mechanical stimuli, generating an electrical signal, the respiratory potential, coupled to breathing. This signal propagates to the brain and modulates neuronal activity across multiple regions, facilitating sensory encoding. Our recent findings indicate that this respiratory potential and its associated neural activity are markedly attenuated during sleep. Electrophysiological recordings show that olfactory bulb activity is significantly reduced during slow-wave states, revealing sensory “gating” at this early processing stage. Moreover, using an artificial sniffing preparation, we demonstrated that nasal airflow is necessary but not sufficient to internalize the respiratory potential, an activated brain state is also required. Further research is needed to determine the precise location and mechanisms of this gating process, and to assess its impact on sensory encoding during sleep.

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Support: NIH Grant DC019505

Title: Subcellular compartmentalization of olfactory bulb dopaminergic interneuron activity

Authors: *E. H. MOSS, P. AMBROSI;
OHSU, Portland, OR

Abstract: Olfactory dysfunction is an early indicator of cognitive decline. Understanding complex mechanisms governing olfactory circuit function, including noncanonical processes like dendrodendritic signaling and neurotransmitter co-transmission from dendrites, is vital. In the olfactory bulb (OB), tyrosine hydroxylase-expressing superficial short axon cells (TH-sSACs) co-release dopamine (DA) and GABA from their extensive dendritic arbors that can span multiple glomeruli. While this allows them to integrate information across glomeruli and modulate circuit activity, how DA release is precisely triggered and controlled at the subcellular level within these arbors is largely unknown. To test the hypothesis that TH-sSAC dendritic

arbors contain compartmentalized regions of depolarization that enable rapid, localized, and heterogeneous DA release across glomeruli we are developing a novel framework for in vivo two-photon (2P) optical physiology in the mouse OB, combining voltage imaging, DA sensor imaging, and optogenetic stimulation of TH-sSACs. Our data demonstrate the feasibility of in vivo 2P voltage imaging with JEDI-2P in TH-sSACs from awake mice and reveal spontaneous subthreshold oscillations in TH-sSAC dendrites. Complementary GCaMP imaging data supports the concept of compartmentalization by showing decorrelated activity in neighboring dendrites from the same neuron. Furthermore, preliminary DA sensor imaging shows the feasibility of visualizing heterogeneous and dynamic DA signals across glomeruli in the OB in response to odors.

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Poster

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Title: Physiological activities in response to the impacts of essential oils on executive functions

Authors: *C. T. Q. TRAN¹, S. KINOSHITA³, S. FUJITA³, T. OKAMOTO^{1,2};
¹Grad. Sch. of Systems Life Sci., ²Fac. of Arts and Sci., Kyushu Univ., Fukuoka City, Fukuoka Prefecture, Japan; ³Olfactory Business Develop. Office, Sony Corp., Tokyo, Japan

Abstract: Executive function skills are the high-level, top-down cognitive processes that allow humans to plan, pay attention, remember instructions, and perform multiple tasks successfully. The core mechanisms of executive functions include working memory, cognitive flexibility, and response inhibition. This study investigated how the human central nervous system responds to essential oils, focusing on the high-level, top-down cognitive ability of executive functions. While essential oils have been reported to positively impact psychophysiological activities, such as anxiety and memory, evidence supporting aromatherapy's effect on comprehensive cognitive skills of executive function remains inconclusive. To address this gap, we evaluated the effects of different essential oils on executive functions through electroencephalograph measurements (EEG) of brain activity. With a between-subjects design, we had 64 participants (30 females, aged 18-37 years) in five odor groups: control, Japanese Cypress, Yuzu, Lavender, and Bay Leaf. Odor stimuli were presented using an olfactometry system, NOS-DX1000 (Sony, Tokyo, Japan), to deliver non-heated, dry, odorant air. Each participant sniffed their assigned odor stimulus for five seconds prior to performing cognitive tasks attributed to the three domains of executive functions. The three cognitive assessments, backward digit span (working memory),

the Wisconsin Card Sorting Test (cognitive flexibility), and Stroop (response inhibition), were implemented to evaluate executive functions comprehensively. One run of all three tasks concluded a set, and participants were required to complete three sets. EEG brain activity was recorded throughout the entire experiment. The study was approved by the ethics review board at Kyushu University. EEG analyses revealed distinct odor-related modulations of neural oscillations across tasks and time. Lavender elevated beta and alpha power toward the last set compared to the control and other groups during the working memory task. In the response inhibition task, Lavender and Bay Leaf showed an increase in beta power, whereas the control group exhibited early-onset alpha and theta dominance. Lavender and Bay Leaf induced significantly higher beta activity over time in the cognitive flexibility task. Japanese Cypress reflected a significant positive frontal alpha asymmetry (FAA) across tasks, suggesting an enhanced approach-related motivation and engagement. Our findings indicate that specific essential oils can modulate neural dynamics underlying executive functions, and their effects vary by domain and temporal progression.

Disclosures: C.T.Q. Tran: None. S. Kinoshita: None. S. Fujita: None. T. Okamoto: None.

Poster

PSTR339: Auditory Processing

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.01/Q13

Topic: E.05. Auditory and Vestibular Systems

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Title: A behavioral and neural method for comparing dynamic aesthetic experiences

Authors: *R. NIU¹, L. ZHU¹, Z. ZHANG², A. S. KAYSER³, M. BARTHOLOMEW⁴, M. HSU⁵;

¹Peking Univ., Beijing, China; ²Univ. of Virginia, Charlottesville, VA; ³Neurol., Univ. of California, San Francisco, San Francisco, CA; ⁴Law Sch., State Univ. of New York at Buffalo, Buffalo, NY; ⁵Univ. of California, Berkeley, Berkeley, CA

Abstract: Characterizing and comparing human aesthetic experiences from the perspective of the average, or ordinary, person is central to legal decision-making, including legal subject areas like copyright law. However, there is little consensus on how it can even be done empirically and scientifically, because of the presumed challenge for people to reflect on and report their own subjective experiences with complex stimuli that unfold over time. To address these challenges, we developed an empirical framework to evaluate music copyright infringement by systematically manipulating song elements, thereby establishing an integrative approach

combining behavioral and neural methods to derive an average group opinion. Specifically, due to the legal requirement for plaintiffs to prove that the allegedly infringing element is both similar to the original and central to the defendant's work, we created modified versions of songs by removing or replacing certain musical elements. We then asked participants to evaluate the subjective distances of these variants from the defendant's original song (N = 151). Our method was first tested on a historical case with a resolved legal judgment, where the behavioral judgments from our approach matched the historical ruling, and it was then applied to a recent, highly controversial case. In both instances, the perceived distances measured behaviorally were consistent with neural distances characterized by cross-brain alpha coherence during a separate EEG passive listening task (N = 43). Taken together, these results demonstrate a practical behavioral framework, supported by neural evidence, for evaluating music infringement, thereby informing legal debates with empirically grounded insights.

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Poster

PSTR339: Auditory Processing

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Topic: E.05. Auditory and Vestibular Systems

Support: JSPS KAKENHI Grant JP23K06797

Title: Fronto-occipital functional coupling in large-scale brain networks supporting audiovisual integration during perceptual alternation

Authors: *S. ZAKERI, N. ONO, R. YOSHITAKE, O. ARAKI;
Tokyo Univ. of Sci., Tokyo, Japan

Abstract: Human perception is often influenced by simultaneous multimodal stimuli, but the neural mechanisms underlying cross-modal interactions in perceptual formation remain poorly understood. This study investigates the functional connectivity and large-scale cortical network dynamics involved in audiovisual integration during bistable auditory perception. Using ABA paired tones with bistable percepts (1-stream or 2-stream) combined with visual flickering, we compared synchronous conditions (the onsets of B tone and flicker were simultaneous) and desynchronized conditions. Participants reported their dominant percept and simultaneity judgments. Electroencephalography (EEG) was recorded from twenty-four participants and preprocessed using a bandpass filter and independent component analysis for artifact removal. Behavioral results showed a significantly higher 2-stream perception rate in the synchronized condition compared to the desynchronized condition (53.2% and 44.9%, respectively; $p < 0.01$), indicating that the integration of audio and visual stimuli enhances 2-stream perception. EEG analysis revealed higher alpha and delta power activity in frontal regions and elevated alpha in

occipital areas during synchronous conditions. Phase-amplitude coupling (PAC) between frontal delta-phase and occipital alpha-amplitude was significantly stronger in the synchronous condition ($p < 0.05$), suggesting that fronto-occipital PAC is a key mechanism in audiovisual integration. Moreover, microstate analysis revealed distinct spatiotemporal dynamics: in the asynchronous condition, frontal alpha power correlated with the duration and transitions of microstate class D ($p < 0.05$), indicating top-down cognitive control. Conversely, in synchronous conditions, occipital delta power correlated with the dynamics of the same microstate class ($p < 0.05$), suggesting bottom-up sensory integration. These findings highlight that audiovisual perception involves both local oscillatory interactions and global network dynamics (fronto-occipital PAC), where synchronous stimuli enhance bottom-up integration, while asynchronous processing relies more on top-down modulation. Together, these results reflect the neural mechanisms by which cross-modal stimuli shape perception through coordinated local and large-scale brain network activity.

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Poster

PSTR339: Auditory Processing

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Topic: E.05. Auditory and Vestibular Systems

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Title: Association between auditory brainstem response under masking conditions and NODDI metrics: links to autism spectrum traits

Authors: *T. TAKEDA^{1,2,3}, N. KASHIDA², K. YAMAMURO², R. ISHIDA¹, H. TAMAKOSHI¹, M. TORITSUKA^{1,3}, N. IWATA¹, M. MAKINODAN^{1,3};

¹Psychiatry, Fujita Hlth. Univ., Toyoake, Japan; ²Psychiatry, Nara Med. Univ., Kashihara, Japan;

³Psychiatry, Kumamoto Univ. Hosp., Kumamoto, Japan

Abstract: Objective: Auditory processing abnormalities have been reported in autism spectrum disorder (ASD), and excessive connections between the thalamus and temporal lobe have been suggested as a possible underlying cause. Such excessive connections may lead to sensory overload and impaired filtering, contributing to distractibility and sensory hypersensitivity. Impaired top-down control from the frontal to the temporal regions may also underlie reduced

filtering capacity. This study focused on Waves VI and VII of the auditory brainstem response (ABR), which originate from the thalamus. By assessing neurite orientation dispersion (ODI) and neurite density (NDI), we aimed to clarify how microstructural neural differences between individuals with ASD and neurotypical adults affect neural activity and relate to ASD traits. Methods: Participants included 12 neurotypical adults (mean age = 37.1 ± 8.0 years) and 15 adults with ASD (mean age = 26.9 ± 8.1 years). ASD traits were assessed using the Autism-Spectrum Quotient (AQ), and sensory processing characteristics were evaluated using the Japanese version of the Adolescent/Adult Sensory Profile (AASP). ABRs were recorded using a forward masking paradigm in which a masking sound precedes the stimulus tone, reducing detectability. Negative peak amplitudes of Waves VI and VII (NVI/NVII) and differential amplitudes from the preceding positive peaks ($\Delta VI/\Delta VII$) were calculated. ODI and NDI were extracted for the thalamus, temporal lobe, and orbitofrontal cortex. Partial correlation analyses were conducted controlling for age and sex.

Results: Significant negative correlations were found between NVII and ODI in the temporal lobe ($r = -0.504$, $p = 0.021$) and thalamus ($r = -0.297$, $p = 0.031$). NVII was also found to be significantly correlated with total AQ scores ($r = -0.495$, $p = 0.012$). In addition, ΔVI showed significant associations with NDI in the orbitofrontal cortex ($r = -0.442$, $p = 0.027$) and with total AASP scores ($r = 0.412$, $p = 0.041$).

Conclusion: We revealed that NVII was meaningfully linked to ODI values in both the thalamus and temporal lobe, and also to traits associated with autism as assessed by the AQ. These findings suggest that the function extending from the thalamus to the temporal lobe may be related to the microstructural characteristics of these regions and to ASD-related traits. Similarly, the association of ΔVI with NDI in the orbitofrontal cortex and with sensory profile measures points to a potential relationship between thalamic activity, prefrontal microstructure, and atypical sensory processing.

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Poster

PSTR339: Auditory Processing

Location: SDCC Halls B-H

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Program #/Poster #: PSTR339.04/Q16

Topic: E.05. Auditory and Vestibular Systems

Support: National Science Foundation Integrative Organismal Systems grant
1121689

Title: Contribution of muscarinic acetylcholine receptors to bottom up amplification of cortical responses to rare oddball tones in rats

Authors: E. GAUNCE, D. TIMUCIN, *M. C. WIEST;
Wellesley Col., Wellesley, MA

Abstract: We previously reported “true” deviance detection in rat cortex, reflected in frontal and parietal local field potential responses to rare tones, and demonstrated that the rare-tone response amplifications could not be accounted for by stimulus-specific adaptation mechanisms alone. Such deviance detection is likely a neural mechanism of bottom-up auditory attention. The cholinergic system is well-known to be involved in attention, but the contributions of different acetylcholine receptor types in bottom-up as opposed to top-down attention is not fully understood. To test the role of muscarinic receptors in amplifying the neural response to rare tones, we recorded local field potentials in medio-dorsal frontal and posterior parietal cortex of awake rats exposed to sequences of frequent standard and rare oddball tones, and compared event-related potentials under 0.75 mg/kg of the muscarinic receptor blocker scopolamine to a control condition (vehicle injection). We analyzed the amplitudes of ERP components—peaks—in order to reveal the contribution of muscarinic receptors to the oddball amplification at each peak. Our preliminary results suggest that muscarinic receptors participate in determining the amplified brain response to rare auditory events.

Disclosures: E. Gaunce: None. D. Timucin: None. M.C. Wiest: None.

Poster

PSTR339: Auditory Processing

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Program #/Poster #: PSTR339.05/R1

Topic: E.05. Auditory and Vestibular Systems

Support: NIH 5T32DC005361-23

Title: Listening in Autism: From Brain-based Assessment to Multisensory Intervention

Authors: *F. AHMED, B. K. LAU;
Univ. of Washington, Seattle, WA

Abstract: Autism affects 1 in 31 children in the United States, and for many of them, perceiving speech in everyday life remains an invisible but persistent struggle. Sensory processing differences, including auditory challenges - particularly in noisy environments - are a core feature of autism that severely impacts their communication and daily functioning. Crucially, these challenges persist despite typical hearing thresholds that often go unnoticed in clinical evaluations and unaddressed in interventions—largely because we lack a clear understanding of how the autistic brain processes complex auditory scenes. As a result, listening difficulties are often identified too late, and there are no objective tools to guide targeted intervention. Current clinical assessments rely heavily on caregiver questionnaires, which, while informative, are limited by subjective interpretation and fail to capture the complexity and individual variability related to auditory processing in autism. This leads us to two critical questions that we are trying to answer in our lab: (1) Can we develop objective, brain-based metrics to identify listening difficulties in autism? (2) Can we design targeted interventions to support those who are

struggling? A prior study in our lab addressed the first question by recording EEG from 38 children aged 7-10-years (19 autistic, 19 neurotypical), who listened to an audiobook under three conditions: Quiet, speech in co-located noise, and spatially segregated noise. A linear modeling approach was used to quantify how well the target speech is encoded in the brain. While neurotypical children showed expected improvement in neural encoding of speech with spatial separation between speech and noise, autistic children demonstrated the opposite effect: speech encoding worsened in the segregated condition. This striking result identified spatially segregated noise as a particularly challenging listening scenario for autistic children—one that may not be adequately captured through standard clinical assessments. Inspired by this finding, here we present a follow-up study to test whether adding visual speech cues can mitigate this difficulty. Using a virtual reality (VR) platform, we deliver real-time visual stimulation alongside auditory input to examine whether multisensory support can improve their speech perception in noise. Together, our work lays the groundwork for objective identifications that go beyond caregiver-reported assessments in clinics and for tailored interventions for listening challenges in autism - moving us one step closer to personalized, neurobiologically informed care.

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Poster

PSTR339: Auditory Processing

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.06/R2

Topic: E.05. Auditory and Vestibular Systems

Title: How do harmonic relationships affect grouping of sounds?

Authors: *J. M. LEEMAN, C. D. KING, J. M. GROH;
Duke Univ., Durham, NC

Abstract: Perceptually, we group sounds from the same source and segregate sounds from different sources. This study characterizes the effects of consonance and dissonance on this process. We used an auditory streaming paradigm: C-AB-C-AB-C-AB etc, with the higher pitch C presented alone and the lower pitches A and B presented simultaneously. The frequency separation between C and B governs whether B groups with A or C (Bregman & Pinker, 1978). Here, we investigated whether the harmonic relationship between A and B affects this grouping by testing two conditions: A and B are two octaves apart (consonant), or A and B are an octave and a major 7th apart (dissonant). Human participants classified sound patterns as to whether B grouped with A or with C as a function of C's frequency. We compared the midpoint of the psychometric curves. The consonant condition had a smaller midpoint, suggesting that consonant intervals may be more likely to fuse. This result will help design stimuli to investigate the neural patterns underlying auditory grouping and segregation. With these stimuli, we can compare neural responses to sounds that differ in their perception as grouped or segregated.

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Poster

PSTR339: Auditory Processing

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Program #/Poster #: PSTR339.07/R3

Topic: E.05. Auditory and Vestibular Systems

Title: The Influence of Linguistic Predictability on the Encoding of Speech in Different Cortical Areas: An Intracranial EEG Study

Authors: *W.-C. LIN, A. RUDAEVA, J. DOU, Z. LI, A. R. NIDIFFER, S. V. NORMAN-HAIGNERE, E. C. LALOR;

Univ. of Rochester, Rochester, NY

Abstract: The idea that top-down, knowledge-based information affects the processing of bottom-up sensory signals has become central to thinking about perception in recent years. And speech perception, in particular, has increasingly been acknowledged to involve the integration of sensory input with expectations based on the context of that speech. However, debate still surrounds the issue of whether or not prior knowledge feeds back to affect early auditory encoding in the lower levels of the speech processing hierarchy, or whether perception can best be explained as a purely feedforward process. Some recent work has provided support for the idea that predictive feedback influences the acoustic-phonetic encoding of speech. Specifically, several scalp EEG studies have reported that the cortical tracking of various acoustic and phonetic speech features appears to be affected by the context-based predictability of words when participants listen to naturalistic (podcast) speech. In the present study, we aim to seek further evidence for this effect - and to see how the effect might vary across different stages of the cortical speech processing hierarchy - by examining neural responses to natural speech using intracranial EEG (iEEG) recordings in neurosurgical patients. Specifically, we analyzed iEEG from patients as they listened to ~12-24 minutes of a podcast. We fitted temporal response functions (TRFs) to model the relationship between acoustic envelope of the speech and the neural data on each iEEG channel (both low frequency activity and high gamma power). We used these TRFs to reconstruct the acoustic envelope on new trials from the recorded iEEG. The reconstruction accuracy served as an index of the low-level acoustic-phonetic encoding of the speech. We then assessed how this index varied for each word as a function of how predictable that word was in the context of the podcast (using an estimate of lexical surprisal computed from a large language model). This analysis revealed that - on many of the iEEG channels - the speech envelope tracking was stronger for words that were more surprising. This finding supports the notion that EEG responses to low level responses to natural speech (at least partially) reflect prediction error. As such, it contributes to our understanding of the neuroscience of perception. And it also provides insights that will be useful in future clinical research, including research involving groups in whom predictive perception is thought to differ.

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Poster

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Title: The pulvinar nucleus of the thalamus may play a crucial role in auditory attention

Authors: *A. N. SIMMONS^{1,2,3,4}, P. BRUNNER^{1,2,3,4}, J. T. WILLIE^{1,2,3,4};

¹Biomed. Engin., Washington Univ. in St. Louis, St. Louis, MO; ²Neurolog. Surgery, ³Div. of Neurotechnology, Washington Univ. Sch. of Med., St. Louis, MO; ⁴Natl. Ctr. for Adaptive Neurotechnologies, St. Louis, MO

Abstract: The pulvinar nucleus of the thalamus plays a crucial role in visual perception and attention. Recent connectivity studies, however, have demonstrated effective connectivity between the pulvinar and the lateral temporal cortex, including the superior temporal gyrus, the primary brain region associated with auditory processing. Despite its connectivity to auditory processing regions, the pulvinar's role in auditory attention remains unclear. Working towards building this understanding, we investigated intracranial auditory evoked potentials (AEP) recorded from four adult neurosurgical patients diagnosed with refractory epilepsy. As part of their standard treatment procedure, all patients were implanted with stereoelectroencephalography (sEEG) electrodes that included contacts located in the pulvinar and brain regions involved in auditory processing, such as the superior temporal gyrus and Heschl's gyrus. To evaluate auditory-related pulvinar function and connectivity, participants underwent a passive auditory language task while AEPs were measured using the implanted sEEG electrodes. All participants understood English and had no history of hearing impairments. Each trial consisted of a 0.7-second auditory stimulus followed by a 1-second silent (baseline) period. Auditory stimuli consisted of 64 words and 64 non-words delivered through speakers. Task-related spectral perturbations were observed at pulvinar contacts with relatively inferior pulvinar channels showing a statistically significant increase in broadband gamma (70-170 Hz) amplitude during the task (Wilcoxon rank-sum test). Additionally, participants underwent electrical stimulation mapping during which single pulse electrical stimulation (SPES) was

delivered to pulvinar contacts and cortico-cortical evoked potentials (CCEPs) were measured at all other electrode contacts. Statistically significant responses (evaluated by comparing pre and post stimulation signal root mean square) were detected across known auditory processing brain regions including Heschl's and the superior temporal gyri. This result aligns with our hypothesis that the pulvinar may play a crucial, yet understudied role in auditory perception.

Disclosures: A.N. Simmons: None. P. Brunner: None. J.T. Willie: None.

Poster

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Topic: E.05. Auditory and Vestibular Systems

Support: Beckman Institute Graduate Fellowship
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Title: Quantifying perceptual inference via an auditory probabilistic deviant detection task

Authors: *W. GAUTHIER, N. JAMES, B. D. AUERBACH;
Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Optimizing sensory decision-making requires the integration of uncertain sensory signals with prior expectations. Disambiguating these bottom-up and top-down contributions to sensory perception, however, remains a difficult challenge in neuroscience. Here we show that a probabilistic oddball detection task provides a useful framework for differentiating these perceptual components. By leveraging an inferential sensory task design, we demonstrate that rats can integrate information that is not directly accessible from the physical sensory stream to predict when a deviant stimulus will occur. Importantly, we show that the current state of a subject's internal model can be observed via covert probe trials and that these models can be manipulated in a manner that is independent from overt task rules and incoming sensory stimuli. This paradigm thus compels rats to form an experimentally specified internal model that can be quantitatively derived from behavior. We further validate these findings by employing a hierarchical drift diffusion model, which reveals parameter changes that specifically correspond to both bottom-up and top-down processes. Modeling this behavior through reinforcement learning and active inference schemes, we further elucidate the relationship of reward and information optimization in inferential auditory decision making. These results align with current theoretical predictions from active inference frameworks, illustrating the utility of this task in parsing the principles of perception.

Disclosures: W. Gauthier: None. N. James: None. B.D. Auerbach: None.

Poster

PSTR339: Auditory Processing**Location:** SDCC Halls B-H**Time:** Tuesday, November 18, 2025, 1:00 PM - 5:00 PM**Program #/Poster #:** PSTR339.10/R6**Topic:** E.05. Auditory and Vestibular Systems**Support:** CCSN funds provided by the McDonnell Center for Systems Neuroscience at Washington University in St. Louis**Title:** Tail of the striatum dopamine signals sensory prediction error during perceptual decisions**Authors:** *E. BANO¹, A. J. CHRISTENSEN¹, A. KEPECS^{1,2};
¹Neurosci., ²Psychiatry, Washington Univ. in St Louis, St. Louis, MO

Abstract: Perceptual decisions depend on internal models of the environment's sensory statistics, which must be continuously updated when predictions fail to match incoming sensory evidence. While dopamine's role in signaling reward prediction errors in the ventral striatum is well established, its involvement in updating sensory expectations is less understood. The tail of the striatum (TS), a sensory-recipient region, has been implicated in threat and, more recently, action prediction errors. However, emerging evidence suggests that TS dopamine also tracks internal perceptual expectations during auditory detection in mice. A unifying computational principle linking these findings has yet to be established. We hypothesize that TS dopamine encodes sensory prediction errors (SPEs)—the mismatch between expected and actual sensory input—providing a common framework that reconciles its diverse roles in perception, action, and threat processing. To test this, we trained mice on an auditory signal detection task in which a near-threshold tone was present or absent with varying probability across uncued blocks, while monitoring dopamine using fiber photometry. We found that mice dynamically shifted their auditory decision threshold in response to blockwise changes in sound probability. These shifts reflected an update in perceptual expectation, not merely reinforcement history. TS dopamine responses to auditory signals scaled with stimulus probability, matching predictions from a Bayesian model of perceptual updating. These transients were distinct from reward-related dopamine signals and exhibited no valence bias, responding similarly to both routine and threatening surprises. To formalize this, we developed an online Bayesian inference model that updates sensory beliefs about stimulus probability based on recent experience. The updating rule adjusted expectations based solely on sensory input, independent of trial outcome, to improve future predictions. The model's prediction errors—differences between expected and actual inputs—accounted for both the animals' behavioral shifts and the TS dopamine responses. Our findings show that TS dopamine signals sensory prediction errors and offer a computational framework that unifies its role in perceptual inference—from routine updates to threat detection—through sensory surprise-driven learning.

Disclosures: E. Bano: None. A.J. Christensen: None. A. Kepecs: None.**Poster**

PSTR339: Auditory Processing**Location:** SDCC Halls B-H**Time:** Tuesday, November 18, 2025, 1:00 PM - 5:00 PM**Program #/Poster #:** PSTR339.11/R7**Topic:** E.05. Auditory and Vestibular Systems**Title:** A novel psychometric toolkit for the improved diagnosis of misophonia**Authors:** S. N. RICO¹, J. R. EBERSBERGER², *A. A. BREWER¹;¹Cognitive Sci., Univ. of California, Irvine, IRVINE, CA; ²Cognitive Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Misophonia is a critically understudied disorder likely affecting a significant population across the globe. The triggers linked to the negative responses of this disorder can cause debilitating downstream effects, and in many cases severely degrade social engagement, outlook, cognitive function, and overall quality of life. Comorbidities may play a role or even be exacerbated by this disorder. Despite the potentially significant reductions in quality of life that sufferers of misophonia may experience, we still lack critical diagnostic criteria as well as nearly any neurological understanding of its cause. Here we have developed a psychometric toolkit that combines behavioral and physiological testing in order to better characterize misophonia, improve diagnosis, and serve as a foundation for our cortical measurements. We conducted an extensive online survey (misophonia.io/participate), combining published misophonia assessment tools, validated psychological measures, and novel questionnaires tailored to what we believe are key metrics in understanding misophonia and its comorbidities (e.g., anxiety, hyperacusis). We paired these questions with a novel diagnostic tool that records participant responses to frequently reported misophonic stimuli. We hope that the development of this novel psychometric toolkit will allow for improved diagnosis of and ultimately treatment for this potentially devastating condition.

Disclosures: S.N. Rico: None. J.R. Ebersberger: None. A.A. Brewer: None.**Poster****PSTR339: Auditory Processing****Location:** SDCC Halls B-H**Time:** Tuesday, November 18, 2025, 1:00 PM - 5:00 PM**Program #/Poster #:** PSTR339.12/R8**Topic:** E.05. Auditory and Vestibular Systems**Support:** National Sawdust**Title:** Eeg study of music engagement: foundations for a premiered neuroart multisensory performance

Authors: *Y. WANG;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: This study builds upon the introduction of NeuroArt at SfN 2024 by establishing a neuroscience foundation for multisensory opera performance design and advancing tools for real-time neurofeedback in music therapy. Understanding how music modulates attention and emotion is critical for both immersive artistic experiences and music therapeutic interventions. Here, we used electroencephalography (EEG) and behavioral data to identify neural markers of listener engagement during music, supporting the development of *COUNTERTENOR*, a premiered multisensory opera performance integrating curated music and smell stimuli to enhance memory encoding during salient musical moments. Ten adult participants (ages 19-40; 6 male, 4 female) passively listened to eight musical excerpts (~55 minutes total), including works by Philip Glass, Antonio Vivaldi, Klaus Nomi, and others. EEG was recorded using a Muse headband (sampling frontal and temporal regions) and preprocessed using MNE-Python. Power spectral density and frontal alpha asymmetry (FAA) were extracted as neural indices of attentional engagement and emotional valence. Behavioral data included timestamped button presses marking emotional shifts and post-listening surveys. Neural and behavioral data were temporally aligned with music to reveal patterns of engagement. Alpha suppression and increased broadband power coincided with climactic musical moments, such as high-pitched vocal lines, tempo changes, and instrumental re-entries. For example, in *Laika*, a mid-piece rhythmic and textural shift corresponded with elevated brain activity and participant-reported excitement. In *Gelido in ogni vena*, a recurring high-pitched motif elicited consistent spectral power increases across listeners. Attention waned during high-frequency instrumental absences (e.g., flute dropout) and rebounded upon re-entry. These findings demonstrate that low-fidelity EEG can meaningfully capture engagement-related brain dynamics in response to music. The results support a neuroaesthetic framework for designing emotionally optimized, multisensory performances and also offer a basis for developing real-time, music-based neurofeedback tools for therapeutic applications.

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Poster

PSTR339: Auditory Processing

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Topic: E.05. Auditory and Vestibular Systems

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Title: Associations between 20-Hz Auditory Steady-State Responses, Musical Sophistication, and Music Reward Sensitivity

Authors: *S. KONDOH^{1,2,3}, K. YOSHIDA^{1,2}, S. TAMURA^{4,5}, S. NAKAJIMA⁶, Y. HIRANO^{4,5}, S. FUJII¹;

¹Fac. of Envrn. and Information Studies, ²Grad. Sch. of Media and Governance, Keio Univ., Kanagawa, Japan; ³Japan Society for the Promotion of Sci., Tokyo, Japan; ⁴Dept. of Psychiatry, Div. of Clin. Neuroscience, Fac. of Med., Univ. of Miyazaki, Miyazaki, Japan; ⁵Dept. of Neuropsychiatry, Grad. Sch. of Med. Sci., Kyushu Univ., Fukuoka, Japan; ⁶Dept. of Neuropsychiatry, Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Auditory steady-state response (ASSR) is an electroencephalogram (EEG) response elicited by repetitive auditory stimuli. The 40-Hz ASSR has been reported to show abnormalities in patients with schizophrenia (Nakanishi et al., 2023) and exhibits individual differences depending on treatment resistance (Ogyu et al., 2023). However, the factors contributing to the individual variability in ASSRs among healthy individuals have not yet been fully elucidated. Neural entrainment to auditory beats below 5 Hz is influenced by musical behavior, such as rhythmic tapping (Nozaradan et al., 2016), and the 40-Hz ASSR appears to correlate with self-reported sound-perception ability, as suggested by our ongoing study (Yoshida et al., in preparation). Accordingly, the 20-Hz ASSR, elicited by auditory stimuli perceived as a “flutter” and occupying an intermediate frequency between rhythmic events (< 10 Hz) and pitch perception (> 30 Hz) (Nourski & Brugge, 2011), may also be linked to musical experience. In this study, we examined whether 20-Hz ASSRs in healthy individuals are associated with musical background. Twelve participants (6 females, 50%; mean age = 25.3 ± 8.2 years) were recruited, and ASSRs were recorded in response to chirp-train stimuli. Inter-trial coherence (ITC) was calculated to assess phase synchronization. The participants’ musical backgrounds were assessed using the Japanese versions of the Goldsmiths Musical Sophistication Index (Gold-MSI; Sadakata et al., 2022) and Barcelona Music Reward Questionnaire (J-BMRQ; Honda et al., 2023). Multiple regression models were constructed with 20-Hz ITC as the response variable and all subscale scores from the Gold-MSI and J-BMRQ as explanatory variables. Stepwise regression identified the optimal model, and variables with high variance inflation factors (VIFs) were removed to address multicollinearity. The final model approached statistical significance ($F(5, 6) = 3.827$, $p = 0.066$, adjusted $R^2 = 0.562$, all VIFs < 4). Among the retained explanatory variables, the music training subscale in the Gold-MSI ($\beta = 0.818$, $p = 0.017$), mood regulation subscale in the J-BMRQ ($\beta = 0.756$, $p = 0.027$), and sensorimotor subscales in the J-BMRQ ($\beta = 0.799$, $p = 0.031$) showed significant positive associations with 20-Hz ITC. Although these preliminary results should be interpreted with caution, they suggest that 20-Hz ASSRs may reflect individual differences in musical sophistication, particularly in formal music training, as well as in sensitivity to music-related rewards, particularly in domains involving sensorimotor processes.

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Poster

PSTR339: Auditory Processing

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.14/R10

Topic: E.05. Auditory and Vestibular Systems

Title: Does the selectivity in pupil responses between relevant and irrelevant background sounds reveal a sound-level dependent sweet spot for attentional control?

Authors: *L. FIEDLER, D. WENDT;
Eriksholm Res. Ctr., Snekkersten, Denmark

Abstract: Auditory attentional control, including sustaining and switching attention to relevant sounds while ignoring irrelevant ones, is crucial for navigating busy auditory scenes. This ability can be compromised by hearing loss, which increases cognitive load under a limited cognitive capacity. We investigated whether manipulating the loudness of both relevant and irrelevant background sounds reveals a sweet spot for attentional control, both in behavior and pupil responses. This sweet spot would manifest as optimal behavioral efficiency and maximal pupil selectivity, defined as the difference between responses to relevant versus irrelevant background sounds. We recorded pupil size from 44 participants (ages 44-83 years; PTA: 7.5 - 64 dB HL) while they performed a dual task. The primary task involved sustained attention to an audiobook, and the secondary task required memorizing spoken numbers (preceded by a cueing sound) from a relevant location, with numbers from an irrelevant location also presented. The levels of the spoken numbers and the cueing sound were manipulated relative to the primary target (-27, -19, -11, -3 dB). Preliminary analysis reveals level-dependent pupil selectivity, peaking at the intermediate background-sound level of -11 dB, suggesting a sweet spot at the group level. Further analysis will explore the relationship between this sweet spot and behavioral performance, as well as cognitive tests of working memory, processing speed, and inhibitory control.

Disclosures: L. Fiedler: A. Employment/Salary (full or part-time): Eriksholm Research Centre, Part of Oticon A/S. D. Wendt: A. Employment/Salary (full or part-time): Eriksholm Research Centre, Part of Oticon A/S.

Poster

PSTR339: Auditory Processing

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.15/R11

Topic: E.05. Auditory and Vestibular Systems

Title: Investigating the role of information variability in hierarchical auditory predictive processing

Authors: *T. DOMZALSKI¹, D. R. QUIROGA-MARTINEZ², A. O. BLENKMANN³, K. BASINSKI¹;

¹Dept. of Psychology, Auditory Neurosci. Lab., Med. Univ. of Gdansk, Gdansk, Poland;

²Psychology, Univ. of Copenhagen, København K, Denmark; ³Dept. of Psychology, Univ. of Oslo, Oslo, Norway

Abstract: Predictive processing theories suggest that perception is shaped by a dynamic interplay between internal generative models and incoming sensory inputs, where discrepancies between predicted and actual input, known as prediction errors (PEs), serve as a signal for updating these models. The influence of PEs on model adjustment is determined by precision weighting. Precision weighting is thought to depend on the entropy (amount of information, or simply predictability) of sensory inputs: low-entropy context allows for more precise predictions and greater influence of PE, while high-entropy context reduces PE updating due to lower estimated precision. The mismatch negativity (MMN) component of the event-related potential (ERP) is considered a neural index of PE signalling in the auditory system. In this study, we investigate how different levels of entropy in auditory sequences affect MMN and P3 complex responses within a local-global paradigm. Participants are presented with three experimental conditions: (a) a low-entropy condition featuring harmonic complex tone sequences, where deviants differ only in fundamental frequency (f_0); (b) a moderate-entropy condition with inharmonic complex tones that are frequency-jittered and occur in a fixed order; (c) a high-entropy condition using inharmonic complex tones that are both frequency-jittered and randomly ordered. This design allows us to separately examine stimulus-based and sequence-based uncertainty effect on the MMN and P3 complex. We hypothesize that MMN amplitudes will reflect the predictability of both local and global auditory structures, with larger MMN/P3 responses observed in low-entropy condition due to higher precision weighting. In contrast, attenuated responses are expected in high-entropy conditions, where increased uncertainty leads to reduced precision estimates. Preliminary results show that local deviants are modulated by inharmonicity for both MMN and P3a responses. However, P3b responses to global deviants are diminished in both low and high-entropy conditions, suggesting that the manipulation of inharmonicity may disrupt the formation or recognition of global-level regularities, making them harder to predict.

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Poster

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.16/R12

Topic: E.05. Auditory and Vestibular Systems

Title: Cortical correlates of effort discounting and listening fatigue in hearing aid users

Authors: *A. SCHULTE^{1,2}, C. J. CARON^{1,2}, M. A. APPS³, D. WENDT^{1,2}, H. INNES-BROWN^{1,2};

¹Dept. of Hlth. Technology, Hearing Systems Section, Tech. Univ. of Denmark, Lyngby,

Denmark; ²Eriksholm Res. Ctr., Oticon, Snekkersten, Denmark; ³Univ. of Oxford, Oxford, United Kingdom

Abstract: Understanding speech in noise requires sustained cognitive effort and often leads to fatigue, especially in individuals with hearing loss. This fatigue can reduce the willingness to use hearing aids or engage in conversations. To address this challenge, it is crucial to understand how fatigue accumulates over time and affects listening decisions. However, self-reports offer only partial insight due to limited conscious awareness of momentary fatigue states. Building on prior work, we applied an existing neurocomputational framework to a listening effort-based decision-making task in order to investigate trial-by-trial listening-related fatigue and its cortical correlates using functional near-infrared spectroscopy. Data from 5 out of 30 hearing aid users have been collected and analyzed so far (male, 74 years mean age). Participants were asked to maximize points which they could collect by choosing to either listen to and repeat back a sentence (high point value) or by resting (low point value). They also rated fatigue on each trial. Task difficulty (SNR) and rewards (point values) varied across trials. We fitted five effort-discounting models to participants' trial-by-trial decisions and fatigue ratings and related model-derived fatigue states to cortical oxygenation changes. Our preliminary data show that decisions to listen and subjective fatigue ratings are best explained by a model that includes both recoverable fatigue (rising with effort, falling with rest), an unrecoverable component (monotonically increasing) and correctness of the response. All 5 participants showed significant activation in prefrontal areas with increasing response amplitudes over time-on-task. In two participants, a left prefrontal subregion was associated with unrecoverable fatigue, consistent with earlier findings of physically induced fatigue (Müller et al., *Nat Commun*, 2021). These preliminary results suggest that listening-related unrecoverable fatigue shares cortical correlates with physical-induced unrecoverable fatigue, validating the effort-discounting framework. Future work may enable monitoring and prediction of fatigue states to inform adaptive adjustments of hearing aid settings or behavioral strategies, helping to prevent mental exhaustion when the subjective value of listening is low.

Disclosures: A. Schulte: None. C.J. Caron: None. M.A. Apps: None. D. Wendt: None. H. Innes-Brown: None.

Poster

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Topic: E.05. Auditory and Vestibular Systems

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)
Chaire Fondation Caroline Durand en audition et vieillissement de
l'Université de Montréal

Title: Dance training shapes the integration of sensory cues for body representation

Authors: *D. PAROMOV¹, C. AZAR¹, M. MAHEU³, B.-A. BACON⁴, F. CHAMPOUX²;
²Sch. of speech language pathology and audiology, ¹Univ. de Montréal, Montreal, QC, Canada;
³Sch. of Speech Language Pathology and Audiol., Univ. de Montréal, Montreal, QC, Canada;
⁴Psychology, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The representation of the body in space, or body schema, results from the integration of multiple sensory inputs (vestibular, visual, somatosensory, auditory). This relationship is bi-directional, with the resulting schema influencing the processing of sensory inputs, and thus for example, auditory spatial processing. While extensive dance training is also known to modulate auditory processing, it is unclear if these supranormal abilities transfer to more accurate body representation. This study aimed to assess the contribution of auditory input to body representation in dancers, both in static and dynamic tasks. A total of 18 dancers with at least 8 years of experience and 14 non-musicians were recruited for this study. Participants were instructed to perform the Fukuda-Unterberger stepping task (dynamic), known to induce involuntary rotation of the body in space, with a static sound source positioned at 0-, 45-, and 90-degrees azimuth as a spatial reference, or without any auditory cues. The same conditions were also repeated in a static task, where participants were asked to stand on a force platform, and sway parameters were derived from the center of pressure measurements. While dancers performed significantly better in tasks without auditory cues, their advantage was less prominent when such spatial cues were added. These results suggest that participants may gain a significant advantage from the addition of auditory cues, but that their experience shapes the weight of these cues in the resulting unified representation.

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Poster

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Topic: E.05. Auditory and Vestibular Systems

Support: NIH Grant DC022778
NIH Grant EY030669

Title: Psychophysical receiver operating characteristic (ROC) curves from a macaque monkey in an auditory spatial discrimination task

Authors: *C. CONROY¹, Y. E. COHEN², R. M. MCPEEK¹;
¹SUNY Col. of Optometry, New York, NY; ²Dept. Otorhinolaryngology-Head Neck Surgery, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Signal detection theory (SDT) posits a role for both sensory and decision factors in the discrimination of sensory stimuli. According to SDT, discriminating between two categories of

sensory stimuli depends on both the observer's internal sensitivity to those stimuli and the decision criterion that is used for the discrimination boundary. The receiver operating characteristic (ROC) curve is a central construct in SDT, therefore, because it shows how performance in a discrimination task changes with the decision criterion. It can also be used to index sensitivity and sheds light on the form of the internal distributions of sensory events that are associated with the two stimulus categories. Neurophysiological investigations of sensory and decision factors in auditory perception, therefore, may benefit from neurophysiological data obtained from behavioral ROC paradigms. Towards this end, we trained a male rhesus macaque monkey on an auditory spatial discrimination task that was designed to yield a behavioral ROC curve. On each trial, the monkey fixated a central point and two visual-saccade targets - one positioned to the left, the other to the right of fixation - were presented. An auditory stimulus (noise) was then presented to either the left or right of the midline. The monkey's task was to decide if the noise was to the left or right of the midline and to report that decision by making a saccade to the left or right visual-saccade target, respectively. Trial-by-trial shifts in the monkey's criterion were induced by manipulating the relative reward size associated with correct-left versus correct-right decisions. The relative reward size was cued to the monkey by the shapes of the visual-saccade targets: they either had different shapes and therefore signaled asymmetric reward potential (and thus were intended to induce relatively liberal or conservative criterion placements) or had the same shapes and signaled even reward potential (and thus were intended to yield a relatively neutral criterion placement). This procedure successfully induced trial-by-trial shifts in the monkey's criterion and yielded a behavioral ROC curve: for example, when the visual cues indicated the potential for a relatively large reward associated with a correct-right decision, the monkey adopted a liberal criterion with respect to right spatial judgements, whereas, the monkey adopted a more conservative criterion when correct-left decisions were associated with a larger reward. Neurophysiological data obtained using this behavioral ROC paradigm could be useful for understanding the neural bases of sensory and decision processes in auditory spatial discrimination.

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Poster

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Program #/Poster #: PSTR339.19/R14

Topic: E.05. Auditory and Vestibular Systems

Support: Mcknight Brain Institute Accelerator Grant

Title: Reconstructing perceived speech using magnetoencephalography and deep learning

Authors: *A. R. VELEZ^{1,5}, L. RU^{2,5}, B. AHMADI^{3,5}, A. BABAJANI-FEREMI^{4,5};

¹Neurosci., ²Electrical and Computer Engin., ³Industrial and Systems Engin., ⁴Dept. of Neurol., Univ. of Florida, Gainesville, FL; ⁵The Norman Fixel Inst. for Neurolog. Dis., Gainesville, FL

Abstract: Millions of individuals lose the ability to move or speak due to a variety of medical circumstances and restoring communication can significantly enhance their quality of life. Brain-computer interface (BCI) research using invasive neural recording methods may encounter surgical complications and limited long-term usability, underscoring the need for non-invasive alternatives. Although electroencephalography (EEG)-based speech decoding has progressed, magnetoencephalography (MEG) remains relatively underutilized. MEG offers greater spatial resolution compared to EEG, and recent advancements, such as optically pumped magnetometer (OPM) MEG, are expanding MEG's potential for clinical and portable applications. In this study, we analyzed 10 hours of MEG data from a single participant listening to audiobooks (Armeni et al., Sci Data. 2022). Preprocessing included artifact removal and sensor-level power extraction across five canonical frequency bands: theta, alpha, low-beta, high-beta, and gamma. We employed a gated recurrent unit (GRU) model in combination with a variational autoencoder (VAE) for data augmentation to apply MEG power in these bands and predict unconstrained sequences of phonemes from a dictionary of 39 phonemes and a silent character. A 125,000-word language model was then used to predict full sentences based on the predicted phoneme sequences. Using only MEG low-beta band power features, our model achieved a phoneme error rate (PER) of 67% and a decoded sentence character error rate (CER) of 69%, well above the chance level accuracy (2.5% for phonemes and 3.7% for characters). These preliminary results demonstrate the feasibility of reconstructing continuous speech from non-invasive neural data using deep learning and language models. Ongoing work aims to further reduce the PER to enable more accurate word- and sentence-level decoding. Improving MEG-based language decoding accuracy is vital for developing BCIs for speech restoration using MEG and other non-invasive modalities. This progress not only supports speech restoration for patients but also enhances our understanding of the overlapping oscillatory dynamics involved in speech perception and imagined speech.

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Poster

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Topic: E.05. Auditory and Vestibular Systems

Support: University at Buffalo start-up funds
University at Minnesota Social Sciences Seed Grant

Title: Multi-channel auditory cortical responses to spatialized speech

Authors: L. MOORE¹, Z. JOCK², *M. DININO¹, E. FISH¹, M. J. POLONENKO³;
¹Univ. at Buffalo, Buffalo, NY; ²Auditory Processing and Perception Lab., Univ. at Buffalo, Waddington, NY; ³Speech-Language-Hearing Sci., Univ. of Minnesota, Minneapolis, MN

Abstract: Electrophysiological measures, such as auditory brainstem responses (ABRs) and cortical auditory evoked potentials (CAEPs) are widely used by both researchers and hearing healthcare professionals to examine auditory neural function. These evoked potentials are traditionally conducted to a brief click stimulus, which does not mimic speech that we hear in everyday listening. Polonenko & Maddox (2021) created a paradigm in which ABRs and CAEPs can be collected in response to continuous speech, which is thought to better assess the neural function that occurs during everyday listening. Spatialized speech, in which speech is presented to the front and to the side, even more accurately represents real-world listening scenarios. However, neural responses to sound are typically examined from electrodes near the top center of the head (Cz or Fz), and responses from other electrodes may be more optimal to assess cortical responses to spatialized speech stimuli. This study aimed to examine cortical responses at different electrode locations in response to both spatialized and non-spatialized speech. Thirty young adults (aged 18-30 years) with verified normal hearing thresholds listened to spatialized and non-spatialized continuous speech while brainstem and cortical responses were recorded. In the non-spatialized condition, two stories spoken by different talkers were played at simulated center. In spatialized conditions, one story was played at simulated center and another was played to the left or right by applying +/- 600 μ s interaural time difference cue. The late latency response, which contains the P1 and N1 event-related potentials, was compared between conditions at different electrode locations, which may reflect different neural generators based on perceived spatial location. Electrode locations were binned as “frontal” (F3, Fz, and F4) or “central” (C3, Cz, and C4). Area under the curve calculation for P1 and N1 responses for the different electrode locations revealed that both P1 and N1 responses differed depending on the speech condition. Interestingly, P1 activity changed across speech conditions in a similar way for the different electrode configurations, but N1 activity was dependent on both speech condition and electrode location. These results suggest that neural activity evoked by spatialized speech conditions is better represented by multiple electrodes rather than the one centralized electrode that is typically used in clinical testing. The findings from this study also provide greater insight into speech processing in the central auditory system.

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Poster

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Topic: E.05. Auditory and Vestibular Systems

Support: University at Buffalo Start-up Funds

Title: Auditory brainstem measures and selective attention deficits in major depressive disorder

Authors: *J. M. MARTINEZ¹, V. WEDGEWOOD¹, M. DININO²;
²Communicative Disorders and Sci., ¹Univ. at Buffalo, Buffalo, NY

Abstract: Auditory selective attention, which allows individuals to focus on a source while suppressing competing sound, is critical for hearing in background noise but depends on accurate neural encoding. Previous research shows that major depressive disorder (MDD) impairs domain-general attentional ability, but the relationship between MDD symptoms and auditory selective attention has not yet been examined. In addition, although the impact of MDD on auditory cortical activity has been thoroughly investigated, the relationship between symptom severity history and auditory brainstem responses (ABRs) remains under-explored. Therefore, we aimed to quantify ABR differences in individuals with MDD and measure how MDD severity and subcortical measures relate to performance on auditory selective attention tasks. We recruited young adults with verified normal hearing thresholds who each completed the Patient Health Questionnaire-8 (PHQ-8) to assess symptom severity, a measurement of persistent MDD symptoms to track symptom longevity, ABR recordings, and an auditory selective attention task requiring subjects to attend to a target stream of syllables while ignoring two distracting streams. Correlation analysis revealed a significant relationship between PHQ-8 responses and persistent depression scores, indicating that individuals with greater depression symptom severity had experienced those symptoms longer. Regression analyses were conducted to examine the relationships between symptoms of depression, ABR metrics, and performance on the auditory selective attention test. Results showed that participants with greater symptom severity and lifetime history of MDD had significantly reduced wave V amplitudes and longer ABR wave V latencies, indicating poorer late subcortical processing relative to individuals with less MDD history and symptom severity. Individuals with a longer history of depressive symptoms were also those who tended to perform worse on the auditory selective attention test. However, ABR metrics alone did not predict auditory attention scores, suggesting that disrupted cortical processing and/or other cognitive factors in individuals with MDD contribute to poorer performance on the task. Our findings suggest that ABR measures may be potential biomarkers of MDD, particularly because symptom persistence significantly increased these effects. This is key for mapping neural pathways that link brainstem processing and cortical control in MDD. Additionally, these findings suggest that future research aimed at strengthening attention in individuals with MDD should focus on higher order cortical and cognitive impairments.

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Poster

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Program #/Poster #: PSTR339.22/S1

Topic: E.05. Auditory and Vestibular Systems

Support: William-Demant Foundation 1018954

Title: Can Music Training Enhance/Affect Working Memory and Speech-in-Noise Perception in Cochlear Implant Users? A Randomized Controlled Study of EEG Measures of Improvement.

Authors: *K. MERTEL¹, A. DIMITRIJEVIC², M. H. THAUT³;

¹Music and Hlth. Res. Collaboration (MaHRC), Univ. of Toronto, Toronto, ON, Canada;

²Cochlear Implant Program, Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; ³Fac. of Music and Fac. of Med., Univ. of Toronto, Toronto, ON, Canada

Abstract: Hearing loss profoundly affects individuals' quality of life, limiting participation in social, professional, and communicative domains. Cochlear implants (CIs) restore access to auditory input for postlingually deafened adults, but understanding speech through a CI relies heavily on neuroplastic adaptation. While many CI users regain the ability to follow conversations in quiet environments, speech-in-noise (SPiN) perception remains a major challenge. Music training has been shown to enhance shared auditory and cognitive neural networks and to improve auditory-motor coupling, which may facilitate SPiN perception in both normal-hearing and hearing-impaired individuals. These findings provide a rationale for using multi-modal Neurologic Music Therapy (NMT) to support auditory rehabilitation in adult CI users. However, research to date has focussed primarily on passive music listening, leaving the effects of structured, active music training largely unexplored. This randomized controlled clinical trial investigates the effects of short-term targeted multi-modal music training on SPiN and working memory (WM) in postlingually deafened adult CI users. Additionally, the study examines neurophysiological mechanisms of change using electroencephalography (EEG), specifically focusing on alpha oscillation modulations during auditory-cognitive tasks. Eighty-one adult CI users are enrolled in a 4-week NMT training program randomly assigned to pitch, rhythm, or timbre-focused conditions. Behavioral outcomes are assessed pre- and post-training using the sentence-final-word-identification-and-recall (SWIR) test; EEG is recorded during all SWIR tasks. Preliminary behavioral results from 27 participants indicate a mild improvement in SPiN perception, with no significant effects on WM and no differences between training types. So far, only behavioral data have been analyzed; EEG analysis is ongoing and will be reported once available. These early findings, based on a small and heterogeneous sample, show promising trends. Further EEG analysis is expected to provide deeper insight into neuroplastic changes, including modulation of alpha activity, potentially supporting music-based strategies to enhance auditory performance and life quality in CI users.

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Poster

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Program #/Poster #: PSTR339.23/S2

Topic: E.05. Auditory and Vestibular Systems

Support: R01DC020097

Title: Quantifying foreground interactions in natural auditory scene speech recognition

Authors: *A. CLONAN¹, I. H. STEVENSON², M. A. ESCABI³;

²Dept. of Psychological Sci., ³Electrical and Computer Engin., ¹Univ. of Connecticut, Storrs, CT

Abstract: Humans excel at recognizing speech in natural auditory scenes despite large variation in speakers' voice quality, intonation, and pitch. Previous work has found that different types of background sounds and their spectrotemporal statistics can improve or worsen speech recognition accuracy. However, how foreground speech features, such as fundamental frequency or formants, influence speech recognition accuracy is not well understood. Here we explore how different speech articulatory features interact with natural and perturbed background sounds to influence speech recognition abilities. Participants (n=10) were tasked with recognizing digit sequences (e.g., 0-9-1, 2-5-7) generated by adult and adolescent male and female talkers in various natural background sounds (e.g., water, babble, construction noise etc.; SNR= -9 dB) and perturbed background variants with distorted spectrum or modulation statistics. We found that different spoken digits had different accuracies depending on both the background sound statistics and the speaker's voice. To explore these speech driven effects, we compared recognition abilities across the fundamental (F0), first formant (F1), and second formant (F2) frequencies of the foreground. Using a generalized linear mixed effects model, we found substantial, statistically significant trends with digit identity, F0, F1, and F2, on recognition accuracy, in addition to background-driven effects. To characterize the unique interactions between the foreground and background sound statistical features, we also used a hierarchical model of the auditory periphery and auditory midbrain, paired with generalized perceptual regression (GPR). GPR models perceptual judgments of single listeners with the neural-acoustic model, and allows perceptual outcomes for arbitrary speech-foreground combinations to be predicted using auditory features. In our data, we find that this sound-computable model captures nearly 76% of the perceptual variance across background and digits interactions, and nearly 88% across background, F0, F1 and F2 interactions. The results identify foreground features that influence speech recognition in natural environmental noise and identify auditory model computations that account for and may underlie perceptual outcomes.

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Poster

PSTR339: Auditory Processing

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Topic: E.05. Auditory and Vestibular Systems

Support: R01DC020097

Title: Brain-inspired, sound-computable models predict discomfort and identify statistical sound cues underlying misophonia perception

Authors: A. CLONAN¹, I. H. STEVENSON², *M. A. ESCABI³;

¹Biomed. Engin., ²Dept. of Psychological Sci., ³Univ. of Connecticut, Storrs, CT

Abstract: Human hearing is critical to everyday communication and the perception of natural auditory scenes. For individuals with misophonia, sounds commonly experienced in daily life can evoke severe discomfort and distress. Aversion is often described in terms of broad sound categories, such as bodily sounds, but what acoustic features cause specific sounds to be aversive or not, within the same category or across different individuals, remains unclear. Here, we explore whether bottom-up statistical sound features processed in the auditory periphery and midbrain can explain aversion to sounds. Using the Free Open-Access Misophonia Stimuli (FOAMS) dataset and a hierarchical model of the auditory system, we find that sound summary statistics can predict discomfort ratings in participants with misophonia. For each listener, the model produces individualized transfer functions that pinpoint specific spectrotemporal modulations that contribute towards sound aversion. Overall, the model explains 76% of the variance in discomfort ratings, and we find substantial differences across participants in which sound features drive aversion. An advantage of the approach here is that it is sound-computable - perceptual ratings can be fit from or predicted for any sound. To illustrate applications of sound-computable models, we consider 1) extrapolation to a large set of untested environmental sounds and 2) personalized trigger detection in continuous audio. Model predictions identify untested sound categories, not in the original FOAMS set, that may also be aversive and suggest that there may be substantial heterogeneity in how aversive specific sounds are within some sound categories. In continuous audio, we show how sound-computable models can identify the timing of potential triggers from sound mixtures. Altogether, our results suggest that acoustic features - spectrotemporal modulations, in particular - can practically be used to characterize the individualized patterns of aversion in participants with misophonia. Future perceptual studies using synthetic sounds and sound sets with more diverse acoustics will allow model predictions to be tested more broadly; however, sound-computable models may already have applications in precision diagnosis and management of misophonia.

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Poster

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Topic: E.05. Auditory and Vestibular Systems

Support: NIH NIDCD-R00-DC018051

Title: Rapid and dynamic construction of acoustically invariant speech representations in the human auditory cortex

Authors: *D. BOEBINGER¹, D. SKRILL¹, G. LIAO¹, K. V. NOURSKI², M. A. HOWARD, III², C. M. GARCIA², T. WYCHOWSKI¹, W. H. PILCHER¹, S. V. NORMAN-HAIGNERE¹;
¹Univ. of Rochester, Rochester, NY; ²The Univ. of Iowa, Iowa City, IA

Abstract: Coding information in speech is computationally challenging in part because environmental factors such as reverberation, background sounds, and imperfect sound transmission can substantially alter the acoustics of speech. Much remains unknown about how the human auditory cortex represents speech information in a manner that is invariant to such acoustic variation, in part due to the coarse resolution of non-invasive neuroimaging methods such as functional MRI. Here, we measured neural responses to a diverse set of spoken sentences with and without common forms of acoustic variation using spatiotemporally precise intracranial recordings from the auditory cortices of neurosurgical patients undergoing chronic invasive monitoring for medically intractable epilepsy. We developed a paradigm to measure the timing and strength of invariance relative to the onset of speech information. We found that the human auditory cortex constructs representations of speech that are strikingly invariant to reverberation, complex background sounds, and spectral variation. Invariance was particularly strong in speech-selective neural populations in non-primary regions of the middle portion of the superior temporal gyrus. While speech-selective electrodes tended to be located further away from primary auditory cortex than non-selective electrodes, the effect of speech-selectivity could not be explained by electrodes' position in the auditory hierarchy. In many neural populations, including speech-selective populations, invariance strength increased rapidly during the first 200 ms after the onset of speech information, indicating rapid and dynamic refinement of an initial acoustic representation. Importantly, the functional organization and time course of invariance could not be predicted by standard spectrotemporal receptive field (STRF) models. Across electrodes, we found that STRFs predicted a narrow range of invariance strengths and thus were unable to account for the variability in invariance strength we observed in different neural populations, including the effect of speech selectivity. Prior research has found that the auditory cortex gradually suppresses responses to stationary properties of sounds, but our results show that the auditory cortex rapidly constructs a highly invariant representation of speech even immediately after a change in acoustic variation. Collectively, these findings indicate that the human auditory cortex uses a rapid and dynamic process to compute a representation of speech-specific structure that is highly robust to many common forms of environmental acoustic variation.

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Poster

PSTR339: Auditory Processing

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.26/S5

Topic: E.05. Auditory and Vestibular Systems

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Freemasons' Royal Arch Research Fellowship with support from the
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NIHR Academic Clinical Fellowship to JW

Title: Pitch-based selective attention is impaired in older “healthy” listeners

Authors: *J. WARD¹, A. FELDMAN², D. SMITH², J. RAMSDEN², A. J. KING³, K. M. WALKER⁴;

¹Nuffield Dept. of Surgical Sci., ³Physiology, Anat. and Genet., ²Univ. of Oxford, Oxford, United Kingdom; ⁴Physiology, Anat. & Genet., Univ. of Oxford, Kidlington, United Kingdom

Abstract: Pitch is our perception of the tonal quality of sounds, and it plays a key role in our ability to selectively attend to a single voice in a crowded room. Multiple acoustical cues, including temporal periodicity and harmonicity, are known to contribute to our perception of pitch, but it is not well understood which of these pitch cues are most important in guiding attentive listening or how this may change with ageing.

We investigated pitch-based selective attention by testing 75 normal-hearing (based on a pure tone audiogram) participants on a psychoacoustical task. Participants were asked to report the final two words of a target sentence and ignore a competing talker with the same voice at a different pitch. We manipulated potential pitch cues in separate testing blocks by either randomly jittering harmonic frequencies, replacing harmonics with noise (simulated whispering), including only “resolved” (2-5th) harmonics, including only “unresolved” (8-20th) harmonics, randomizing harmonic phase alignment, or adding reverberation.

We found that older healthy listeners (>65y) performed worse than younger adults (18-40y) in all stimulus conditions (2-way ANOVA; $p < 0.001$). Both age groups performed significantly better using resolved harmonics compared to unresolved harmonics (Tukey’s t-test; $p < 0.001$), but older listeners performed particularly poorly when resolved harmonics were removed.

Interestingly, reverberation had a detrimental effect on the performance of younger listeners, but more subtle effects on elderly participants, compared to their performance with natural speech. Misaligning harmonic phase did not significantly impact task performance for either age group, suggesting that perhaps this grouping cue does not play a large role in attentive listening to speech.

Overall, our results show that older adults struggle to use pitch cues to selective attend in a multi-talker environment, even when standard audiological criteria do not describe them as having a “hearing impairment”. Our findings further suggest that resolved harmonics are more important than unresolved harmonics in selective listening for speech, but especially so for older listeners, and these cues should be a priority for the future design of corrective hearing devices.

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Poster

PSTR339: Auditory Processing

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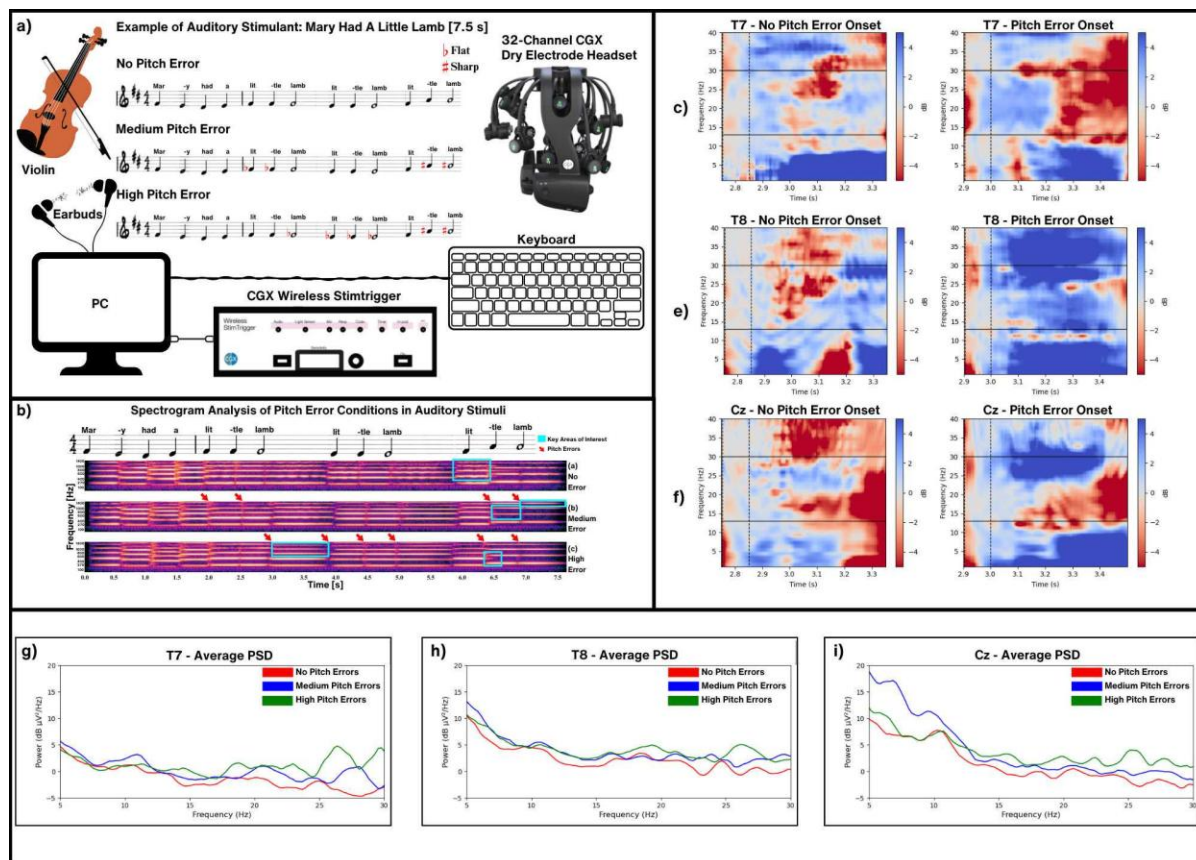
Topic: E.05. Auditory and Vestibular Systems

Title: Neural dynamics of pitch errors in music perception: beta-band activity reveals error detection and severity evaluation during passive listening

Authors: *A. KIM^{1,2}, A. UPPAL¹, K. GANO¹, G. CAUWENBERGHS¹;

¹Chien-Lay Dept. of Bioengineering, UC San Diego, San Diego, CA; ²Torrey Pines High Sch., San Diego, CA

Abstract: Pitch errors offer a controlled window into auditory surprise, revealing neural mechanisms underlying music perception. Although not central to everyday listening—which often involves the buildup and resolution of musical expectations—pitch errors serve as a useful paradigm for studying the brain’s real-time response to auditory irregularities. Prior EEG studies, such as Maidhof et al. (2013) and Ruiz et al. (2011), have demonstrated that beta-band oscillations in musicians reflect the detection and correction of self-generated pitch deviations during piano performance. However, the neural dynamics involved in processing pitch errors embedded in external stimuli during passive listening, particularly with complex musical pitch errors, are less characterized. This study investigated brain responses to pitch deviations in monophonic violin excerpts of nursery rhymes (e.g., "Mary Had a Little Lamb") using a portable dry-electrode EEG system (CGX Quick-32r). A trained musician (N=1) passively listened to seven 10-second excerpts with eyes closed. Pitch errors (0-6 semitones, sharp and flat) were varied across three conditions: no errors (true control), medium errors (2-4 semitones), and high errors (5-6 semitones), each repeated three times per excerpt (21 trials/condition). EEG data from 32 channels were analyzed at T7, T8, and Cz for beta-band (15-30 Hz) activity after preprocessing (1 Hz filter, 60 Hz noise removal, artifact correction). Power Spectral Density (PSD) analysis showed a 2.4 dB beta power increase at T8 from no-error to high-error conditions. Event-Related Desynchronization/Synchronization (ERDS) revealed desynchronization at T7 (~250 ms post-error), synchronization at T8, and synchronization at Cz (~400 ms post-error), suggesting three neural processes: (i) error detection (left temporal), (ii) severity evaluation (right temporal), and (iii) predictive adjustment (central).



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Poster

PSTR339: Auditory Processing

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR339.28/S7

Topic: E.05. Auditory and Vestibular Systems

Title: Low doses of ketamine and propofol shift multimodal noninvasive metrics of excitation-inhibition balance and cardiovascular response

Authors: *J. KUNZE¹, J. STUDENT², S. TUNE¹, A. HERRMANN¹, M. GÖTTLICH¹, H. OSTER¹, A. TZABAZIS², B. LORENZ², C. NAU², J. OBLESER¹;

¹Dept. of Psychology, Univ. of Luebeck, Luebeck, Germany; ²Dept. of Anesthesiol. and Intensive Care Med., Univ. Med. Ctr. Schleswig-Holstein, Campus Luebeck, Luebeck, Germany

Abstract: Ketamine, a glutamatergic N-methyl-D-aspartate receptor (NMDAR) antagonist, models schizophrenia-related hypofunction by shifting neural excitation-inhibition (E-I) balance towards excitation, potentially increasing sensory and cognitive noise. How such NMDAR-driven excitability changes manifest themselves in noninvasive proxies of E-I balance in human EEG remains to be clarified. In a single-blind, crossover pharmacological challenge study participants (N=17; currently 74 EEG sessions) performed an auditory decision-making task while receiving, in separate sessions, a low-dose infusion of ketamine (expected to increase E-I), propofol (a GABA-A agonist, expected to decrease E-I), or placebo. GABA and glutamate/glutamine (Glx) levels in the left auditory and anterior cingulate cortex (ACC) were assessed 24 h later using magnetic resonance spectroscopy (MRS). Target-controlled infusions based on the Domino and Schnider model were applied to achieve and maintain predicted plasma concentrations of 0.15 µg/ml and 0.5 µg/ml for ketamine and propofol, respectively. Drug effects on vital signs were as follows: ketamine increased systolic blood pressure (BP) on average by 13 mmHg and heart rate (HR) by 8 bpm; propofol reduced BP by 5 mmHg and HR by 4 bpm. As expected, ketamine reliably increased dissociative symptoms. Notably, however, higher systolic BP predicted elevated dissociation scores independent of drug condition, suggesting a link between cardiovascular arousal and dissociative experience. In resting-state EEG, ketamine significantly decreased the 1/f slope (spectral exponent), indicating a shift toward excitation. Propofol showed a trend in the opposite direction. Both drugs reduced alpha power, consistent with their neuromodulatory profiles. In MRS, both drugs increased interregional Glx correlation across Heschl's gyrus and ACC. These parallel changes suggest a shared systems-level effect on cortical neurochemistry. Our findings demonstrate that low doses of ketamine and propofol modulate EEG- and MRS-based markers of E-I balance and highlight cardiovascular changes as predictors of dissociative experience.

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Poster

PSTR339: Auditory Processing

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Program #/Poster #: PSTR339.29/S8

Topic: E.05. Auditory and Vestibular Systems

Title: Perturbing the neurophysiological excitation-inhibition balance of auditory decision-making

Authors: *S. TUNE¹, J. KUNZE², J. STUDENT³, C. NAU³, J. OBLESER²;

²Dept. of Psychology, ¹Univ. of Lübeck, Lübeck, Germany; ³Dept. of Anaesthesiology and Intensive Care, Univ. Med. Ctr. Schleswig-Holstein, Campus Lübeck, Lübeck, Germany

Abstract: Perceptual decisions and metacognitive judgements emerge from a dynamic interplay of external sensory evidence and internal psychological states. How fluctuations in neurophysiological excitation-inhibition (E-I) balance mediate their interaction in auditory decision-making remains poorly understood. We tested this brain-behaviour link in a single-blind crossover intervention EEG study where healthy human adults (N=17) performed an adapted version of the ‘Bernoulli clicks’ auditory decision-making task [Keung et al. 2019]. Probabilistic cues and variable sensory evidence strength modulated prior expectation and perceptual uncertainty, respectively. In three separate sessions, participants received a target-controlled low-dose infusion of ketamine (a glutamatergic N-methyl-D-aspartate receptor (NMDAR) antagonist; expected to increase excitation), propofol (a GABA-A agonist; expected to increase inhibition), or placebo. We expected impulsive decision-making under increased excitation, and indecisive decision-making under increased inhibition. We aimed at understanding how both drug-specific alterations and moment-to-moment fluctuations in cortical E-I balance shape sensory encoding, evidence accumulation, and ultimately perceptual choices, thereby giving rise to distinct behavioural phenotypes. To this end, we combined a multi-faceted behavioural characterization based on continuous joystick traces with psychophysical and neural sensory encoding modelling. Comparison of pre- to post-task resting-state EEG spectra revealed the predicted modulation of cortical E-I balance: ketamine flattened the 1/f slope, indicating relative excitation, whereas propofol showed a trend toward steepening. In line with E-I alterations, we observed dissociable sources of suboptimality in behaviour: Under ketamine, behaviour was characterized by an increase in premature responses and a tendency to respond in line with an emerging overall bias to the right side. In contrast, propofol induced a more uniform decrement in behavioural performance with reduced sensitivity and slower reaction times, consistent with decision hesitancy under enhanced inhibition. Our results on how pharmacologically induced alterations of cortical E-I balance are linked to dissociable behavioural phenotypes underscores the critical role of E:I balance for adaptive behaviour, and directly inform computational theories linking cortical gain control to perceptual and metacognitive fidelity.

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Poster

PSTR339: Auditory Processing

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Topic: E.05. Auditory and Vestibular Systems

Support: DFG Ob 352/2-2
DFG He 7857/1-1
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Title: Noise gating: Charting the impact of sensory versus perceptual uncertainty on auditory encoding

Authors: *J. OBLESER¹, L. WASCHKE², M. ORF³, B. HERRMANN⁵, S. TUNE⁴;

¹Univ. of Lübeck, Luebeck, Germany; ²Max-Planck-Institute for Human Develop., Berlin, Germany; ³Univ. of Luebeck, Lübeck, Germany; ⁴Univ. of Luebeck, Luebeck, Germany;

⁵Rotman Res. Inst., Toronto, ON, Canada

Abstract: Assessing internal noise by probing a system with varying external noise is a well-established method in systems identification and psychophysics. Curiously, it aligns with item-response theory, where internal observer uncertainty (θ) and stimulus-induced uncertainty (δ) are estimated on a shared latent scale. Yet, in neuroscience, sensory uncertainty (e.g., stochastic afferent encoding) and perceptual uncertainty (e.g., fluctuations beyond encoding) remain conceptually separable but are rarely being dissociated empirically. In this project, we aim to quantify sensory and perceptual uncertainty using LFP recordings from N=29 rodent A1 neurons (Dewese & Zador, 2004) for sensory-level analysis, and EEG from N=25 humans performing pitch discrimination (Waschke et al., eLife 2019) for the perceptual-level and behavioural analyses. In rodent A1, internal noise should ideally sit at an intermediate level balancing excitability and sensitivity. In line with this, we observed the largest single-trial evoked responses (± 25 ms, and late negative LFP at ± 200 ms) at intermediate levels of internal noise, here expressed as pre-stimulus baseline variability σ (linear mixed model, LMM, on >30,000 trials, controlling for average baseline activity). This variability σ appeared embedded in a slow delta-range (1-4 Hz) rhythm. At the human perceptual level, pre-stimulus perceptual noise should ‘gate’ the impact of incoming sensory evidence: When stimuli are low in uncertainty, baseline noise should have little effect; but when stimuli approach the perceptual threshold, internal noise should come to dominate shaping auditory encoding. In spatially-filtered human EEG (reflecting auditory cortex), we thus modelled single-trial N1 amplitude as a joint function of pre-stimulus σ and stimulus uncertainty δ (from no to several semitones pitch difference). N1 amplitude showed a significant $\sigma \times \delta$ interaction (LMM, >10,000 trials, controlling for average baseline activity): The smaller or even absent the pitch deviation from 1 kHz (i.e., high perceptual uncertainty), the more pre-stimulus σ predicted N1 amplitude. This effect diminished linearly with increasing perceptual evidence. Finally, treating N1 amplitude as an aggregate proxy of internal noise up to a perceptual stage, we modeled pitch decisions as a function of stimulus evidence (relative to an implicit 1 kHz standard) and encoding quality. A stronger N1 and, less robustly, lower pre-stimulus σ enhanced pitch sensitivity. These findings highlight how internal uncertainty and external evidence jointly shape auditory perception and motivate a formalised, multilevel model of auditory uncertainty.

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Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR340.01/S10

Topic: E.06. Vision

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NIH Grant R01NS122742
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Title: Deep-learning-assisted simulation of a cortical circuit: integrating anatomy, physiology, and function

Authors: *S. ITO¹, D. HAUFLER¹, J. GALVÁN FRAILE², K. DAI¹, J. AMAN¹, O. ZOBEIRI¹, G. CHEN³, C. MIRASSO², W. MAASS⁴, A. ARKHIPOV¹;
¹Allen Inst., Seattle, WA; ²IFISC, Univ. de les Illes Balears, Palma de Mallorca, Spain; ³Peking Univ., Beijing, China; ⁴Graz Univ. of Technol., Graz, Austria

Abstract: We present a biologically realistic model of mouse primary visual cortex (V1) that integrates anatomical and physiological constraints, with model parameters optimized via deep-learning methods to align simulated neural activity with in vivo data. The model incorporates recent synaptic physiology data (Campagnola, Seeman et al., *Science*, 2022; portal.brain-map.org/connectivity/synaptic-physiology) and electron microscopy connectomics data from the IARPA MICrONS project (The MICrONS Consortium, *Nature*, 2025; www.microns-explorer.org), constraining connectivity structure, degree distributions, synaptic weight statistics, and temporal synaptic properties. Importantly, the model exhibits stable activity prior to any optimization, highlighting the importance of biologically grounded model construction. To improve correspondence with functional recordings, we apply backpropagation through time to optimize synaptic weights to match experimentally observed activity statistics—such as firing rates, orientation and direction selectivity, and neuronal synchronization—while maintaining the experimentally observed distribution of synaptic weights. Structure-function relationships, such as like-to-like connectivity, emerge through this optimization. By integrating biological constraints with a deep-learning-like training strategy, the model achieves close alignment with experimental observations in both structure and function. To support community use and collaboration, we will publicly release the model and the full codebase for model construction and optimization.

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Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR340.02/S11

Topic: E.06. Vision

Support: NIH R01NS120289

Title: Functional connectivity analysis of spontaneous activity reflects circuit function in mouse visual cortex

Authors: *R. SANCHEZ-ROMERO, S. AKYUZ, M. RILEY, P.-O. POLACK, B. KREKELBERG;
Rutgers Univ. Newark Ctr. for Mol. and Behavioral Neurosci., Newark, NJ

Abstract: Two-photon imaging (TPI) has revolutionized neuroscience by dramatically increasing the data yield from in vivo experiments, leading to deeper insights into neural function. Most TPI studies have prioritized single-neuron analyses—characterizing tuning properties, response amplitudes, and trial-averaged activity—rather than the interactions between neurons. It is increasingly recognized, however, that these interactions are key to understanding neural circuitry and computations. Building on tools developed in the fMRI literature, we present an approach using multivariate functional connectivity (FC) and empirically-constrained computational modeling (actflow) to study circuitry and computations with TPI.

We imaged neurons in layers 2/3 of left V1 in six mice (CamKII;Ai163) expressing GCaMP6s in pyramidal neurons (fov 0.5 x 0.7 mm, sampling rate 15 Hz, head-restrained on spherical treadmill). Trials (300 per session) consisted of 1.5 s visual stimulus presentation (oriented grating) followed by 1.5 s inter-trial interval (ITI; grey screen). We used suite2p to extract fluorescence traces and selected a subset of highly responsive neurons for analysis.

Using Lasso, we estimated FC from spontaneous activity in the ITI. Lasso estimates sparse FC controlling for confounding and indirect connections (properties missing in traditional FC based on Pearson correlation). We found that pairs of neurons with similar orientation preference exhibited more and stronger positive FC and fewer and weaker negative FC, than pairs with dissimilar orientation preference. Although ground truth connectivity of these neurons is unknown, these patterns match the standard view of connectivity in V1 circuits, suggesting that our FC approach can extract underlying connectivity from spontaneous activity.

We provide additional support for this view with actflow modeling. Specifically, we predict each neuron's tuning curve by computing the dot product between its FC and the stimulus-evoked activity of all other neurons, separately for each oriented grating. Actflow predictions were strongly correlated with empirical tuning curves ($r = 0.69$ (0.31), mean (std) across all mice and neurons). For comparison, we repeated the analysis using Pearson correlation FC; this resulted in primarily positive connections and a substantially lower actflow accuracy ($r = 0.25$ (0.46)).

Taken together, these results demonstrate that multivariate FC methods that control for confounding and indirect connections can extract meaningful connectivity from spontaneous activity, and that this captures the computations performed by local circuits supporting orientation selectivity.

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Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR340.03/S12

Topic: E.06. Vision

Title: Coordination of modular spontaneous activity across brain regions in the developing cortex

Authors: *C. J. APGAR, G. B. SMITH;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: During early development, cortical networks exhibit highly organized patterns of spontaneous activity that are thought to play a key role in establishing mature functional architecture. In the visual cortex (V1) of ferrets, spontaneous activity prior to eye opening is highly modular, with millimeter-scale correlations that predict features of visually evoked responses that emerge later in development. This millimeter-scale correlated activity appears to emerge through self-organization within local intracortical networks, and does not require feed-forward inputs. Recently, it was found that similar patterns of millimeter-scale modular correlated networks are also evident in spontaneous activity throughout the cortex, including both other sensory areas and higher order cortices, suggesting that similar local mechanisms may operate independently in all regions of the developing brain. Alternatively, modular activity in V1 may be relayed to other cortical regions, or result from a common driver. To address this, we performed simultaneous widefield calcium imaging of GCaMP6s in ferret V1 and auditory cortex (A1) in animals prior to eye opening. We find that the average activity within V1 and A1 was only weakly related over time. Surprisingly, when examining the relatively rare periods of simultaneously occurring spontaneous events in both A1 and V1, we found a statistically significant relationship between the modular spatial patterns of activity across regions. During these simultaneous events, distributed and modular locations within A1 (or V1) exhibited significant correlations with particular locations in V1 (or A1), indicating the presence of co-occurring modular patterns of activity across widely separated brain regions. These results suggest the presence of cross-modal interactions in spontaneous activity during early development, that may provide an organizing mechanism for future integration of multisensory features.

Disclosures: C.J. Apgar: None. G.B. Smith: None.

Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

Location: SDCC Halls B-H

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Program #/Poster #: PSTR340.04/T1

Topic: E.06. Vision

Support: NIH R01EY030893
NSF IIS-2011542
BMBF 01GQ2002

Title: Selective amplification of recurrent subnetworks in the developing visual cortex

Authors: H. N. MULHOLLAND¹, S. TRÄGENAP³, M. KASCHUBE⁴, *G. B. SMITH²;
¹Neurosci., ²Univ. of Minnesota, Minneapolis, MN; ³Frankfurt Inst. for Advanced Studies,
Frankfurt am Main, Germany; ⁴FIAS, Frankfurt am Main, Germany

Abstract: The selective amplification of specific activity patterns is proposed to be a major functional consequence of the strongly recurrent circuits found in the cortex. However, this has not been tested in development in the millimeter-scale networks that form the basis for future sensory representations. Using patterned optogenetic stimulation and calcium imaging in immature ferret visual cortex, we found that activation with stimuli derived from endogenous activity evoked responses that were both more reliable and more similar to their input pattern compared to random stimuli with similar spatial statistics. The degree of reliability and stimulus similarity was predicted by the overlap of the stimulus input pattern with the leading principal components of spontaneous activity. These results indicate that already before eye opening, early cortical networks preferentially amplify inputs that align with the endogenous network architecture, thus supporting a potential mechanism for building and refining stimulus specific representations during development.

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Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

Location: SDCC Halls B-H

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Program #/Poster #: PSTR340.05/T2

Topic: E.06. Vision

Title: A primary visual cortical layer network model with diffusion reveals an emergence of spatially correlated activity.

Authors: *M. SAITO, M. IWAMOTO;
Toyota Central R&D Labs., Inc., Nagakute, Japan

Abstract: Neurons in the primary visual cortex exhibit orientation selectivity, responding strongly to stimuli with specific orientations. Neighboring neurons often share similar orientation preferences, forming a structured orientation selectivity map. In convolutional neural networks (CNNs), orientation selectivity is represented by feature maps generated via convolution with orientation-specific kernels. In contrast, in the visual cortex, cells responsive to various orientations are spatially mixed within a single layer. The advantage and developmental origin of

this structure remain unclear. Recent studies show that spatial patterns of correlated spontaneous activity shortly after birth can predict mature orientation map, even without visual input. This suggests that early spontaneous activity forms a basis for map development. We propose a cortical layer network model composed of excitatory and inhibitory populations to examine whether such activity patterns can emerge. Based on the canonical microcircuit model, excitatory and inhibitory layers interact vertically. We added diffusion to extend the model from a single population unit to a spatial network. The addition enabled neuronal activity in each layer to interact horizontally and to spread within a two-dimensional cortical plane. Random thalamic input simulates early spontaneous activity before eye opening. Simulations showed that when inhibitory neurons have higher diffusion coefficient than excitatory ones, maze-like spatial correlation patterns emerge, resembling patterns observed in the ferret cortex. Equal diffusion coefficients, however, produce weaker and more uniform correlations. These results are consistent with the Turing mechanism, where differing diffusion rates between two components produce spatially heterogeneous patterns. In our case, diffusion induces spatial patterning in the cortical model. Such pattern formation, possibly combined with Hebbian plasticity, may underlie the development of orientation selectivity maps.

Disclosures: **M. Saito:** A. Employment/Salary (full or part-time): Toyota Central R&D Labs., Inc. **M. Iwamoto:** A. Employment/Salary (full or part-time): Toyota Central R&D Labs., Inc..

Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR340.06/T3

Topic: E.06. Vision

Support: Takahashi Industrial and Economic Research Foundation Grant

Title: A mathematical model for synaptic rewiring explains postnatal development of spatiotemporal receptive fields of visual cortical neurons and orientation-direction joint maps.

Authors: ***M. MIYASHITA**¹, S. TANAKA²;

¹Natl. Inst. of Technol., Numazu, Shizuoka, Japan; ²Natl. Inst. of Tech., Numazu, Shizuoka, Japan

Abstract: It is widely accepted that neural circuits in the visual cortex are elaborated postnatally in an experience-dependent manner. Such a self-organization process is caused by the morphological changes of dendritic spines. We previously built a mathematical model for the activity-dependent synaptic rewiring of afferent inputs from lateral geniculate nucleus (LGN), taking into account molecular mechanisms regarding Rho-GTPases-mediated polymerization/depolymerization of cytoskeletal actin filaments. We assumed the presence of four types of neurons in the LGN: lagged- and nonlagged types for ON- and OFF-center cells. Computer simulation based on this model reproduced simple-cell-like receptive fields of

individual cortical neurons and orientation-direction joint maps in the visual cortex. We also obtained developmental profiles of synapse number, orientation selectivity and direction selectivity. The total number of synapses increased in an early phase of simulation and decreased in a late phase. Orientation selectivity of model cortical neurons rapidly increased immediately after visual stimulation and then reached a plateau. Iso-orientation domains began segregated sharply in the early phase. In contrast, direction selectivity increased gradually later than orientation selectivity, and finally most of neurons became unidirectional cells. The unimodal profile of the synapse number and the growth of direction selectivity later than orientation selectivity closely agree with those that have been observed in experimental studies. For the rewiring of LGN cell inputs to single cortical neurons, ON- and OFF-center cells that sent synaptic inputs to individual cortical neurons were aligned separately in the visual field first, resulting in the formation of spatiotemporal separable receptive fields. Then, lagged and nonlagged cells that sent inputs to individual neurons were segregated to form subfields, and spatiotemporal inseparable receptive fields were completed. In addition, direction maps matured with the reduction in the total number of LGN inputs in the late phase. In simulations performed with weak intracortical interactions, iso-direction domains were hardly delineated because neurons of opposite preferred directions were likely to be arranged next to each other in the cortex. Taken together, the following are suggested: (1) direction selectivity develops later than orientation selectivity; (2) the pruning of inappropriate synapses shapes direction selectivity; and (3) the intracortical interaction plays a pivotal role particularly for direction map formation.

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Poster

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Title: Single-cell optogenetic perturbations reveal stimulus-dependent network interactions

Authors: D. KONG¹, J. BARRETO², M. KASCHUBE³, *B. SCHOLL⁴;

¹Frankfurt Inst. for Advanced Studies, FRANKFURT AM MAIN, Germany; ²Physiol. and Biophysics, Univ. of Colorado - Anschutz Med. Campus, Aurora, CO; ³FIAS, Frankfurt am Main, Germany; ⁴Physiol. and Biophysics, CU Anschutz, Denver, CO

Abstract: Cortical computations depend on dynamic interactions between neurons that vary with sensory context. Theoretical models propose that cortical networks can shift between amplifying

or suppressing activity based on input strength, yet how such state-dependent dynamics manifest in topographically organized circuits remains poorly understood. We investigated recurrent excitatory interactions in ferret primary visual cortex (V1), which exhibits columnar organization, using a combination of two-photon calcium imaging and two-photon optogenetics to stimulate individual layer 2/3 excitatory neurons during visual stimulation. Responses to drifting gratings of varying contrast were recorded, and single-cell perturbation effects were quantified using Poisson generalized linear models to estimate each cell's influence on surrounding neurons. We found that excitatory neurons exerted net suppressive effects on others, with suppression magnitude increasing with cortical distance. Notably, suppression weakened at low contrast and transformed into facilitation specifically among highly noise-correlated neuron pairs. These contrast- and correlation-dependent effects indicate that shared functional input modulates whether recurrent excitation facilitates or suppresses neighbors, consistent with stabilized supralinear network (SSN) models. Furthermore, decoding analyses revealed improved stimulus discriminability as contrast increased, coinciding with the observed shift toward suppression. Our findings demonstrate that excitatory cortical circuits dynamically reorganize their influence depending on stimulus strength and correlation structure, suggesting a flexible recurrent mechanism that enhances sensory coding efficiency in columnar visual cortex.

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Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

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Title: Year-long functional ultrasound mapping of the cat visual cortex

Authors: ***D. A. HORVATH**^{1,3}, K. CSIKÓS^{2,4}, Á. PETIK^{1,3}, B. ROSKA⁵, A. URBAN^{6,7,8}, D. HILLIER^{2,3};

²Inst. of Cognitive Neurosci. and Psychology, ¹HUN-REN Res. Ctr. for Natural Sci., Budapest, Hungary; ³Fac. of Information Technol. and Bionics, Pazmany Peter Catholic Univ., Budapest, Hungary; ⁴János Szentágothai Neurosci. Doctoral Sch., Semmelweis Univ., Budapest, Hungary;

⁵Friedrich Miescher Inst. For Biomed. Resear, Basel, Switzerland; ⁶NERF, Gent, ; ⁷KU Leuven, Leuven, Belgium; ⁸VIB, Leuven, Belgium

Abstract: Understanding the functional architecture of the visual cortex in large-brained species requires access to both large-scale organization and fine-grained domain structure over time. Functional ultrasound imaging (fUSI) offers a spatial resolution between fMRI and electrophysiology (~150 μ m) across extended cortical volumes, but its use in longitudinal experiments in large-brained species has been limited. We developed a protocol combining anesthesia with fUSI to enable chronic, high-resolution functional mapping in cats. Cats underwent repeated fUSI sessions over several months under a finely titrated anesthesia regimen optimized for stability of physiology and neurovascular coupling. Visual stimuli elicited cortical responses used to reconstruct retinotopic and orientation preference maps across primary and higher visual areas. We consistently mapped functional domains across >1500 mm³ of cortex over months. Orientation and retinotopy maps revealed robust iso-orientation domains embedded within global visual field representations, enabling the observation of interactions between local and large-scale cortical structure. The anesthesia protocol supported stable recordings across sessions and enabled integration of additional modalities, such as ECoG. This work establishes a scalable platform for chronic, multimodal functional imaging in large-brained species. The combination of stable anesthesia and high-resolution fUSI enables detailed reconstruction of mesoscale cortical architecture and its dynamics over time, supporting systems-level investigations in translational neuroscience.

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Poster

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Topic: E.06. Vision

Support: NIH R01EY020950

Title: Functional organization of binocular response properties in tree shrew primary visual cortex

Authors: ***L. YANG**, J. REN, J. MCDANIEL, S. TANABE, J. CANG;
Univ. of Virginia, Charlottesville, VA

Abstract: The visual cortex is spatially organized into maps such that nearby neurons are often tuned to similar features of the visual images. For example, orientation maps form pinwheels in the primary visual cortex (V1) of many mammals, such as primates, cats, and tree shrews. Interestingly, geniculocortical terminals in tree shrews are organized into eye-specific sublayers of layer 4, which is different from the ocular dominance columns in primates and cats.

Projections from layer 4 to layers 2/3 are also organized along cortical depth. These unique anatomical features suggest that tree shrew V1 may possess distinct functional organization for binocular properties, including ocular dominance and potentially binocular disparity selectivity, along cortical depths.

In this study, we used in vivo 2-photon calcium imaging and electrophysiology recording with Ultra-High-Density Neuropixels probes in awake tree shrew V1 to investigate the functional organization of binocular properties both across the surface (horizontally) and across depths and layers (perpendicularly). In addition, we characterized the spatial relationship between binocular disparity selectivity and the well-studied orientation maps, which is currently unknown in any species.

With 2-photon imaging of layer 2/3 neurons, we observed robust orientation columns in tree shrew V1 with characteristic pinwheel structures throughout the depths, consistent with previous work. Interestingly, for a single imaging plane at a given depth, each orientation domain displayed a narrow range of phase disparity preferences. The phase disparity preference varied smoothly from one orientation domain to the next. Notably, the pattern of tight coupling appeared to shift as we moved the imaging plane to deeper depths. Using Neuropixels recording across all layers within an orientation column, the results appear to show a systematic change in ocular dominance along the depth. The recording data also allow us to reconstruct the cellular functional connectivity network between pairs of recorded neurons across layers within the orientation map. Together, our findings suggest that orientation and binocular properties may be systematically mapped in tree shrew V1, which could be a biological solution for representing the 3-dimensional environment onto a volume of cortex.

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Poster

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Topic: E.06. Vision

Support: NIH R01EY020950

Title: Transformation of monocular receptive field properties to binocular disparity tuning in tree shrew and mouse V1

Authors: *J. MCDANIEL, L. YANG, J. REN, S. TANABE, J. CANG;
Univ. of Virginia, Charlottesville, VA

Abstract: The visual projections of the 3-dimensional environment produce 2 slightly offset images onto the left-eye and right-eye retinæ, respectively. The difference between the two images, i.e., binocular disparity, is encoded by neurons in the primary visual cortex (V1) where

signals originating in the two eyes are first integrated. In the current model of binocular integration, a neuron's sensitivity to binocular interactions is explained by the summation of monocular receptive fields (RFs) followed by an output nonlinearity. Only specific disparities that align with the offset of left-eye and right-eye RFs would cause the neuron to discharge, thereby generating disparity tuning. Despite this model being an elegant solution to explain monocular to binocular transformation, how the integration process is implemented in V1 neurons has not been directly tested. Here we aim to fill this gap by studying monocular to binocular transformation using two animal models, the tree shrew and mouse. For these experiments we utilized both 2-photon calcium imaging and ultra-high density Neuropixels recording to study visually evoked responses in V1 neurons. When presented with binocular drifting gratings, V1 neurons in both animal models had strong tuning for binocular phase disparity, consistent with previous findings. We then mapped the monocular receptive fields by using static gratings of varying orientations and phases. From the mapped receptive fields, we generated the prediction for the neuron's binocular phase disparity preference. We tested this prediction against the observed phase disparity tuning of each neuron. With these data we are able to directly test the mechanism underlying how V1 neurons integrate monocular input to generate binocular disparity. Furthermore, our study can provide insight into how the brain combines multiple streams of input to create useful signals for perception and sensory-guided behaviors.

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Poster

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Title: Orientation Encoding in the Primate Visual Hierarchy Reveals a Functional Hub Network for Visual Processing

Authors: *B. KARAMI^{1,2,3,4}, T. NIGAM^{5,4,3,7}, C. M. SCHWIEDRZIK^{6,4,3,7};

¹NIH, Bethesda, MD; ²Donders Ctr. for Cognitive Neuroimaging, Nijmegen, Netherlands;

³German Primate Ctr., Goettingen, Germany; ⁴European Neurosci. Inst., Goettingen, Germany;

⁶Cognitive NeuroBiology, ⁵Res. Ctr. One Health, Ruhr-University Bochum, Bochum, Germany;

⁷Leibniz Sci. Campus, Goettingen, Germany

Abstract: The primate visual system is a hierarchical cascade of brain areas that transform retinal inputs into rich percepts. It is traditionally believed that simple features, such as orientation, are encoded only in early visual areas, while higher-order regions like inferotemporal

(IT) cortex are specialized for complex object representations. However, a brain-wide systematic quantification of orientation representation is still lacking. In this study, we used extensive fMRI scanning and multivoxel pattern analysis (MVPA) in macaque monkeys to map orientation encoding across the entire visual hierarchy. We found robust orientation information not only in early areas (V1-V2), but also throughout mid- (V3-V4) and high-level regions including IT cortex. In cortical space, orientation representation followed an hourglass-shaped topography: clusters converged into larger ones in mid-level areas, then diverged again into smaller, more scattered patches in IT. In representational space, orientation encoding became increasingly distributed along the hierarchy, shifting from meso-scale coding in early areas to widespread, distributed encoding in higher areas. To examine whether this unexpected orientation representation in IT relates to its role in object processing, we measured BOLD responses during blocks of face, body, and object images. IT voxels then were categorized as *pure* (responsive to one category) or *mix* (responsive to multiple categories) responsive clusters. Notably, *mix* voxels carried significantly more orientation information than *pure* voxels and showed stronger functional connectivity both within IT and with upstream visual areas. We propose that these *mix*-responsive voxels contain a class of “hub” neurons that integrate low-level and high-level features through enhanced connectivity. Supporting this hypothesis, we observed a similar relationship between object selectivity and orientation encoding in deep convolutional neural networks with high brain similarity. Together, our results reveal that orientation re-emerges in IT cortex not through redundancy but as a computational byproduct of functional diversity and connectivity, highlighting a new class of hub-like neurons that could support flexible and integrative visual processing beyond object recognition.

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Poster

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Title: Precise Functional Localization of the Foveolar Representation in Anesthetized Macaque Visual Cortex

Authors: *M. CHEN¹, M. QIAN², P. LI¹, A. W. ROE²;

¹Zhejiang Univ., Hangzhou, China; ²Translational Neurosci. Lab. Division, Ctr. for Biomed. Imaging and Neuromodulation (C-BIN), Nathan S. Kline Inst. for Psychiatric Res., New York, NY

Abstract: Background: The primate foveola, which represents the central $\sim 1^\circ$ of the visual field, is critical for high-acuity vision, color perception, and gaze-directed attention. In foveal cortex, Qian (2025 Nature Neurosci), using ultrahigh field 7T MRI in awake fixating macaques, discovered a cortical specialization comprising multiple (8) foveolar loci encircling a large 'foveolar core'. To study this foveolar area in anesthetized monkeys, we devised a method for determining exactly where the foveola is directed on a monitor in anesthetized animals.

Methods: Functional EPI data (0.8 mm³ isotropic, 7T MRI, 16-channel RF coil) were acquired monocularly. We used a progressive method of imaging with large to narrow stimuli to triangulate the center of gaze. Initially, wide (4.5°) (vertical or horizontal) bars containing drifting gratings were presented, at 3 adjacent positions; as cortical magnification is greatest at the center, the bar that induced the strongest response (amplitude, voxel number) was chosen as the most central. This was repeated with successively narrower bars (1.5°, 0.5°, 0.25°, 0.1° wide) presented at 3 positions within each successively determined centralmost location. Three 0.2° spots were presented across the estimated foveal coordinate (intersection of the centralmost 0.5° horizontal and vertical bars) to further confirm the centralmost location. **Results:** Retinotopic shifts in activation corresponded well with stimulus position. The most central vertical bar stimulus activated the vertical meridian at the V1/V2 border; off-center bars produced parallel activations on either side of the border. When stimuli crossed into the ipsilateral hemifield, activation shifted to the other hemisphere as predicted. For horizontal meridian, the centralmost stimulus produced the largest areal activation and was marked by activation of the both the dorsal and ventral V2/V3 borders. Foveolar spot stimuli exactly at the center produced bilateral foveolar activations. **Summary & Significance:** These results confirm the feasibility of precise (0.1°) determination of foveolar viewing location on a monitor in anesthetized macaques using highfield fMRI and provide a foundation for further studies of foveolar cortex using fMRI and other recording and stimulation methodologies.

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Poster

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Research to Prevent Blindness

Title: Functional tuning of parvalbumin-expressing interneurons across the layers of primate primary visual cortex

Authors: *W. DAI¹, A. M. CLARK², A. VAFAEI², F. C. FEDERER², A. ANGELUCCI²;
²Dept. of Ophthalmology and Visual Science, Moran Eye Inst., ¹Univ. of Utah, Salt Lake City, UT

Abstract: Diverse inhibitory neuron (IN) subtypes shape neural circuit function in the mammalian neocortex, but their tuning properties in primates remain poorly understood. Using cell-type-specific viral labeling (AAV-PHP.eB-S5E2-C1V1) and high-density Neuropixel recordings, we investigated the functional tuning and laminar organization of parvalbumin-expressing (PV) interneurons, the largest IN population, in the primary visual cortex (V1) of anesthetized marmosets. We performed opto-tagging to identify PV neurons and characterized their tuning for visual stimulus orientation, direction, spatial frequency (SF), size, and contrast, across four penetrations. Across the population of recorded single units, compared to putative excitatory neurons (EN), PV neurons showed broader orientation tuning, weaker direction selectivity, and higher preferred SF, but similar SF bandwidth. The proportions of tuned cells, optimal size and contrast tuning did not differ significantly between PV and EN populations. However, tuning properties varied with laminar location and cell type. Notably, for ENs, orientation selectivity (OSI) increased, and orientation tuning sharpened (orientation tuning width decreased), from L4 to L2/3, whereas orientation selectivity and tuning of PV neuron were similar across layers. Accordingly, in L2/3 EN were more sharply tuned than PV cells. These results are consistent with our previous reports that in marmoset V1 optogenetic manipulations of PV neurons alters the orientation tuning of ENs in L2/3 but not in L4 (Vafaei et al. SFN 2024). We suggest that broad input pooling shapes the functional tuning of PV neurons, leading to sharpening of orientation selectivity and tuning of L2/3 ENs. These results underscore the importance of laminar circuits in visual processing.

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Poster

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Research to Prevent Blindness

Title: Laminar specificity and coverage of enhancer-AAV-mediated gene expression restricted to subpopulations of somatostatin and parvalbumin inhibitory interneurons in primate V1

Authors: *M. R. GIELOW¹, F. C. FEDERER², A. JAYAKUMAR¹, Z. CALDWELL¹, C. WINEBRENNER¹, X. XU⁴, A. ANGELUCCI³;
²Moran Eye Inst., ³Ophthalmol, Moran Eye Inst., ¹Univ. of Utah, Salt Lake City, UT; ⁴Anat. and Neurobio., Univ. California, Irvine, Irvine, CA

Abstract: Primary visual cortex (V1) GABAergic interneurons are integral to V1 computations, dynamics and visual processing. The various functions of inhibition are mediated by 4 major classes of inhibitory neurons (INs), among which are the somatostatin (SST) and parvalbumin (PV) classes. These INs have been studied extensively in mouse, but the direct applicability of results gained from mouse to primate remains an open question. Recent advances in viral technology have led to new enhancer-AAVs for cell-specific access. We have recently validated in marmoset V1 the S5E2 enhancer, and found it to transduce PV neurons in this species with high specificity and efficiency (Federer et al, eLife 2024). However, PV-INs, like all other IN classes, consist of multiple sub-types, which differ morphologically, physiologically and transcriptomically, and are differentially enriched in distinct layers. Recently, enhancer-AAVs for specific access to PV and SST interneuron subtypes have been developed (Furlanis et al, Neuron 2025). Here, utilizing HCR-FISH, we have characterized the distribution, specificity and coverage of transporter protein expression mediated by two such enhancer-AAVs in marmoset V1: AAV-PHP.eB-BiPVe3 and AAV-PHP.eB-S9E10 for specific access to PV basket cells (BC) and SST non-Martinotti cells (NMC), respectively. Results. PV-mRNA in marmoset V1 is present in all layers but peaks in L2/3 and L4C. The laminar distribution of reporter expression mediated by the BC-enhancer virus resembles that of PV-mRNA, also peaking in L2/3 and 4C, but in L5 it is higher than PV-mRNA. Specificity for PV-HCR-FISH of BC-enhancer is 94-97% in L2/3-4C but it is lower in L5 and L6 (60-80%). BC-enhancer covered ~63% of V1 PV cells overall. SST-mRNA in marmoset V1 is most prominent in L2, followed by L3-4B and L5, and is sparse in L4C and L6. Reporter expression mediated by the NMC-enhancer resembles the normal distribution of SST-mRNA in L2-4B, but is higher in L4C and lower in L5/6. Specificity for SST-HCR-FISH of NMC-enhancer is 85-87% in all layers but L4C, where it is 41%. NMC-enhancer covered ~80% of V1 SST cells overall (93% in L2-4C, and 42% in L5/6). Our results show these two enhancer-AAVs as promising tools for studying BC and NMC function and connectivity in primate cortex.

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Poster

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Research for the Prevention of Blindness

Title: Laminar organization of cortico-cortical feedback to primary visual cortex in the primate

Authors: *S. JAYAKUMAR¹, F. C. FEDERER², M. R. GIELOW¹, M. NGUYEN¹, C. GARRETT¹, A. ANGELUCCI³;

²Moran Eye Inst., ³Ophthalmol, Moran Eye Inst., ¹Univ. of Utah, Salt Lake City, UT

Abstract: INTRODUCTION. The mammalian neocortex consists of hierarchically organized areas reciprocally connected via feedforward (FF) and feedback (FB) circuits. FB circuits have been implicated in attention, prediction, and sensory context. In primate visual cortex, FB connections arise from superficial (SL) and deep layers (DL) in proportions that depend on the hierarchical distance between areas; for example, the fraction of SL neurons sending FB to V1 is 42% for V2, 30% for V4, and 17% for MT (Markov et al., 2014). FB connections terminate outside layer (L) 4, but are densest in L1/2 and L5/6. However, the differential contribution of SL vs. DL FB projections to SL and DL terminations is unknown. Anatomical and physiological evidence suggests that FB connections are heterogeneous. Theoretical models have postulated the existence of two, possibly more, FB systems, arising from distinct layers. Predictive Coding models have postulated a SL-to-SL and DL-to-DL type of connectivity (e.g. Shipp 2016; Bastos & Mscic-Flogel 2018). Here, we have investigated the laminar organization of FB connections to V1 arising from area V2 in macaque monkey. **METHODS.** We made epipial deposits of the retrograde tracer fast blue (Fb), and small intracortical injections of the retrograde tracer CTB-alexa, to restrict injections to specific V1 layers (n=5 hemispheres), and mapped the resulting cell label across V2 layers. **RESULTS.** Following Fb injections in L1, ~70% of V2 labeled cells were located in the SL and the remainder in DL, with >80% of DL labeled cells located in L5. Fb injections encompassing L1-2A also resulted in ~70% of labeled cells in V2 SL, but the DL label was located in both L5 and L6 in similar proportions. Fb injections encompassing L1 to L5, labeled cells in SL and DL in approximately equal proportion, with equal contribution from L5 and L6. CTB-alexa injections confined to V1 L4B or L6 labeled V2 cells almost exclusively (92-93%) in SL, and only 6-8% in L6, but not in L5. Injections confined to V1 L3 labeled cells mostly (70%) in V2 SL and the remainder in both L5 and 6. In summary, our results so far indicate that V2 SL project to all V1 layers except L4C. FB from L5 and L6 both project to L3 and L5, but only L5-FB projects to L1, and only L6-FB projects to L4B and 6. However, V1 SL receive most of their FB projections from V2 SL, while the V1 DL receives FB inputs from both V2 SL and DL. We are performing additional DL injections to understand whether any bias exists in the V2 SL vs DL FB projections to V1 DL. **CONCLUSIONS.** Our results demonstrate a more complex laminar organization of FB connections than a simple SL-to-SL and DL-to-DL connectivity, and suggest that L5 and L6 represent two specialized DL FB systems.

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Poster

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Topic: E.06. Vision

Support: NEI R01EY031959
Research for the Prevention of Blindness

Title: Modeling the effects of optogenetic manipulation of parvalbumin-expressing inhibitory neurons on orientation selectivity and response gain in primate V1

Authors: *A. VAFAEI¹, A. M. CLARK², F. C. FEDERER³, A. ANGELUCCI⁴;
²Dept. of Ophthalmology and Visual Sci., ³Moran Eye Inst., ⁴Ophthalmol, Moran Eye Inst.,
¹Univ. of Utah, Salt Lake City, UT

Abstract: The cerebral cortex is composed of excitatory neurons (ENs) and inhibitory interneurons (INs), which collectively shape functional responses. While ENs propagate sensory information, INs play crucial roles in modulating gain, controlling input noise, refining tuning, and regulating the timing of EN activity. Despite extensive investigation in mouse models, the computational function of parvalbumin-expressing (PV) INs—the predominant IN subtype (Federer et al. 2024)—in primate primary visual cortex (V1) remains unclear, partly due to contradictory findings in earlier mouse studies (Lee et al., 2014). To address this, we performed bidirectional optogenetic manipulation of PV INs in marmoset V1, using AAV-PhP.eB-S5E2 expressing C1V1 or stGtACR2 for excitation and inhibition, respectively. We identified two key effects: (1) a multiplicative (gain-modulating) effect in the input layer that preserves orientation tuning bandwidth (HBW), and (2) a nonlinear effect outside input layers, where PV excitation sharpens tuning (reduced HBW) while PV inhibition broadens it (increased HBW) (Vafaei et al, SFN 2024). While a linear threshold model (Atallah et al., 2012) explains the gain effect, it fails to account for the nonlinear tuning changes, nor can a floor effect explain the observed tuning sharpening. To uncover the mechanisms underlying both linear and non-linear effects, we developed and fit to our data a computational model incorporating previously characterized properties of V1 neurons (Priebe et al. 2008). In this model, the intrinsic threshold nonlinearities of ENs are captured using an exponential input-output (I/O) function with separate parameters controlling exponential growth (β) and threshold (θ). Adjusting θ yields a multiplicative effect on model cells' orientation tuning (gain modulation), while increasing β sharpens the I/O relationship, effectively transforming a soft threshold into a hard threshold. This introduces a nonlinear effect, whereby non-preferred orientations elicit a weaker response in model cells, resulting in a narrower orientation tuning curve. Notably, an increase in β corresponds to a reduction in input noise to the cell (Gerstner et al., 2014). Thus, our model successfully accounts

for both gain control and noise modulation by PV INs. It suggests PV cells primarily regulate input gain in the input layer and reduce input noise, sharpening orientation tuning, in other layers. These findings provide new insight into the layer-specific computational functions of PV INs in primate V1 and highlight the importance of non-linear mechanisms in shaping sensory representations.

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Poster

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Title: Relationship between cortical interneuron classification schemes for rodents and primates

Authors: *A. M. BRIGANDE, A. A. DISNEY;
Neurobio., Duke Univ., Durham, NC

Abstract: Rodent and primate cortical interneurons are classified differently at the protein level. In macaque primary visual cortex (V1), interneurons form functionally distinct populations that express the calcium-binding proteins parvalbumin (PV), calbindin (CB), or calretinin (CR) with minimal overlap. In mouse V1, however, interneurons often co-express CB and CR, and so interneuron populations are classified by expression of either PV, somatostatin (SST), or vasoactive intestinal peptide (VIP). The relationship (if any) between CB+ and CR+ interneurons and SST+ and VIP+ interneurons in macaque is unclear. In the present study, we performed 4-channel RNAscope experiments to detect various combinations of GAD1, VGAT, PV, SST, VIP, CB, and CR mRNA transcripts in fresh-frozen tissue from V1 and secondary visual cortex (V2) of an adult female macaque. We acquired 2D confocal tile scans spanning all cortical layers, and counted single-, double-, triple-, and quadruple-labeled cells using a mixture of machine learning and manual curation. In a preliminary analysis of V1 (n = 1,165 interneurons), we found that over 90% of VIP+ interneurons express CR (and not CB), and approximately 70% of SST+ interneurons express CB (and not CR). These data provide insight into the relationship between rodent and primate classification schemes for interneurons in early visual cortex.

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Poster

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Title: A two-photon calcium imaging study of spatial variation in responses to cross-orientation stimuli in macaque primary visual cortex

Authors: *M. AGARWAL¹, G. HATANAKA², S. CHATTERJEE⁴, K. TAKASAKI⁴, T. KIM², C. DYLLA², A. K. PASUPATHY^{2,3}, J. WATERS⁴, R. REID⁴, W. BAIR^{2,3};
²Neurobio. & Biophysics, ³Washington Natl. Primate Res. Ctr., ¹Univ. of Washington, Seattle, WA; ⁴Allen Inst. for Brain Sci., Seattle, WA

Abstract: The selectivity of neurons in macaque primary visual cortex (V1) is known to be clustered in space for several visual features, including orientation, spatial frequency (SF) and chromatic tuning. Here we examine whether the cortex shows spatial organization in terms of responses to cross-orientation stimuli (a sum of two orthogonal sine gratings - a plaid) in comparison to responses to single gratings. The phenomenon of cross-orientation suppression has been studied extensively in the visual cortex, and it is known that the strength of suppression created by adding an orthogonal grating to an optimally oriented grating can vary substantially across neurons; however, to our knowledge, no study has tested for spatial organization of this property. To address this, we analyzed the visual responses of nearly 5000 ROIs (detected with Suite2p) across 28 fields of view (FOVs) spanning 400 μm of depth in layer 2/3 of the anesthetized macaque (2-photon Ca^{2+} imaging, PHP.eB-CAG-GCaMP6s). In addition to presenting cross-orientation stimuli (sine gratings and plaids drifting at 4 Hz, 4 cyc/deg, 2 deg patch), we mapped receptive fields with light/dark spots and measured direction and SF tuning. Assuming that responses to plaid stimuli can be predicted by the linear sum of responses to the individual grating components, we computed a predicted plaid orientation tuning curve. To quantify deviations from this linear prediction (e.g., the degree of suppression), we used two metrics: S, the average difference between the predicted and observed plaid tuning curves, and R, the Pearson correlation between them. We found a statistically significant decrease in the average value of S with increasing cortical depth of the FOV ($r = -0.71$, $p < 0.0001$, $n=28$) and a significant increase in R with depth ($r = 0.78$, $p < 0.0001$, $n=28$). S was on average positive at shallow depths but negative below about 400 μm . This suggests that cross-orientation suppression becomes stronger with depth, but it will be important to eliminate other explanations, for example, an interaction between changes in orientation tuning bandwidth and nonlinearities inherent in Ca^{2+} imaging. We also observed apparent spatial clustering of S within single FOVs. These trends held even as we varied a threshold to exclude ROIs with low signal-to-noise ratio tuning curves. Our results raise an intriguing question about how basic properties of orientation tuning and response normalization depend on depth within the cortex. We will

present potential explanations in the context of predictions from linear-nonlinear Gabor filter models of orientation selective simple and complex cells, followed by plausible imaging nonlinearities.

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Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR340.19/T16

Topic: E.06. Vision

Support: NIMH; Grant ZIA MH002909
NEI; Grant ZIA EY000511

Title: Data driven translation of functional activity patterns between monkeys and humans

Authors: ***K. BRAUNLICH**¹, M. DUYCK³, S. DUFFIELD⁴, A. BEHEL⁵, B. R. CONWAY⁶, C. I. BAKER²;

²Lab. Brain and Cognition, ¹NIH, Bethesda, MD; ³NIH, Natl. Eye Inst. (NEI), Bethesda, MD;

⁴Emory Univ. Sch. of Med., Decatur, GA; ⁵Lab. of Brain and Cognition, NIMH, Bethesda, MD;

⁶NEI, Natl. Inst. of Hlth., Bethesda, MD

Abstract: The macaque is a critical model for studying the human brain, but its validity depends on the similarity of the brains of the two species. While the visual behavior of the two species is comparable, the degree to which brain areas are homologous is less well established, especially for higher-order cortical regions such as inferior temporal cortex, but even for visual areas early in the putative hierarchical processing pathway (V1, V2, MT). This challenge stems from differences in methods (invasive vs. non-invasive), limited understanding of higher-order representations, and differences in the tasks that each species can perform. Identifying shared representational structure is a foundational step for understanding the evolutionary basis of uniquely human capacities, but training macaques on tasks unique to human is not feasible. To achieve this, we collected fMRI data from both species (7T in nine humans, 3T in three macaques) while they viewed temporally synchronized stimuli rich in perceptual, cognitive, and affective content (e.g., movies). To identify representational structure shared between species, we extended hyperalignment—a data-driven technique typically used to reveal shared structure across human participants. This approach is attractive because it derives cross-species projection matrices that enable direct translation of results between species and facilitate generalization beyond the training stimuli. We first examined representational signatures in V1, V2, V3, V4, and MT. The strongest correspondence across species was observed for matched regions, indicating successful recovery of region-specific representational structure. We also found a

hemispheric effect; cross-species information sharing was greatest when comparing regions within the same hemisphere. Building on these validating results, we then used an atlas-based approach to identify shared information across the brain. We found that information shared with humans spanned widespread macaque brain regions, including the frontal and parietal lobes, whereas in humans, information shared with macaques was mostly confined to the occipital and temporal cortices. Collectively, our results demonstrate a quantitative method for comparing representational structure across species, providing key insight into functional homologies and divergences.

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Poster

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Location: SDCC Halls B-H

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Topic: E.06. Vision

Support: NIH Grant EY029663

Title: Nicotine delivery in 4C causes mixed effects across layers in V1 that are predicted by a normalization model

Authors: *V. C. GALVIN¹, A. A. DISNEY²;
¹Neurobio., ²Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: The effect of acetylcholine acting at various cholinergic receptors on neuronal activity has been well documented across primary visual cortex (V1) in primates, where there is strong cholinergic innervation and precise knowledge of receptor distribution patterns. Previous work has shown that activation of the highly selective nicotinic $\beta 2$ subunit-containing receptors with nicotine *in vivo* increases responsiveness and lowers contrast thresholds of neurons in layer 4C, effectively altering gain of input to visual cortex. We sought to determine whether these local 4C effects propagate through the visual circuit by recording across a visual column while delivering nicotine to layer 4C. We observed both enhancement and suppression across cortical layers following nicotine delivery in 4C, which was not well explained by observed variation in receptive field size, position, or preferred orientation compared to the properties of the presented stimuli. Interestingly, the normalization model of attention proposed by Reynolds and Heeger (2009) predicts such mixed effects depending on the overlap of both stimulus properties and the size of an attention 'field' in tuning space. We find our results are very well predicted by this model, suggesting normalization alone can account for both enhancement and suppression within a visual circuit.

Disclosures: V.C. Galvin: None. A.A. Disney: None.

Poster

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Topic: E.06. Vision

Support: National Eye Institute EY022122
Brandeis Funds

Title: Tuning fields from geometric spectral synthesis: closed-form derivation of V1 simple cell tuning properties using canonical parameterizations of retino-geniculate inputs and stimuli.

Authors: *R. L. RODRIGUEZ, IV;
Neurosci., Brandeis Univ., Waltham, MA

Abstract: Simple cells in the input layer (L4) of primary visual cortex (V1) perform a critical role as initial feature extractors; understanding how their tuning properties arise from convergent feed-forward circuitry represents a significant step toward understanding mammalian vision. Existing models tend to express purely independent (i.e. bandpass) tuning profiles, wherein preference in one stimulus dimension does not condition on the values of another. This characterization is incongruent with empirical support for at least two variants of joint tuning in simple cells: TF-Direction dependence and speed tuning (SF-TF dependence).

To resolve these inconsistencies, we introduce Geometric Spectral Synthesis (GSS), a novel frequency-domain framework for computing V1 simple cell responses. GSS builds directly upon the classical Linear-Nonlinear (LN) approach by modeling LGN receptive fields (RFs) as spatiotemporally separable filters, but rather than fully applying the convolution theorem to compute each input response, we recognize that each “intermediate” integral transform constitutes a complex-valued transfer function over a spectral domain whose axes map directly onto the parametric basis for drifting gratings. The linear drive to each V1 cell is thus the sum of these responses. To eliminate non-physiological destructive interference between out-of-phase LGN inputs, we compose the pairwise coefficients in the sum with a thresholding function, the “floor” of which is an amplitude ratio interpreted as a geometric invariant w.r.t. the phase difference between input pairs.

We refer to the full formulation as the Cortical Tuning Field (CTF). The frequency scaling present in each phase coherence term results in a kind of “spectral fingerprint”; a unique reflection of the implicit spatiotemporal geometry between inputs. This feature facilitates mechanistic explanation of diverse joint tuning profiles as systematic variations in pairwise phase differences with stimulus parameters: iso-coherence contour orientations in the SF-TF plane (determined by spatiotemporal latencies between inputs) predict speed tuning, while direction-dependent spatial offset projections account for TF preference shifts across directions.

Disclosures: R.L. Rodriguez: None.

Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

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Topic: E.06. Vision

Support: AFOSR MURI Grant FA9550-21-1-0230
Center for the Co-Design of Cognitive Systems (CoCoSys)

Title: A functional model of V1 horizontal connections based on denoising structure in natural images

Authors: *A. BELSTEN, Z. YUN, B. A. OLSHAUSEN;
Redwood Ctr. for Theoretical Neurosci., Univ. of California, Berkeley, Berkeley, CA

Abstract: A striking feature of V1 architecture is the long-range, excitatory horizontal connections among neurons in layers 2+3. It has been hypothesized that these connections facilitate integration of related visual features, such as aligned edges (Field et al., 1993). However, developing a working model of such a system capable of functioning in a stable and beneficial manner on natural images has remained an outstanding problem. Here we build on the sparse coding model of V1 (Olshausen & Field, 1996) by adding to the sparse prior coupling term that allows for facilitatory interactions among latent variables. These connections are then jointly learned with the basis functions (receptive fields) and sparsity parameters. Standard learning methods, such as Maximum Likelihood Estimation (MLE), are intractable in this setting without additional assumptions. To address this, we leverage the denoising score matching framework (Vincent, 2011), which provides a simpler yet efficient way to train the model than previous methods and enables principled model evaluation.

When this model is trained on a diverse set of natural images, we find that the learned excitatory connections are spatially localized and preferentially link similarly oriented units, while inhibitory connections are less localized. We evaluate model quality via score matching and show that models with learned horizontal connections better capture image statistics than those without. These results provide insight into both training energy-based latent variable models, such as sparse coding, and the functional role of horizontal connections in V1.

Disclosures: A. Belsten: None. Z. Yun: None. B.A. Olshausen: None.

Poster

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Program #/Poster #: PSTR340.23/U4

Topic: E.06. Vision

Support: Kempner Institute Graduate Fellowship
NSF 12-599

Title: Hierarchical development, learning, and robustness in deep networks as a model for the primate visual stream

Authors: *D. C. HIDALGO^{1,2}, G. KREIMAN³;

¹Neurobio., Harvard Med. Sch., Boston, MA; ²Kempner Institute, Boston, MA; ³Boston Children's Hosp., Boston, MA

Abstract: Understanding how hierarchical processing, maturation, and robustness emerge in the primate visual system is a central challenge in neuroscience. Directly studying these processes in biological neural circuits remains difficult due to current technological limitations. Here, we use deep convolutional neural networks (DCNNs) as biologically inspired models of the primate visual system, leveraging their accessible connectivity patterns, activations, and performance metrics to generate concrete, experimentally testable predictions about cortical function. We investigate three biological hypotheses using DCNNs: (1) hierarchical maturation of visual areas during development, (2) structured changes in network representations during learning new visual tasks (plasticity), and (3) robustness differences across cortical areas when facing synaptic or neuronal perturbations. Our findings reveal that hierarchical maturation emerges naturally in DCNNs, with earlier visual layers maturing faster than later ones—consistent with developmental patterns observed in primates. Furthermore, during transfer learning, higher layers adapt more readily to new tasks, suggesting a hierarchical structure in visual cortical plasticity. Finally, robustness tests demonstrate that higher-level representations in strictly feedforward architectures (e.g., AlexNet) are significantly more resilient to synaptic and node perturbations compared to lower layers, whereas architectures with skip connections (e.g., ResNet) show robustness dependent on circuit connectivity rather than strict hierarchical depth. These findings hold consistently across multiple datasets and training methods, including alternative biologically plausible training schemes such as forward direct feedback alignment (FDFA). By bridging biological theory and deep learning, our results propose testable predictions about visual cortical development, learning, and robustness, guiding future experimental approaches in NeuroAI research.

Disclosures: D.C. Hidalgo: None. G. Kreiman: None.

Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR340.24/U5

Topic: E.06. Vision

Support: NSF grant CRCNS 2208362

Title: Modeling divisive normalization in visual cortex

Authors: *G. W. COTTRELL¹, Y. SHAH², X. ZHI³, K. LONG³;

¹UCSD, La Jolla, CA; ²Stanford Univ., Sunnyvale, CA, ; ³USDC, La Jolla, CA

Abstract: CNNs embody priors about the visual world: Locality, stationary statistics, translation invariance and compositionality. Similarly, CNNs implement the retinotopy of visual cortex - nearby pixels are processed by nearby neurons. A common cortical computation not usually included in CNNs is divisive normalization. It has been shown that divisive normalization of gabor filters results in more independent responses. More recently, Miller et al. showed that a complex form of divisive normalization applied to AlexNet resulted in improved accuracy. Here, we employ a simpler, more efficient version of divisive normalization inspired by Carandini and Heeger's model. We show that this version of divisive normalization is more stably optimized, takes less computation, and results in greater improvement in performance over the version used by Miller et al. The Alexnet prediction accuracy with a divisive normalization layer added is 3.3% better than Alexnet without divisive normalization, and 2.0% better than the Miller et al. version of divisive normalization. Remarkably, on AlexNet, our model works best by using divisive normalization as the sole activation function, without ReLU. Finally, we find that divisive normalization improves robustness to all 19 image corruptions in the Imagenet-C.

Disclosures: G.W. Cottrell: None. X. Zhi: None. K. Long: None.

Poster

PSTR340: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR340.25/U6

Topic: E.06. Vision

Support: NIH R44-MH129278
NIH U24-NS129949

Title: Whole-brain, cerebral blood volume weighted imaging of cortical networks using the Next Generation (NexGen) 7T scanner

Authors: *E. WALKER;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Mapping functional brain circuits at the levels of cortical columns and layers requires mesoscale (submillimeter) resolution, sufficient temporal resolution to measure relevant signals, and a functional imaging contrast specific to the area and depth of cortical activity. While recent advances have allowed for laminar imaging in limited field of view slabs, meeting these criteria with whole-brain coverage is technically very challenging, with previous approaches being limited in achievable temporal resolution.

The Next Generation (NexGen) 7T scanner is specifically designed for high-resolution cortical

neuroimaging. Here, the dedicated hardware was leveraged to achieve cerebral blood volume (CBV) weighted functional imaging using VAScular Space Occupancy (VASO), with whole-brain coverage, submillimeter resolution and sufficient temporal resolution to reveal cortical networks in a movie watching paradigm. The high-performance Impulse head gradient coil allowed the echo-spacing to be shortened, which combined with high-acceleration factors achievable a high-channel count RF array yielded very fast 3D image readouts, allowing for single-shot CBV weighted VASO imaging with whole-brain coverage at mesoscale resolutions. Images were collected using a Skipped-CAIPI 3D EPI VASO sequence, using a dual-polarity correction technique to overcome “fuzzy ripple” artifacts seen when pushing to high resolutions, image acceleration factors and imaging bandwidths (0.85mm isotropic resolution, whole volume TR 5s, TE 16.2ms, TI 1128.5/3071.6ms, Partial Fourier 7/8, Skipped-CAIPI 4x4z2 (total acceleration 16), Bandwidth 1766 Hz, Echo Spacing 0.63ms). Participants undertook 3-5 runs (15-75 min) of a movie watching task (using a 15-min collection of movie clips from the 7T HCP study (a.k.a. MOVIE1)). Data were denoised using NORDIC, motion corrected using ANTS, and the fuzzy-ripple artifact was removed using dual-polarity read-out calibration approach. After averaging across runs MELODIC ICA was run on the BOLD and BOLD-corrected VASO time-series at the subject level. The expected components from movie watching could be resolved in both the BOLD and VASO time-series, demonstrating this approach can be used to study whole-brain networks and circuits.

Disclosures: E. Walker: A. Employment/Salary (full or part-time): Advanced MRI Technologies.

Poster

PSTR341: Visual Cognition: Decision Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR341.01/U7

Topic: E.06. Vision

Title: Modulation of Perceptual Noise Can Explain the Apparent Selective Effect of Prefrontal Damage on Conscious Visibility

Authors: *D. BREDIKHIN¹, A. SCHURGER²;

¹Inst. for Interdisciplinary Brain and Behavioral Sci., Chapman Univ., Orange, CA; ²Brain Inst., Chapman Univ. Brain Inst., Orange, CA

Abstract: Previous studies on conscious visual access have suggested that the prefrontal cortex selectively affects conscious visibility but not objective performance in detection and discrimination tasks. In Del Cul et al. (2009), this conclusion was reached based on subjective reports in a backward masking task in patients with focal prefrontal lesions. The specificity of the effect on conscious visual access was demonstrated using a simulation with the dual-route model, which includes separate routes for conscious and non-conscious processing. Here, we tested the idea that prefrontal damage may not be selective for conscious perception and that

previous results can be explained solely by differences in perceptual decision-making. To test this hypothesis, we replicated the original simulation using a simpler single-route sensory evidence accumulator model, with variable levels of perceptual noise for the two experimental groups. As a result, the main features of the empirical data and the dual-route model simulation from Del Cul et al. (2009) were reproduced using the single-route model, without assuming a selective effect of the prefrontal cortex on conscious perception. The optimal set of parameters identified in the simulations indicated a stronger presence of perceptual noise in the patient group. Accordingly, based on our simulation results, we propose a simple experimental approach to test this hypothesis empirically by presenting variable levels of perceptual noise to participants in the two experimental groups. Overall, our simulation results showed that the pattern of findings reported in Del Cul et al. (2009) could be reproduced by varying the strength of perceptual noise in the experimental stimuli. If confirmed empirically, the selective causal role of the prefrontal cortex in visual consciousness will not be supported by this line of research.

Disclosures: D. Bredikhin: None. A. Schurger: None.

Poster

PSTR341: Visual Cognition: Decision Making

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Program #/Poster #: PSTR341.02/U8

Topic: E.06. Vision

Support: DFG-SFB1528 (A01)

Title: Neural correlates of continuous perceptual decisions in area MT and LIP of rhesus monkeys in solo and social contexts

Authors: *F. SCHNEIDER, A. NAZARI, A. CALAPAI, S. MOELLER, A. GAIL, I. KAGAN, S. TREUE;

Cognitive Neurosci. Lab., German Primate Ctr., Göttingen, Germany

Abstract: Access to social information during perceptual decision-making changes the behavioral responses of humans. We seek to determine the neural correlates of this social information integration in the brain of behaving rhesus macaques. To that end, we developed a continuous perceptual report (CPR) task with peri-decision wagering to determine if and how direction-selective neurons in brain areas MT/MST and LIP integrate sensory and social information about noisy random dot motion (RDM) stimuli. In humans, this task allows real-time assessment of perceptual accuracy and confidence in solo and social contexts (Schneider et al. 2024). Here, social context consisted of side-by-side monkey-human confederate dyads. On a shared display, the stimulus and the continuous perceptual reports of the monkey subject and the partner were shown. To deliver precisely controlled social information to the monkey, the confederate followed an accurate computer agent who expressed varying levels of confidence, scaled with RDM coherence. We used two 32-channel Plexon V-probes to record extracellular

spiking activity along the superior temporal and intraparietal sulcus in the right hemisphere of one rhesus monkey. First results indicate behavioral and neural differences between dyadic and solo conditions. This approach provides initial evidence that neurons in MT/MST and LIP are involved in integrating social cues with sensory evidence during perceptual decision-making. Schneider, Calapai, Mundry, Báez-Mendoza, Gail, Kagan, Treue (2024) Confidence over competence: Real-time integration of social information in human continuous perceptual decision-making. eLife13:RP101021 <https://doi.org/10.7554/eLife.101021.1>
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Poster

PSTR341: Visual Cognition: Decision Making

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Program #/Poster #: PSTR341.03/U9

Topic: E.06. Vision

Support: ST12030-Major Projects (2021ZD0203800)
NSFC32171036

Title: Differential roles of oculomotor areas in perceptual decision-making for social and nonsocial stimuli

Authors: *S. YANG^{1,2}, Y. ZHOU^{1,2,3};

¹Peking Univ., Beijing, China; ²Peking-Tsinghua Ctr. for Life Sciences, Peking Univ., Beijing, China; ³Sch. of Psychological and Cognitive Sciences, Peking Univ., Beijing, China

Abstract: Perceptual decision-making (DM) involves transforming sensory inputs into motor outputs, yet the distinct roles of oculomotor regions and their contributions to learning DM tasks remain unclear—especially in social versus nonsocial contexts. Here, we simultaneously recorded neuronal activity from the lateral intraparietal area (LIP), frontal eye field (FEF), superior colliculus (SC), and caudate nucleus in macaques performing saccade-based choices involving either social or nonsocial stimuli. By presenting stimuli within neuronal response fields, we isolated the sensory components of DM. SC showed the strongest stimulus and earliest choice representations, along with signals of choice uncertainty, indicating a central role in simple perceptual DM. FEF and LIP also encoded stimuli and choices, with FEF showing stronger stimulus but weaker choice encoding in social tasks, and LIP displaying the weakest stimulus representation under social conditions. The caudate exhibited the latest encoding of stimulus and choice, with stronger stimulus encoding in social versus nonsocial contexts. Notably, while choice representations were similar across conditions, stimulus encoding varied by content but was not categorically separated by sociality, suggesting no dedicated "social"

module in brain. During learning, stimulus and choice encoding gradually increased in FEF, LIP, and SC, with FEF showing the earliest enhancements, suggesting FEF's important role during the learning process. Together, these findings reveal distinct and dynamic roles of oculomotor areas in perceptual decision-making and learning.

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Poster

PSTR341: Visual Cognition: Decision Making

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Program #/Poster #: PSTR341.04/U10

Topic: E.06. Vision

Support: Wellcome Trust 216324
Simons Foundation

Title: Towards a mesoscopic cortical map of neural activity during visual decision-making

Authors: C. QUADRADO¹, I. BRAIN LAB¹, M. CARANDINI¹, M. FAULKNER¹, K. D. HARRIS¹, M. KRUMIN¹, G. RAISER³, M. J. WELLS¹, S. J. WEST², *S. A. M. PICARD¹;
²Sainsbury Wellcome Ctr., ¹UCL, London, United Kingdom; ³Champalimaud Ctr. for the Unknown, Lisbon, Portugal

Abstract: The International Brain Laboratory (IBL) recently produced a brainwide map of electrophysiological activity during a visual decision-making task, revealing rich dynamics across cortical and subcortical regions (www.internationalbrainlab.com/data). While powerful, acute neuropixels recordings have limited yield, particularly in the neocortex, and cannot capture the stability of neural representations over time. To address these gaps, we used large-scale two-photon calcium imaging to build a dense, cellular-resolution map of neural activity across the dorsal cortex during decision-making.

We trained mice on the IBL's standardized decision-making task: mice turned a wheel to indicate whether a visual grating appeared on the left or the right, with prior probability alternating between 0.2 and 0.8 in blocks of variable length. After the task, we presented a battery of passive stimuli (task events, sparse noise and full-field movies). Meanwhile, we used a two-photon random-access mesoscope to simultaneously image calcium activity of ~10,000 excitatory neurons per session across 6 to 12 regions of the dorsal cortex. In a subset of mice, we tracked up to 80% of these neurons across multiple weeks to investigate longitudinal changes in neural coding, and their relationship to behaviour.

We have so far acquired data from approximately 1 million good-quality excitatory neurons, covering nearly all regions of the dorsal cortex. Visual cortical neurons with reliable responses to passive stimuli within a session, had similarly reliable responses across two sessions, validating our longitudinal tracking. Consistent with previous electrophysiology, during task performance, visual stimulus-related signals were reliably detected in mainly visual areas; choice-related

signals were much sparser, but present in multiple cortical regions; and movement-related signals were widespread across the cortex. In expert animals, pre-stimulus population activity sparsely encoded the blockwise prior across several cortical regions, including primary visual cortex. This encoding was stronger in sessions where mice behaviorally leveraged the bias-blocks.

Our standardized longitudinal two-photon imaging approach reveals stable sensory representations, and sparse encoding of priors across the dorsal cortex, which tracks behavioural block-bias. This dense cortical map complements and extends brainwide electrophysiological findings, and offers a unique, publicly available dataset for studying large-scale cortical dynamics underlying decision-making and learning.

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Poster

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Topic: E.06. Vision

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Whitehall Foundation
E. Matilda Ziegler Foundation for the Blind

Title: Dynamics of visual, vestibular, and decision signals in multisensory cortical areas during a reaction-time heading discrimination task

Authors: *Y.-C. CHIANG¹, Y. R. HAILE², C. R. FETSCH²;

¹Dept. of Neurosci. & Mind/Brain Inst., ²Neurosci. & Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: In natural environments, organisms must rapidly integrate uncertain information from multiple modalities to guide action. While the neural basis of multisensory integration has been extensively studied, very little is known about the dynamics of multisensory decisions under speeded conditions. From the neural perspective, it is unclear how the brain is able to extract dynamic multimodal information distributed across a large cortical network, as is the case for visual and vestibular signals pertinent to self-motion perception. From the behavioral perspective, most prior studies used fixed-duration tasks without reaction time measurements, limiting our understanding of decision formation and termination. We trained rhesus monkeys to perform a reaction-time heading discrimination task within a VR ‘flight-simulator’ apparatus. Translational heading stimuli were delivered via optic flow, inertial motion, or both. Animals reported their binary heading judgment (left or right) via a saccadic eye movement as soon as

they were ready, followed by a binary confidence judgment with a second saccade (post-decision wagering). While animals performed the task, we recorded separately or simultaneously from the dorsal medial superior temporal area (MSTd) and visual posterior sylvian area (VPS) using 32-channel linear arrays (N=440 units in 2 monkeys) or Neuropixels probes (N=6 sessions in 1 monkey, 50-100 units/session). Both areas carried linearly decodable information about stimulus, choice, and confidence, though their temporal profiles and relative decoding strengths varied by condition. Choice decoding was slow to emerge after stimulus onset but became robust prior to the first saccade, with a pre-choice 'ramping' profile extending into the post-decision epoch. Surprisingly, encoding of the stimulus itself (heading angle) was strikingly weak, despite previous work showing robust tuning and causal links to heading perception in both areas. Fewer than 20% of neurons in our sample showed significant heading selectivity in a timeframe that could plausibly support decision formation, even when restricting analyses to neurons with significant heading tuning in a passive fixation task. The lack of robust stimulus information arriving fast enough to support the decision suggests that either (1) momentary evidence for speeded heading decisions resides elsewhere, or (2) readout of decision evidence occurs from a small subset of neurons and only in a brief window before the choice. We consider these results in the context of alternatives to the standard evidence accumulation framework, including a 'snapshot' model combined with temporal prediction.

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Poster

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Support: NEI EY025872

Title: Neural networks rely on expectations under conditions of uncertainty

Authors: *H. KULAR¹, J. SERENCES¹, R. KIM², N. RUNGRATSAMEETAWEEEMANA³;

¹Psychology, UCSD, La Jolla, CA; ²Neurol., Cedars-Sinai Med. Ctr., Los Angeles, CA;

³Biomed. Engin., Columbia Univ., New York, NY

Abstract: Statistical regularities found in natural visual environments can be used to form expectations that enable predictions that guide behavior. However, it remains unclear how the top-down information from expectation modulates information processing along the cortical hierarchy in visual processing. Here, we test the hypothesis that expectations can shape processing at all levels of information processing, particularly when stimulus uncertainty is high. Using a three-layer hierarchical continuous-time recurrent neural network (RNN) trained on a perceptual decision-making task we isolated effects of expectation while simultaneously manipulating external noise (stimulus uncertainty) and internal noise (encoding variability). We

established top-down expectations in the network by training RNNs with biased stimulus probability where the expected stimulus was presented more frequently than others. For the control condition, we trained a separate set of RNNs under conditions of uniform stimulus probability where each stimulus identity was equally likely during training. To investigate how expectations interact with multiple sources of uncertainty, we systematically manipulated both external and internal sources of noise and evaluated RNN behavior under these conditions. We show that the RNN extracts sensory statistics and develops expectations that improve processing when stimuli are uncertain. Linear SVM classifiers were trained on RNN hidden layer activity to decode stimulus identity. Decoding accuracy was highest for the expected stimuli, particularly under conditions of uncertainty - indicating that expectations selectively enhance representations when sensory input is degraded. Moreover, the expected stimulus could be more readily decoded from activity in the final hidden layer under high uncertainty conditions, suggesting a strong top-down influence from higher layers. These findings show that the RNNs developed expectations that facilitated performance when external sensory information and internal representational fidelity were reduced, supporting the hypothesis that expectations improve processing efficiency across the cortical hierarchy.

Disclosures: H. Kular: None. J. Serences: None. R. Kim: None. N. Rungratsameetaweemana: None.

Poster

PSTR341: Visual Cognition: Decision Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR341.07/U13

Topic: E.06. Vision

Support: ERC-682426 (VISONby3DSTIM)
GINOP-2016
NKP-2017
VKE-2018
GINOP-2021-00061
PM/20453-15/2020
KFI-2018-097

Title: Mculus: an immersive virtual reality system for mice incorporating stereo vision

Authors: *B. TARJÁN¹, H. SZEBIK², B. ROZSA^{3,2}, G. SZALAY^{4,2}, K. ÓCSAI¹, Z. SZADAI²;
¹BrainVisionCenter Res. Inst. and Competence Ctr., Budapest, Hungary; ²BrainVisionCenter Res. Inst. and Competence Centre, Hungary, Budapest, Hungary; ³Inst. Exptl. Med. of HUN-REN, Budapest, Hungary; ⁴Lab. of 3D functional network and dendritic imaging, Femtonics Ltd., Budapest, Hungary

Abstract: Due to technical roadblocks, it is unclear how visual circuits represent multiple features or how behaviorally relevant representations are selected for long-term memory. Here we developed Moccus, a head-mounted virtual reality platform for mice that covers the entire visual field, and allows binocular depth perception and full visual immersion. This controllable environment, with three-dimensional (3D) corridors and 3D objects, in combination with 3D acousto-optical imaging, affords rapid visual learning and the uncovering of circuit substrates in one measurement session. Both the control and reinforcement-associated visual cue coding neuronal assemblies are transiently expanded by reinforcement feedback to near-saturation levels. This increases computational capability and allows competition among assemblies that encode behaviorally relevant information. The coding assemblies form partially orthogonal and overlapping clusters centered around hub cells with higher and earlier ramp-like responses, as well as locally increased functional connectivity.

Disclosures: **B. Tarján:** 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.; 2020-2.1.1-ED-2022-00208. **H. Szebik:** 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.; 2020-2.1.1-ED-2022-00208. **B. Rozsa:** 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.; 2020-2.1.1-ED-2022-00208. **G. Szalay:** 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.; 2020-2.1.1-ED-2022-00208. **K. Ócsai:** 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.; 2020-2.1.1-ED-2022-00208. **Z. Szadai:** 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.; 2020-2.1.1-ED-2022-00208.

Poster

PSTR341: Visual Cognition: Decision Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR341.08/U14

Topic: E.06. Vision

Support: NEI 1R01EY035791-01A1
NEI: 1R01EY032101
NIGMS: P20GM103650 (Cellular and Molecular Imaging Core facility)

Title: Strain vs. Strain: Divergent Sensory Strategies in Mouse Prey Capture

Authors: *K. HUANG¹, S. NECAISE², S. ROUT³, J. HOY⁴;

¹Univ. of Reno Nevada, Reno, NV; ²Univ. of Nevada, Reno, NV; ³Univ. of Nevada, Reno, NV; ⁴Univ. of Nevada, Reno Integrative Neurosci. Grad. Program, Reno, NV

Abstract: Prey capture in mice depends on the integration of visual and auditory cues, yet the influence of genetic background and sensory map alignment on this behavior remains unclear. We compared live prey capture performance between three genetically distinct mouse strains—C57BL/6 (WT), CBA/CaJ (CBA), and B6.CAST-Cdh23 (B6.CAST)—across conditions that isolated or combined sensory modalities while hunting crickets: full sensory input (light), vision-only (via bilateral earplugs), and audition-only (in darkness). After habituation to the paradigm, fasted mice were tested across multiple days to assess how each strain relied on sensory inputs for successful prey capture. Strain-specific differences emerged, particularly under full sensory conditions, suggesting that genetic background shapes the relative weighting of visual and auditory cues during ethologically relevant behavior. Notably, certain strains maintained robust performance even when one sensory modality was removed, highlighting potential differences in sensory processing strategies or topographic map alignment. These findings point to a key role for genetic and circuit-level factors in multisensory integration during orienting and approach behaviors.

Disclosures: K. Huang: None. S. Necaise: None. S. Rout: None. J. Hoy: None.

Poster

PSTR341: Visual Cognition: Decision Making

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Topic: I.05. Working Memory

Support: National Natural Science Foundation of China (T2421004 to F.F., A.L. and H.L.)
the National Science and Technology Innovation STI2030-Major Project 2021ZD0204100 (2021ZD0204103 to H.L.)

Title: Feedback modulates serial dependence in perceptual decision-making

Authors: *Y. ZHENG¹, H. ZHANG², H. LUO³;

²Sch. of Psychological and Cognitive Sci., ³Sch. of Psychological and Cognitive Sci., ¹Peking Univ., Beijing, China

Abstract: Our brains constantly use past to guide present—a fundamental aspect of adaptive behavior. A striking example is serial dependence, where current perception and decisions are automatically biased by seemingly irrelevant information from previous trials. This phenomenon occurs throughout the cascade of information processing, from sensory encoding to motor

responses. While feedback provides objective teaching signals to calibrate cognitive systems to the environment, its role in shaping serial dependence remains largely unknown.

We investigated this question using a near-threshold orientation discrimination task, in which perceptual reports and motor responses were dissociated through trial-wise random choice-response mappings, with veridical feedback provided on each trial. Human observers exhibited opposing serial dependence effects: perceptual reports were biased toward the previous choice (attractive bias) and motor responses alternated across trials (repulsive bias). Critically, feedback modulated serial dependence across multiple dimensions. First, feedback prolonged the attractive perceptual bias, with effects persisting up to ten trials back. Second, the attractive perceptual bias occurred following both positive and negative feedback, though effects were stronger after negative feedback. Third, motor bias was short-lived, limited to the immediately preceding trial, and significant only after negative feedback.

To account for these effects, we developed a Bayesian model incorporating trial-wise updates of perceptual priors based on previous choices and response alternation, fitting it to both our dataset and a previously published one without feedback. We hypothesized that feedback modulates perceptual bias by changing the updating weight and/or representational uncertainty of the immediately preceding choice. Models with the historical information component outperformed baseline models, with parameters revealing key insights into feedback's role. Compared to the no-feedback condition, feedback slowed perceptual prior updates, making perceptual bias weaker but more persistent. Moreover, feedback type influenced serial dependence through distinct pathways: positive feedback reduced perceptual uncertainty while negative feedback enhanced motor alternation rates.

Together, our findings reveal that feedback modulates serial dependence across perceptual and motor domains through distinct mechanisms. This study provides both empirical evidence and computational insights into how cognitive systems flexibly incorporate historical information for adaptive, efficient processing.

Disclosures: Y. Zheng: None. H. Zhang: None. H. Luo: None.

Poster

PSTR341: Visual Cognition: Decision Making

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Program #/Poster #: PSTR341.10/U16

Topic: I.05. Working Memory

Support: National Natural Science Foundation of China T2421004
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2021ZD0204100

Title: Structural bias in network learning: Spontaneous emergence hierarchy from homogeneity

Authors: *Z. YANG^{1,3,4}, H. LUO^{2,3,4}, M. WANG^{2,5,3,4};

²Sch. of Psychological and Cognitive Sci., ¹Peking Univ., Beijing, China; ³Peking Univ., PKU-

IDG/McGovern Inst. for Brain Res., Beijing, China; ⁴Peking Univ., Beijing Key Lab. of Behavior and Mental Hlth., Beijing, China; ⁵Applied Computat. Psychiatry Lab, Max Planck UCL Ctr. for Computat. Psychiatry, Queen Square Inst. of Neurology, UCL, London, United Kingdom

Abstract: Human cognition can efficiently organize fragmented information into abstract structures that support sense making and memorization. Employing prior structural knowledge during organization, from meta-learning and Bayesian perspectives, can serve to facilitate learning and generalization, though this process may introduce systematic biases. Notably, as heterogeneous and hierarchical organization prevails in real-world knowledge networks, we examined whether humans apply such heterogeneous structural priors when acquiring new knowledge. Our central hypothesis posits that if this structural bias exists, learners should manifest heterogeneous and hierarchical organization in behavior, even when learning a homogeneous network structure. Here participants learned a perfectly homogeneous lattice network. The behavioral findings support our hypothesis. First, participants exhibited significant heterogeneous choice preferences, favoring some nodes over others. Second, participants formed structured preferences—nodes with higher choice preferences are clustered within the network rather than being randomly distributed. Furthermore, participants formed a hierarchical organization—choice preferences decay with distance from hub. In post-learning tests conducted on a separate day, participants judged whether there is a connection between two successive images, with their magnetoencephalography (MEG) activities recorded. Neural responses systematically tracked the ‘biased’ network topology formed in the previous day. For the first presented image, global field power (GFP) magnitude encoded node centrality, with higher-preference nodes evoking stronger responses localized in the left posterior cingulate cortex (PCC), while images structurally closer to hub nodes elicited larger GFP. For the second image, we observed distance-dependent suppression of GFP, where amplitudes decreased monotonically with shorter path lengths to the first image, indicating implicit retrieval of network structure. In summary, we provide converging behavioral and neural evidence supporting that humans spontaneously impose heterogeneous and hierarchical structural prior -- even when confronted with objectively homogeneous knowledge network. This structural bias may serve as an adaptive mechanism to overcome inherent capacity limits in human cognition.

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Poster

PSTR341: Visual Cognition: Decision Making

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Program #/Poster #: PSTR341.11/V1

Topic: E.06. Vision

Support: IRC Grant GOIPD/2024/870
Wellcome Trust 219572/Z/19/Z

Title: A novel perceptual decision making task may allow joint measurement of sensory signals from feedforward and recurrent visual activity

Authors: *K. S. MOHR¹, S. KELLY²;

¹Univ. Col. Dublin, Dublin 4, Ireland; ²Sch. of Electrical and Electronic Engin., Univ. Col. Dublin, Dublin, Ireland

Abstract: The study, in humans, of the neural mechanisms underlying perceptual decision making has been facilitated by task paradigms that allow EEG signals relating to decision formation to be measured alongside those related to sensory evidence encoding. However, the latter, most often a steady-state potential, does not distinguish between bottom-up and top-down contributions to sensory evidence encoding, although we know that visual perception entails an interplay between these two. Here, we present a novel decision making paradigm where sensory signals consist of two components that can be measured separately, both from one another and from downstream decision-related signals. These two sensory components, the C1 and C2 components of the visual evoked potential (VEP), are thought to reflect feedforward and recurrent visual activity, respectively. The task paradigm involves presenting orthogonal sequences of rapid stimulus pulses at various locations in the visual field, which elicits a train of VEPs over time. We implemented two versions of this task, one in which targets were defined as increases in luminance for pulses in either the left or right visual field, and the other in which pulses were gratings of random orientation with targets defined as a preponderance of pulses in which gratings were +/-45 deg. In both cases, we found that the C1 and C2 exhibited a counter correlated sequence of amplitude variations, with a boosted C1 and suppressed C2 at approximately 500 ms after target onset, and a boosted C2 and suppressed C1 approximately 500 ms later. Both stages of this sequence were stronger for hits compared with misses and when response times were faster. The second phase was stronger for choice congruent stimulus pulses in the luminance task, and in the orientation task overall compared with the luminance task. We suggest that this reflects an initial boosting of bottom-up stimulus processing followed by a suppression of bottom-up activity in favour of top-down driven stimulus processing. Although future research will be needed to elucidate the precise roles of these signal components more precisely, their distinct relationships with task performance demonstrated here suggest that this novel paradigm will facilitate a richer understanding of sensory evidence encoding in perceptual decisions.

Disclosures: K.S. Mohr: None. S. Kelly: None.

Poster

PSTR341: Visual Cognition: Decision Making

Location: SDCC Halls B-H

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Topic: I.05. Working Memory

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Title: Implicit learning of higher-order network structure through a 'Whack-a-Mole' game paradigm

Authors: *H. YANG^{1,2,3}, M. WANG^{4,2,3,5}, H. LUO^{4,2,3};

¹Sch. of Psychological and Cognitive Sciences, Peking Univ., Beijing, China; ²PKU-IDG/McGovern Inst. for Brain Res., ³Beijing Key Lab. of Behavior and Mental Hlth., ⁴Sch. of Psychological and Cognitive Sci., Peking Univ., Beijing, China; ⁵Applied Computat. Psychiatry Lab, Max Planck UCL Ctr. for Computat. Psychiatry, Queen Square Inst. of Neurology, UCL, London, United Kingdom

Abstract: Understanding how humans construct network structures through discrete pieces of knowledge offers key insights into universal learning mechanisms. While our previous research has established that higher-order structures that transcend local experiences could be extracted through network learning, whether this higher-order learning would occur implicitly remains unknown. Here we developed a novel “Whack-a-Mole” gaming paradigm to tackle the question. Specifically, participants tapped moles that appeared on screen as quickly and accurately as possible to earn coins. Unbeknownst to them, the spatial sequence of mole appearances was generated by a random walk on an underlying scale-free network. During the subsequent testing phase designed to assess learning effects, participants engaged with the same gaming paradigm, while spatial sequences were randomized instead of following a network. First, responses were faster for transitions between directly connected (vs. unconnected) nodes during the testing phase, confirming acquisition of low-order structure. Most importantly, subjects’ behavior (i.e., RT) was modulated by higher-order network properties (i.e., communicability index), even after controlling for local adjacency and recency effects. Critically, this higher-order dependence still held for node pairs that have not been experienced in the learning phase, suggesting topology-driven generalization beyond local experience. In contrast, a lattice network that lacks heterogeneity properties did not show the behavioral dependence on communicability index. These findings collectively demonstrate that the properties of network heterogeneity can be acquired through implicit learning, thereby enhancing both network learning capacity and generalization ability beyond locally acquired experiences in humans. Furthermore, our innovative gaming paradigm establishes an ecologically valid experimental platform for investigating structural learning across diverse populations.

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Poster

PSTR341: Visual Cognition: Decision Making

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under Grant Agreement No. 899287 (NeuraViPeR) to EF
Alfred P. Sloan Foundation Research Fellowship to TCS

Title: Stimulus-selective spiking activity associated with working memory in human early visual cortex

Authors: *L. M. TURKSTRA¹, J. GRANLEY², H. STONE¹, F. GRANI³, L. SOO³, G. POGONCHEFF², C. SOTO-SÁNCHEZ³, T. C. SPRAGUE¹, E. FERNANDEZ³, M. BEYELER²;

¹Psychological and Brain Sci., ²Computer Sci., UC Santa Barbara, Santa Barbara, CA; ³Inst. de Bioingeniería, Univ. Miguel Hernández de Elche, Elche, Spain

Abstract: Current studies of the neural basis of visual working memory (VWM) in the early visual cortex (EVC) demonstrate spiking correlates in nonhuman primates. As analogous efforts in humans have previously been limited to noninvasive imaging methods, spiking activity associated with VWM in human EVC remains poorly understood. To this end, we utilized neural recordings from a blind individual implanted with a 96-channel visual prosthesis in parafoveal V1/V2 to directly test whether stimulus-specific spiking activity persists in EVC during VWM. The participant performed 465 trials of a delayed match-to-sample task utilizing distinct phosphenes evoked by stimulation of one of three electrodes. They reported whether the first phosphene matched a second phosphene after a 5s delay period, throughout which multi-unit spiking activity was recorded. The participant achieved an average task accuracy of 93.2%. Significant differences in firing rates were observed between pre-stimulation, stimulation, delay and spontaneous periods ($p < 0.05$, t test). Despite variability in day-to-day stimulation-evoked responses, each stimulating electrode’s neural signature was stable across sessions and remained unique and decodable across the delay period (Fig 1).

These results present the first direct evidence for sustained, stimulus-selective spiking in human EVC during VWM, suggesting a shared mnemonic subspace distinct from the full sensory response and underscoring the distributed nature of WM. These findings highlight the recruitment of EVC for the active maintenance of visual content at the spiking level, and offer a foundation for future cortical visual prostheses that interface, rather than interfere, with endogenous cognitive dynamics.

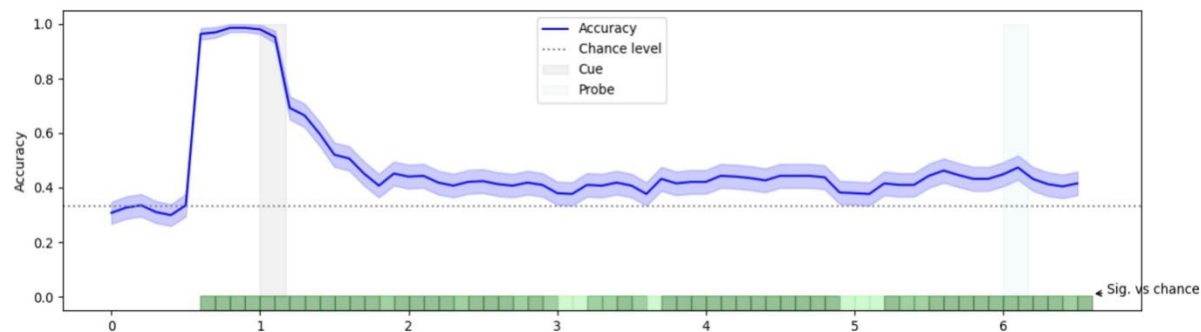


Fig. 1: 42% decoder accuracy persists across 100% of delay period. X axis represents trial duration (s). Blue line represents decoding accuracy via linear SVC on mean channel activity (100ms stride, LOOCV per 500ms sliding window). Green boxes denote accuracy significantly above chance ($p < 0.05$, permutation test).

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Poster

PSTR341: Visual Cognition: Decision Making

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Program #/Poster #: PSTR341.14/V4

Topic: I.05. Working Memory

Support: NSF Grant 2419387
University of Tampa Summer Undergraduate Research Fellowship
University of Tampa Professional Development Award

Title: High competitiveness enhances both speed and accuracy in working memory on a value-directed remembering task

Authors: *A. Y. SCHULTZ, S. B. FESTINI;
Univ. of Tampa, Tampa, FL

Abstract: Competitiveness is defined as an individual's drive to outperform others and achieve greater success. While prior research has explored the effects of competitiveness on various cognitive domains, there is limited research on its influence on laboratory-evaluated memory processes. We predicted that competitiveness would facilitate working memory (WM) and executive functioning. The present study assessed whether self-reported competitiveness impacted WM and long-term memory (LTM) performance, in a value-directed remembering WM paradigm with a surprise LTM test. Participants studied sets of words on both sides of the

computer screen for several seconds. Following encoding, these words were assigned either positive or negative point values corresponding to the side of the screen on which they were displayed. After a short delay, participants were shown a single word and asked to identify whether it was a positive-value word that they studied on that trial. Participants would lose points for endorsing a negative-value word. Occasionally, participants were presented with a recent item that had appeared on the prior WM trial as opposed to the current trial, inducing proactive interference (PI). Participants performed 60 WM trials, a surprise LTM assessment, and then completed a 9-item competitiveness index. Across all participants, results revealed significantly less PI for negative-value-recent than positive-value-recent items, suggesting the appropriate use of executive control to remove negative-value information from mind. ANCOVAs investigating the impact of competitiveness and value-type on performance showed a significant negative relationship between competitiveness and response time (RT) as well as a significant positive relationship between competitiveness and accuracy in WM. A significant main effect of value type (positive or negative) on RT was also observed, along with a significant interaction between value type and competitiveness. The interaction occurred because competitiveness exhibited a stronger relationship with RT for positive-value-recent items. In contrast, there was no influence of competitiveness on LTM, likely due to its incidental nature; participants were not explicitly instructed to remember the words for long-term testing, limiting their ability to focus their competitive efforts. Overall, these findings suggest that competitiveness enhances WM performance by improving both speed and accuracy of memory recognition, potentially through more effective engagement of cognitive control processes.

Disclosures: A.Y. Schultz: None. S.B. Festini: None.

Poster

PSTR341: Visual Cognition: Decision Making

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Topic: I.05. Working Memory

Support: the National Natural Science Foundation of China Grant 31930052
the China Postdoctoral Science Foundation Grant 2023M740124

Title: Oculomotor Signatures Reveal Hierarchical Scaffolding of Sequences in Working Memory

Authors: *J. LI^{1,2,3}, Y. FAN^{1,2,3}, H. LUO^{1,2,3};

¹Sch. of Psychological and Cognitive Sci., ²PKU-IDG/McGovern Inst. for Brain Res., ³Beijing Key Lab. of Behavior and Mental Hlth., Peking Univ., Beijing, China

Abstract: Human cognition inherently transforms one-dimensional (1D) sequences—such as speech, movement, and music—into structured representations with multiple hierarchical levels. Our recent EEG and MEG studies demonstrate that when humans maintain complex syllable

sequences in working memory (WM), these 1D sequences are dynamically reorganized into two-dimensional (2D) neural geometries, reflecting a 1D-to-2D WM folding mechanism. Building upon these neurophysiological findings and established relationships between oculomotor patterns and simple sequence processing, here we conducted four experiments to investigate whether eye movements serve as behavioral markers of this hierarchical scaffolding during complex sequence retention. Participants maintained auditorily presented syllable sequences in WM while recalling the ordinal positions of a cuing syllable, with their eye movements recorded without constraints on a blank screen. Analysis of eye movements time-locked to the cued syllable revealed systematic spatial organization as a function of ordinal position. For 5-syllable random sequences, we observed a characteristic inverted U-shaped profile ("rainbow pattern") along the horizontal axis (leftward bias for initial positions, rightward for final positions) and a circular vertical distribution (elevated position for middle rank, lower position for initial/final positions). Most interestingly, when retaining 10-syllable complex sequences (two 5-syllable sentences), the gaze pattern revealed two partially overlapping rainbow patterns, with each 5-syllable sentence occupying one rainbow. The pattern further generalized to 14-syllable sequences (two 7-syllable sentences) and 9-syllable sequences (three 3-syllable phrases), with the latter showing three partially overlapping rainbow patterns. In summary, our findings provide novel oculomotor evidence for hierarchical reorganization in WM, demonstrating that spatial configurations serve as a structural scaffold for maintaining complex sequences.

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Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

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Program #/Poster #: PSTR342.01/V6

Topic: F.02. Cerebellum

Support: NSF Grant 1912557
NIH Grant R01 NS-058659
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Title: Contrasting neuronal activity in cerebellar dentate and interpositus nuclei during vision-guided stepping

Authors: *I. N. BELOOZEROVA¹, W. U. NILAWEERA², V. MARLINSKI³;

¹Georgia Inst. of Technol., Atlanta, GA; ²Ellsworth Community Col., Iowa Falls, IA; ³Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The cerebellar dentate (DN) and interpositus (IN) nuclei are the major sources of afferent input to the ventrolateral thalamus and further the motor cortex, and to the midbrain red nucleus and further the spinal cord. Signals conveyed via these pathways are essential for control of movements, including vision-guided stepping on a complex terrain. Neuronal mechanisms of

visual control of steps are not well understood. Here we analyzed the firing activity of neurons in the feline DN and IN related to visual guidance of feet to desired locations on a complex surface. Experiments were done on chronically implanted cats using extracellular single-neuron recordings. We found that the average firing of DN cells ($n=157$) was 37 spikes/s, while that of IN cells ($n=46$) was 55 spikes/s. During locomotion on a flat surface, a task that did not require visual control of steps, 61-63% of neurons in both DN and IN fired one burst of spikes per stride, while 19-28% had two. On transition to vision-guided stepping on a horizontally placed ladder, the firing of 88% of DN cells changed either in the phase (63% neurons), depth of stride-related modulation (59%), firing rate (54%), and/or temporal precision (30%). In contrast, the firing of only 50% of IN cells changed either in the depth of modulation (31%), rate (22%), phase (21%), and/or precision (16%). All fractions but the last were smaller in IN than DN ($p<.001$). We found that regardless of the complexity of the locomotor task, 43% of IN neurons (dark blue arrow in Fig.1a) conveyed only basic locomotor movement-related information. In contrast, 33% of DN neurons during locomotion on a complex surface conveyed a combined movement- and vision-related information signals in which visual information dominated (orange arrow in Fig.1b). We suggest that IN and DN play different roles in movement control during vision-guided stepping. IN neurons provide their projection targets with signals reflecting the basic canvas of the locomotor rhythm. DN cells provide these targets with information for particular adjustments specific for the behavioral goal of ongoing locomotion.

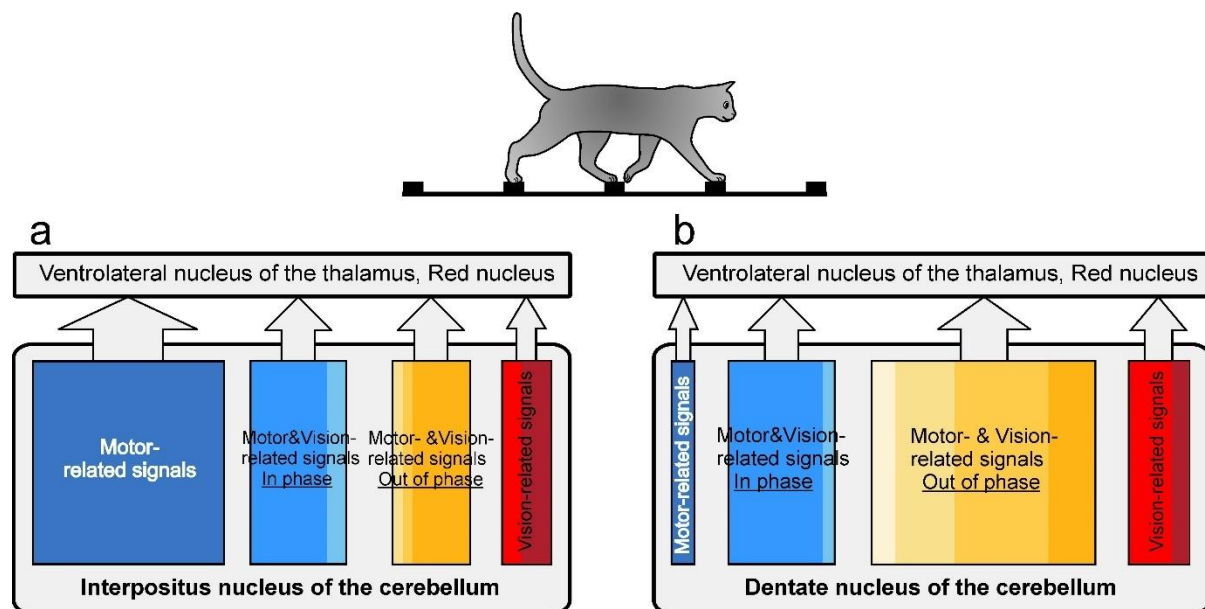


Figure 1

Disclosures: I.N. Beloozerova: None. W.U. Nilaweera: None. V. Marlinski: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

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JST SPRING JPMJSP2110

Title: Parallel acquisition of counteracting memories highlighted by limited reinforcement experience in eyeblink conditioning

Authors: *R. IWASE, S.-Y. KAWAGUCHI;
Grad. Sch. of Sci. , Kyoto Univ., Kyoto, Japan

Abstract: Pavlovian eyeblink conditioning is one of the major models of cerebellum-dependent motor learning. Animals receive two sensory inputs: innately neutral conditioned stimulus (CS: e.g., tone) and eyeblink-eliciting unconditioned stimulus (US: e.g., air puff to cornea), and repeated paired presentation of CS & US makes animals learn to blink in response to initially neutral tone. In many studies, a large amount of CS-US pairing has been used for conditioning, and little is known about the effects of limiting experience of paired sensory inputs. To address this issue, here we analyzed mice behaviors in two groups: 90%_{CS-US} (90 paired & 10 tone-alone trials/day) and 10%_{CS-US} (10 paired & 90 tone-alone trials/day). Mice were head-fixed on a treadmill during experiments, and given daily 100 trials for consecutive 10 days. Air puff was given 250 ms after the tone onset and they co-terminated in 40 ms. Eyelid movements were monitored by a high-speed camera. Unexpectedly, in spite of only ~11 % reinforcement experience, the 10%_{CS-US} group robustly acquired conditioned responses (CRs), although the final probability of CRs were slightly smaller, with similarly well-timed eyelid movements as those in the 90%_{CS-US}. Surprisingly, the 10%_{CS-US} group showed ~6 times more efficient learning. To understand the underlying mechanism, we next conducted daily 11 trials training composed of 10 paired & 1 tone-alone trials, and found that 11 trials were enough for the acquisition of as much CRs as the 90%_{CS-US} group with high learning efficiency. These results imply that too condensed training does not always lead to high performance in learning, and massive tone-alone trials only lower CR probability without affecting efficiency of CR acquisition. Pharmacological inhibition of neural activity in deep cerebellar nuclei revealed that cerebellar circuits are important for learning in the 10%_{CS-US} group as in the 90%_{CS-US} group. Finally, we attempted to develop a simple learning model based on Rescorla-Wagner model to explain the CR probability behavior during acquisition and extinction with distinct experience patterns. Simulation of the model succeeded in reproducing the experimental observations and suggested that two opposite memories for CR execution and its suppression are independently generated and their interaction defines the probability of CRs.

Disclosures: R. Iwase: None. S. Kawaguchi: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.03/V8

Topic: F.02. Cerebellum

Support: NSTC 112-2628-B-002-011
NSTC 113-2628-B-002-002

Title: Dissect cerebellar mechanism and therapeutics of tremor subtypes by spatio-temporal neural dynamics

Authors: *L. CHEN^{1,2}, M.-K. PAN³;

¹Natl. Taiwan Univ., Taipei, Taiwan; ³Inst. of Pharmacol., ²Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstract: Essential tremor (ET) is the most common movement disorder among the elderly, with fewer than half of patients achieving satisfactory outcomes from available pharmacological treatments. This limited efficacy is thought to reflect the heterogeneity of ET, which has been clinically attributed to at least two distinct pathophysiological mechanisms: harmaline toxicity and cerebellar climbing fiber (CF) overgrowth. In this study, we show that differences in the spatial synchrony of cerebellar Purkinje cells (PCs) contribute to the distinct therapeutic responses observed in mouse models of tremor. Using in vivo electrophysiology and two-photon calcium imaging, we investigated the spatiotemporal neural dynamics in aged harmaline-treated mice and *Grid2^{dupE3}* mice, representing harmaline toxicity and CF overgrowth, respectively. Harmaline-induced tremors responded to both propranolol and primidone, whereas *Grid2^{dupE3}* mice exhibited resistance to both drugs. Single-unit recordings revealed a shared mechanism of tremor-frequency coding across models, while calcium imaging identified contrasting spatial synchrony patterns: harmaline mice exhibited regionally restricted PC synchrony, whereas *Grid2^{dupE3}* mice showed globally synchronized activity. Computational modeling indicated that cerebellar oscillations in harmaline mice depended on the pacemaking properties of HCN and T-type calcium channels in inferior olivary (IO) neurons. In contrast, CF overgrowth in *Grid2^{dupE3}* mice produced sustained olivocerebellar oscillations through global PC synchrony, independent of IO pacemaking. These predictions were validated in a preclinical drug trial, where a T-type calcium channel blocker alleviated tremor in harmaline mice but had no effect in *Grid2^{dupE3}* mice. Together, these findings provide a mechanistic explanation for pharmacological failures in ET and offer guidance for the development of more targeted therapeutic strategies.

Disclosures: L. Chen: None. M. Pan: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.04/V9

Topic: F.02. Cerebellum

Support: NIH R37-NS128416

Title: Sensory and reward prediction errors in the climbing fiber input to the cerebellum

Authors: ***J. DOOSTMOHAMMADI**¹, N. MOHAMMADREZAEI², M. FAKHARIAN¹, A. SHOUP¹, P. HAGE⁴, R. SHADMEHR³;

¹Biomed. Engin., ²Bionedical Engin., ³Dept Biomed. Eng, Johns Hopkins Univ., Baltimore, MD;

⁴Johns Hopkins Univ. Sch. of Med., Baltimore, MD.

Abstract: Recent studies suggest a role for the cerebellum in learning from reward. Viral tracing shows connections of cerebellum with midbrain and basal ganglia reward centers. Yet, direct evidence in primates is limited. To address this, we used a 2×2 paradigm to differentiate how climbing fiber input separately encodes sensory and reward prediction errors (SPE, RPE). Two marmosets were trained to saccade to abstract visual stimuli linked to high or low rewards. In standard trials, they received the expected reward. Sensory prediction errors (SPE) were introduced by shifting target location. In negative RPE trials, a high-reward target was replaced with a low one; in positive RPE trials, the reverse occurred. Some trials combined both SPE and RPE. We recorded from lobule VI & VII of the vermis and isolated 900 Purkinje cells. To assess effects of reward on saccade kinematics, we measured reaction time (RT), peak velocity, end-point accuracy, and vigor. High-reward targets led to faster, more vigorous, and more accurate saccades than low-reward ones. Positive RPEs further enhanced speed and accuracy, while negative RPEs slowed responses and increased errors, especially in corrective saccades. These results suggest that reward expectations enhance motor performance, leading to faster and more accurate movements. To examine cerebellar encoding of prediction errors, we analyzed complex spike (CS) activity of Purkinje cells. Baseline CS rates were ~1 Hz, rising to ~2 Hz during saccades to high-reward targets, but not low-reward ones. At saccade offset, positive RPEs increased CS rates by ~50%, while negative RPEs suppressed them. CS activity was strongest in each cell's CS-ON direction, particularly in positive RPE trials, suggesting directionally tuned encoding of reward prediction errors. Our results show that in the primate cerebellum, climbing fibers encode both SPE and RPE. These neural signals coincide with changes in saccade timing, speed, accuracy, and vigor. Directionally tuned CS responses to reward suggest a role in movement planning, extending the cerebellum's function beyond motor and sensory control.

Disclosures: **J. Doostmohammadi:** None. **N. Mohammadrezaei:** None. **M. Fakharian:** None. **A. Shoup:** None. **R. Shadmehr:** None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.05/V10

Topic: F.02. Cerebellum

Title: Selective Cerebellar Ultrasound Stimulation Differentially Enhances Cognitive and Motor Performance

Authors: *Y. CHEN¹, A. SANSARE², Y.-H. WANG¹, Y. LEI¹;

²Kinesiology, ¹Texas A&M Univ., College Station, TX

Abstract: The multi-domain contributions of the cerebellum to cognitive and motor behavior, and its extensive connections with the cerebral cortex, make it an optimal target for interventions aimed at improving human performance. However, current non-invasive cerebellar neuromodulation techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), struggle to selectively stimulate deeper motor areas of the cerebellum without concurrently affecting superficial regions involved in cognitive function. Transcranial focused ultrasound stimulation (TUS) offers a promising alternative, with its ability to precisely target specific brain regions. The objective of this study was to investigate whether selective TUS of the motor (posterior lobule) or cognitive (anterior lobule) cerebellar regions could differentially improve motor and cognitive behavior. We conducted a sham-controlled study in young adults, who were randomly allocated to either a motor stimulation, cognitive stimulation, or sham group. Motor performance was measured using an auditory tapping task, where participants synchronized key presses with a periodic auditory tone sequence. Cognitive changes were assessed using the 2-back task (verbal letters or object shapes), where participants determined if a stimulus matched one seen two items back. A 0-back task served as a control. Our results revealed distinct effects: a single session of active TUS to the posterior lobe significantly improved reaction time on the auditory tapping motor task compared to sham. Similarly, a single session of active TUS to the anterior lobe significantly improved accuracy on the 2-back task compared to sham. Importantly, improvements in the tapping motor task following posterior lobe stimulation were not accompanied by improvements on the 2-back test. Conversely, improvements in 2-back performance following anterior lobe stimulation were not accompanied by improvements on the tapping task. These findings demonstrate that selective cerebellar neuromodulation can be achieved using TUS, enabling specific improvements in motor function without affecting cognition when motor areas are targeted, and vice versa for cognitive areas. Overall, these results support the neuroplastic potential of the cerebellum for restoring motor and cognitive function and identify it as a promising target for future interventions.

Disclosures: Y. Chen: None. A. Sansare: None. Y. Wang: None. Y. Lei: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.06/V11

Topic: F.02. Cerebellum

Support:

NIH R37-NS128416

Title: Identifying potent vectors for the neurons of the cerebellum**Authors:** *H. Y. ELSEWEIFI¹, M. FAKHARIAN¹, A. SHOUP¹, P. HAGE¹, R. SHADMEHR²;¹Biomed. Engin., ²Dept Biomed. Eng, Johns Hopkins Univ., Baltimore, MD

Abstract: The contribution of neurons to control of movements is often understood at the population level. In this framework, each neuron's activity affects behavior via a potent vector, an estimate of the downstream influence of that neuron on behavior. In the cerebral cortex, these vectors are currently found by fitting a linear model that maps neuronal activity to behavior. In the cerebellum, however, the potent vector for a Purkinje cell (P-cell) can be identified via its climbing fiber input (complex spikes, CSs). This input can act as a stochastic perturbation by completely suppressing the simple spikes (SSs), allowing for spike-triggered averaging that identifies how that P-cell's activity affects movements. Thus, in the cerebellum, finding potent vectors benefits from this special biophysical and anatomical property. However, can we identify the potent vector for neurons that lack such a robust perturbation mechanism? For example, is there a way to find potent vectors for P-cells for which we have not recorded CSs? If such a procedure could be found, it may generalize to other regions of the brain and allow for finding of potent vectors without fitting to linear models. Here, we re-examined our previously recorded electrophysiological data, comprising 270 P-cells (154 definitive; 116 putative with no CS) recorded from the oculomotor vermis in lobules VI and VII of the cerebellum in two marmoset monkeys (*Callithrix jacchus*) as they performed goal-directed saccadic eye movements. Rather than relying on their CSs, we describe two novel methods for detecting "surprise events," defined as stochastic and transient changes in SS activity during movements that deviate from the expected statistics. We show that surprise SS pauses, which are not elicited by CSs, produce a force that pulls saccades along the same vector as encoded by CSs. Importantly, when the surprise SS event is an excessive burst, the recovered potent vector points in the opposite direction. We then use the fact that simultaneously recorded cells in the cerebellar cortex can be organized into clusters, or cliques, defined by the strength of their spike interactions, and that P-cells within the same clique tend to share the same potent vector. Remarkably, the recovered potent vector for even the putative P-cells matches the potent vector of the definitive P-cells within the same clique. In summary, our results reveal a robust approach for detecting the potent vector of P-cells in the cerebellum by finding events in which a neuron exhibited a stochastic, yet transient deviation from its expected neural activity. This technique provides a new way with which one may find potent vectors for neurons in the brain.

Disclosures: H.Y. Elseweifi: None. M. Fakharian: None. A. Shoup: None. P. Hage: None. R. Shadmehr: None.

Poster**PSTR342: Sensorimotor and Learning in the Cerebellum****Location:** SDCC Halls B-H**Time:** Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.07/W1

Topic: F.02. Cerebellum

Support: NIH Grant NS136954

Title: Predictive parallel fiber ramping activity facilitates LTD in the in vivo mouse cerebellum

Authors: *C. HANSEL;
Univ. of Chicago, Chicago, IL

Abstract: Classic cerebellar learning models suggest that synaptic strength at parallel fiber (PF) to Purkinje cell (PC) synapses is modified based on the timing of repeated PF and climbing fiber (CF) activation. However, the timing requirements observed in brain slice preparations do not strictly reflect those observed in vivo during associative learning. For instance, in slices, single-pulse PF+CF pairing delivered at 1Hz for 5min successfully evokes long-term depression (LTD) as long as the bath calcium concentration is high enough. Using a physiologically realistic ionic milieu, a prolonged interval and CF burst activity are required for LTD induction. To determine whether a similar protocol induces LTD in vivo, we used two-photon imaging to monitor jRCaMP7f-encoded calcium transients in PC dendrites of awake mice as a measure of postsynaptic activation. PF bundles and CF inputs were selectively activated using saline-filled glass pipettes. We found that single PF pulses paired with CF bursts, delivered at 1Hz for 5min, did not reliably induce LTD. In contrast, a burst of five PF pulses at 10Hz (400ms duration), with the last pulse coinciding with the onset of the CF burst, successfully induced LTD—even when delivered at 1Hz for only 1min. This prolonged PF input preceding the CF burst is reminiscent of recent studies showing that ramping granule cell activity often precedes anticipated reward in cerebellar circuits by hundreds of milliseconds. To mimic this ramping pattern more closely, we applied three PF pulses with 100ms intervals, followed by a fourth pulse after 50ms and a fifth pulse after 20ms, resulting in a total duration of 370ms. Repeating this ramping PF pattern at 1Hz for 1min, with each burst paired with CF burst, caused the most pronounced LTD. In contrast, a short burst—three PF pulses with 20ms intervals—paired with the CF burst (1Hz, 1min) did not cause LTD, indicating that the full ramping protocol, combining prolonged PF activity and final high-frequency burst, is most efficient to trigger LTD. Analysis of the first 10 sec of each conditioning protocol revealed that PF ramping elicited the largest calcium transients, followed by constant 10Hz burst, while single-pulse stimulation produced the weakest responses. Notably, only the ramping protocol caused observable calcium build-up during this initial period. Our findings suggest that PF ramping is an efficient, naturalistic pattern that facilitates LTD induction. Together, these findings suggest that subthreshold calcium accumulation, likely driven by PF ramping, lowers the LTD threshold.

Disclosures: C. Hansel: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.08/W2

Topic: F.02. Cerebellum

Support: R01 DC004154
R01 NS072406
3R01DC004154-22S1
NC-GB-CULM-00002796-02
R01 EY031972

Title: Evidence for cannabinoid-driven metaplasticity reversing learning deficits in a Fragile X Syndrome mouse model

Authors: *A. M. SHAKHAWAT, N. E. CANTU, J. L. RAYMOND;
Neurobio., Stanford Univ., Stanford, CA

Abstract: Altered synaptic plasticity is a hallmark of many neurodevelopmental disorders. In a mouse model of Fragile X Syndrome (FXS), enhanced associative long-term depression (LTD) at cerebellar parallel fiber-Purkinje cell (PF-PC) synapses is, paradoxically, associated with impaired motor learning (Koekkoek et al., 2005). We hypothesize that enhanced plasticity can impair learning through a history-dependent increase in its threshold—a concept known as metaplasticity (Bienenstock et al., 1982; Nguyen-Vu et al., 2017; Shakhawat et al., 2024). Here, we tested whether the recent history of LTD induction alters the capacity for subsequent LTD-dependent learning. More specifically, we tested whether pharmacological suppression of LTD during a period before training could restore subsequent learning in mice with excessive LTD. Mice with the Fragile X gene *FMR1* selectively deleted from cerebellar Purkinje cells (L7-Fmr1 KO mice) have enhanced PF-PC LTD but were selectively impaired on oculomotor learning tasks that are known to depend on PF-PC LTD. If this impairment reflects aberrantly high recruitment of LTD during the period before training inducing an increase in the LTD threshold, then transient suppression of LTD should lower the induction threshold and restore the capacity of the PF-PC synapses to support learning. To suppress PF-PC LTD, a single systemic dose of the CB1 receptor antagonist AM251 was administered 18-24 hours prior to training. This AM251 pretreatment rescued the oculomotor learning deficits in L7-Fmr1 KO mice. The rescue was specific to tasks known to depend on PF-PC LTD. AM251 pre-treatment had no effect on LTD-independent learning tasks, nor did it enhance learning in wild-type mice. Our results suggest that the history of LTD induction modulates the availability of synapses for further LTD-dependent learning, suggesting a role for threshold metaplasticity in regulating learning. This work highlights the relevance of CB1R-mediated signaling to FXS pathophysiology. More broadly, it underscores the role of recent experience in shaping the capacity for learning and the potential for therapeutic modulation of synaptic thresholds to reverse cognitive deficits in neurodevelopmental disorders.

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Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.09/W3

Topic: F.02. Cerebellum

Support: NIH Grant 5R01NS128054

Title: Dynamic Modulation of Complex and Simple Spikes in Reward-Based Cerebellar Learning

Authors: *S. ÖZTÜRK¹, M. E. HEMELT², C. HULL³;

¹Neurobio., Duke Univ. Sch. of Med., Durham, NC; ²Lampe Joint Dept. of Biomed. Engin., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; ³Neurobio. Dept, Duke Univ., Durham, NC

Abstract: The cerebellum utilizes climbing fiber (CF) driven complex spikes (Cspks) as error-based teaching signals to modulate simple spike (Sspk) output, thereby facilitating the learning of precise motor timing. Recent work has also revealed that the cerebellum encodes reward-based CF teaching signals, suggesting a broader role that extends beyond classical error-driven learning. In support of this idea, a recent study has shown that disruption of CF reward signals impairs reward-based learning, suggesting their critical contribution to cerebellar function. In the present study, we employed *in vivo* electrophysiological recordings using high-density silicon probes (Neuropixels) to investigate how reward-related teaching signals are represented in the cerebellum. We recorded neuronal activity during a reward-based higher-order Pavlovian conditioning task. Initially, Cspk responses were observed in response to an auditory tone (A1) that preceded the unconditioned stimulus (US). Following the introduction of a second auditory tone (A2) earlier in the trial structure, Cspk responses to A1 were attenuated, while novel Cspk responses emerged in response to A2, indicative of second-order neural learning. These findings reveal that Cspk responses are dynamically modulated by experience to encode reward-predictive sensory cues. Notably, Sspks that were temporally coupled to reward-responsive Cspks and exhibited minimal modulation in naïve animals underwent progressive refinement with learning, developing structured firing patterns aligned with rhythmic licking behavior. Overall, Cspk activity was modulated both in magnitude and temporal dynamics based on the hierarchical structure of sensory cues, while their paired Sspk activity underwent changes to reflect preparatory ramping and rhythmic motor output. Together, these results demonstrate that reward-driven complex spikes can represent higher order reward predictive cues, highlighting the cerebellum's contribution to predictive sensory processing. Moreover, simple spikes, by integrating input from their associated reward-driven complex spike pairs, are transformed to more precisely reflect motor output related to lick bouts, thereby supporting adaptive motor timing.

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Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

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Program #/Poster #: PSTR342.10/W4

Topic: F.02. Cerebellum

Support: NIH Grant DC002390
NIH Grant DC018061

Title: Dynamic encoding of motion and orientation by cerebellar Purkinje cells in the nodulus/uvula during voluntary behavior

Authors: *R. L. MILDREN, K. E. CULLEN;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: The cerebellar nodulus and uvula (NU) is known for its role in integrating vestibular signals from the semicircular canals and otolith organs to encode head motion and orientation relative to gravity. It has long been proposed that NU Purkinje cells implement internal forward models to suppress predictable, self-generated vestibular input, selectively encoding externally imposed, passive motion. However, this central tenet has not been directly tested in behaving primates. We recorded single-unit activity from Purkinje cells in the NU of two alert rhesus monkeys during passive head movements, active voluntary head translations and tilts, combined active-passive motion, and motor command-only trials in which head motion was prevented. Contrary to predictive coding models, NU Purkinje cells exhibited comparable firing rate modulation during active and passive movements, with matched sensitivities and phases. When active and passive motion occurred concurrently, neuronal responses reflected the total head movement in space, without distinguishing the source. In contrast, when neck motor commands were issued but movement was blocked, Purkinje cell activity remained unchanged, indicating no contribution from efference copy signals. Additionally, NU Purkinje cells reliably encoded both the dynamic and static components of changes in head orientation relative to gravity, mirroring responses during passive tilts. These findings demonstrate that NU Purkinje cells do not suppress reafferent input, but instead provide a stable, context-invariant representation of self-motion and orientation relative to gravity. This robust encoding likely supports essential vestibular functions—including postural stabilization, autonomic regulation, and spatial perception—and distinguishes the NU's computational role within the cerebellar vermis. Our results challenge long-standing internal model frameworks and redefine the contribution of the NU to sensorimotor integration during voluntary behavior.

Disclosures: R.L. Mildren: None. K.E. Cullen: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

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Topic: F.02. Cerebellum

Support: Simons Collaboration on the Global Brain 543031
National Institutes of Health R01 DC004154
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Title: Adaptive tuning of oculomotor integrator dynamics in mice

Authors: *A. P. ADAM, K. VU, B. ANGELES, S. JAYABAL, J. L. RAYMOND;
Neurobio., Stanford Univ., Stanford, CA

Abstract: Neural integration is a computational process that converts transient inputs into sustained neural activity, supporting various brain functions, including decision-making, working memory, and motor control. One specific example is the oculomotor integrator, which enables a subject to maintain the eye stably at a given position for an extended period, thereby supporting vision. Previous work in goldfish has shown that the oculomotor integrator can be adaptively modified using visual feedback (Major et al., 2004). Here, we developed training protocols for adaptively modifying the oculomotor integrator in mice. To create conditions that required the dynamics of the oculomotor integrator to be altered to stabilize the images on the retina, an optokinetic drum surrounding the mouse was moved at a velocity contingent upon eye position. The ratio of visual stimulus velocity to eye position during training (training gain) was varied, as was the range of eye positions around the null position that were excluded from training. For all training gains, visual feedback training designed to induce unstable oculomotor integrator dynamics was effective at 1) eliciting eye movements to track the visual stimulus during training and 2) inducing a learned increase in the instability of the oculomotor integrator as tested in the dark after training. In contrast, for training designed to induce leaky integrator dynamics, the tracking eye movements and learning induced were more variable across mice and across training parameters.

Disclosures: A.P. Adam: None. K. Vu: None. B. Angeles: None. S. Jayabal: None. J.L. Raymond: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

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Topic: F.02. Cerebellum

Support: ERC-CoG-866237
Simons Foundation 717106
FCT SFRH/BPD/119404/2016

Title: Nonlinear mixed selectivity in cerebellar Purkinje cells facilitates flexible decoding of locomotor coordination

Authors: J. E. RAMÍREZ-BURITICÁ, H. G. MARQUES, D. DUARTE, *M. R. CAREY;
Champalimaud Fndn., Lisboa, Portugal

Abstract: The cerebellum plays a crucial role in coordinating movement across the body. During locomotion, it is particularly important for establishing and maintaining interlimb coordination. However, it has not been clear how cerebellar activity could be read out to achieve this. We have performed cell-attached recordings of Purkinje cell activity from head-fixed mice locomoting on a freely rotating wheel, together with high-speed 3D tracking of limb and body kinematics. These recordings revealed that the vast majority of Purkinje cells simultaneously encode movements of multiple body parts to provide precise representations of temporal coordination across the body. We further found that many Purkinje cells are significantly modulated by non-linear interactions across limbs. Here, we took an encoder-decoder approach to understand how Purkinje cell population activity could be read out to control locomotor coordination. We simulated the firing rates of a pseudopopulation of Purkinje cells by summing the tunings of each neuron to the movement of each limb, plus a nonlinear interaction term across limbs, interpolated for the same segments of locomotor behavior. We then asked how well various features of locomotor behavior could be decoded from the population. We find that the kinematics of individual paws can be read out effectively from models that include sensitivity only to linear combinations of limbs. In contrast, nonlinear mixed selectivity greatly enhances the ability to decode interlimb coordination, especially interlimb timing and deviations from a canonical coordination pattern. Nonlinear mixed selectivity also facilitates the flexibility of decoding by reducing the representational distance across locomotor features. In conclusion, we find that the high prevalence of non-linear mixed selectivity across the Purkinje cell population helps optimize readouts of interlimb coordination by a simple linear decoder. Taken together, these findings can explain how the cerebellum flexibly coordinates whole-body movements for dynamic environments.

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Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

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Program #/Poster #: PSTR342.13/W7

Topic: F.02. Cerebellum

Support: NIH Grant R01NS128054
NIH Grant R01NS112917

Title: Climbing fibers selectively recruit disinhibitory interneurons to enhance dendritic calcium signaling in cerebellar Purkinje cells.

Authors: *F. SANTOS-VALENCIA¹, E. LACKEY³, A. NORTON⁴, C. HULL², W. REGEHR⁵;

¹Duke Univ., Durham, NC; ²Duke Univ., Durham, NC, ; ³Baylor Col. of Med., Houston, TX, ;

⁴Northeastern Univ., Boston, MA, ; ⁵Harvard Med. Sch., Boston, MA.

Abstract: Climbing fiber (CF) inputs to Purkinje cells (PCs) instruct plasticity and learning in the cerebellum. Paradoxically, CFs also excite molecular layer interneurons (MLIs), a cell-type that inhibits PCs and can restrict plasticity and learning. However, two types of MLIs with opposing influences have recently been identified: MLI1s inhibit PCs, reduce dendritic calcium signals, and suppress plasticity of granule cell to PC synapses, whereas MLI2s inhibit MLI1s and disinhibit PCs. To determine how CFs can activate MLIs without also suppressing the PC calcium signals necessary for plasticity and learning, we investigated the specificity of CF inputs onto MLIs. Serial EM reconstructions indicate that CFs contact both MLI subtypes without making conventional synapses, but more CFs contact each MLI2 via more sites with larger contact areas. Slice experiments indicate that CFs preferentially excite MLI2s via glutamate spillover. In agreement with these anatomical and slice experiments, in vivo Neuropixels recordings show that spontaneous CF activity excites MLI2s, inhibits MLI1s, and disinhibits PCs. In contrast, learning-related sensory stimulation produced more complex responses, driving convergent CF and granule cell inputs that could either activate or suppress MLI1s. This balance was robustly shifted toward MLI1 suppression when CFs were synchronously active, in turn elevating the PC dendritic calcium signals necessary for LTD. These data provide mechanistic insight into why CF synchrony can be highly effective at inducing cerebellar learning^{2,3} by revealing a critical disinhibitory circuit that allows CFs to act through MLIs to enhance PC dendritic calcium signals necessary for plasticity.

Disclosures: F. Santos-Valencia: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.14/W8

Topic: F.02. Cerebellum

Title: Heterogeneous synaptic dynamics extend the bandwidth of sensory-motor representations and facilitate temporal learning in a cerebellar cortex model

Authors: *A. MAHDAVI OMASELAN OLYA^{1,2}, A. BARRI³, D. A. DIGREGORIO¹;

¹Dept. of Physiol. and Biophysics, Univ. of Colorado, Anschutz Med. Campus, Aurora, CO;

²Dept. of Physiol. and Biophysics, Univ. of Colorado, Anschutz, Aurora, CO; ³Inst. Pasteur, Paris, France

Abstract: Dynamic patterns of neural population activity support precise timing of motor behavior. In the cerebellum, granule cells (GCs) are thought to generate high-dimensional spatiotemporal representations of sensory-motor contexts, enabling well-timed associative learning. Yet the mechanisms by which GCs produce such temporally rich dynamics remain incompletely understood. Recent work from our lab showed that heterogeneity in short-term synaptic plasticity (STP) at mossy fiber-granule cell (MF-GC) synapses can endow a feedforward network with temporal structure, enabling the generation of Purkinje cell (PC) pauses in a model of delay eyelid conditioning when MF inputs are static (Barri et al., 2022). Complementary modeling suggests that temporally structured MF activity alone can also support GC dynamics required for learning (Gilmer et al., 2022). However, whether STP heterogeneity enhances learning in the presence of dynamic MF inputs remains unknown. To address this, we estimated trial-averaged MF firing rate dynamics during a forelimb reach-to-grasp task. We used these patterns to drive a two-layer rate-based perceptron model with either static or STP-modulated synapses. Synaptic weights between GCs and PC were trained using gradient descent. Learning performance was evaluated as a function of the spectral overlap between GC responses and target PC activity. We found that successful learning required GC responses to match or exceed the spectral content of the target signal. Compared to homogeneous or absent STP configurations, diverse STP profiles preserved high temporal sparsity and extended the bandwidth of GC activity. This broadened the range of GC thresholds, yielding accurate learning and reduced output error. In effect, STP heterogeneity increased the robustness and flexibility of the GC basis set. These results demonstrate that combining dynamic MF inputs with heterogeneous STP produces richer GC dynamics, enabling more effective encoding of temporally precise PC activity in tasks such as delay conditioning and reach execution. Our findings provide a mechanistic understanding of how synaptic diversity enables flexible and accurate temporal learning in the cerebellum.

Disclosures: A. Mahdavi Omaselan Olya: None. A. Barri: None. D.A. DiGregorio: None.

Poster

PSTR342: Sensorimotor and Learning in the Cerebellum

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR342.15/W9

Topic: F.02. Cerebellum

Support: R01 MH093727-07A1

Title: State-dependent reconfiguration of mossy fiber sensory representation during cerebellar learning

Authors: *X. CHEN¹, J. F. MEDINA²;

²Dept. of Neurosci., ¹Baylor Col. of Med., Houston, TX

Abstract: Locomotion profoundly modulates neuronal activity across the brain, including sensory cortices where it alters response gain and enhances perceptual performance. In the cerebellum, locomotion has been shown to accelerate learning in sensorimotor tasks such as eyeblink conditioning. The prevailing hypothesis is that this enhancement arises from a locomotion-induced elevation in the baseline “tone” of mossy fiber inputs, thereby facilitating cerebellar plasticity. However, the underlying circuit mechanisms remain unclear. Here, we used high-density Neuropixels probes to examine how locomotion affects sensory encoding in cerebellar mossy fibers during eyeblink conditioning. Mice were trained under conditions that required them to either remain stationary or locomote on a running wheel while we recorded mossy fiber activity across training phases. Behavioral performance was strongly state-dependent: as in previous studies, performance was higher during locomotion than during stationary conditions, but mice trained while stationary performed poorly when switched to locomotion, and vice versa. At the neural level, locomotion elevated baseline mossy fiber activity but did not simply modulate sensory response gain. Instead, it triggered a striking reconfiguration of sensory responses at the level of individual mossy fibers: some responded only during locomotion, others only when mice were stationary, and some reversed their response polarity across states. Trial-to-trial variability increased during locomotion, resulting in lower SNR of single mossy fibers. Despite this increased variability, population-level analyses revealed enhanced stimulus encoding during locomotion. Post-stimulus trajectories in PCA space were longer during locomotion periods, and baseline and post-stimulus trajectories were close to orthogonal in both conditions. Critically, the trial-to-trial variability along the post-stimulus trajectory was comparable across states. These findings imply that locomotion increases the separation between baseline and stimulus-evoked activity at the population level without increasing noise along the stimulus-relevant axis, thereby improving the effective SNR of the population code. Together, our results demonstrate that locomotion reshapes sensory representations in the cerebellum by creating a distinct neural context, which may account for the strong state-dependence of behavioral performance. Although noisier at the level of individual mossy fibers, locomotion enhances the separability of population responses in a way that may facilitate faster learning and improve cerebellar function.

Disclosures: X. Chen: None. J.F. Medina: None.

Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR343.01/W10

Topic: F.04. Voluntary Movements

Support: JSPS JP23H05488
JSPS JP21K17633

Title: Motor adaptation to optogenetically induced internal reflex errors in primate reaching

Authors: *A. KOSUGI¹, M. KUDO¹, S. EGAWA^{1,2}, K.-I. INOUE³, M. TAKADA^{3,4}, K. SEKI¹;
¹NCNP, Tokyo, Japan; ²Kyushu Univ., Fukuoka, Japan; ³Kyoto Univ., Aichi, Japan; ⁴Osaka Univ., Osaka, Japan

Abstract: A key question in motor adaptation is whether the motor system identifies the error sources and adapts accordingly. Previous studies suggest that the brain performs credit assignment: if the error arises from internal factor, such as changes in body dynamics, it may update internal model; if the error is external, such as imposed force, it may adapt motor command. However, since most studies have focused on externally imposed errors, this credit assignment framework remains largely untested in the context of internally generated errors. Here, we developed a novel paradigm in which a reflex-based perturbation via optogenetics during a monkey's reaching movement, enabling investigation of adaptation to errors from internal spinal reflex pathways. First, we examined the optimal serotype and administration route of adeno-associated virus (AAV) vectors for selective targeting of large-diameter primary afferents associated with tactile and proprioceptive sensations in common marmosets. Immunohistochemistry revealed that intravenous injection of the capsid variant of a serotype-9 AAV vector (AAV-PHP.B) selectively transduced channelrhodopsin 2 into large-diameter primary afferents. Next, we tested the effect of optogenetic modulation on voluntary movement. Two animals were trained to perform a visually-guided reaching task. During the task, blue light was delivered via an optical fiber to the medial elbow to transdermally stimulate median and ulnar nerves. When stimulation was triggered by EMG activity of the agonistic muscle for reaching (triceps brachii [TB]), animals exhibited undershot reaching and failed to reach the target. This was due to a significant reduction in TB activity, with short-latency (<20 ms) inhibition after stimulus onset, suggesting recruitment of heteronymous inhibitory reflex. Finally, we tested how the animal adapts to this reflex-based perturbation. After 20 reaches, undershooting diminished, and the animals reached the target, indicating motor adaptation. This was due to a significant increase in TB activity after adaptation, compensating for reflex-induced inhibition. Notably, the inhibition magnitude remained unchanged, suggesting reflex gain was unaffected. Instead, ECoG recordings showed a significant increase in high-gamma band power in the motor cortex, indicating a change in motor command. These findings suggest that animals adapt to internally generated errors by adjusting motor commands. This indicates that the brain failed to identify the error as arising from internal spinal reflex pathway, and therefore did not update internal model, instead treating it as if it had arisen externally.

Disclosures: A. Kosugi: None. M. Kudo: None. S. Egawa: None. K. Inoue: None. M. Takada: None. K. Seki: None.

Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR343.02/W11

Topic: F.04. Voluntary Movements

Support: RF1 NS132913

Title: Comparison of neural population activity between mPFC and M2 in a reach-to-grasp task

Authors: *Z. WANG, L. HE, K. GANGULY;
Univ. of California, San Francisco, San Francisco, CA

Abstract: Motor skill acquisition not only refers to the refinement of movements with practice but also includes the understanding of contextual information, which is a comprehension of how to act and when to act. Previous studies have demonstrated that motor cortex plays a key role in the establishment and execution of motor skills involving prehension, while prefrontal cortex encodes abstract rules and associations among cues, actions and outcomes. However, little is known about how these two adjacent cortex regions are functionally distinguished and connected during sensorimotor learning. Here, using calcium imaging, we compare the population firing patterns of medial prefrontal cortex (mPFC) and secondary motor cortex (M2) in mice performing a reach-to-grasp task. We show that mPFC has longer timescales of neural activity, particularly during inter-trial intervals between reach-to-grasp actions, while M2 has higher movement-modulated activity, suggesting their distinct contributions to motor skill learning. Moreover, using an antegrade tracer, we show that mPFC directly sends projections to M2. Interestingly, by conducting recordings on neurons identified using retrograde GCaMP labeling in mPFC, we notice that M2 projection neurons in mPFC appear to “transfer” information such as cue timing and the history of trial outcome (success versus failure). Together, these results suggest a model in which neural activity with different timescales distributed across mPFC and M2 enables the refinement of when and how to act during skill learning.

Disclosures: Z. Wang: None. L. He: None. K. Ganguly: None.

Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR343.03/W12

Topic: F.04. Voluntary Movements

Support: A Grant-in-Aid for Scientific Research from MEXT to RY (KAKENHI (B) no. 21H02798)

Title: Inactivation of caudal pontine reticular nucleus (PnC) impairs hand dexterity in reach-and-grasp tasks in non-human primates

Authors: *Y. SUN, R. YAMAGUCHI, T. ISA;
Dept. of Neurosci., Kyoto Univ., Kyoto, Japan

Abstract: Dexterous finger control is important for daily hand functions in nonhuman primates and humans. Maintaining balanced muscle strength and tone is necessary for finger precision,

but its control mechanisms are less clear compared to the mechanisms of fractionation of individual fingers, primarily attributed to the corticospinal tract. Recent evidence suggests that the reticulospinal neurons (RSNs) plays a critical role in this process. In this study, we used two healthy nonhuman primates (*Macaca fuscata*) and recorded single-unit activity to localize the startle-reaction neurons, presumably the RSNs, in the caudal pontine reticular formation (PnC). The identified location was consistent with previous studies. Based on this anatomical information, we manipulated the RSNs by microinjections of muscimol, a GABA_A receptor agonist, into the PnC to investigate the contributions of the RSNs to hand dexterity. In addition, we developed an innovative, markerless 3D kinematic reconstruction method using DeepLabCut™ to quantitatively analyze behavioral changes in reach-and-grasp tasks before and after muscimol injection. Our findings revealed substantial impairments in hand dexterity without obvious posture change following muscimol injection, including increased grasping time and impaired thumb-index finger coordination during various grasping tasks. Specifically, based on monkeys' strategies across tasks, we found that the distance between the tips of the index finger and thumb during the pre-shaping phase changed distinctly across the monkeys. Also, we observed that the thumb abduction movement decreased after muscimol injection in both monkeys during the pre-shaping phase, which may reflect a reduction in antigravity muscle tone. These results may suggest that the RSNs modulate fine finger control by coordinating the strength and tone of finger muscles. Future investigations of the physiological mechanisms underlying these effects will further clarify the RSN's contributions to sensorimotor integration and motor control of dexterous movements.

Disclosures: Y. Sun: None. R. Yamaguchi: None. T. Isa: None.

Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR343.04/W13

Topic: F.04. Voluntary Movements

Support: HKRGC- GRF 14112423
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HKRGC- CRF C4012-22GF
CityU SGP Grant 9380157

Title: A paradigm of complex movement sequence learning in mice

Authors: *Y. ZHENG^{1,2}, J. HUANG², Y. KE², W. YUNG¹;

¹City Univ. of Hong Kong, Hong Kong, China; ²The Chinese Univ. of Hong Kong, Hong Kong, China

Abstract: Complex motor skills typically involve linking multiple basic movements into defined, rapid sequences with precise transitions between movement components. Disruptions in

sequential motor control are common in brain disorders such as Parkinson's disease and stroke, even when the ability to perform simple, fragmented motor acts is preserved. Despite its importance, the neural mechanisms underlying sequential motor learning remain incompletely understood. As a first step to tackle this question, we developed an original dexterous motor task in which mice learn to pull a lever through a trajectory involving multiple sequential movements with defined turning points. The task design involves gradual shaping: initially training mice to perform straight pulls, followed by incremental introduction of turning phases and systematic tightening of temporal constraints on movement initiation and execution. Over a four-week training period, mice demonstrated significant improvements in task performance, with success rates increasing to nearly 100% and cue-to-reward response times decreasing to about 1 second. Lever trajectory analysis showed enhanced smoothness and consistency as learning progressed. Fine-scale forelimb movements, including elbow, paw, and finger positions, were tracked using DeepLabCut, providing detailed kinematic profiles that revealed progressive refinement in movement strategies. This paradigm is compatible with neuronal recording techniques for deciphering the neural mechanisms underlying the learning of complex movement sequences.

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Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR343.05/W14

Topic: F.04. Voluntary Movements

Title: Beyond reward: dopaminergic effort prediction errors for guiding energy-efficient movement

Authors: *Q. A. LI¹, Q. CHEVY², S. REN³, R. SHADMEHR⁴, A. KEPECS⁵;

¹WashU in St. Louis, St Louis, MO; ²Washington Univ. Sch. of Med., Providence, RI;

³Washington Univ. Sch. of Med., Saint Louis, MO; ⁴Dept Biomed. Eng, Johns Hopkins Univ., Baltimore, MD; ⁵Neurosci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: From predators pacing their chase to ensure a successful hunt, to migratory birds optimizing wingbeats to reach distant breeding grounds, longevity and reproductive success depend on choosing actions that yield sufficient benefit for their energetic cost. Producing such actions requires the brain to anticipate both the associated reward value and physical effort cost. While dopamine in the ventral striatum supports reward-based learning via reward prediction errors, how does the brain learn to predict the effort cost of an action? Here, we show that the nigrostriatal dopamine pathway to the dorsolateral striatum (DLS-DA), which degenerates in Parkinson's disease, encodes effort prediction errors that guide the search for movements that minimize effort. We developed a cued effort-based forelimb reaching task, where mice encountered external force-field that disrupted their preferred reach trajectories, leading to inefficient performance. Through trial-and-error, mice learned to minimize effort through

predictive steering to counteract the anticipated force field. This behavioral strategy was tracked by phasic DLS-DA release before reach onset, signaling expected effort and predicting movement vigor and success. While unexpected force-field changes occurred mid-movement, nigrostriatal dopamine encoded bidirectional effort prediction errors, reflecting deviations between predicted and actual effort. These errors acted as teaching signals to guide proportional updates of effort expectation and movements in the subsequent trial. Our findings expand prediction error coding beyond reward, revealing parallel dopamine systems for learning about rewards and movement costs, with implications for disrupted effort processing underlying motor deficits in Parkinson's disease.

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Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

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Topic: F.04. Voluntary Movements

Support: VA Grant I01RX001640
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Title: Condition-invariant oscillations in correlated spiking activity link cortical and subcortical regions during reaching in mice

Authors: *C. L. WOODARD¹, K. GANGULY²;

¹Univ. of California San Francisco, San Francisco, CA; ²Neurol., UCSF, San Francisco, CA

Abstract: The mammalian brain relies on a distributed network of cortical and subcortical regions, including the motor cortex, basal ganglia, cerebellum, and brainstem, to generate and refine voluntary movements. These structures contribute uniquely to motor planning and execution via distinct descending pathways, such as the pyramidal and extrapyramidal tracts. While their individual roles have been extensively studied, evidence suggests that effective motor control also requires dynamic coordination across these regions at fast timescales. For instance, learning of skilled motor and brain-machine interface tasks has been linked to enhanced oscillatory coupling between primary motor cortex (M1) and dorsolateral striatum (DLS). However, the specifics of this inter-area coordination are still unclear, and it is not known whether similar mechanisms extend to other subcortical structures involved in extrapyramidal control of movement. Moreover, it is yet unresolved whether cross-region coupling reflects a general-purpose action network or is finely tuned to specifics of movement (e.g. reach direction). To address these questions, we performed high-density electrophysiological recordings in mice engaged in a directional reaching task, targeting M1, DLS, and the medullary reticular formation (MedRF) of the brainstem. As animals learned the task, we observed the emergence of robust,

reach-related spiking correlations between M1 and subcortical regions that were largely independent of reach direction. Preliminary results from electromyographic (EMG) recordings in forelimb muscles (triceps and biceps) revealed that oscillatory components of this inter-regional coupling were temporally aligned with muscle recruitment during reaching. These findings suggest that learning promotes the formation of a broadly coordinated action network spanning cortical and subcortical structures. Understanding the structure and dynamics of this network is essential for informing the design of brain stimulation therapies aimed at restoring voluntary movement after neurological injury.

Disclosures: C.L. Woodard: None. K. Ganguly: None.

Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR343.07/X1

Topic: F.04. Voluntary Movements

Support: 1R01DA060790

Title: Motor commands embedded in neural activity of cerebellar and motor cortex populations during reach assessed through physiological muscle simulations

Authors: *J. GILMER;

Univ. of Colorado - Sch. of Med., Aurora, CO

Abstract: Dexterous motor control requires well timed, coordinated, and accurate movements. To achieve successful movements, a multitude of motor centers within the brain and spine coordinate activity to plan and execute descending commands. While there is a longstanding effort to understand movement through the lens of neural recordings during behavior, it is often difficult or impossible to capture the full suite of ongoing muscle activity. Therefore, discrimination between planning, predicting, or commanding motor activity in high-level motor control regions of the brain is impeded by our lack of knowledge concerning muscle patterning. We developed a musculoskeletal model of the murine forearm based on optical dissection of muscular anatomy and validated the activity of muscle patterning using reach kinematics with paired electromyographic (EMG) recordings (*Gilmer et al. 2025*). Here, we leverage the simulation of limb musculature to examine population neural recordings from primary motor cortex (MCX) and cerebellar cortex (CBX) during reaching movements to determine what degree of motor command-like activity arises in the neural populations of these structures. We replicated recorded kinematics in our forelimb model and then used the resulting predictions of muscle patterns to align neural activity, both in a trial-by-trial manner and in the mean activity of cell activity. We found that principal cells of MCX and CBX (pyramidal and Purkinje cells, respectively) were well aligned to activity of muscles involved in limb movements, such as *biceps brachii*, and that individual movement variation in muscle activity timing and amplitude

had a covariant relationship to neural firing patterns and rate. These results suggest that subpopulations within motor control structures may perform distinct roles, such as directing individual muscles or coordinating muscle synergies, during the generation of movement. Although physiological muscle activity tends to be highly variable, estimation of mean muscle patterning can be valuable to finding relationships between neural activity and movements by examining them through the lens of descending motor commands, and may help researchers to further our understanding of the role of motor regions of the brain in producing behavior.

Disclosures: J. Gilmer: None.

Poster

PSTR343: Reaching Control: Motor Learning: Animal

Location: SDCC Halls B-H

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Topic: F.04. Voluntary Movements

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Eric and Wendy Schmit AI in Science Fellowship
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Title: Local synaptic potentiation underlies the reorganization of cortical circuit dynamics during motor learning

Authors: ***W. J. WRIGHT**¹, E. C.-Y. CHEN¹, B. ZHOU⁴, J. WANG², Z. LIU⁴, B. MORALES⁴, T. KOMIYAMA³;

²Neurobio. Department, Sch. of Biol. Sci., ³Ctr. for Neural Circuits and Behavior Rm 304,

¹UCSD, La Jolla, CA; ⁴Neurobio., Univ. of California, La Jolla, CA

Abstract: A fundamental function of the brain is to control the flexible acquisition of new behaviors through learning. For this to occur, learning must induce modifications within neural circuits that alter their output in a way that ultimately leads to the generation of a novel behavior. Synaptic plasticity is hypothesized to fulfill this role by reshaping synaptic inputs to transform neural output in response to specific stimuli. However, changes in neural activity may arise from several different processes, such as shifts in inhibition, altered intrinsic excitability, or may simply be inherited from upstream inputs. Therefore, the precise role synaptic plasticity plays in the reorganization of neural circuit activity during learning remains unclear. Here, we used a combination of optical tools to block the induction of long-term potentiation (LTP) and *in vivo* two-photon calcium imaging to determine how synaptic plasticity reorganizes the activity dynamics of L2/3 pyramidal neurons in the primary motor cortex (M1) of adult mice as they

underwent motor learning. We found that blocking the induction of LTP within M1 prevented mice from developing a stable, stereotyped movement pattern over the course of training, demonstrating a critical role for local LTP within M1 for motor learning. We also found that blocking LTP disrupted the reorganization of activity dynamics of L2/3 pyramidal neurons. Specifically, we found that blocking LTP prevented the emergence of reproducible spatiotemporal activity patterns during the execution of the learned movements. The emergence of this reproducible activity pattern was associated with a specific increase in the functional connectivity between movement-encoding neurons, which was also disrupted by blocking LTP. Overall, these findings suggest that local synaptic plasticity within M1 acts to reorganize neural circuit activity into reproducible spatiotemporal dynamics that contribute to the acquisition of stable movement patterns over the course of learning.

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Poster

PSTR343: Reaching Control: Motor Learning: Animal

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Program #/Poster #: PSTR343.09/X3

Topic: F.04. Voluntary Movements

Support: ERC-2012-SyG_318987
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JHS AddOn Fellowship

Title: Optogenetic restoration of neuron subtype-specific cortical activity ameliorates motor deficits in Huntington's Disease mice

Authors: *S. BLUMENSTOCK^{1,2}, D. ARAKELYAN¹, N. A. DEL GROSSO², S. SCHNEIDER², Y. SHAO¹, E. GJONI¹, R. KLEIN², I. DUDANOVA³, T. KOMIYAMA¹; ¹Ctr. for Neural Circuits and Behavior, UCSD, La Jolla, CA; ²Max Planck Inst. for Biol. Intelligence, Martinsried, Germany; ³Fac. of Med. and Univ. Hosp. Cologne, Ctr. of Anat., Cologne, Germany

Abstract: Huntington's disease (HD) is a devastating movement disorder without a current cure. Although the monogenic basis of HD is well-defined, the complex downstream effects that underlie behavioral symptoms are poorly understood. These effects include cortical dysfunctions, yet the involvement of specific cortical excitatory and inhibitory neuronal populations in HD remains largely unexplored. To dissect the contributions of major cortical neuron subtypes, we used longitudinal *in vivo* two-photon calcium imaging to examine the activity of three cortical inhibitory neuron (IN) subtypes and excitatory corticostriatal (CStr) projection neurons in the motor cortex of R6/2 and zQ175DN HD mouse models throughout disease progression and during both spontaneous and skilled motor behaviors. Using pose estimation and behavior

classification, we show that gait and behavioral state dynamics in HD mice progressively diverge from healthy controls. Furthermore, we found that motor deficits in HD mice were accompanied by neuron type-specific abnormalities in movement-related activity, including hypoactivity of vasoactive intestinal peptide (VIP)-INs and CStr neurons. Optogenetic activation of VIP-INs in R6/2 mice restored healthy levels of activity in VIP-INs and their downstream CStr neurons and ameliorated motor deficits in R6/2 mice. Our findings highlight cortical INs as a potential therapeutic target for HD and possibly other neurological diseases.

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Poster

PSTR343: Reaching Control: Motor Learning: Animal

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Topic: F.04. Voluntary Movements

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Simons Collaboration on the Global Brain Pilot Award

Title: Targeted stimulation of motor cortex neural ensembles drives learned movements

Authors: *Q. CHEN^{1,2,3,4}, A. WU^{1,2,3,4}, B. YU^{1,2,3,4,5}, S. CHAE^{1,2,3,4}, Z. TAN^{1,2,3,4}, A. RAMOT^{1,2,3,4}, J. ALJADEFF^{1,2,4}, T. KOMIYAMA^{1,2,3,4,6},
²Neurobio., ³Neurosciences, ⁴Ctr. for Neural Circuits and Behavior, ⁵Electrical and Computer Engin., ⁶Halicioğlu Data Sci. Inst., ¹UC San Diego, La Jolla, CA

Abstract: During the execution of learned motor skills, the neural population in the layer 2/3 (L2/3) of the primary motor cortex (M1) expresses a reproducible spatiotemporal activity pattern. It is debated whether M1 actively participates in generating this activity pattern and whether this learned pattern causally drives the learned movement. To address these questions, we utilized in vivo two-photon calcium imaging combined with holographic optogenetic stimulation of specific M1 L2/3 neuronal ensembles in mice performing a skilled lever-pressing task. We briefly and synchronously stimulated ~20 neurons whose activity onset in voluntary trials precedes movement onsets. This stimulation, despite lacking temporal patterns, induced movements that resembled the learned movement, while producing spatiotemporal activity patterns in non-target M1 neurons that resembled the activity during the voluntary learned movement. Crucially, the effectiveness of this stimulation was highly task-dependent, often

failing to generate any movement outside the task context. Moreover, trial-by-trial variability of optogenetically triggered population activity in the non-target neurons correlated with the variability in the induced movements. These trial-by-trial variabilities were predicted by the initial state of M1 population activity immediately preceding the optogenetic stimulation. Stimulation of the neurons whose activity followed voluntary movement onsets, or those not related to movement, failed to induce the learned movement. Taken together, the learned activity pattern in M1 L2/3 can be generated when the M1 network is prepared at the optimal initial state and receives precise triggering inputs, supporting the active role of M1 in learned activity generation. The resulting activity pattern then drives the learned movement in a context-specific manner.

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Poster

PSTR343: Reaching Control: Motor Learning: Animal

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Program #/Poster #: PSTR343.11/X5

Topic: F.04. Voluntary Movements

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NIH Grant F32NS140212-01

Title: Learning-induced synaptic plasticity in corticospinal output neurons of motor cortex

Authors: *J. Y. LI¹, W. WRIGHT², T. KOMIYAMA³;

¹Univ. of California San Diego, San Diego, CA; ²Neurobio., ³Ctr. for Neural Circuits and Behavior Rm 304, UCSD, La Jolla, CA

Abstract: Motor learning is a fundamental feature of the brain that enables action to be informed by prior experience. During motor learning, movement patterns can be refined to give rise to skilled movements that effectively achieve a specific goal. Understanding how neural circuits are modified through mechanisms such as synaptic plasticity will provide important insight into how behavior can be adapted in light of changing environmental demands. Primary motor cortex (M1) is a key brain area involved in the learning and execution of skilled movements. Prior studies have demonstrated that motor learning induces robust changes in the population activity of neurons in deeper layer 5 (L5) of M1 that project to the spinal cord to drive movement (Peters et al., 2017). These L5 neurons exhibit synaptic plasticity during motor learning (Xu et al., 2010), but the rules that govern which L5 dendritic spines are enlarged or shrunken during motor learning have yet to be elucidated. Here, we test the hypothesis that learning induces selective synaptic plasticity at inputs to M1 L5 corticospinal output neurons (CSNs) that are involved in driving the task-related movement. To test this, we imaged synaptic glutamate activity and

dendritic calcium activity in the apical dendrites of L5 M1 CSNs over two weeks as mice learned a forelimb-based lever press task. M1 L5 CSNs were co-labeled with a glutamate sensor (iGluSnFr3) and calcium sensor (RCaMP2) via retrograde Cre injection in the spinal cord. Using previously validated approaches (Hedrick et al., 2022), we measured spine volumes to classify spines as undergoing structural LTD, LTP, or remaining stable. In support of our hypothesis, dendrites reliably active during rewarded lever pressing movements had significantly greater proportion of spines undergoing structural LTP. Spines that were subsequently enlarged were active more selectively during rewarded movements, compared to neighboring spines that underwent LTD. These features were not observed in M1 L2/3 neurons. Moreover, the plasticity rules that our lab has recently identified for layer 2/3 neurons (Wright et al., 2025) do not predict structural changes in L5 CSNs. Therefore, we conclude that cell-type specific synaptic plasticity at L5 CSNs has a distinct function in the M1 circuit which serves to reinforce recruitment of the subset of neurons that were active during successful movements.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR344.01/X6

Topic: F.07. Rhythmic Pattern Generation

Title: Characterizing Firing Profiles of Spinal V3 Interneurons During Drug-Evoked Rhythmic Locomotor-Like Activity

Authors: *R. MOLYNEAUX¹, N. SHAHSAVANI², K. C. COWLEY¹, J. W. CHOPEK¹;
¹Physiol. and Pathophysiology, ²Physiol. and pathophysiology, Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: V3 interneurons (INs) are a genetically defined population of glutamatergic spinal neurons implicated in regulating robustness of locomotion. Previous studies using computational modeling have postulated that V3 INs play a critical role in maintaining robust, coordinated alternation during rhythmic motor output. However, to date, no studies have directly recorded from identified V3 INs during rhythmic locomotor-like activity in the mammalian spinal cord. Therefore, the purpose of this study is to characterize the intrinsic electrophysiological properties and activity patterns of V3 INs during rhythmic motor activity using whole-cell patch-clamp recordings in Sim1Cre/TdTomato P1-P5 mouse spinal cord preparations. Electrophysiological recordings were made prior to and performed during pharmacologically induced tonic and rhythmic locomotor like ventral root (VR) activity via perfusion of serotonin (5-HT) and N-methyl-D-aspartate (NMDA). Analysis to date indicate that most V3 INs are quiescent at baseline, although some demonstrate spontaneous activity. Heterogenous responses were recorded from V3 INs drug evoked tonic VR activity. Specifically, following 5-HT and NMDA application and the emergence of tonic VR activity, most V3 INs recorded to date displayed an

increase in excitability. This was characterized by a depolarized resting membrane potential and an increased firing rate correlating with the onset of tonic activity in VR recordings. Conversely, some V3 INs demonstrated decreased activity during drug evoked tonic VR activity, suggesting functional diversity within the V3 INs recorded. Analysis is ongoing to examine whether firing patterns in recorded V3 INs correlate with either flexor or extensor-related discharge during rhythmic VR activity. These findings will provide critical insights into the cellular mechanisms and contributions of V3 INs to the generation and modulation of spinal locomotor circuits.

Disclosures: **R. Molyneaux:** None. **N. Shahsavani:** None. **K.C. Cowley:** None. **J.W. Chopek:** None.

Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR344.02/X7

Topic: F.07. Rhythmic Pattern Generation

Support: U01NS131086

Title: In Vivo Lumbar Spinal Recordings During Discrete and Rhythmic Movements

Authors: ***B. TEMPLE**¹, **Y. WU**², **J. ALJADEFF**³, **C. XIE**², **L. LUAN**², **S. L. PFAFF**¹;
¹Salk Inst., La Jolla, CA; ²Rice Univ., Houston, TX; ³Section of Neurobiology, Div. of Biol. Sci., UC San Diego, La Jolla, CA

Abstract: Assemblies of interneurons in the lumbar spinal cord regulate both rhythmic and discrete movements of the hindlimbs. However, historically, it has been challenging to monitor the activity of these cells in vivo because the spinal tissue moves considerably during behavior. Here, to address that challenge, we present extracellular recordings from lumbar interneurons acquired in freely-moving mice using a novel, ultrathin multi-channel electrode. We compare the spiking of neurons during locomotion to their activity during a brief perturbation to the position of one hindlimb. Clustering analyses on the population of bursting profiles suggest distinct functional categories within each behavioral context. Additionally, using optogenetic tagging, we label V1 spinal interneurons and their putative output targets. These early studies suggest within-behavior and within-interneuron class heterogeneity in ventral spinal circuits.

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Poster

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Program #/Poster #: PSTR344.03/X8

Topic: F.07. Rhythmic Pattern Generation

Support: (NSF) DBI 2015317

Title: A Continuous Neural Model for Rhythmic Locomotion Control

Authors: *C. B. JACKSON¹, R. D. QUINN², W. R. P. NOURSE²;

²Mechanical Engin., ¹Case Western Reserve Univ., Cleveland, OH

Abstract: Traditional modeling of the mammalian spinal cord relies on rhythmic activity generated by central pattern generators (CPG's) modeled as half-center oscillators. However, recent studies have shown that the neural activity in the spinal cord exhibits a wave-like propagation rather than a flip-flop behavior. In this work, we present a computational model of rhythmic network which produces this wave of activity using a non-spiking leaky integrator neural model. The model is designed as a winner-take-all network with modulatory effects which determine the speed and direction of the wave of activity. We first use the full network, composed of 50 interconnected nodes, to control a simplified musculoskeletal model of rat hindlimbs with antagonistic muscle pairs controlling the hip, knee, and ankle joints in the sagittal plane. The resulting joint trajectories exhibit stable, smooth, and coordinated walking behavior. We then reduce the network to a simplified two-node version that reproduces alternating activity, similar to that of a half-center oscillator. Finally, we implement a two-layer CPG model with half-center oscillators. The continuous network provides greater flexibility for modulation and enables finer control of limb dynamics. While this level of control is not strictly necessary for the simplified model with antagonistic muscle pairs, it becomes increasingly valuable when scaling to more complex musculoskeletal systems and tasks. These results suggest that continuously active spinal networks offer a more adaptable and biologically grounded framework for generating detailed locomotor behavior, particularly in systems requiring coordination across many muscles and joints.

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Chong Xie, Lan Luan, and Pavlo Zolotavin hold equity in NeuralThread Inc., an entity that commercializes electrodes.

Title: High density intraspinal recording with ultraflexible electrodes reveals heterogeneous neural dynamics of diverse limb movements in behaving animals

Authors: ***J. ZHANG**¹, L. DE LA PALMA PIEDRAS¹, S. WANG¹, Y. WU¹, P. ZOLOTAVIN¹, B. TEMPLE², C. THÖRN PEREZ², S. L. PFAFF², C. XIE¹, L. LUAN¹;
¹Rice Univ., Houston, TX; ²Salk Inst., La Jolla, CA

Abstract: The spinal cord contains a diverse array of genetically and functionally heterogeneous cell types that are highly interconnected and spatially intermingled, forming complex circuits that compute limb movements. From a computational perspective, motor control represents an enormous challenge, as even the simplest motor actions require precise coordination of muscle contraction timing and force across joints that have multiple degrees of freedom. However, understanding how the spinal cord processes motor commands for meaningful limb movements requires recording a large population of diverse cell types intraspinally during natural movements, which has been technologically challenging. Here we report intraspinal recording using ultraflexible nano-electronic threads (NETs) of 128 recording sites per thread to monitor neuronal activity in the spinal cord of awake mice under multiple hindlimb movements, including walking, trotting, and air-puff-induced reflex. The ultraflexibility of NETs minimizes tissue-electrode displacement, enabling high-quality single-unit isolation during free movement. Additionally, NETs can be co-sectioned with tissue, allowing precise post-mortem verification of electrode placement in the spinal laminae, enabling potential correlation between function and anatomical location. Our goal is to decipher the modularity vs. multi-functionality of spinal circuits, examining to what extent the same neurons or subpopulation participate in different limb movements. We analyze neural functions at three levels: single unit tuning, network connectivity, and population dynamics in the latent space. Our preliminary results suggest that the behavioral tuning of each single-unit is consistent across different behavioral contexts. At the network level, pairwise connections are composed of both shared subsets across behaviors and behavior-unique connections. Consistently, population dynamics in the reduced-dimensionality space reveal a heterogeneity of latent components in shared and behavior-unique subspaces. These preliminary results suggest that overlapping neuronal populations participate in multiple limb movements and coordinate dynamically with each other to support diverse motor patterns.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

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Topic: F.07. Rhythmic Pattern Generation

Support: NIH BRAIN U19NS137920
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Title: Revealing cell type-specific connectivity motifs in orofacial premotor circuits

Authors: *J. VINCENT¹, P. CHEN², J. BIRNBAUM³, S. KRICK³, J. TAKATOH⁴, M. MOYA³, F. WANG⁵, M. ECONOMO³;

²Biol., ³Biomed. Engin., ¹Boston Univ., Boston, MA; ⁴Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; ⁵Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Orofacial movements are the product of convergent, descending synaptic input from higher-level motor centers to lower-level premotor circuits in the intermediate and parvocellular reticular nuclei (IRN/PARN) of the brainstem. The IRN and PARN contain central pattern generators and complex feedback loops that, while often flexibly driven by higher-level motor centers, are also capable of organizing basic orofacial motor output independent of descending input. Yet, due in large part to a fundamental lack of knowledge about the cellular and circuit structure of the IRN and PARN, how these circuits integrate control signals from higher-level motor centers to produce flexible, goal-directed movements remains unknown. Progress in connectomic mapping of medullary reticular nuclei like the IRN and PARN has lagged behind other brain regions due to these structures' exceptional cellular diversity and cytoarchitectonic disorganization. Recently, landmark developments in spatial and single-cell whole-brain transcriptomic atlases have uncovered the precise transcriptomic identity and spatial arrangement of cell types within medullary reticular nuclei, enabling positive identification of cell types within these structures for the first time. Here, we use this information to develop a highly optimized gene panel for multiplexed fluorescence in situ hybridization (mFISH) capable of accurately discriminating up to 70 unique neuronal cell types in the IRN and PARN with just 24 genes. We combine this novel capacity for high-throughput cell type identification with cutting edge anterograde and retrograde trans-synaptic viral labeling constructs like AAV1, CTB, and pseudotyped rabies virus to answer fundamental questions about cell type-specific circuit structure within the IRN and PARN. Specifically, we identify cell type-specific motifs governing connectivity between the IRN and PARN and higher order motor centers (motor cortex, superior colliculus, substantia nigra, cerebellum), output motor nuclei (trigeminal, hypoglossal), and internally between contralateral hemispheres as well as pre-premotor and premotor neurons.

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Poster

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Title: Examining lumbar locomotor V3 neuron ascending projections to spinal cord and brainstem autonomic and motor centres

Authors: *C. CHACON, T. W. CHENG, N. SHAHSAVANI, K. C. COWLEY, J. W. CHOPEK;
Physiol. & Pathophysiology, Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Sustained locomotion and exercise require coordination between motor and sympathetic systems to maintain appropriate metabolic support and body homeostasis. Lumbar V3 interneurons (INs) are a genetically defined excitatory spinal neuron population important for maintaining stable and robust locomotion. We recently showed that V3 INs provide direct, excitatory synaptic input onto thoracic sympathetic preganglionic neurons, demonstrating the presence of an ascending excitatory coupling between lumbar locomotor and thoracic sympathetic autonomic circuitry. Here, we examined whether lumbar V3 INs also provide direct, ascending input to brainstem centres regulating autonomic and/or motor functions. To test whether brainstem nuclei receive rostrocaudal input from lumbar V3 INs, 1 μ L of AAV2/9-GFP anterograde (N=7) tracer was injected into rostral (L1-L2, n=3), intermediate (L3-L4, n=2) or caudal (L4-L5, n=2) spinal segments of adult Sim1CreTdTomato mice. Mice were post-operatively monitored for 3 weeks and then spinal cords and brainstems were dissected and harvested for analysis. We observed double-labelled V3 IN puncta (TdTom⁺/GFP⁺) in the rostral ventrolateral medulla (RVLM), important for sympathetic tone and locomotion. Investigation of double-labelled V3 IN puncta in other brainstem regions including the gigantocellular reticular nucleus (GRN) and the dorsal motor nucleus of the vagus (DMX), important for the initiation/regulation of locomotion and parasympathetic functions, respectively, are ongoing. In a second series of injections (same survival period of 3 weeks), 200-800 nL of AAV2-retrograde-GFP tracer (N=6) was injected into the GRN (n=3), the RVLM (n=1) or the DMX (n=2) to test which subpopulations of V3 INs project to brainstem regions important for motor and/or autonomic control. We observed retrogradely double-labeled V3 IN cell bodies (TdTom⁺/GFP⁺) across all lumbar segments (L1-L6), with projections from dorsal, intermediate and ventral V3 IN subpopulations. The distribution of projections from the different V3 IN subpopulations in the spinal cord to the GRN, RVLM and DMX nuclei are ongoing. Overall, these findings suggest that coordination and integration between motor and autonomic circuits exists at the spinal and brainstem level and ascending spinal projections contribute to this spinal-brainstem circuitry.

Ultimately, this ascending spinal-brainstem circuitry may provide feedback during ongoing locomotion to sustain locomotor activity and to fine-tune motor output.

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Poster

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Support: NIH grant R01HD099200
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Title: Brain Connectivity During Walking and Obstacle Avoidance in Persons with Multiple Sclerosis and Healthy Controls: Preliminary EEG Study

Authors: *F. AL-SHARGIE¹, M. GLASSEN², J. DELUCA³, S. H. SALEH⁴;
¹Rehabil. and Movement Sci., Rutgers Univ., Newark, NJ; ²New Jersey Inst. Of Technol., hillsborough, NJ; ³SVP, Kessler Fndn., West Orange, NJ; ⁴Rehabil. and Movement Sci., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Persons with multiple sclerosis (pwMS) face debilitating motor-cognitive impairments during real-world tasks like obstacle avoidance, yet the neural mechanisms underlying these deficits remain poorly understood. This study addresses this gap by characterizing effective connectivity and hemispheric asymmetry in pwMS (n=8) versus healthy controls (HC, n=9) during walking alone and unpredictable obstacle avoidance, using high-density electroencephalography (EEG). Directed functional connectivity was quantified via partial directed coherence (PDC) in beta (13-30 Hz), alpha (8-12 Hz), and theta (4-7 Hz) bands, with rigorous artifact rejection and blinding to group status during preprocessing. In healthy controls, obstacle avoidance increased connectivity in motor and cognitive regions including left central (LC), left temporal (LT), and right frontal (RF) regions, $p < 0.0014$. In contrast, pwMS demonstrated weaker and more localized connectivity, primarily in the left central regions (sensorimotor cortices) $p < 0.0013$, suggesting reduced efficiency in brain networks and compensatory mechanisms to maintain task performance. Further, pwMS showed left laterality toward the central region during both walking conditions compared to HC, $p < 0.05$. Correlational analysis revealed that connectivity during obstacle avoidance in HC positively correlated with comfortable walking speed ($r = 0.57$), indicating efficient neural pathways. In pwMS, connectivity showed a negative correlation with walking speed ($r = -0.65$), indicating compensatory but inefficient neural engagement. These findings reveal MS-related disruptions in dynamic network integration during complex walking, advancing our understanding of

compensatory mechanisms and providing a biomarker-driven foundation for targeted neuromodulation therapies to improve gait and functional independence.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

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Title: Innovations in spinal cord cell type heterogeneity during vertebrate evolution

Authors: Y. IGNATYEV¹, S. PAPADOPOULOS¹, M. SORETIC², J. YEUNG¹, A. LEVINE³, E. M. TANAKA⁴, M. I. GABITTO⁵, *L. B. SWEENEY¹;

¹Inst. of Sci. and Technol. Austria (ISTA), Klosterneuburg, Austria; ²ETH Zurich, Zurich, Switzerland; ³NIH, Bethesda, MD; ⁴Inst. of Mol. Biotech., Vienna, Austria; ⁵Allen Inst., Seattle, WA

Abstract: Four-limbed locomotion constitutes a highly conserved trait within vertebrates, with tetrapods sharing basic limb architecture and a suite of homologous motor behaviors, including sensory-driven escape responses and coordinated movements involved in feeding and reproduction. This conserved motor repertoire raises the fundamental questions of to what extent a shared spinal circuit architecture underlies motor control across tetrapods, and how it might have adapted for divergent, species-specific behaviors. Recent studies have detailed spinal cell-type architecture in mammals, best exemplified in mice and humans. However, a comparable, high-resolution atlas of spinal cell types in non-mammalian tetrapods is lacking. Here, we focus on one of the most primitive amphibians, the frog *Xenopus laevis*, and evaluate conservation of spinal cell-type architecture for limb locomotion between frogs, mice and humans. Across species, our analysis defines a core program of cell type specification during development, which segregates spinal neurons into nearly identical cardinal classes and subtypes in both amphibians and mammals. This starkly contrasts with adult stages, when spinal cell-type composition is similar at a coarse level but diverges at the level of subpopulations between frogs and mammals. Using spatial transcriptomics, we localize this species divergence to the superficial dorsal spinal cord, where variant neuropeptide expression defines mammalian-specific cell types. The dorsal spinal cord thus emerges as a recently evolved hub for sensory integration in mammals, a neo-spinal cord analogous to the neocortex.

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Poster

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Title: Mechanisms of sniff generation in the preBötzinger Complex

Authors: *B. THADARI¹, K. KAM²;

¹Neurosci., Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; ²Cell Biol. and Anat., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Breathing at rest (eupnea) is intermixed with slower and faster inspiratory patterns such as sighs and sniffs, each with a distinct function. A key region for inspiratory rhythm and pattern generation is the preBötzinger Complex (preBötC), located in the ventrolateral medulla. In the preBötC, a slower sigh rhythm can be elicited by bombesin-like neuropeptides; however, the mechanisms for generating faster breathing patterns, such as sniffing, remain unknown. We hypothesized that modulating ionic currents involved in burst generation is necessary for producing variations in burst shape and frequency that underlie sniffing and higher frequency breathing. We first focused on persistent sodium current (INaP), a major determinant of inspiratory burst shape in vitro. In rhythmically active transverse medullary slices (550-650 μm) from neonatal mice (P0-P5 of either sex) containing preBötC, bath application of the INaP blocker phenytoin (100 μM) decreased inspiratory burst duration and frequency. In some preparations, the slower inspiratory rhythm (freq. ~ 0.1 Hz) was interspersed with fast “runs” of 2-3 bursts resembling in vivo sniffs, which we therefore termed “fictive sniffs” (freq. ~ 1 Hz). With INaP blocked, robust fictive sniffing was elicited by norepinephrine (10 μM), a neuromodulator involved in arousal, attention, and exploratory behaviors. Other excitatory neuromodulators, such as Substance P, serotonin, and Neuromedin B, also had modest effects in generating fictive sniffs when applied with phenytoin. Decreasing extracellular K⁺ from 9 mM to 3 mM or bath application of the μ -opioid receptor agonist DAMGO (30 nM) increased the intervals between fictive sniff bouts, but did not affect the intra-sniff interval within a fictive sniff bout. Blocking hyperpolarization-activated cation current (I_h) with ZD 7288 (30 μM) abolished high frequency fictive sniffing, while the eupneic rhythm remained intact. We therefore challenge the longstanding view that sniffing engages eupneic rhythmogenic

mechanisms, merely driven faster. Instead, we conclude that sniffing and fast breathing rely on an I_h -dependent pacemaking mechanism, distinct from the emergent rhythmogenic mechanism for eupnea.

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Poster

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Topic: F.07. Rhythmic Pattern Generation

Support: NIH R01 HD90642

Title: The muscle spindle behaves as a tunable feedback controller during perturbed locomotion

Authors: *S. SIMHA¹, G. S. SAWICKI², T. C. COPE³, L. H. TING⁴;

¹Emory Univ., Atlanta, GA; ²Mechanical Engin., ³Applied Physiol. and Engin., Georgia Inst. of Technol., Atlanta, GA; ⁴Biomed. Engin., Emory Univ. and Georgia Tech., Atlanta, GA

Abstract: Why do we have two independent gamma motor drives (static, dynamic) to muscle spindles and what are the implications for motor control? Muscle spindles—sensory organs within skeletal muscles—receive two motor drives that are independently modulated from each other and from the alpha motor drive to the parent muscle. However, we are technologically limited from directly probing this sophisticated motor control system. Here, we investigated the role of each gamma motor drive during rhythmic movement. We use a biophysical computational model of muscle spindle Ia afferent firing that can generalize to a wide range of conditions. When the model was provided with the sinusoidal parent muscle length changes and Ia firing measured experimentally from cat locomotion, it predicted the patterns of each gamma motor drive measured empirically from the same experiment. The model reproduced the profound differences in Ia firing for the same parent muscle stretch during locomotion versus when the muscle is relaxed. Leveraging the model's generalizable nature, we demonstrate that independent gamma drives during locomotor-like conditions enable Ia firing to function as position-and-velocity-error feedback control, potentially explaining the functional role of Ia monosynaptic stretch reflexes and higher-order sensorimotor responses to perturbations. Specifically, we find that *gamma-dynamic* onset timing minimally affects Ia firing during unperturbed locomotion but, the empirically measured, *gamma-dynamic* activity during muscle shortening and silence at stretch onset increases Ia sensitivity to perturbation during leg swing. In contrast, the relative phase and magnitude of sinusoidal *gamma-static* activity in relation to muscle kinematics enables the mechanical computation of the error between the intended and actual muscle length and velocity. When modeling an external load causing the parent muscle length to deviate from the intended trajectory, the simulated Ia firing reflected an additional firing pattern that was predicted by a linear combination of error in position and velocity of the

muscle length, and tuned by *gamma-static* phase and amplitude. Such error-feedback tuning by *gamma static* drive can allow the nervous system to distinguish muscle length change that is due to self-generated motor activity versus an external load, serving as a potential signal to guide sensorimotor adaptation. At minimum, these differences in spindle Ia firing arising solely from varying gamma drives highlight that we cannot estimate muscle spindle firing without understanding how neural control sculpts muscle spindle firing in a task-specific manner.

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Poster

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Title: Identifying the optimal prestimulus phase from ongoing oscillations for effective intracranial stimulation of primary motor cortex

Authors: S. D'SOUZA¹, A. KO², J. A. HERRON³, J. OJEMANN¹, B. L. GRANNAN⁶, V. MARTINEZ⁴, J. T. MILES⁷, *K. E. WEAVER⁵;

²Neurolog. Surgery, ³Dept. of Neurolog. Surgery, ¹Univ. of Washington, Seattle, WA; ⁴Univ. of Washington, Seattle, WA, ; ⁵Radiology, Univ. of Washington, Seattle, WA; ⁶Neurolog. Surgery, Univ. of Washington Sch. Of Med., Seattle, WA; ⁷Seattle Children's Res. Inst., Seattle, WA.

Abstract: The commonly observed dominant frequency band within the broadband cortical spectrum varies regionally and is associated with dynamic variations in brain state, behavior, and basic physiology such as excitability and connectivity. Electrophysiological investigations exploring such parameters linked with idling cortical rhythmicity often focus on band-limited responses as defined by established, canonical frequency bands. For example, the phase of idling alpha band activity just prior to afferent stimulation is associated with enhanced detection of environmental stimuli and modulation of the motor evoked potential (MEP) through transcranial magnetic stimulation. In this effort, we examined the hypothesis that intracranial stimulation delivered at distinct phase cycles from specific motor cortical frequencies is associated with MEP response variability. In 12 movement disorders patients, we recorded from and delivered electrical stimulation to primary motor cortex (M1) through intraoperative electrocorticography (ECoG). MEP monopolar stimulation was delivered as a train of 5 pulses. 10 MEP trials were acquired at 6 MEP stimulation amplitudes around an established MEP threshold. EMG responses were captured through needle electrodes from upper-extremity muscles. Three categories of intrinsic a priori band-limited activity were identified from a separate 2-min resting-state period: 1) alpha / mu (8-12 Hz), 2) beta (13-30 Hz) bands, and 3) an individually identified, dominant frequency. Pre-stimulus phase angle was extracted for each stimulation trial by truncating at +50

ms following stimulation onset, applying bandpass-filter (fourth-order Butterworth IIR at each frequency category). The Hilbert transform established the phase angle at stimulation onset. This method was repeated for the dominant frequency response, established through a multi-taper spectral decomposition and removal of the background 1/f power spectrum. Positive local maxima in the resultant curves facilitated the identification of dominant peak widths from a 2 and 32 Hz response range. Preliminary results indicate that MEP stimuli delivered to the falling phase interval of the trough were most closely associated with larger peak-to-peak EMG amplitudes. Additionally, circular-linear correlation analyses indicate this association is strongest when coupled to phase components of the individually defined, data-driven dominant frequency band. These results will be discussed in the context of identifying optimal frequency and phase elements for parameter selection of intracranial protocols dependent on M1 stimulation, such as therapeutic neuromodulation.

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Poster

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Title: A neurorobotic model for exploring locomotor control after recovery from thoracic spinal cord injury

Authors: *A. B. LOCKHART¹, H. CHU^{2,3}, S. TATA RAMALINGASETTY¹, N. A. SHEVTSOVA¹, D. S. MAGNUSON⁴, S. M. DANNER¹;
¹Drexel Univ. Col. Of Med., Philadelphia, PA; ²Drexel Univ. Collge of Med., Philadelphia, PA;
³Drexel Univ. Col. of Med., Philadelphia, PA; ⁴Neurolog. Surgery, Univ. of Louisville, Louisville, KY

Abstract: Thoracic spinal cord contusion disrupts cervical to lumbar connectivity but leaves both enlargements intact. Despite this injury, rats recover locomotor function, though with reduced speed and altered speed-dependent gait expression. Our previous computational model of spinal locomotor circuitry reproduced these observed gait adaptations, linking them to impaired long propriospinal connectivity and lumbar circuitry reorganization, likely involving increased reliance on afferent feedback. To investigate the role of sensory feedback in locomotion and explore circuit reorganization post-contusion, we developed a neurorobotic model of quadrupedal locomotion in which the spinal circuitry was embedded in a body capable

of interacting with the environment. We extended our previous neural network model of spinal locomotor circuitry to control locomotion of a simulated Unitree Go1 quadrupedal robot. The model includes four rhythm generators, one per limb, interconnected by commissural and long propriospinal neurons. Activity from each rhythm generator controls a pattern formation network that coordinates muscle activation in each limb. Hill-type muscles convert this activation into torque to actuate the motors and allow for calculation of proprioceptive feedback, which interacts with all levels of the spinal circuitry. Connection weights of proprioceptive, vestibular, and pattern forming neurons are optimized using covariance matrix adaptation evolution strategy to produce adaptive locomotion. The optimized model produces stable locomotion across a range of target speeds. Integration of muscle states and environmental information through proprioceptive, cutaneous, and vestibular neurons allows the model to traverse rough terrain consisting of variable slopes and ground friction. To simulate contusion injury, we reduced the connection weights of inter-enlargement long propriospinal neurons, which resulted in altered gaits. The model provides a testbed for linking neuronal manipulations to changes in locomotion and behavior. By comparing locomotor gaits across models—those undergoing a second round of optimization post-contusion, those that have not, and experimental results from rats—we can identify and analyze critical neuronal connections involved in recovery. Using this approach, we will further investigate how circuit reorganization can contribute to locomotor recovery after thoracic spinal cord contusion.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR344.14/Y2

Topic: F.07. Rhythmic Pattern Generation

Support: Funded by Aligning Science Across Parkinson's [ASAP-020572] through the Michael J. Fox Foundation for Parkinson's Research (MJFF).

Title: Variability and Degeneracy In Simulations of Primary Motor Cortex Pyramidal Tract Neurons

Authors: A. NEWTON¹, D. W. DOHERTY², L. CHEN³, H.-Y. CHU⁴, *W. W. LYTTON⁵;

¹Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; ²Dept. of Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Pittsburgh, PA; ³Neurodegenerative disorder department, Van Andel Res. Inst., Grand Rapids, MI; ⁴Georgetown, Washington DC, DC;

⁵DHSU, Brooklyn, NY

Abstract: Excitability of layer 5B pyramidal tract (PT5B) neurons is reduced in the 6-OHDA parkinsonian mouse model. However there is substantial variability among voltage traces from

PT5B neurons, which makes it difficult to distinguish between neurons from healthy controls and 6-OHDA parkinsonian mice. We also suspect there is considerable degeneracy, where multiple configurations of ion channels can produce similar responses. This variability and degeneracy may be related to the susceptibility or robustness of cortical activity to Parkinson's disease (PD). We used BluePyOpt to optimize parameters of conductance-based multicompartment neuron models for individual fits to in vitro 6-level somatic current clamp data from PT5B neurons (120 from healthy controls, 16 from 6-OHDA parkinsonian mice). To explore potential degeneracy, we optimized parameters independently 20 times for the same experimental response to produce an ensemble of neuron models. As perturbations can reveal the underlying differences, we modeled the effects of both; low dose tetrodotoxin (persistent sodium channel blocker) and paxillin (big potassium channel blocker) and compared with experimental findings. To assess the potential role of variability in PD pathology, we tuned the synaptic responses and embedded these populations of neurons in a NetPyNE model of mouse primary motor cortex (M1). The network model was perturbed with changes to ion channel conductances to model the effects of norepinephrine, as well as increasing thalamic inputs, to switch between states of quiet restfulness and active movement.

Parameter optimization allowed simulations to reproduce many features of the in vitro voltage traces, capturing the excitability of both individual neurons and the population as a whole. Similar voltage traces could be produced with a wide variety of model parameters, with substantial variability in the contribution of calcium and dendritic currents. Such underlying degeneracy has been shown to play a role in the survival of invertebrates and may be relevant to varying resistance to parkinsonism or to other brain pathologies.

Disclosures: A. Newton: None. D.W. Doherty: None. L. Chen: None. H. Chu: None. W.W. Lytton: None.

Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR344.15/Y3

Topic: F.07. Rhythmic Pattern Generation

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NIH P51-OD011132 Emory National Primate Research Center
NIH R01NS121371 H.Y.C.

Title: Network dynamics in simulated primary motor cortex of 6-OHDA-treated mice

Authors: *D. W. DOHERTY¹, L. CHEN², Y. SMITH³, T. WICHMANN⁴, H.-Y. CHU², W. W. LYTTON⁵;

¹SUNY Downstate Hlth. Sci. Univ., Pittsburgh, PA; ²Dept. of Pharmacol. and Physiol., Georgetown Univ. Med. Ctr., Washington, DC; ³Emory Primate Center, Dept Neurol., Emory

University, Emory Natl. Primate Res. Ctr., Atlanta, GA; ⁴Dept Neurol, Emory Univ. Sch. Med., Atlanta, GA; ⁵Physiology/Pharmacology, DHSU, Brooklyn, NY

Abstract: Primary motor cortex (M1) layer 5B pyramidal tract neurons (PT5B) have decreased intrinsic excitability in mouse models of Parkinson's disease (PD). We previously demonstrated that reducing intrinsic PT5B excitability by the ~64% shown in 6-OHDA animal studies altered activity in an M1 circuit simulation, producing increased local field potential (LFP) beta-band power and increased PT5B spike rates. While increased beta band power is a signature of the pathophysiology of PD, in vivo recordings have reported decreased PT5B rates in PD animal models. We hypothesized that recent reports of decreased motor thalamic synaptic strength on layer 5 dendrites of PT5B neurons might resolve this discrepancy. To test this hypothesis, additional simulations were carried out. Our network model of M1 in the parkinsonian state was changed in two ways: 1. We altered ion channel densities in the multicompartment PT5B model to decrease excitability by 64%; 2. We decreased motor thalamic synaptic strength on PT5B by 50%. Under these conditions, PT5B spiking was reduced instead of increased. Rather than concentrated LFP beta band power around 15 Hz in the resting state (as found in the original model), the oscillatory power shifted to 18-30 Hz (~24 bursts/s). We thus demonstrate that a decrease in motor thalamic input to layer 5 dendrites of the PT5B neurons lowers spike rates in PT5B neurons in our model and increases beta band oscillations rates by 3-5 Hz while moderating the large increase in beta power seen when only decreased PT5B excitability is included in the M1 simulation.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

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Program #/Poster #: PSTR344.16/Y4

Topic: F.07. Rhythmic Pattern Generation

Support: NIH Grant NS134170

Title: Ionotropic Glutamate Receptors Differentially Regulate Spinal Locomotor Activity

Authors: A. LEWIS¹, S. HIGLEY¹, B. MERCIER², *M. A. MASINO³;

²Neurosci., ¹Univ. of Minnesota, Twin Cities, Minneapolis, MN; ³Univ. of Minnesota, Minneapolis, MN

Abstract: Spinal locomotor networks have the intrinsic capacity to convert unorganized excitatory drive into organized locomotor output, even in the absence of sensory input and higher-order input from the brain. Although it is well established that vertebrate spinal locomotor circuits are activated by glutamatergic-mediated excitation, our understanding of glutamatergic signaling is incomplete. Specifically, the roles that subtypes of ionotropic glutamate receptors

(iGluRs; *i.e.*, NMDARs and AMPARs) play in spinal locomotor circuit function need to be better defined. To address this gap in knowledge, we utilized a complement of tools, including optogenetic activation of spinal locomotor networks, pharmacology, and high-speed videography to investigate the roles of NMDARs and AMPARs in spinal locomotor network function. We compared the properties of locomotor activity before (Control) and during glutamate receptor antagonism (Treatment) in spinalized transgenic larvae expressing ChR2 in glutamatergic neurons. Photostimulation (10 s; 470 nm; ~17 mw/cm²/nm) was applied to induce locomotor activity every 10 min (t₀ - t₆) and high-speed videography was used to measure body/tail kinematics (locomotor properties) in tethered preparations. Within the control group, a significant increase in tail bend frequency ($F=7.56$, $p<0.001$) and a decrease in tail beat amplitude ($F=4.09$, $p=0.005$) were found. In response to NMDAR antagonism (50 μ M APV), significant decreases in the number of episodes ($F=3.94$, $p=0.003$), episode durations ($F=4.07$, $p=0.003$), number of tail bends ($F=7.42$, $p<0.001$), and tail beat amplitude ($z=-2.80$, $p=0.002$) were found. These results demonstrated that NMDA receptor antagonism reduced the power of locomotor output. In response to AMPAR antagonism (50 μ M CNQX), a significant decrease in tail bend frequency ($z=-2.68$, $p=0.005$) was found. In addition, AMPAR antagonism disrupted the rostrocaudal progression of activity. These results demonstrated that AMPAR antagonism reduced locomotor power (tail bend frequency) and perturbed metachronal locomotor activity. In summary, subtype glutamate receptor signaling differentially affects the power of locomotor output; NMDARs play a role in episode duration, episode number, and tail bend amplitude, while AMPARs play a role in tail bend frequency and coordination.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

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Program #/Poster #: PSTR344.17/Y5

Topic: F.07. Rhythmic Pattern Generation

Support: 1R21NS135780-01A1

Title: Differential expression of Dopamine 2-like receptors and levels of endogenous dopamine contribute to the maturation of locomotion in larval zebrafish

Authors: *B. MERCIER¹, M. L. BECKMAN², S. M. GARRAWAY³, M. A. MASINO⁴;

¹Univ. of Minnesota, Twin Cities, Minneapolis, MN; ²Augsburg Col. Biopsychology, Minneapolis, MN; ³Cell Biol., Sch. of Med., Atlanta, GA; ⁴Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The developmental refinement of gross motor skills, such as locomotion, is conserved across vertebrate species. Previously we reported, in larval zebrafish, that dopaminergic (DAergic) signaling through dopamine 4 receptors (D4Rs) was necessary for locomotor

maturation from an immature pattern (erratic, long duration swim episodes) to a mature pattern (goal-directed, short duration swim episodes) between 3 and 4 days post-fertilization (dpf) (Lambert et al., 2012). While the neurons that are the primary source of spinal dopamine (DA) are present as early as 1dpf and project the extent of the spinal cord by 3 dpf (Takada et al., 1988; Kastenhuber et al., 2010), and both DA transporter and tyrosine hydroxylase are present at 3 dpf (Xi et al., 2011), it is unclear why the maturation doesn't occur until 4 dpf. Here we tested the hypothesis that the maturation of locomotor activity was due to a change in D2-like receptor expression and/or a change in DA levels.

First, to test if functional DARs were present in immature larvae, NMDA was used to induce fictive locomotor activity in spinalized preparations. Locomotor properties were measured and compared to baseline. NMDA-only controls (n=6) were stable over the course of 60 min with no significant change in episodes (t=-2.6 p=0.051), episode duration (z=-0.73 p=0.56), or episode frequency (t=-2.0 p=0.11). Application of D4 receptor agonist (PD 168 077) (n=7) increased both the number of episodes, (t=-3.0 p=0.02) and episode frequency (t=-2.7 p=0.03) but decreased episode duration (t=3.1 p=0.02). Thus, DARs were present and functioned to transform the activity of immature larvae to mimic mature larvae. Next, to quantify DRD2-like mRNA transcript levels at different developmental stages, we performed qRT-PCR on clutch-matched 3 and 5 dpf zebrafish larvae (3 biological replicates, 2 technical replicates). Results showed that 3 dpf larvae had significantly lower transcript levels of most DRD2-like receptors (DRD2a t=3.0 p=0.02; DRD2b t=3.1 p=0.021; DRD4r-s t=4.3 p=0.01; DRD4a t=3.2 p=0.02; DRD4b t=3.5 p=0.01). Finally, to quantify DA levels at different developmental stages, we performed liquid chromatography-mass spectrometry on clutch-matched 3 and 5 dpf larval zebrafish (3 biological replicates, 4 technical replicates). DA levels were significantly lower in 3 dpf larvae (t=-13.6 p<0.001), which demonstrated that more DA was available for release in mature larvae. These results support the hypotheses that an increase in DA available for release and increase in DAR expression between immature and mature larvae correlate with the DAergic-mediated maturation of locomotor activity during development.

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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR344.18/Web Only

Topic: F.07. Rhythmic Pattern Generation

Title: Effect of early kainate receptor activation on behaviour and corticosterone levels in adult rats

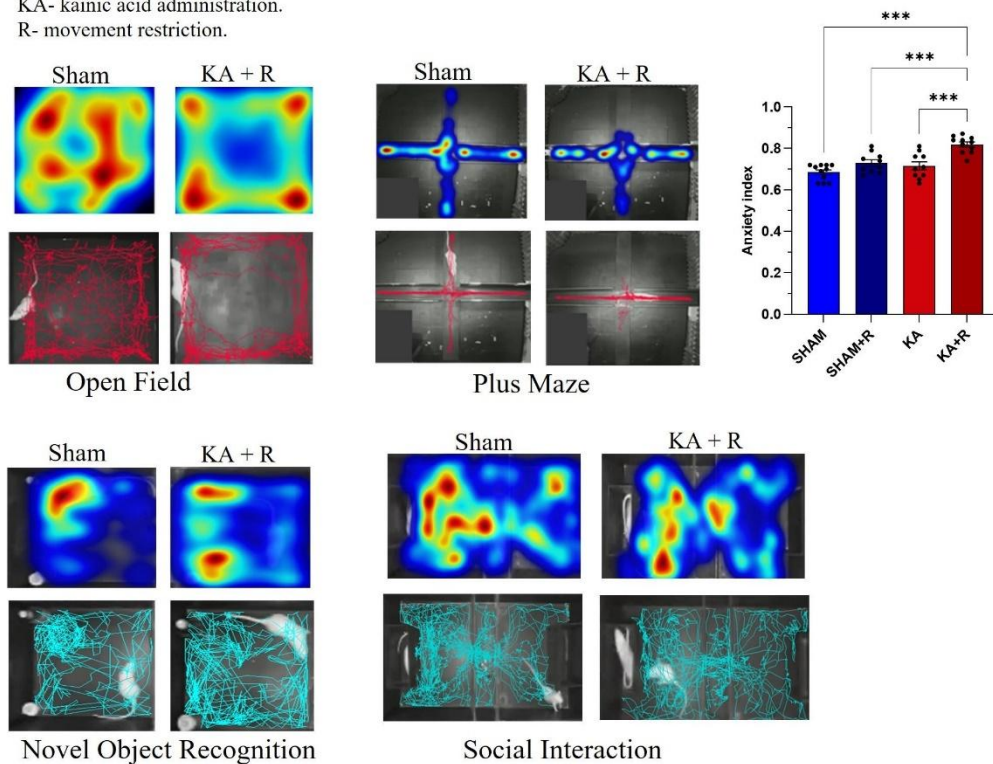
Authors: *L. GARCÍA MIRANDA^{1,2}, J. V. NEGRETE DIAZ²;

¹Dept. of Physiology, Biophysics and Neurosci., Ctr. for Res. and Advanced Studies of the Natl.

Polytechnic Inst. (CINVESTAV), Mexico City, Mexico; ²División de Ciencias de la Salud e Ingenierías, Univ. de Guanajuato, Celaya, Mexico

Abstract: Mental disorders are a current problem in Mexico and the world and are considered an important factor that impacts socially and economically. Unfortunately, few is known about the predisposition to mental illness in childhood and about the triggers that start these behaviors. The stress vulnerability model in neurodevelopment focuses on critical stages of neuronal development and the importance of events that modify behavior and establish mental illness. Using this model in rats, two hits are performed: the first, at seven postnatal days of the rat, homologous to second/third trimester of pregnancy human; this performing a neuromodulation of a kainate-type glutamatergic receptor, inhibiting glutamate release in a reversible manner, and at twenty-eight postnatal days, which is comparable to human preadolescence; through a low-stress event performed with movement restraints. Behavioral tests were performed in an adult stage evaluating affective and cognitive processes, as well as locomotion, as well as chronic stress evaluated in hair using corticosterone concentration. It was found that inhibition of glutamate release at neonatal age childhood and low stress in adolescence for a prolonged period, results in impairments in recognition memory, learning, locomotion and leads to anxiety-like behavior, and an increase in chronic stress levels. These results provide further information about a novel and functional model of stress vulnerability in neurodevelopment through glutamatergic modulation of the kainate-type receptor, in addition to contributing to the understanding of the importance of mental health in childhood and adolescence.

KA- kainic acid administration.
R- movement restriction.



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Poster

PSTR344: Rhythmic Pattern Generation Mammals and Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR344.19/Y7

Topic: F.07. Rhythmic Pattern Generation

Support: NIH BRAIN U19NS137920
NIH R01NS121409
NSF CAREER 2239412

Title: Cortical and Subcortical Descending Control of Motor Initiation and Adaptation

Authors: *Y. HAN, S. ZYLKA, M. ECONOMO;
Boston Univ., Boston, MA

Abstract: Goal-directed behavior emerges from the continuous mapping of time-varying inputs onto flexible motor outputs. While the parameters of this mapping can be distributed across multiple layers of the brain's hierarchy, a key outstanding question is how descending motor pathways flexibly shape motor outputs. Here, we simultaneously recorded the activity of descending neurons in the motor cortex, midbrain, and cerebellum that project to low-level motor circuits in the medulla and spinal cord and tested how perturbation of these neurons affects flexible parameters of movement. We examined the descending control of orofacial movements in mice performing a head-fixed licking task in which animals adapted their movements across two contexts: when movements are self-initiated or linked to an external cue. We found that mice exhibited kinematically distinct forms of movement initiation across contexts and rapidly adjusted the direction, amplitude, and duration of individual licks within a licking bout. Our analysis showed that a large portion of subcortical descending neurons exhibited rapid modulation around movement initiation and remained persistently tuned to ongoing kinematic changes throughout a licking bout, whereas many cortical descending neurons were more slowly modulated and relatively insensitive to changes in ongoing kinematics. Optogenetic inhibition experiments revealed that subcortical descending pathways provide both online input drive and directional control signals to their medullary downstream. Together, our results suggest distinct roles for cortical and subcortical descending pathways in motor control, emphasizing the online contributions of subcortical pathways to motor initiation and adaptation.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

Location: SDCC Halls B-H

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Program #/Poster #: PSTR345.01/Y8

Topic: F.07. Rhythmic Pattern Generation

Support: INBRE 2P20GM103423

Title: The effects of temperature and modulation on a small motor network

Authors: *D. J. POWELL, C. GARCIA, Y. ALTUG;
Bowdoin Col., Brunswick, ME

Abstract: Nervous systems must be able to maintain function despite fluctuations in ambient temperature. Because temperature affects the kinetics of all cellular processes (e.g., ion channel function), it is not immediately clear how temperature compensation is achieved at the level of single neurons or whole circuits (i.e., how does a nervous system maintain stability despite temperature change). This is particularly true for organisms that do not homeostatically regulate their internal temperature. In some cases, nervous systems are exposed to fluctuations $> 10^{\circ}\text{C}$ in a day. The nervous systems of the American lobster (*H. americanus*) can maintain function across a wide range of temperature ($\sim 7\text{-}30^{\circ}\text{C}$). Neuromodulators (e.g., amines, peptides, hormones, and gases) can influence this temperature range such that some modulators limit this range while others extend it. Here we use the cardiac neuromuscular system of the American lobster; this includes the cardiac ganglion (CG), a nine-neuron circuit that generates the heartbeat, and the associated cardiac muscles that the CG motor neurons innervate. The heart will continue to beat after being dissected from the animal so long as physiological saline is continuously perfused through it. For these experiments, we perfused the neuromodulatory peptide myosuppressin (dissolved in physiological saline) at four temperature steps (7, 10, 13, and 16°C) to understand how temperature influences the cardiac response to this modulator. We chose myosuppressin as it modulates cardiac muscle physiology and CG output differently. Here we show how the temperature dependencies of these hearts differed in physiological saline (control) as well as myosuppressin across this temperature range. Interestingly, the hearts of animals collected in the winter showed temperature compensation in control while the myosuppressin modulated heartbeat was not temperature compensated. However, hearts collected from summer animals were not temperature compensated in control, and myosuppressin enabled a temperature compensated heartbeat.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

Location: SDCC Halls B-H

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Program #/Poster #: PSTR345.02/Y9

Topic: F.07. Rhythmic Pattern Generation

Support: NIH NINDS R15-NS128619 (DMB)
NIH NINDS NS029436 (MPN)

Title: Modulation of CPG feedback changes rhythm generation locus

Authors: B. GNANABHARATHI¹, M. J. COLEMAN², T. AKAY³, M. P. NUSBAUM⁴, *D. M. BLITZ¹;

¹Miami Univ., Oxford, OH; ²Natural Sci. Dept., Pitzer and Scripps Colleges, Claremont, CA;

³Dept. of Med. Neurosci., Dalhousie Univ., Halifax, NS, Canada; ⁴Dept. of Neurosci., Perelman SOM, Univ. Pennsylvania, Philadelphia, PA

Abstract: Central pattern generator (CPG) networks underlying rhythmic behaviors can produce distinct outputs due to descending projection neuron activation. Projection neurons also receive feedback from their target CPG, and changing the feedback strength can switch CPG output (Coleman & Blitz, SfN 2024). We now test the hypothesis that altering CPG feedback strength also changes the spatial distribution of the rhythm generating circuit, enabling a distinct rhythm generation mechanism. We examined this issue using the gastric mill (chewing) CPG in the stomatogastric ganglion (STG) of the crab *Cancer borealis*. Many gastric mill rhythm versions are driven by the reciprocally inhibitory rhythm generator neurons LG and Int1. Int1 also has a long-distance inhibitory feedback synapse onto projection neuron CPN2, the strength of which is modulated by sensory pathways to elicit distinct CPG outputs (Blitz, J Neurophys 2017; Coleman & Blitz, SfN 2024). CPN2 drives LG activity (Blitz, 2017). To test if the spatial rhythm generator distribution is distinct between different modulatory states, we blocked the Int1 to LG synapse in the STG by injecting 4M KAcetate into Int1 (Coleman et al, Nature 1995). When this synapse was blocked, a similar gastric mill rhythm was still elicited if Int1 to CPN2 feedback was in the strong state (LG cycle period $p=0.47$, LG burst duration $p=0.68$, $n=5$). However, no gastric mill rhythm was elicited when Int1 to CPN2 feedback was in the weak state ($n=3/3$). We hypothesized that, in the Feedback-strong state, the half-center rhythm generator is not necessary because the rhythm generator circuit also includes the Int1 to CPN2 feedback synapse, enabling Int1 to indirectly inhibit LG. To test this idea, in the Feedback-weak state, with the Int1 to LG synapse blocked and no gastric mill rhythm, we increased Int1 activity to enhance its feedback inhibition of CPN2. If the Int1 inhibition was sufficient to trigger CPN2 rebound bursts as in the Feedback-strong state (Blitz, 2017), a Feedback-strong gastric mill version was initiated ($n=3/3$). Returning Int1 activity below that necessary to trigger CPN2 rebound eliminated the gastric mill rhythm ($n=3/3$). This indicates a shift from the Int1:LG half-center rhythm generator to a tri-synaptic Int1:CPN2:LG circuit when Int1 to CPN2 feedback is strong. We further found that the redistribution of rhythm generation has functional consequences. In the Feedback-strong state, CPN2 activity drove stronger motor neuron activity ($p=0.004$, $n=6$), which elicited larger muscle electrical responses ($p<0.001$, $n=7$). Thus, distinct modulatory states can use different rhythm generating circuits for different CPG outputs.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR345.03/Y10

Topic: F.07. Rhythmic Pattern Generation

Support: R35 NS097343

Title: A new variant synaptic release model for characterizing temperature-driven changes in synaptic plasticity

Authors: *J. ZENG, K. JACQUERIE, E. E. MARDER;
Brandeis Univ., Waltham, MA

Abstract: Synapses alter their responses to stimuli in a history-dependent manner. Short-term plasticity is commonly studied using paired-pulse or train stimuli, which require long recordings to capture a broad dynamic range. An alternative approach employs Poisson stimulation to construct a phenomenological model (Sen et al., 1996). To investigate how synaptic processes are influenced by temperature, we develop a model to extract synaptic properties from a crustacean neuromuscular junction responding to Poisson stimuli. We use the neuromuscular synapses of the LG neuron to the gastric muscles gm5b and gm6 in the crab *Cancer borealis*. Stimuli are applied to the nerve lvn, and excitatory junctional potentials (EJPs) are recorded intracellularly. EJPs evoked by 0.1 Hz and Poisson stimuli are fit with a model incorporating the following synaptic parameters: initial probability of synaptic vesicle release (p_0), facilitation time constant, depression time constant, a simplified term representing scaled presynaptic calcium (Ca^{2+}), and the Hill coefficient for cooperativity of calcium binding. Facilitation is modeled as the accumulation of presynaptic Ca^{2+} and its cooperative binding with the synaptic vesicle release machinery. Calcium increases stepwise following each stimulus and decays exponentially with a facilitation time constant. The release probability (p) follows a Hill equation as a function of Ca^{2+} , and the facilitation factor is calculated as p/p_0 . Depression is modeled as a result of vesicle depletion and retrieval. The depression factor decreases stepwise after each stimulus and recovers exponentially with the depression time constant. The net peak of each EJP is modeled as the product of the baseline EJP peak, the facilitation factor, and the depression factor. The absolute peak amplitude of each EJP is the sum of the residual decaying tail from the previous EJP and the net peak of the current pulse. We fit the model to EJP traces at multiple temperatures to quantify synaptic parameters that define facilitation and depression, using a genetic algorithm. Although both gm5b and gm6 are innervated by the same LG neuron, they exhibit distinct synaptic properties. At 11 °C, gm5b displays significantly higher facilitation time constant, depression time constant, and initial release probability compared to gm6. Despite these baseline differences, both muscles show similar temperature-dependent responses: increasing temperature reduces both facilitation and depression time constants, while the initial release probability increases. This model provides a valuable tool for comparing synaptic properties across different conditions.

Disclosures: J. Zeng: None. K. Jacquerie: None. E.E. Marder: None.

Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

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Program #/Poster #: PSTR345.04/Y11

Topic: F.07. Rhythmic Pattern Generation

Support: BAEF

Title: Temperature sensitivity of the neuromuscular junction of the crab *Cancer borealis*

Authors: *K. JACQUERIE¹, J. DIMARTINO¹, A. POGHOSYAN¹, A. DALAL¹, D. J. SCHULZ², E. MARDER¹;

¹Brandeis Univ., Waltham, MA; ²Div. of Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO

Abstract: Understanding how warming affects animal physiology is critical as ocean temperatures rise at unprecedented rates. Our study focuses on the stomatogastric nervous system of the crab *Cancer borealis*, which includes the pylorus and gastric mill responsible for filtering and grinding food. Muscle contraction in this system depends on several temperature-sensitive components, from ion channels and neuromuscular junctions to the contractile machinery. Temperature influences all of those processes and challenges the maintenance of stereotyped neuronal activity essential for digestion. Previous studies show that pyloric and gastric rhythm frequencies of the nerve activity increase with temperature while preserving phase relationships (Tang et al. 2012), until the rhythm “crashes” at extreme temperatures. Here, we extend this work to the neuromuscular junction and muscle contraction to identify the system’s weak link—the least resilient component from neural activity to muscle output. We first simultaneously record STG nerve activity and excitatory junctional potentials (EJPs) in gastric and pyloric muscles (gm5a, gm5b, gm6, cpv4, cpv6, p1, p2) during acute temperature ramps until the nerve activity crashes. Then, we record nerve-evoked EJPs using 2-second stimulation trains from 1 to 10 Hz across temperatures. We use a new variant of a synaptic release model to compare facilitation and depression, characterizing temperature-dependent changes in presynaptic calcium dynamics and vesicle depletion and retrieval. Finally, we record nerve-evoked contraction in gm5b/gm6 using realistic spike trains recorded at 11°C (low-frequency) and 21°C (high-frequency), as well as in cpv4-6 and p1. We observe (i) a decrease in EJP amplitude, (ii) a consistent hyperpolarization of ~8-10 mV across a 10°C increase (11°C to 21°C), (iii) faster facilitation and depression kinetics, and (iv) reduced contraction. The EJP amplitude reduction correlates with a decrease in muscle input resistance. Two-electrode voltage clamp recordings from gm5b revealed a temperature- and pH-sensitive potassium current. PCR confirmed the expression of two-pore domain K⁺ channels homologous to TASK-1 and TASK-3. Notably, high-frequency stimulation, mimicking warmer STG activity, partially rescued contraction at 21°C, suggesting that increased neuronal drive can compensate for temperature-induced reductions in muscle performance and enhance neuromuscular robustness.

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Poster

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Program #/Poster #: PSTR345.05/Y12

Topic: F.07. Rhythmic Pattern Generation

Support: NIH Grant R90 DA060341
NIH Grant R35 NS097343

Title: Effects of temperature acclimation at the neuromuscular junction in the crab *Cancer borealis*

Authors: *J. M. DIMARTINO¹, K. JACQUERIE¹, A. DALAL¹, D. J. SCHULZ², E. E. MARDER¹;

¹Biol., Brandeis Univ., Waltham, MA; ²Div. of Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO

Abstract: Temperature influences all biological processes and presents a challenge in maintaining stereotyped activity within neuronal circuits. As ecosystems change with the warming climate, understanding the effects of temperature fluctuations on neural systems becomes increasingly important. Our study focuses on the stomatogastric nervous system (STNS) of the crab *Cancer borealis*, a model for studying temperature robustness in neural circuits. The STNS, responsible for generating chewing and filtering rhythms for digestion, comprises well-identified neurons and muscles. Previous studies show that while the baseline rhythm at 11°C is preserved and frequency increases during temperature ramps, long-term acclimation extends the functional temperature range by shifting the crash point to higher temperatures (Tang et al., 2012). We expand this research to determine how acclimation shapes neuromuscular junction (NMJ) properties and muscle contractility. Crabs were acclimated to 4°C, 11°C, or 18°C for four weeks. At 11°C, we record nerve-evoked excitatory junctional potentials (EJPs) in the gastric mill muscle 5b (gm5b) using 2-second trains at 1, 3, 5, and 10 Hz. Two-electrode voltage clamp recordings show that (i) input resistance is 50% lower in 4°C-acclimated animals compared to 18°C-acclimated animals, (ii) both first and last EJP amplitudes decrease about 30% with 4°C acclimation, and (iii) warmer-acclimated animals display longer EJP decay time constants (100, 150, and 200 ms for 4 °C, 11 °C, and 18 °C acclimated animals, respectively). To assess the temperature sensitivity of the NMJ, we increase the temperature and observe a decrease in EJP amplitude along with a hyperpolarization of the resting membrane potential (about -8 mV) for all animals. These effects are consistent with the activation of temperature-sensitive two-pore potassium (KCNK) channels, recently identified in this muscle (Jacquerie et al., 2025). However, PCR analysis reveals a 50% downregulation of KCNK channel expression in 18 °C-acclimated crabs compared to those acclimated to 11 °C. We next use a force-displacement transducer to measure contraction dynamics in gm5b and gm6 using realistic spike trains recorded in LG neuron at 11°C and 21°C. Preliminary results suggest that the temperature for optimal contraction shifts toward the acclimation temperature. Finally, neurotransmitters are directly applied to the muscle across temperatures to isolate acclimation effects on the contractile machinery. This integrative approach reveals how plasticity across neural, synaptic, and muscular levels supports functional resilience.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

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Program #/Poster #: PSTR345.06/Y13

Topic: F.07. Rhythmic Pattern Generation

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Title: Sifamide modulates muscles of the stomatogastric system of the crab *Cancer borealis*

Authors: *A. POGHOSYAN, K. JACQUERIE, J. ZENG, E. E. MARDER;
Brandeis Univ., Waltham, MA

Abstract: Neuromodulation is one of the many methods crustaceans use to maintain homeostasis in their oceanic environments. SIFamide is one of these neuromodulators that has been identified in the stomatogastric nervous system of the *Cancer borealis* crab, particularly involved in the crab's regulation of its gastric functions and movements, including digestion. The two isomers of SIFamide, VYRKPPFNGSIFamide (Val1-SIFamide) and GYRKPPFNGSIFamide (Gly1-SIFamide), have previously been tested on the cardiac ganglion and heart muscle of the *Cancer borealis* and increase the frequency and amplitude of heart contractions. In addition, Val1-SIFamide applied on the stomatogastric ganglion of the *Homarus americanus* increased the burst amplitude and duration in the rhythm produced by the PD neuron. Yet, the role of SIFamide at the neuromuscular junction (NMJ) of the gastric and pyloric muscles of the *Cancer borealis* is unknown. In these experiments, we studied the effects of SIFamide on the gastric and pyloric muscles of *Cancer borealis*. We investigated how the NMJ is modulated by SIFamide using its naturally occurring isomer (TRKLPPFNGSIFamide from *Aedes aegypti*) by applying it to a preparation of muscles. SIFamide was dissolved in physiological saline solution at varying concentrations between 10^{-9} M and 10^{-6} M. Modulation induced by the concentrations of peptide was monitored in gastric muscles gm5b, 6ab, innervated by the lateral gastric neuron, and pyloric muscle cpv1a, innervated by the pyloric dilator neuron, from the crab's stomatogastric system. The recorded excitatory junction potentials (EJPs) produced by the muscles at 11°C were a result of nerve stimulation at 1Hz, 3Hz, 5Hz, and 10Hz via a glass suction electrode. SIFamide increased the amplitude of the EJPs produced by the muscles. In addition, we have seen increased muscle contractility in higher concentrations. We conclude that SIFamide increases muscle activity at the neuromuscular junction, initiating larger EJPs to create muscle contractions. By understanding the modulation that occurs at the NMJ by peptides such as SIFamide, we will have a better understanding of how neuropeptides modulate the muscle response of crustaceans in a variety of environmental conditions.

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Poster

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Program #/Poster #: PSTR345.07/Y14

Topic: F.07. Rhythmic Pattern Generation

Support: GSU Brains & Behavior Seed Grants Program

Title: Neuromodulatory mechanisms protecting functional bursting activity against sodium dynamics perturbation

Authors: *Y. O. SHAMS¹, R. L. CALABRESE², G. S. CYMBALYUK¹;

¹Neurosci., Georgia State Univ., Atlanta, GA; ²Biol., Emory Univ., Atlanta, GA

Abstract: Central pattern generators (CPGs) control rhythmic behaviors and are tuned by neuromodulation. The leech heartbeat CPG consists of mutually inhibitory heart interneurons (HNs) forming half-center oscillators (HCOs). Myomodulin (Myo) speeds up the HCO by increasing the h-current (I_h) and decreasing Na/K pump current (I_{Pump}). We mapped HN and HCO models' activity under variation of maximal I_{Pump} ($I_{PumpMax}$) and maximal h-current conductance (G_h). In HCO, we found dysfunctional regimes: silence (S) at lower G_h , plateau-containing bursting (PCB) at lower $I_{PumpMax}$, and asymmetric bursting (ASB) at higher $I_{PumpMax}$, where one cell's burst is longer than the other's. At mid-range $I_{PumpMax}$, we identified two functional regimes—a typical low-frequency bursting (LFB) and a high-frequency bursting (HFB)—such that, as $I_{PumpMax}$ increases, the HFB transitions into the LFB. In HN, we found S at low G_h , PCB at low $I_{PumpMax}$, and LFB and HFB at intermediate $I_{PumpMax}$. In LFB, decreasing $I_{PumpMax}$ speeds up bursting, while in HFB, increasing $I_{PumpMax}$ speeds up bursting. In HFB, the burst duration and interburst interval shorten together due to a special role of I_{Pump} . On HCO map, equi-period lines show LFB is sensitive to G_h , while HFB is sensitive to $I_{PumpMax}$. Experiments with different Myo concentrations (0, 1, 10, 100 μ M) were matched at HCO map as four points (M0-M3) in the LFB domain, defining Myo comodulation path between PCB and ASB. On HN map, M0 and M1 support LFB, while M2 and M3 support HFB. M0-M3 have $G_h = [1.1, 4.6, 6.2, 9.9]$ nS, respectively, increasing along the path. We investigated the robustness of bursting against perturbation of the intracellular Na^+ concentration ($[Na^+]_i$). It was previously shown in living HNs and HCOs, that monensin raises $[Na^+]_i$ and speeds up bursting by increasing Na/K pump activity. Monensin silences HNs with I_h blocked with Cs^+ . To simulate monensin effects, we implemented a monensin rate factor (F) representing the monensin-mediated Na^+ diffusion. We investigated the effects of F for M0-M3 points. LFB at M0 transitioned to silence with small increase in F, while bursting at modulated points withstood increase of F by an order of magnitude higher. Obtained for higher F, HN and HCO maps show that the S domain progressively expanded along with the PCB domain reduction. At higher F, PCB transforms to functional bursting which becomes restricted to higher G_h . In this domain,

I_{pump} plays a pivotal role which is evident from the relatively hyperpolarized membrane potential reaching below K^+ reversal potential. Our results highlight the protective role of I_h in Myo modulation against perturbed Na^+ dynamics.

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Poster

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Topic: F.07. Rhythmic Pattern Generation

Support: GSU Brains and Behavior seed grant

Title: Dynamical mechanisms of episodic bursting activity

Authors: *G. S. CYMBALYUK¹, O. BURLKO², O. KOVALENKO³;

¹Georgia State Univ., Atlanta, GA; ²Inst. of Mathematics, NAS of Ukraine, Kyiv, Ukraine;

³NGO NEURO-UNIVERSE, Kyiv, Ukraine

Abstract: Rhythmic movements such as breathing and locomotion are generated by specialized oscillatory circuits known as Central Pattern Generators (CPGs). Conventionally, CPG activity has been characterized by either continuous bursting, corresponding to uninterrupted behaviors like breathing, walking, or swimming, or a silent regime, which corresponds to stillness. Until recently, episodic bursting, *i.e.*, activity consisting of intervals of repetitive bursting separated by intervals of quiescence, was not recognized as a basic CPG pattern. However, studies in isolated spinal cords from larval zebrafish and neonatal mice have demonstrated that these CPGs can generate episodic bursting [1,2], adding a new level of complexity to the repertoire of CPG regimes.

Here, we consider the upgraded repertoire: silence, sub-threshold oscillations, tonic spiking, bursting, and episodic bursting. We describe two qualitatively distinct types of episodic bursting—elliptic episodic bursting and square-wave episodic bursting and construct low-dimensional Hodgkin-Huxley-style models to compare their properties. Both models highlight the role of the h-current (I_h) in generating episodes and utilize an inward current with slow inactivation kinetics, similar to the persistent Na^+ current (I_{NaP}), in burst generation. We demonstrate each type of episodic bursting in a corresponding single-neuron model. In both cases, a decrease in the voltage of half-activation or maximal conductance of I_h leads to elongation of the inter-episode interval and transition into silence. Knowledge of the transitions (bifurcations) between regimes predicts the responses of these neurons to short perturbations: silent neurons respond with a single episode, which is very similar to the episodes from the activity right before the transition. This feature typifies saddle-node bifurcations [3]. We describe mechanisms highlighting co-existence of episodic bursting and continuous bursting, as well as transitions between them, along with changes of I_h , I_{NaP} , and non-inactivating K^+ current. These

dynamical mechanisms are generic and could govern the episodic bursting regimes in rhythmic neuronal networks such as central pattern generators.

References

1. Wiggin, et al. (2012) J Neurophysiol, **108**(3): p. 925-34.
2. Sharples, et al. (2021) Front Cell Neurosci, **15**: p. 715427.
3. Barnett and Cymbalyuk (2014) PLoS One, **9**(1): p. e85451.

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Poster

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Title: Gradual locomotor recovery during spinal cord regeneration after complete spinal cord transection in salamanders

Authors: I. KHSIME¹, L. BLANCHÉ², A. JOVEN ARAUS⁵, Z. TONELLI GOMBALOVA⁶, C. VAN DER ZOUWEN³, J. SWIEGERS³, K. MEDINA-ORTIZ⁴, A. IJSPEERT⁸, A. SIMON⁷, *D. RYCZKO²;

¹Pharmacologie et Physiologie, ³Pharmacology-Physiology, ⁴Dept. de pharmacologie-physiologie, ²Univ. de Sherbrooke, Sherbrooke, QC, Canada; ⁵Cell and Mol. Biol., Karolinska Inst., Solna, Sweden; ⁶Karolinska Institutet, 17165, Sweden; ⁷Karolinska Institutet, Stockholm, Sweden; ⁸EPFL, Lausanne, Switzerland

Abstract: Salamanders can recover voluntary locomotion after full spinal transection, and this is associated with spinal cord regeneration. However, a detailed analysis of the gradual recovery of swimming and walking movements during spinal cord regeneration is lacking. Here we used video recordings and a deep-learning based software (DeepLabCut) to analyze the locomotor movements of salamanders before and after a full spinal transection at low thoracic level. To measure spontaneous locomotor activity, we recorded 20 salamanders in an open field arena at

30 fps, before injury and from 1 to 17 weeks post-injury. The spontaneous locomotor activity gradually recovered during spinal cord regeneration until 17 weeks post-injury. Using a qualitative recovery scale in 59 salamanders recorded in a transparent corridor, we found that walking and swimming recover gradually as animals progress through successive recovery stages. To precisely measure locomotor movements, we tracked 25 anatomical points distributed on the body axis, limbs and head in 17 salamanders. To analyze axial movements, we measured the amplitude of the angular excursions along the body axis as a function of time. During swimming, a traveling wave of axial movements was propagated from head to tail. The amplitude of the angular excursions increased rostrocaudally. To establish the footfall pattern during walking, we measured the angular excursion of limb movements. Salamanders mostly displayed a lateral sequence walk, and this was coordinated with a standing wave of axial movements in the trunk. In the first week after spinal cord injury, the body parts below lesion were paralyzed, and the coordination between body parts above and below the lesion was lost. During the following weeks, the hindlimb excursion angle gradually recovered, and coordination between forelimb and hindlimb movements was restored during walking. Swimming also recovered, but more slowly than walking. Walking scores reached 80% of the control level around 8 weeks, while swimming reached a similar level around 11 weeks. Propagated waves of axial undulation were restored and coordinated along the axial body parts. Such locomotor recovery was associated with tissue regrowth at the spinal lesion site. Altogether, our results show that walking and swimming motor patterns are robustly restored after a complete spinal cord injury at a low thoracic level. This study represents the first high-resolution analysis of this recovery process.

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Poster

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Title: Regeneration of descending brainstem pathways following complete spinal cord injury in salamanders

Authors: *J. SWIEGERS¹, L. BLANCHÉ¹, R. NEGOTA¹, K. MEDINA-ORTIZ¹, I. KHSIME¹, C. VAN DER ZOUWEN¹, A. JOVEN ARAUS², Y. LAVOIE¹, M. TOSCHES³, A. J. IJSPEERT⁴, A. SIMON⁵, **D. RYCZKO**¹;

¹Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Cell and Mol. Biol., Karolinska Inst., Solna, Sweden; ³Dept. of Biol. Sci., Columbia Univ., New York, NY; ⁴EPFL, Lausanne, Switzerland; ⁵Karolinska Institutet, Stockholm, Sweden

Abstract: Salamanders can regenerate their nervous system and recover voluntary locomotion after a complete spinal cord transection. However, the neural circuits involved in this recovery remain unclear. In vertebrates, the mesencephalic locomotor region (MLR) controls locomotion via the reticular formation, which in turn activates the spinal central pattern generator. While salamanders possess an MLR, the circuits through which it influences spinal cord activity are not fully understood. Here, we investigated the pathways through which the MLR controls spinal cord circuits before and after spinal cord injury. In intact animals, we used whole-cell patch-clamp recordings in an isolated brainstem-spinal cord preparation and found that electrical stimulation of the MLR evoked synaptic responses in reticular neurons. Increasing stimulation intensity enhanced these synaptic responses. Trains of MLR stimulation elicited spiking activity in both reticular and spinal neurons located in forelimb-innervating segments. In some cases, MLR stimulation evoked rhythmic bursting in those neurons at frequencies compatible with those observed during locomotion. Next, we examined whether brainstem circuits regain control of the spinal cord below the lesion following complete spinal cord injury. Using video recordings and movement analysis before and after a complete transection at the low thoracic level, we observed that paralysis gradually resolved. Locomotor movements began to return between 4 and 10 weeks post-injury and were fully restored by 17 weeks. Tracer injections below the lesion revealed regrowth of reticulospinal axons during the early stages of locomotor recovery. To examine the effects of MLR stimulation on the spinal cord below the regeneration site, we used transgenic salamanders expressing GCaMP6 under the hSyn promoter. At 4-10 weeks post-transection, when locomotor abilities were beginning to return, we performed two-photon calcium imaging in brainstem-spinal cord preparations. MLR stimulation increased calcium activity in both reticular and spinal neurons located below the lesion. Local application of glutamatergic antagonists in the reticular formation abolished the MLR-induced spinal cord activity below the regeneration site. Altogether, our findings indicate that the MLR reactivates spinal circuits below the lesion early during locomotor recovery after complete spinal cord transection in salamanders.

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Poster

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Topic: F.07. Rhythmic Pattern Generation

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Title: Investigation of connectivity between limb and trunk spinal segments using patch-clamp recordings in salamanders

Authors: *R. NEGOTA¹, J. SWIEGERS², L. BLANCHÉ³, A. J. IJSPEERT⁵, A. SIMON⁶, D. RYCZKO⁴;

¹Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Pharmacology-Physiology, ⁴Dept. de Pharmacologie-Physiologie, ³Univ. de Sherbrooke, Sherbrooke, QC, Canada; ⁵EPFL, Lausanne, Switzerland; ⁶Karolinska Institutet, Stockholm, Sweden

Abstract: In quadrupeds, locomotion involves coordination between the limbs and the trunk. In salamanders, coordinated trunk movements contribute to increased stride length during walking. During the stance phase, forelimb extension is synchronized with activation of trunk muscles on the same side, allowing the contralateral forelimb to reach a more advanced position. To explain this coordination, modeling studies have proposed the existence of propriospinal connections between spinal segments that innervate the limbs and those that control the trunk. Supporting this idea, fictive locomotion studies in salamanders have shown coordinated activity between limb and trunk spinal segments in the absence of brain input and sensory feedback, suggesting the presence of intrinsic spinal circuitry. However, these spinal connections have not yet been studied at the cellular level. To investigate these, we developed an isolated spinal cord preparation of salamanders to examine connectivity between cervical segments (forelimbs) and thoracic segments (trunk). To access the ventral horn, which contains motoneurons and premotor interneurons, we surgically removed the dorsal portion of the spinal cord. Using whole-cell patch-clamp recordings, we characterized the electrophysiological properties of 12 ventral horn neurons in the trunk segments, including input resistance, membrane time constant, firing frequencies, action potential amplitude etc. To explore interactions between forelimb and trunk segments, we placed a stimulation electrode in segment 3 (innervating forelimb extensors) and performed whole-cell recordings in trunk segment 7. Single-pulse stimulation (0.1 Hz, 2-13 μ A, 2 ms pulses) of the forelimb segment evoked robust excitatory postsynaptic potentials (~2-11 mV, ~50-100 ms to peak response) in 7 out of 10 ipsilateral trunk neurons. The remaining 3 neurons showed either weak excitatory or no response with similar stimulation parameters (5-11 μ A). A train of electrical stimulation (3-5 Hz, 3-13 μ A, 10 s, 2 ms pulses) applied to the forelimb

extensor segment induced spiking activity in all ipsilateral trunk neurons tested (8/8), including in two neurons that previously showed no synaptic response, suggesting a polysynaptic pathway in those cases. Altogether, our findings demonstrate the presence of an excitatory descending propriospinal connection from forelimb to trunk segments that is strong enough to drive spiking activity. This may provide a well-positioned substrate for the model-predicted pathways that coordinate limb and trunk movements during locomotion.

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Poster

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Title: Interactions between the basal ganglia and brainstem locomotor circuits in the salamander

Authors: ***D. X. REID**¹, J. SWIEGERS¹, K. MEDINA-ORTIZ¹, L. BLANCHÉ¹, A. JOVEN ARAUS², M. TOSCHES³, A. J. IJSPEERT⁴, A. SIMON⁵, D. RYCZKO¹;

¹Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Cell and Mol. Biol., Karolinska Inst., Solna, Sweden; ³Biol. Sci., Columbia Univ., New York, NY; ⁴École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; ⁵Karolinska Institutet, Stockholm, Sweden

Abstract: Locomotor control is influenced by interactions between the basal ganglia and brainstem circuits. Dopaminergic input to the striatum, the entry point of the basal ganglia, is essential for initiating locomotion, and the loss of dopaminergic neurons leads to impaired locomotor function. Interestingly, salamanders regain locomotion within weeks following the destruction of dopaminergic neurons. However, the striatum in salamanders has been relatively unexplored, and the interactions between the basal ganglia and brainstem locomotor circuits remain largely unknown. Here, we examine the striatum and the pathways through which the basal ganglia influence brainstem circuits in salamanders. Using immunofluorescence against tyrosine-hydroxylase on brain sections, we confirmed the presence of presumably dopaminergic

projections to the striatum. To target this region, we developed a salamander striatal slice preparation for whole-cell patch-clamp recordings. We report the electrophysiological properties of salamander striatal neurons, including cell resistance, membrane time constant, firing frequencies, action potential amplitude etc. To investigate the interactions between the striatum and brainstem locomotor circuits, we used two-photon calcium imaging in isolated brains from transgenic salamanders expressing the calcium sensor GCaMP6s under the hSyn neuronal promoter. Preliminary two-photon imaging indicates that electrical stimulation of the striatum induces calcium responses in neurons of the reticular formation, where many reticulospinal neurons receiving input from the Mesencephalic Locomotor Region are located. Furthermore, preliminary patch-clamp recordings indicate that striatal stimulation can produce varying effects in reticular neurons depending on the preparation, either inducing rhythmic firing or suppressing ongoing spiking activity. Altogether our findings reveal a functional relationship in salamanders between the striatum (the entry station of the basal ganglia) and the reticular formation, which contains reticulospinal neurons involved in locomotor control.

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Poster

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Title: D₂ receptor-expressing neurons in the gigantocellularis nucleus modulate locomotion and head movements in mice

Authors: *J. SURESH, A. JUÁREZ TELLO, C. VAN DER ZOUWEN, J. JUNG, J. SWIEGERS, K. MEDINA-ORTIZ, J. DUQUE-YATE, D. RYCZKO;
Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: The reticular formation plays a key role in motor control, containing the command systems for initiating, stopping, and directing locomotion, with these commands transmitted to spinal motor circuits via reticulospinal neurons. In lampreys, these reticular regions have been shown to be targets of descending dopaminergic pathways (Ryczko et al. 2020 J Neurosci). However, in mammals, the specific neurons targeted by dopamine within the reticular formation and their behavioral roles remain unknown. We are currently investigating in mice the behavioral role of neurons that express dopaminergic D2 receptors in the gigantocellular reticular nucleus (Gi), a region known to contain command neurons involved in stopping and turning. Using RNAscope in brainstem slices from two mice, we identified cells expressing the D2 receptor in the Gi. To activate these D2-positive neurons in vivo, we unilaterally injected a virus into the Gi of transgenic D2-Cre mice, enabling Cre-dependent expression of channelrhodopsin, a light-sensitive cation channel. An optical fiber was implanted above the injection site to allow optogenetic stimulation with blue light (470 nm) in freely moving mice. Behavior was recorded in an open field and analyzed using deep learning-based software (DeepLabCut). Video recordings were used to track the speed of the mouse's body center before, during, and after optogenetic stimulation. Preliminary data from three mice indicate that a brief (1-second) unilateral optogenetic activation of D2-expressing neurons in the Gi reduces locomotor speed. Additionally, head tracking revealed optogenetically-evoked head rotations toward the stimulated side in the same animals. These behavioral effects were not observed when blue light was replaced with red light (589 nm). Altogether, our findings indicate that activation of D2-positive neurons in the gigantocellular reticular nucleus (Gi) decreases locomotor speed and induces ipsilateral head turning.

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Title: In vivo role of lumbar sensory feedback in the regulation of walking movements in salamanders

Authors: *L. BLANCHÉ¹, I. KHSIME¹, J. SWIEGERS¹, C. VAN DER ZOUWEN¹, A. SIMON², A. J. IJSPEERT³, D. RYCZKO¹;

¹Univ. de Sherbrooke, Sherbrooke, QC, Canada; ²Cell and Mol. Biol., Karolinska Institutet, Stockholm, Sweden; ³EPFL, Lausanne, Switzerland

Abstract: Locomotion results from complex interactions between the central pattern generator, descending brain commands, sensory feedback and body mechanics. In salamanders, modelling studies suggest that limb proprioceptive sensory feedback plays a role in regulating limb movements during walking in salamanders. Merkel cells (mechanoreceptors) were found in salamander skin. In their muscles, muscle spindles-like fibers were observed, and the stretching of the muscles induced electrophysiological responses, comparable to what has been seen in mammals. The *Egr3* gene, expressed in muscle spindles in mammals, is present in salamanders. However, there have been no studies investigating the role of sensory feedback during walking in salamanders. Here, we compared walking movements before and 1 week after acute bilateral surgical lesions of the dorsal roots in the four spinal segments that innervate the hindlimbs (segments 15-18). The loss of sensory feedback was confirmed by the loss of the paw withdrawal reflex by mechanical stimulation with von Frey filaments. Locomotor movements were recorded from below at 300 fps in a transparent corridor in 7 salamanders. To analyze movements, we tracked 25 markerless anatomical points on the four limbs and on axial body parts using deep learning-based software (DeepLabCut). A group of 6 sham animals underwent surgery without the lesioning of dorsal roots. On ground, before the dorsal root lesion, salamanders used a lateral sequence walk. After the dorsal root lesion, such walking pattern was still present, though with subtle modifications. At the hindlimb level, we observed decreased amplitude of limb angular excursion during protraction, decreased stride length, decreased limb movement frequency and decreased walking speed. In the sham group, these walking locomotor parameters were not affected. Altogether, our results indicate that lumbar sensory feedback regulates limb angular excursion, stride length, movement frequency and walking speed.

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Poster

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Title: Cellular diversity of brainstem locomotor circuits revealed by cross-species transcriptomics in mouse and salamander

Authors: ***K. MEDINA-ORTIZ**¹, A. JOVEN ARAUS³, F. PRATESI², Z. TONELLI GOMBALOVA³, J. DUQUE-YATE¹, K. J. FERNANDES², A. J. IJSPEERT⁴, A. SIMON³, D. RYCZKO¹;

¹Dept. de Pharmacologie-Physiologie, ²Dept. de médecine, Univ. de Sherbrooke, Sherbrooke, QC, Canada; ³Cell and Mol. Biol., Karolinska Inst., Solna, Sweden; ⁴École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Abstract: Salamander models such as *Pleurodeles waltl* are among the only tetrapods capable of regenerating both the brain and spinal cord, offering a unique opportunity to investigate the cellular and molecular programs underlying motor control and regeneration from an evolutionary perspective. In vertebrates, the general organization of neural circuits controlling locomotion is conserved. The mesencephalic locomotor region (MLR) initiates movement through reticulospinal neurons, which project to central pattern generators (CPGs) in the spinal cord. However, the composition and identity of brainstem cell types, along with their spinal projections and homologous counterparts in mammalian models like the mouse, are not yet resolved. Using single-nucleus/cell transcriptomics, we compared the full salamander brainstem (from the MLR to the inferior reticular nucleus) with seven anatomically defined motor-related regions in the mouse: the cuneiform nucleus (CnF), pedunculopontine nucleus (PPN), pontine reticular nucleus oralis (PnO), caudal pontine reticular nucleus (PnC), gigantocellular reticular nucleus (Gi), lateral paragigantocellular nucleus (LPGi), and ventral medullary reticular formation (MdV). We identified both conserved and species-specific cell type features in *Pleurodeles waltl* and mouse. Cross-species integration was performed using scVI, scANVI, Seurat CCA, Harmony, BBKNN, and SAMap, each evaluated for biological relevance and integration quality. All methods consistently clustered broad cell types such as neurons, oligodendrocytes, and microglia across species, indicating conserved molecular identities, with species-specific diversity observed within these groups. Glutamatergic neurons, predominant in salamander clusters, showed strong correspondence with mouse populations, while inhibitory salamander neurons (GABAergic and glycinergic) showed more limited alignment. Cell type diversity was assessed using Shannon entropy, and regional composition and proportions were analyzed with compositional methods. These results reveal conserved brainstem cell types across species, with species-specific differences within neuronal subtypes potentially linked to differences in motor control.

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Poster

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Title: Development and evolution of rostrocaudal spinal networks for limb control

Authors: *S. PAPADOPOULOS^{1,3}, F. TOMA², D. LE RAY⁴, L. B. SWEENEY³;
¹Inst. of Sci. and Technol. Austria, Vienna, Austria; ²Inst. of Sci. and Technol. Austria,
Klosterneuburg, Austria; ³Inst. of Sci. and Technol. Austria (ISTA), Klosterneuburg, Austria;
⁴Univ. Bordeaux CNRS Umr5287 - INCIA, Bordeaux, France

Abstract: The development of limbs was a major landmark in vertebrate evolution, yet the specialization of spinal interneuron circuits for limb, as opposed to axial or torso, control still remains poorly understood. Amphibians, the first tetrapods to walk on land, transition to limb locomotion during metamorphosis and offer a unique opportunity to characterize the underlying spinal cord changes for limb locomotion. To address this question, we first take a comparative approach, using tadpoles, frogs and mice to define novel spinal interneuron rostrocaudal architecture patterns at both developing and mature stages. We uncover stereotypical, limb-biased distributions for ventral interneurons but highly variable dorsal organization profiles. We then demonstrate with EdU birthdating that neurogenesis drives these divergent rostrocaudal patterns. Previous studies in mice have demonstrated that Hox genes regulate rostrocaudal spinal neuron subtype identity, providing a powerful means to manipulate these patterns and assess their functional contribution. Using CRISPR to generate HoxC9 mutant frogs, we find that the HoxC9 mutation transforms the thoracic spinal cord to be limb-like. This transformation is evident at a cellular, anatomical and functional level for all cardinal classes, demonstrating a near universal role of the Hox code in specifying rostrocaudal interneuron identity. In addition, both architecture and proliferation are affected: thoracic ventral roots are not only redirected to the limbs, but also exhibit atypical, bilaterally synchronous activity. This provides the first evidence of the functional consequences of thoracic-to-limb transformation of premotor circuits. Our study thus generates new insight into how spinal neurons differ in their number, type and function at limb versus non-limb levels across species, and pinpoints a global Hox-dependent mechanism that orchestrates these differences.

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Poster

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Support: NSF Career Award IOS-1943276
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Title: Inhibitory Premotor Circuits Coordinate Rhythmic Leg Actions and Behavior Transitions in *Drosophila* Grooming

Authors: *D. S. SYED, P. RAVBAR, J. H. SIMPSON;
Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: How central circuits coordinate multi-joint limb movements to produce discrete motor actions and govern the transitions between them remains a fundamental question in neuroscience. Both excitatory and inhibitory premotor neurons have been identified in vertebrate and invertebrate motor circuits, and sensory feedback is known to modulate central control, but a full mechanistic understanding is lacking in any system. Here, we identify a key role for inhibitory premotor circuits in organizing rhythmic grooming actions and transitions in *Drosophila*.

Fly grooming consists of alternating head sweeps and leg rubs. We first characterized the movement of individual legs during these rhythmic behaviors. We find that joint angles and dynamics are distinct between the two actions: we can accurately classify them from very short, context-free video segments using diagnostic features. Some leg movements are shared and may represent motor primitives, while others are modified and specific to each grooming movement. Next, we analyzed transitions between head grooming and leg rubbing. Transition states differ: intermediate limb positions and dynamics are directional, and a head to leg transition does not resemble a leg to head transition in reverse. This lack of mirror-symmetry suggests differences in neural control.

GABAergic neurons in the ventral nerve cord contribute to both limb positioning within head sweeps/leg rubs and normal transitions between these actions. A behavioral screen identified neurons from the 13A and 13B hemilineages as essential for grooming. Connectome analysis revealed diverse morphologies and spatially organized connectivity, supporting both joint-specific and multi-joint control. Some 13A neurons directly inhibit motor pools and their antagonists, enabling disinhibition. Additional motifs—including reciprocal inhibition among 13A neurons and interactions with 13Bs—form a layered control architecture. Proprioceptive inputs onto 13As further support their role in sensorimotor integration.

Optogenetic manipulation of specific inhibitory subsets altered leg trajectories, joint dynamics, and bout durations—affecting both action execution and transitions. Pulsed activation of a 13A subset was sufficient to evoke rhythmic, alternating leg movements.

Together, our behavioral experiments and modeling reveal that inhibition is not merely permissive but instructive—playing an active role in shaping patterned motor output and organizing behavioral transitions.

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Poster

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Title: New genetic tools for assessing the behavioral roles of spinal V2a neurons in larval zebrafish

Authors: Z. LUO, *M. KOYAMA;
Univ. of Toronto, Scarborough, Toronto, ON, Canada

Abstract: Physiological and anatomical studies of spinal V2a neurons across vertebrate models indicate that this interneuron type is essential in generating rhythmic motor output during locomotion. However, their behavioral contribution to rhythmic motor output remains controversial. Genetic ablation of V2a neurons in mice results in the co-activation of the left and right ventral root activity in the isolated spinal cord and a change in the gate pattern at fast locomotor speed, but leaves rhythm generation intact. On the other hand, two-photon ablation of a portion of spinal V2a neurons in larval zebrafish results in an increase in the induction threshold of swimming activity by electrical and pharmacological stimulation and the reduction of the swimming duration and beat frequency when induced, suggesting an essential role of this cell type in rhythm generation. These studies differ not only in the model systems employed but also in the techniques used, each with a different set of caveats, including developmental compensation, off-target or incomplete ablation, and acute effects. To dissociate these confounding factors and pinpoint the exact behavioral role of spinal V2a neurons, we established two distinct genetic approaches for cellular ablation. One approach is based on Diphtheria Toxin A-chain (DTA) as in the mouse studies. However, its expression is restricted to the spinal cord using a spinal cord-specific Cre line. The toxin is expressed from the endogenous locus of the *vsx2* gene, which is active in differentiating V2a neurons. Thus, this approach is subject to potential developmental compensation. The other approach utilizes a recently established HaloTag photosensitizer, Janelia Fluor 570 (JF₅₇₀), which generates free radicals upon exposure to red light. By localizing HaloTag to mitochondria, we can optically induce apoptosis in a genetically defined population. This approach offers distinct advantages over previous approaches since we control the timing, location, and cell type of ablation, allowing us to minimize developmental compensation, imprecise ablation, and acute effects. Preliminary experiments based on the DTA-based approach showed that the treatment group can still produce escape and spontaneous swimming, but with significant effects on bend amplitude and interval. Comparison with our new chemigenetic approach should allow us to isolate the contribution of

developmental compensation and reveal the true extent of the role of spinal V2a neurons in rhythm generation.

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Poster

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Topic: F.07. Rhythmic Pattern Generation

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Title: Tensor component analysis (TCA) of egestive priming in *Aplysia*

Authors: *A. GUPTA¹, C. NEVEU², E. C. CROPPER³, J. H. BYRNE⁴;

¹Dept. of Neurobio. and Anat., McGovern Med. Sch. at the Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; ²Neurobio. and Anat., UTHealth, Houston, TX; ³Neurosci., Icahn Schl Med/Mt Sinai, New York, NY; ⁴Dept. of Neurobio. and Anat., McGovern Med. Sch. at UTHealth Houston, Houston, TX

Abstract: Feeding behavior of *Aplysia* is mediated by a multifunctional central pattern generating circuit capable of producing two types of behavior: ingestive, moving food into the gut; and egestive, moving food out. Each behavior consists of forward movement of the radula (protraction) followed by backward movement of the radula (retraction). The direction of food movement is determined by the timing of radular closure. Previous work has shown progressive strengthening in feeding response efficacy as a result of repetition priming. In isolated buccal ganglia, egestive repetition priming (EP) leads to increased closure activity during protraction. The changes in neuronal activity underlying EP have not been fully elucidated in this system. To address this question, tensor component analysis (TCA) was used to deconstruct neuronal activity recorded by large-scale, single-neuron resolution voltage-sensitive dye imaging to obtain low-dimensional descriptions of EP both within and across fictive motor patterns. Four tensor components were extracted from each of 12 experiments. All 48 components were grouped into four clusters, revealing early and late protraction and retraction clusters. An increase in the across-pattern (learning) factor was observed in the early retraction cluster, suggesting that changes in this cluster could be a correlate of EP. TCA also revealed differences between ingestive and egestive pattern selection. Ingestive patterns featured early and late protraction and retraction clusters, whereas egestive patterns featured a single protraction cluster and early, middle, and late retraction clusters. Differences in the spatial distribution of the TCA components across the ganglia were also investigated. Neurons contributing to the protraction and late retraction clusters in both patterns were spatially compact, whereas neurons contributing to the early and middle retraction clusters were more broadly distributed. Moreover, EP led to an increase in the learning factor of the early retraction cluster for egestive patterns, but not

ingestive patterns. These findings suggest that the neural populations underlying early retraction are important for motor pattern selection and EP. This work demonstrates how TCA can reveal general principles of motor pattern selection and learning.

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Poster

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Topic: F.07. Rhythmic Pattern Generation

Support: NS101356

Title: Disentangling the effects of multi-site plasticity of operant reward learning

Authors: *C. NEVEU¹, Y. MOMOHARA², J. H. BYRNE³;

¹UTHealth, Houston, TX; ²Neurobio. and Anat., McGovern Med. Sch. at The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; ³Dept. of Neurobio. and Anat., McGovern Med. Sch. at UTHealth Houston, Houston, TX

Abstract: Reinforcement of a behavior during operant reward learning involves widespread changes across the neuronal network that mediates the behavior. The consequences of changing a network at multiple levels of a network are challenging to investigate due to recurrent feed-forward and feed-back excitatory and inhibitory pathways. We used an *in vitro* paradigm of operant reward learning of *Aplysia* feeding behavior to investigate the effects of widespread plasticity across a complex neuronal network. We identified additional sites of plasticity underlying operant reward learning. These sites include increased intrinsic excitability of the pattern-generating neuron B64 ($t_{(10)}=2.679$, $p=0.023$, $n=11$), decreased intrinsic excitability, sag potential, and inhibitory synaptic connection of neuron B52 (excitability, $t_{(11)}=5.135$, $p<0.001$, $n=12$; sag potential, $t_{(10)}=4.122$, $p<0.002$, $n=11$; synaptic connection to B51, $t_{(10)}=3.664$, $p=0.004$, $n=11$), decreased intrinsic excitability and synaptic connection of B20 (excitability, $t_{(9)}=2.733$, $p=0.023$, $n=10$; synaptic connection to B8, $t_{(8)}=2.866$, $p=0.021$, $n=9$), and increased intrinsic excitability of motor neurons B6 ($t_{(11)}=-4.047$, $p=0.002$, $n=12$) and B9 ($t_{(6)}=-2.717$, $p=0.035$, $n=7$). In addition to these changes, previous research suggests that operant reward learning increases the excitability and electrical coupling of neurons B30, B63, and B65, and increases the excitability of B51 and decreases the excitability and inhibitory synaptic connection of neuron B4. The widespread changes across a complex network present a challenging analytic problem. We inserted these changes into an empirically constrained conductance-based model of the feeding CPG to investigate the ways in which widespread plasticity affects a complex recurrent network. Consistent with the empirical data, simulations indicate that these changes reconfigure the activity to produce the reinforced motor pattern. These analyses will provide insight into the ways complex networks with widespread plasticity mediate learning.

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Poster

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Title: Motor mechanisms of switching between opposite behaviors during *Aplysia* feeding

Authors: *J. M. MCMANUS¹, H. J. CHIEL²;

¹Biol., Case Western Reserve Univ., Cleveland, OH; ²Biol., Case Western Res. Univ., Cleveland, OH

Abstract: Switching between behaviors is critical as animals survive and navigate through changing environments featuring changing stimuli. In some cases, such as going from forward to backward locomotion, the switch is between motions that are, to a first approximation, opposites. How do nervous systems produce different behaviors with the same musculature and achieve this behavioral switching? In vertebrates, it is difficult to address this issue at the level of single identified neurons. The marine mollusk *Aplysia californica* is a tractable system for such studies. *Aplysia*'s feeding system produces several distinct behaviors using the same musculature, including ingestive (drawing food into the mouth) and egestive (pushing an object out of the mouth) behaviors. It was originally thought that these opposite motions were discrete behavioral modes: ingestion is a response to edible food and egestion is a response to inedible material. More recent work suggests that an animal also uses egestion to grapple with food that is edible but tough, and that flexibly switching between ingestion and egestion is important for the animal to successfully feed in its natural environment. By analyzing recordings from intact, behaving animals, we confirmed that *Aplysia* regularly switch between ingestion and egestion when attempting to feed on tough stimuli, in contrast to their producing almost solely ingestive patterns when feeding on regular seaweed strips. Multi-action neurons B4/B5 often increase their activity prior to a switch from ingestion to egestion. We additionally observed behavioral switching in a semi-intact preparation capable of producing feeding movements, allowing more insight into both muscle movements and the activity of neurons causing those movements. We previously showed that during swallowing movements in which the animal is ingesting food, it alternates between holding the food with the radula (food grasper) and with the jaws. The jaw pinch is caused by the B38 motor neuron during the protraction phase; grasper closure is caused by the B8 motor neurons during the retraction phase. Here we show a similar handoff between grasper closure and jaw pinch during egestion, but with the phases switched such that grasper closure occurs during protraction and jaw pinch during retraction. Intriguingly, the phase switch of grasper closure is mediated by changing the B8 neurons' activation phase, whereas the phase

switch of jaw pinch is mediated by deploying a different jaw pinch motor neuron, B39. Thus, we reveal the actions of several identified neurons in achieving flexible switching between opposite behaviors, a common motor control problem for many animals.

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Poster

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Title: Modeling Mechanisms of Pattern Generator Variability

Authors: I. HURWITZ¹, U. DICKMAN³, P. J. THOMAS⁴, H. J. CHIEL⁵, *A. J. SUSSWEIN²;

¹Bar-Ilan Univ., Ramat Gan, Israel; ²Bar-Ilan Univ., Ramat-Gan, Israel; ³Univ. of California-Santa Barbara, Santa Barbara, CA; ⁴Mathematics, Applied Mathematics, and Statistics, Case Western Reserve Univ., Cleveland, OH; ⁵Biol., Case Western Res. Univ., University Heights, OH

Abstract: Many pattern generators show cycle-to-cycle variability in intact, behaving animals. What are the mechanisms underlying these variable responses? We studied this question in the *Aplysia* feeding system. An *Aplysia* feeding act consists of radula protraction and retraction, and a variable delay before feeding is again initiated. The delay allows animals time to determine whether and when to respond. A small number of key neurons with unusual properties and complex interconnections organize repeated protraction-retraction cycles. To determine how these properties and connections contribute to the delay between feeding acts, and to variability in protraction-retraction sequences, we constructed a simplified simulation of the circuit containing these neurons, partially based on previous voltage clamp measurements. The simulation produced patterned activity similar to that during feeding. We then systematically analyzed the roles of model components. Protraction is controlled by motor neurons B31/B32 innervating the protractor muscle I2. B31/B32 somata are inexcitable, and have a low input impedance. B31/B32 somata undergo a slow depolarization of variable length, during which the system is not yet committed to initiating behavior. Sufficient depolarization induces a sustained, plateau-like depolarization during which the B31/B32 axons fire, producing the first stage of behavior, protraction. B63 are conventionally spiking neurons producing both fast and slow facilitating cholinergic EPSPs onto B31/B32, as well as being electrically coupled to them. Depolarizing B63 readily initiates protraction. The simulations showed that the delay preceding protraction is critically dependent on the amplitude of the depolarization of B63, and thus this is a critical control point for variation in time between responses. Even small fluctuations in the voltage of B63 can affect the delay before the initiation of protractions. The combination of the

facilitating slow EPSP and electrical coupling underlie the slow depolarization preceding protraction, and also produce the functional equivalent of a plateau potential that underlies the sustained depolarization driving protraction. Adding spikes to the B31/B32 somata reduces protraction delay. Neuron B64 produces a switch from protraction to retraction, by inhibiting B63 and B31/B32 and exciting retraction motor neurons. A slow EPSP from B63 to B64 can drive B64 with a longer delay than that preceding protraction, thereby producing the change from protraction to retraction, and potentially serving as another control point for variable intervals in the pattern.

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Title: Neuronpyxl: bridging from SNNAP to NEURON models for fast, flexible, Python-integrated simulation of biophysical neural networks with complex plastic synapses

Authors: *U. DICKMAN¹, C. NEVEU², H. J. CHIEL³, P. J. THOMAS⁴;

¹Mechanical Engin., Univ. of California-Santa Barbara, Goleta, CA; ²Neurobio. and Anat., UTHealth, Houston, TX; ³Biol., Case Western Res. Univ., Cleveland, OH; ⁴Mathematics, Applied Mathemtics, and Statistics, Case Western Reserve Univ., Cleveland, OH

Abstract: Conductance-based modeling has been essential to understanding neuronal networks that mediate behavior. NEURON has been widely used as an experimentally-based simulation tool. Successful simulations in NEURON require understanding the underlying biology, computer programming and numerical methods. Previous work has used the Simulator for Neural Networks and Action Potentials (SNNAP) to create detailed Central Pattern Generator (CPG) neural circuit models. The CPGs that model feeding in *Aplysia californica* are highly useful because these models incorporate ion pool dynamics, second messengers, synaptic conductances, and channel noise, in addition to typical conductance-based modeling assets. However, SNNAP is no longer under active development, and as a standalone tool, it is not Python-compatible. We introduce a Python-based tool called NEURONpyxl, which constructs and runs simulations on full neural networks with biophysical noise in NEURON by reading parameters from a spreadsheet, which removes the programming requisite for complex computational modeling of neural networks and ensures that SNNAP and NEURONpyxl models are interchangeable. Test simulations from well-understood neuronal networks were created in NEURONpyxl, and were observed to be essentially identical to simulations of the same network

in SNNAP. We then used NEURONpyxl to conduct a parameter grid search to optimize conductances in a previously developed model of Aplysia feeding. Through this method, we located parameter values that generated simulated motor patterns with durations of protraction and retraction that matched real feeding behavior under different conditions of mechanical load. We found that both the conductance of the slow potassium channel in B64 and the conductance of the slow chemical synaptic connection from B63 to B31 needed to be increased from the original model to match feeding behavior in either loaded or unloaded conditions. Under mechanical load, the slow potassium channel was increased relative to unloaded conditions, whereas the synaptic conductance remained almost the same in models that matched the unloaded and loaded conditions. The duration of protraction was independent of the slow potassium channel in B64, whereas the retraction duration was independent of the synaptic conductance. The protraction duration was much more sensitive to conductance changes than was retraction. NEURONpyxl lowers the barrier to building and simulating complex biophysical neural networks. Future work could integrate modern machine learning algorithms for more efficient parameter optimization.

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Poster

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Title: Tradeoffs in the energetic value of neuromodulation in a closed-loop neuromechanical system

Authors: *Z. YU¹, Y. WANG², P. J. THOMAS³, H. J. CHIEL⁴;

¹Dept. of Psychology & Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; ²Dept. of Mathematics, Brandeis Univ., Waltham, MA; ³Mathematics, Applied Mathematics, and Statistics,

⁴Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Rhythmic motor behaviors controlled by neuromechanical systems, consisting of central neural circuitry, biomechanics, and sensory feedback, show efficiency in energy expenditure. The biomechanical elements (e.g., muscles) are modulated by peripheral neuromodulation which may improve their strength and speed properties. However, there are relatively few studies on neuromodulatory control of muscle function and metabolic mechanical efficiency in neuromechanical systems. To investigate the role of neuromodulation on the system's mechanical efficiency, we consider a neuromuscular model of motor patterns for feeding in the marine mollusk *Aplysia californica*. By incorporating muscle energetics and neuromodulatory effects into the model, we demonstrate tradeoffs in the energy efficiency of

Aplysia's rhythmic swallowing behavior as a function of the level of neuromodulation. A robust efficiency optimum arises from an intermediate level of neuromodulation, and excessive neuromodulation may be inefficient and disadvantageous to an animal's metabolism. This optimum emerges from physiological constraints, i.e., the effects of serotonin on the length-tension (peak force) and force-frequency (muscle relaxation time constant) of the muscles, which defines serotonergic modulation trajectories on the energy efficiency landscape (see Figure 1). Our results may lead to experimentally testable hypotheses of the role of neuromodulation in rhythmic motor control.

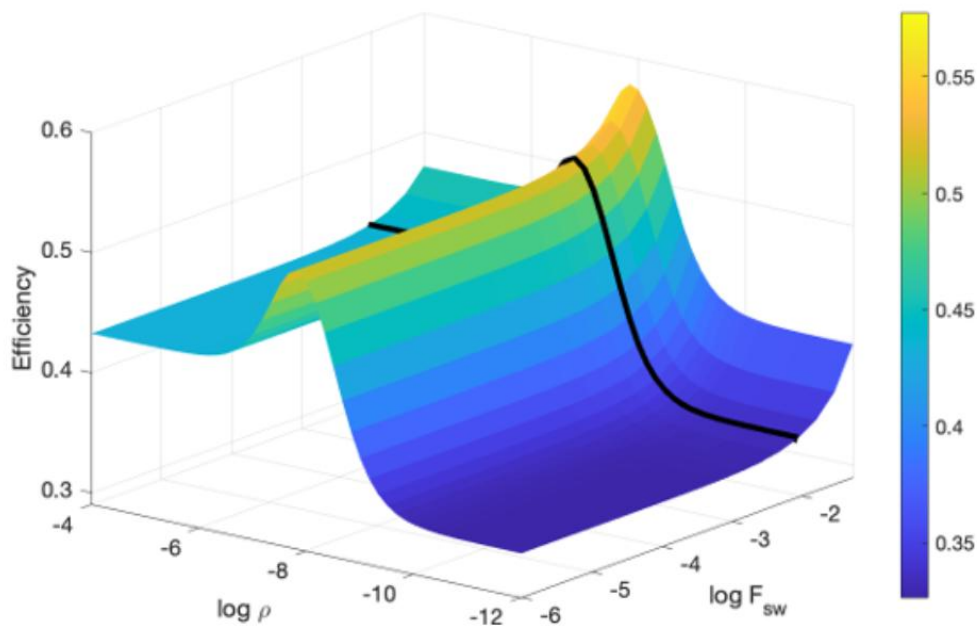


Figure 1: Effects of serotonin concentration ρ on the energy efficiency with changing seaweed force F_{sw} . Black curve corresponds to the default system with $F_{sw} = 0.01$ N. Similar plateaus and optima arise in response to different values of F_{sw} , which are relatively insensitive to the change of F_{sw} .

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Poster

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Title: Fast/slow dissection and dimension reduction for a model of pattern generator variability

Authors: U. DICKMAN¹, Q. IP MORAIS², I. HURWITZ⁴, H. J. CHIEL⁶, A. J. SUSSWEIN⁵,
*P. J. THOMAS³;

¹Univ. of California-Santa Barbara, Woodside, CA; ³Math, Applied Math, and Statistics, ²Case Western Reserve Univ., Cleveland, OH; ⁴Bar-Ilan Univ., Ramat Gan, Israel; ⁵Life Sci., Bar-Ilan Univ., Ramat-Gan, Israel; ⁶Biol., Case Western Res. Univ., University Heights, OH

Abstract: How are pattern generators capable of responding to variations in the environment with variable intervals between behavioral acts and within components of a behavioral act? Variability is observed in a variety of pattern generators, and one that is tractable for study at both the behavioral and cellular level is the neural circuit controlling feeding in the marine mollusk *Aplysia californica*. Susswein et al have proposed a model for the feeding circuit comprising the B31/B32, B63, and B64 neurons regulating the protraction and retraction movements of the *Aplysia* feeding apparatus, which is critical for all the behavioral acts of feeding. The model comprises 48 dynamical variables (voltages, ionic and synaptic gating variables). It produces repetitive trajectories exhibiting a burst of activity in B63 followed by a plateau-like potential in B31/B32 (which is responsible, in part, for the motor activity that generates protraction), followed by a burst of activity in B64 (corresponding to retraction), after which there is a pause prior to the next feeding motor pattern. Experimental recordings show significant variability in the interval between the end of retraction (termination of the B64 burst) and the start of protraction (initiation of the B63 burst), ranging from less than a second to over a minute. As a preliminary investigation into mechanisms regulating the duration of this interval, we identified fast, slow and extremely slow variables using the Diekmann heuristic (Diekmann et al (2017) J. Neurophys. 118.4:2194). We found seven extremely slow variables, with time scales greater than 100 msec. We systematically reduced the dimensionality of the slow subsystem to three extremely slow variables by exploiting approximate model symmetries analogous to the Fitzhugh-Rinzel reduction of the Hodgkin-Huxley equations. Finally, we studied the sensitivity of the interval of interest to variation of initial values of fast, slow, and extremely slow variables. We test the hypothesis that the variables most influential in lengthening or shortening the interval should fall within the subset of extremely slow dynamical variables. Preliminary results appear to contradict this hypothesis: the system is exquisitely sensitive to the actual number of spikes that occur within the B64 burst, and the depolarization of B63; this in turn may be consistent with the results observed in the complete model and the experimental data.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR345.27/AA5

Topic: F.07. Rhythmic Pattern Generation

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Title: Paired spike sorting method for identifying individual neurons from multi-channel nerve recordings

Authors: *M. C. JENCKES¹, J. M. MCMANUS², L. Y. XIONG², H. J. CHIEL²;
¹Biomed. Engin., ²Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Animals activate different combinations of neurons to produce adjustable behaviors that are optimized to meet a wide variety of task requirements. Understanding the role of individual neurons in these changing combinations often requires intracellular recordings, which can be technically difficult and lack context associated with other neural components. Extracellular nerve recordings are easier to obtain and generally more stable, and they contain information about patterns of neural activity. Methods for sorting extracellular signals to extract data on individual inputs have been applied to various neural systems, from invertebrate ganglia to the human brain. Particularly in vertebrates, the focus has often been on multi-electrode recordings in cortex, where a single input may appear simultaneously on many channels. Spike sorting using multiple recording sites on a nerve has less commonly been attempted. This approach provides information on activity of the motor pool for the muscle the nerve innervates, and unlike for intracortical recordings, time delays can be used to pair signals with the same somatic source. With only about 20,000 neurons and several well-characterized adaptive behaviors, the sea slug *Aplysia californica* is a useful model for linking neural components with their effects on motor outputs. Across an extracellular nerve recording, the spikes associated with a particular neuron have consistent shape and amplitude characteristics. To maximize shape information associated with a particular neuron, nerve recordings were obtained from (1) an electrode on the main branch of a nerve and another electrode on a sub branch after a bifurcation, or (2) two different types of electrode on the same nerve: one hooked around the nerve body and one suctioned to the end of the cut nerve. Spikes associated with the same neuron were paired using temporal proximity of peaks, and the total set of spike pairs was clustered using principal component analysis of the cross-correlation of amplitude data surrounding the peaks (descriptive of spike shape). This spike sorting method efficiently identified distinct clusters that closely matched template-based neuron identification from previous research. Clustering using data from one channel produced less discriminative results than clustering using two channels, indicating that multiple channels do provide more information for distinguishing signals. This method for extracting data about specific neurons from nerve recordings has potential broad applicability for datasets where the same inputs project non-instantaneously to multiple channels.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

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Topic: F.07. Rhythmic Pattern Generation

Support: NSF DBI2015317

Title: A neuromechanical model of *Aplysia*'s feeding system with the beam hinge mechanism and Synthetic Nervous System control

Authors: *Y. LI¹, M. BENNINGTON³, V. A. WEBSTER-WOOD⁴, H. J. CHIEL⁵, R. D. QUINN²;

²Mechanical Engin., ¹Case Western Reserve Univ., Cleveland, OH; ³Dept. of Mechanical Engin.,

⁴Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA; ⁵Biol., Case Western Res. Univ., University Heights, OH

Abstract: Humans exhibit dexterous manipulation due to their sophisticated body structure and nervous systems, while some animals still achieve complex control through compliant apparatus and relatively small control circuits. For example, the sea slug *Aplysia californica*, with a soft muscular periphery and around 2000 neurons in its buccal ganglia, can conduct multifunctional and adaptive feeding behaviors, including biting, swallowing, and rejection. To understand the mechanisms underlying *Aplysia*'s multifunctionality, we previously developed a series of simplified neuromechanical models that can qualitatively reproduce *Aplysia*'s key feeding behaviors, behavioral switching in response to sensory stimuli, and adaptability to external load. However, these models treated the "hinge," the interconnection of the odontophore and the luminal structure, as a spring-like element, which underestimated retraction force during the retraction phase of biting and rejection. To improve the model, a more detailed biomechanical model describing the hinge as a geometrically exact beam has been developed. In this study, we integrate this beam hinge model with a bio-inspired neural network model called Synthetic Nervous Systems (SNSs). The structure of the circuitry is based on existing literature. We have tuned the parameters of key neurons so that they can capture the features of the neural dynamics observed in animals. Additionally, we have hand-tuned parameters in synaptic connections and feedback pathways, allowing the neuromuscular model to generate animal-like feeding behaviors. We have quantitatively compared the model outputs with animal data to verify the integrated model. We show that the contribution of the beam hinge muscle model is stronger than that of spring-like hinge muscles in previous models. We also find that the beam hinge mechanism allows the model to generate enough retraction during biting and rejection via hinge muscle force. Earlier models relied on strong B6/B9/B3 activity and I3 muscle force to achieve similar behaviors, which conflict with animal data. Together, these findings suggest that the model can quantitatively reproduce *Aplysia*'s feeding behaviors and its accuracy can be further improved by incorporating more biologically plausible elements.

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Poster

PSTR345: Rhythmic Pattern Generation Invertebrates

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Title: Multi-layered intersegmental connectivity underlies rhythmic propagation and phase lag in motor control

Authors: *G. I. KEARNEY¹, L. SZCZUPAK^{1,2};

¹Inst. de Fisiología, Biología Mol. y Neurociencias (UBA - CONICET), Ciudad Autónoma de Buenos Aires, Argentina; ²Fisiología, Biología Mol. y Neurociencias, Facultad de Ciencias Exactas y Naturales (UBA), Buenos Aires, Argentina

Abstract: Animal rhythmic behaviors are controlled by widespread neural networks. The nerve cord contains segmental central pattern generators (CPGs) and pathways for intersegmental coordination along the body's longitudinal axis. Our goal is to study the nature of intersegmental interactions. Leeches crawl by producing sequential waves of elongation and contraction of their body. Each of the 21 segmental ganglia can individually or collectively generate fictive crawling (*crawling*) when exposed to dopamine (DA). In this study we isolated chains of three ganglia and stimulated them with DA in three different configurations: DA applied to all three ganglia (global-DA) or DA applied only to the anterior or the posterior ganglion of the chain, leaving the other two ganglia untreated. *Crawling* was monitored in each ganglion via extracellular recordings of an identified motoneuron (DE-3), which controls segmental contraction. Coordination across the three ganglia was assessed using cross-correlation index (CCI) and anteroposterior phase lag (lag/period). We hypothesized that local DA stimulation in either the anterior or posterior ganglion would activate (switch on the CPGs) and/or entrain (non-autonomous) rhythmic activity in the rest of the chain, depending on the intersegmental circuitry. Under global-DA stimulation, all three ganglia exhibited coordinated rhythmic activity, with mean CCI above 0.7, phase lags around 0.1/ganglion, and similar DE-3 firing frequencies across the chain. With local DA application (anterior or posterior only), *crawling* was observed in the treated ganglion and rhythmic activity in untreated ones. The DA-treated ganglion and its adjacent untreated neighbor showed high coordination (mean CCI > 0.6) and phase lags comparable to those under global-DA. However, the two untreated ganglia showed no lag between them, and DE-3 firing frequency decreased linearly with distance from the DA-treated ganglion. Severing the connection between the DA-treated ganglion and the rest of the chain abolished rhythmic activity in the untreated ganglia, although *crawling* persisted in the DA-treated one. These results show that activation of a single segmental CPG can entrain correlated activity both forward and backward along the chain. However, this signal alone is insufficient to activate adjacent CPGs or establish an intersegmental phase lag. The findings suggest that intersegmental connectivity includes multiple layers. A basic layer can propagate rhythmic activity to motoneurons without encoding the phase lag, while activation of local CPGs is required not only to initiate autonomous oscillations but also the proper metachronal order.

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Poster

PSTR346: Vocal/Social Communication

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Support: VIEP-BUAP to CA in Neuroendocrinology (BUAP-CA-288)

Title: High-yawning respond better to dopaminergic D2 agonists than low-yawning young adult male rats

Authors: *J. R. EGUIBAR¹, C. CORTES², A. UGARTE¹;

¹Inst. of Physiol., Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; ²Inst. of Physiol., B. Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: Yawning is a stereotyped behavioral pattern that is characterized by deep inspiration followed by short expiration across all vertebrate species. It is relevant that yawning frequency is extremely low, approximately 1 yawn/h. We have two sublines of Sprague-Dawley rats that differ in their spontaneous yawning frequency, the high-yawning (HY) yawns 20 times/h, and the low-yawning just 2 times/h. The HY rats also had more spontaneous penile erections (PE) that are time correlated with yawning. Physiologically penile erection occurs in response to cavernous smooth muscle relaxation, increased blood flow to the penis, and restriction of venous outflow. The aim of this study was to analyze the effects of three dopaminergic D2 agonists on the frequency of yawning and penile erections injected systemically with different doses in young adult HY and LY male rats. Our results showed that 7-OH-DPAT, PD 128,907 and pramipexol significantly increased the yawning frequency in HY ($P < 0.05$), with higher effect in HY with respect to LY rats ($P < 0.05$). Importantly, all D2 agonists increased PE frequencies but they do not differ between sublines. In conclusion, dopaminergic D2 agonists in the HY subline induced higher yawning frequencies probably due to their higher sensibility to the action of D2 agonists, but it is not the case in PE frequency supporting that different dopaminergic neuronal circuits in these two innate behaviors. This study was partially grant from VIEP-BUAP to CA in Neuroendocrinology (BUAP-CA-288).

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Poster

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Topic: G.01. Neuroethology

Support: NSFC Grant 32271083

Title: Relative and causal contributions of vocal acoustic control among the prefrontal cortex, motor cortex, and midbrain in marmoset monkeys

Authors: *Y. XU^{1,3,4}, J. TSUNADA^{2,4};

¹Chinese Inst. for Brain Research, Beijing, Beijing, China; ²Chinese Inst. for Brain Research, Beijing, Beijing, China; ³State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China; ⁴Beijing Inst. for Brain Research, Chinese Acad. of Med. Sci. & Peking Union Med. Col., Beijing, China

Abstract: Human speech exhibit remarkable flexibility, allowing control over not only content but also fine acoustic structures. Two brain pathways have been proposed to govern fine vocal control in humans and non-human primates: 1) one descending from the orofacial motor cortex to motoneuron pools, and 2) another descending from the anterior cingulate and prefrontal cortices via the periaqueductal gray (PAG) to motoneuron pools. While the role of the former pathway in fine vocal motor control has been studied—neural activity in the speech motor cortex can be explained by linear combinations of speech apparatus engagements—the latter pathway's role and relative contributions of these two pathways remain unknown. To address this question, we investigated the neural mechanisms of vocal acoustic control in marmoset monkeys, a New World primate species capable of sophisticated regulation of vocal timing, sound intensity, and acoustic frequencies. Specifically, by using pharmacological inactivation and electrical stimulation, we perturbed activity in the orofacial motor cortex, prefrontal cortex and PAG, and analyzed their effects on vocal acoustics. Consistent with previous lesion studies, pharmacological inactivation of the orofacial motor cortex and prefrontal cortex using GABA_A agonist muscimol did not alter acoustic structures and call rates. However, orofacial motor cortex inactivation biased call type usage. Additionally, electrical stimulation of the orofacial motor cortex modulated the fundamental frequency (F0) of vocalizations in a current- and timing-dependent manner, although stimulation alone did not evoke vocalizations. In contrast, PAG stimulation reliably evoked vocalizations with short latencies (700 ± 160 ms), consistent with its established role in vocal triggering. We also confirmed that different PAG subregions preferentially associated with specific call types. Importantly, evoked calls exhibited significantly elevated F0 compared to spontaneous calls, and F0 and sound intensity positively correlated with stimulation power, revealing a novel mechanism for parametrically controlled vocal acoustics. These findings suggest 1) a differential role of the orofacial motor cortex in fine acoustic control between humans and monkeys, and 2) the existence of a midbrain circuit in the PAG that preprograms fine acoustic parameters prior to vocal production.

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Poster

PSTR346: Vocal/Social Communication

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Title: A social-vocal norm in a non-human primate?

Authors: *N. PHANIRAJ¹, J. M. BURKART^{1,2};

¹Inst. of Evolutionary Anthropol. and Neurosci. Ctr. Zurich, ²Ctr. for the Interdisciplinary Study of Language Evolution (ISLE), Univ. of Zurich, Zurich, Switzerland

Abstract: Human language is structured by a complex system of social norms that govern when, how, and what we communicate. In ethological terms, for a behavior to qualify as a social norm, it must be consistent across individuals, elicit conformity, be socially reinforced, and serve a functional role in the group. Vocal accommodation in the highly social and vocal common marmoset (*Callithrix jacchus*) - a phenomenon where individuals modify their call structure to resemble that of their social partners - is a compelling candidate for such a norm. This phenomenon is widespread across primates, including humans, and is thought to facilitate social bonding. Mechanistically, it is thought to resemble the template-matching vocal learning observed in songbirds and may represent an evolutionary precursor to the more complex vocal learning that underlies human language. While most marmosets predominantly exhibit convergence in vocal accommodation, rare instances of divergence suggest a social pressure to conform. However, the mechanisms by which this conformity is socially reinforced is unknown. To investigate this, we leveraged the species' antiphonal calling behavior in a closed-loop interactive acoustic playback experiment. Twenty individuals were tested in three playback conditions: (1) their partner's natural calls, (2) partner-like synthetic calls that gradually converged acoustically toward their own calls, and (3) partner-like synthetic calls that diverged from their own calls. Marmosets showed a significantly higher response probability to converging calls and a lower response probability to diverging calls, compared to their partner's calls, with no sex or age effects. Notably, divergent calls also consistently triggered an increased rate of agonistic tsik vocalizations, providing behavioral evidence of negative social reinforcement. Together, these findings reveal that vocal accommodation in marmosets is not merely adaptive mimicry, but a socially regulated behavior with normative properties, marking the first evidence from the vocal domain in a non-human primate species. This opens new neuroethological avenues for investigating how socially guided vocal plasticity may have played a foundational role in the evolutionary trajectory leading to human language.

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Poster

PSTR346: Vocal/Social Communication

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Topic: G.01. Neuroethology

Support: DFG LO3184/1-1

Title: The dynamics of vocal production in marmoset monkeys

Authors: *J. LÖSCHNER^{1,2}, S. R. HAGE^{1,2};

¹Neurobio. of Social Communication, Dept. of Otolaryngology – Head and Neck Surgery, Univ. of Tübingen, Med. Ctr., Tübingen, Germany; ²Werner Reichardt Ctr. for Integrative Neuroscience, Univ. of Tübingen, Tübingen, Germany

Abstract: Primate vocal production is increasingly recognized as a dynamic and flexible process, challenging traditional views that emphasize stereotypy and fixed signal forms. In common marmosets (*Callithrix jacchus*), our previous work identified distinct acoustic subunits within long-distance phee calls, indicating that these calls are composed of smaller building blocks rather than existing as holistic fixed action patterns. This finding suggests a more modular organization of vocal production than traditionally assumed. In the current study, we investigate whether this subunit-based compositional structure extends across call types. We analyzed acoustic data from freely moving marmosets with recordings under experimentally controlled auditory perturbations. Our preliminary results reveal consistent patterns of amplitude modulation and temporal dynamics that align with the presence of discrete vocal units, supporting the hypothesis of a modular vocal architecture. These findings have important implications for understanding the neural substrates that enable such structured vocal production and raise intriguing questions about the sensorimotor mechanisms underlying vocal flexibility in primates.

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Poster

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JPMJMS2012

Title: Macro-scale traveling waves coordinate vocal communication in common marmosets (*Callithrix jacchus*)

Authors: *D. YI^{1,2}, M. KOMATSU³, J. TSUNADA^{4,1};

¹Chinese Inst. for Brain Res., Beijing, China; ²Acad. for Advanced Interdisciplinary Studies, Peking Univ., Beijing, China; ³Inst. of Integrated Res., Inst. of Sci. Tokyo, Tokyo, Japan;

⁴Beijing Inst. for Brain Res., Chinese Acad. of Med. Sci. & Peking Union Med. Col., Beijing, China

Abstract: Vocal communication involves a series of cognitive processes, which can be broadly categorized into three components: perceiving others' vocalizations; deciding whether and how to respond, encompassing decision-making and vocal planning; and producing vocal motor outputs. These processes must work harmoniously, with integration and bridging between components being crucial for effective communication. Previous research has typically focused on specific brain regions or isolated cognitive functions, often lacking a holistic perspective of macro-scale, whole-cortical dynamics and their role in the complete communication process. Therefore, although the cortical areas associated with each cognitive component have been localized in humans, the macro-scale cortical dynamics underlying the integration of these cognitive processes remain unknown. Building on recent findings linking macro-scale cortical dynamics to behavioral performance, we hypothesized that traveling wave like cross-areal interactions play a role in integrating the three communicative components. To test this hypothesis, we recorded whole-cortical activity using epidural electrocorticography (ECoG) while subject marmosets vocally interacted with partners. We found theta-band activation in several cortical areas, including the parietal and auditory cortices, while listening to partner's calls. This activity was further modulated depending on whether the subjects engaged in vocal interactions, potentially representing the integration of sensory processing with decision-making and vocal motor preparation. Given the widespread nature of this modulation, we next characterized whole-brain activity patterns by employing a novel analytical method, Weakly Orthogonal Conjugate Contrast Analysis (WOCCA). This analysis revealed that cortical activity could be decomposed into two distinct traveling wave like propagation patterns: a rotational wave corresponding to vocal motor preparation and a translational wave to decision-making. Notably, the energy of the translational wave during listening to partner's calls correlated with the vocal production-induced suppression of high-gamma-band activity, particularly in the prefrontal and auditory cortices. As vocalization-induced suppression is believed to reflect sensory prediction, the translational wave may propagate specific decision-related or acoustic information associated with subsequent vocal production to local cortical areas. These findings suggest that the brain orchestrates the sequential cognitive processes underlying vocal communication through macro-scale traveling waves.

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Poster

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Title: Social modulation of an internal clock in the singing mice

Authors: *Y. THAPA, X. ZHENG, M. DAVIS, C. HARPOLE, A. BANERJEE, B. R. COWLEY;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Social behaviors require continuous integration of internal rhythms and external cues across multiple timescales. However, most behavioral paradigms used in systems neuroscience tend to study these factors in isolation. As a result, the interplay between internal dynamics and external stimuli and the underlying neural mechanisms during social interactions remain poorly understood. This highlights the need for a tractable model system with a behavioral readout that spans a large range of timescales and varies both with internal state and external stimuli of a social partner. Here, we present the vocal behavior of Alston's singing mouse (*Scotinomys teguina*) as this tractable model. The singing mice produce dozens of human-audible vocalizations in isolation everyday and also engage in rapid turn-taking in a social setting. Why they produce so many songs and how they pattern the timing of their songs remains an open question. To address this, we continuously recorded 18 mice across 2 weeks in both isolated and paired social conditions. We found that their singing behavior is strongly influenced by their internal state, unfolding across multiple timescales, including circadian (days), ultradian (hours), as well as minutes (timing between songs), seconds, and milliseconds (timing between song notes). In isolation, mice exhibit robust diurnal and ultradian rhythms. Remarkably, in the minute timescales, these mice space their songs with clock-like precision with the time between songs determined by the mouse's internal state. To characterize the presence and duration of these clock-like states, we use a statistical model (GLM-HMM) to infer the animal's ongoing internal states—periods of sleep, randomly-timed song, and clock-like song. In a social setting, a mouse may override its clock-like song behavior to instantly respond to a conspecific's song. During these social interactions, an asymmetry emerges: A "follower" mouse will override its internal state to respond immediately to a conspecific's song, while the other "leader" mouse does not alter its song behavior from isolation. The mice establish their leader and follower roles only after a few songs, suggesting internal state be a determining factor in this asymmetry. We again use the GLM-HMM to identify these leader-follower roles and how song behavior changes from isolation to social settings. Overall, our work proposes the singing mouse as a tractable model system to study how a single behavioral readout evolves over a large range of time scales, internal states, and social settings. This sets the stage to uncover the neural underpinnings of this remarkable behavior.

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Poster

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Title: The role of midbrain PAG in vocal repertoire expansion in singing mice

Authors: *C. HARPOLE¹, X. ZHENG¹, M. DAVIS¹, A. BANERJEE²;
²Neurosci., ¹Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: How neural circuits generate diverse behaviors is a fundamental question in neuroscience. Distinct behavioral outputs may arise from either dedicated motor circuits or shared circuits operating in different functional states. While multifunctional circuits offer an efficient solution for behavioral flexibility and may drive rapid evolutionary adaptations, their neural mechanisms remain poorly understood, especially in mammals. Here, we leverage the rich vocal repertoire of the singing mouse *Scotinomys teguina* to investigate the organizational logic of multifunctional motor circuits. We developed a behavioral assay (PAIRId) that enables precise attribution of vocalizations during social interactions. This paradigm revealed two distinct vocal modes: soft, variable, ultrasonic vocalizations (USVs) ancestral to rodents used for short-range communication, and loud, rhythmic, human-audible songs unique to the singing mouse lineage used for long-range communication. Despite their substantial acoustic and contextual differences, we found that USVs and songs do not arise from parallel pathways. Instead, they share the same sound production mechanism, phonatory-respiratory coupling, and vocal gating from the midbrain caudolateral periaqueductal gray (clPAG). To understand the mechanism governing song production, we combined mathematical modeling of song rhythm with synaptic silencing of clPAG, which progressively reduced song amplitude and duration. We demonstrate that song duration decreases via a single parameter controlling its termination. Notably, this mechanism also accounts for sexual dimorphism in songs, identifying clPAG as a key locus for driving natural behavioral variability. Our findings reveal how parametric tuning of a central circuit node produces distinct vocal modes, providing a mechanistic basis for rapid behavioral evolution in mammals. In ongoing experiments, we are identifying additional neural loci underlying the evolution of the novel song behavior using a combination of tetanus light chain toxin-mediated synaptic silencing, chemogenetic manipulations, and chronic electrophysiology.

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Poster

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Title: Neural mechanisms underlying the vocal ontogeny of the singing mouse

Authors: *K. SHAW¹, X. ZHENG¹, M. DAVIS¹, C. HARPOLE¹, A. BANERJEE²;
²Neurosci., ¹Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Vocalizations serve as a cornerstone of social behaviors. While peripheral sound production mechanisms are well-studied, neural circuit mechanisms underlying the development and evolution of mammalian vocalizations are largely unknown. We leverage the Alston's singing mouse (*Scotinomys teguina*) which exhibits both a novel specialized vocalization (songs) and the ancestral ultrasonic vocalizations (USVs) ubiquitous among rodents. Therefore, evaluating the ontogeny of songs and USVs in the same animal gives us a window into how vocal repertoires can be diversified over rapid evolutionary time. We hypothesize that early in development, acoustic features of songs and USVs will be similar; however they will progressively diverge reaching very different adult forms. We conducted longitudinal behavioral recordings from postnatal day 5 to adulthood. We observed a gradual maturation of songs, progressing from short proto-songs at P5 to long mature adult songs at P80. In line with our hypothesis, song acoustics progressively diverged from USVs across development to reach the adult form. To get mechanistic insights, we parameterized the song rhythm across development using a simple linear model with three parameters: maximum note rate, minimum note rate, and cadence. We found that each of these three features followed a unique developmental trajectory. For instance, the minimum note rate decreased from P30 to P80, with a more pronounced effect in males. This divergence results in a sexual dimorphism in adult songs, where females produce, on average, shorter songs than males. We subsequently explored the neural mechanisms underlying this sexual dimorphism. Previous experiments have demonstrated the posterior periaqueductal gray (PAG) expresses androgen receptor and modulates minimum note rate in *S. teguina* songs. To determine if PAG androgen receptors are necessary for sexual differences in song duration, we used a viral strategy to specifically target the androgen receptor in the PAG using CRISPR. We found that the knockdown of androgen receptor in the PAG of male singing mice increases minimum note rate mimicking female songs, demonstrating a causal role of the midbrain PAG in the development of sex-specific vocalizations. Our results suggest that the ontogeny of songs in *S. teguina* involves the progressive developmental elaboration of specific acoustic parameters from the ancestral USV template, with androgen receptor signaling in the

PAG driving sex-specific maturation of song duration. These findings reveal that behavioral innovation can rapidly emerge through the differential maturation of neural circuits controlling distinct acoustic features.

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Poster

PSTR346: Vocal/Social Communication

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.10/AA16

Topic: G.01. Neuroethology

Title: The Encoding of Pair-Bond Information in the Hippocampus of *Peromyscus californicus*

Authors: *K. HERNANDEZ¹, S. A. SIEGELBAUM², A. BENDESKY³;

¹Zuckerman Mind Brain Behavior Inst., ²Zuckerman Mind Brain Behavior Institute; Dept. of Neuroscience; Dept. of Pharmacol., ³Zuckerman Mind Brain Behavior Institute; Dept. of Ecology, Evolution and Environ. Biol., Columbia Univ., New York, NY

Abstract: For many species, having strong social bonds is essential for survival. Social bonds allow species to accomplish common goals, such as home building and rearing offspring. However, it is not well understood what role social memory has in establishing and maintaining these bonds. Previous work has identified the hippocampus, and in particular the CA2 region, as being important for social memory, enabling an animal to discriminate whether a conspecific is novel or familiar. The neural circuitry supporting the memory and recognition of a pair-bonded mate is, however, uncertain. To understand the neural circuits involved in social bonds, the strong pair-bond observed in the monogamous California mouse (*Peromyscus californicus*) presents a useful model. We sought to determine whether CA2 and neighboring CA1 participate in the memorization and recognition of a pair-bonded mate and how it computes this recognition in the California mouse. We utilized 64-channel silicone probes to record from the hippocampus of freely behaving animals as they engaged in social recognition task. Test mice were given the option to explore either its opposite-sex partner or an opposite-sex novel conspecific. Neural activity patterns in both CA1 and CA2 differed during initial exploration of novel opposite-sex conspecifics. Co-housing with a potential partner for 3-7 days led to a change in representations in both CA1 and CA2. At the completion of the 7 days of recordings, California mice were tested in a pair-bond task to examine pair-bond behaviors. When presented with a partner or novel mouse in a tether pair-bond option task, test mice preferentially displayed affiliative behavior towards the partner mouse compared to a novel opposite-sex mouse. Correlations of behavior with neural responses shows promise to provide new insights into the neural encoding of social bond information in the hippocampus.

Disclosures: K. Hernandez: None. S.A. Siegelbaum: None. A. Bendesky: None.

Poster

PSTR346: Vocal/Social Communication

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.11/BB1

Topic: G.01. Neuroethology

Title: Identification of midbrain-projecting forebrain neurons that may regulate the production of mouse ultrasonic vocalizations during courtship vs. female-female interactions

Authors: *C. MALONE^{1,2}, P. ZIOBRO², K. A. TSCHIDA²;
²Psychology, ¹Cornell Univ., Ithaca, NY

Abstract: Animals vocalize only in certain behavioral contexts, but the neural circuits that enable the context-dependent regulation of vocal communication remain poorly understood. The midbrain periaqueductal gray (PAG) has been identified as a critical gate for vocal production, and in the current study, we seek to identify forebrain inputs to the PAG that may promote the production of ultrasonic vocalizations (USVs) in *Mus musculus*, during courtship (male USVs) vs. during female-female interactions (female USVs). To this end, we combined retrograde tracing from the PAG with immediate early gene mapping (Fos immunostaining) in the forebrain to identify populations of PAG-projecting forebrain neurons that upregulate Fos expression in a manner that scales with USV production. Preliminary results suggest that PAG-projecting neurons in the anterior cingulate cortex may play a role in regulating USV production in males, and work is ongoing to examine this neuronal population in females and to identify other neuronal populations of interest. Ultimately, this study will lay the groundwork for manipulations of neural activity in PAG-projecting forebrain neurons to evaluate their functional contributions to USV production and to social behavior more generally during same-sex and opposite-sex social interactions.

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Poster

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Program #/Poster #: PSTR346.12/BB2

Topic: G.01. Neuroethology

Support: EMBO ALTF 1084-2021
C.V. Starr Fellowship

Title: Vocal patterning is dynamically updated during naturalistic courtship behavior in mice

Authors: ***B. MIMICA**^{1,2}, J. ZHANG³, T. TSIN², J. JIANG², R. E. PETERSON⁴, A. TANELUS⁶, S. N. OLIVE², A. H. WILLIAMS⁵, A. L. FALKNER², M. MURTHY²;
¹Princeton Neurosci. Inst., Princeton, NJ; ²Princeton Neurosci. Inst., ³Quantitative & Computat. Biol., Princeton Univ., Princeton, NJ; ⁴Ctr. for Neural Sci., ⁵New York Univ., New York, NY; ⁶Flatiron Inst., New York, NY

Abstract: In numerous species, courtship is an intricate social interplay between opposite-sex individuals involving reciprocal exchange of sensory information, and often facilitated by rich acoustic communication. Both female and male house mice, for instance, emit ultrasonic vocalizations (USVs) in the presence of an opposite-sex conspecific. The periaqueductal grey (PAG), which maps sensory and state information from the hypothalamus to downstream motor outputs, is known to play a role in this process. Our objective is to learn whether mice use ongoing social sensory feedback to pattern vocal production during courtship and how brain activity in the PAG performs this mapping. Here, we present a novel framework for assessing naturalistic dyadic interactions, employing 3D multi-animal pose tracking, DNN-assisted USV assignment, and simultaneous bilateral electrophysiological recordings of >1000 single units from the male PAG and adjacent midbrain areas. We parametrize the tracked 3D poses and quantify moment-to-moment changes in features specific to individual mice, such as Euler angles and movements of the head, back, and tail, as well as distances and angles between relevant partner body parts. We also identify, assign, and phenotype individual USVs. Our behavioral modeling results suggest male and female mice rely on different cues when planning their upcoming calls. While USVs of both sexes are primarily driven by nose-to-nose distance, males additionally rely more on orientation towards females and their partner's speed. Females, conversely, more strongly consider male vocal output and certain postural aspects, like male tail curvature. Moreover, PAG ensemble activity recorded in males during courtship exhibits a striking balance between encoding sensory cues relevant for USV production—like distance between and orientation towards the partner—and motor commands for generating vocalizations or executing various head or body turns. In summary, our work uncovers sex-specific strategies for synthesizing sensory inputs to sculpt vocal responses. Both sexes weigh distance cues the most, but place differing importance on other features, such as their partner's song production history. We also provide preliminary evidence that the PAG serves as a sensorimotor transformation hub in the male mouse brain, simultaneously able to encode the flux of incoming sensory information and craft a response pattern predictive of impending orienting movements and vocal output.

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Poster

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Topic: G.01. Neuroethology

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Title: Prefrontal representations of social information and complex group dynamics

Authors: A. M. BENJAMIN¹, S. ANPILOV^{4,2}, O. IZHAKI², T. BIAGINI², Y. SHEMESH², I. SARAF-SINIK², A. RUBIN³, A. CHEN², O. YIZHAR²;
¹Brain Sci. dept. and Mol. Neurosci. dept., ²Weizmann Inst. of Sci., Rehovot, Israel; ³Weizmann Inst. of Sci., Rehovot, ; ⁴Max Planck Inst. for Metabolism Res., Köln, Germany

Abstract: The medial prefrontal cortex (mPFC) is strongly linked with social cognitive functions and dominance related behavior. However, since most studies focus on short and simplified social interactions between pairs of individuals in impoverished environments, we have limited understanding of how mPFC neurons process and represent more naturalistic social information and coordinate complex social interactions in groups of freely interacting animals. We therefore used wireless head-mounted neural loggers and wireless optogenetics, to simultaneously silence and electrophysiologically record mPFC activity, with single-unit resolution, in 17 groups of three or four outbred male mice, freely interacting in an enriched environment. Overall, we recorded 5,516 neurons from 59 mice and ~500h of simultaneous neural-behavioral data. Rigorous analysis of single-unit tuning curves showed that most mPFC neurons represent different aspects of the subjects' and/or the other group members' behavior, e.g., location, 3D postures and/or movement kinematics, in the past, present and/or future. Interestingly, different neurons represented this information with different tuning properties and reference frames (e.g., cartesian vs polar, allocentric vs egocentric). Using machine learning encoding models and feature attribution methods showed that these neurons represent (1) social variables per se, beyond what could be explained by the subjects' own behavior, and (2) the other group members' identity, beyond what could be explained by the dynamics of the social interactions. Furthermore, the mice exhibit prominent and stable social dominance hierarchies, and a large fraction of mPFC neurons represent the social rank of both the subject and the other group members. Leveraging our neural recordings from all the group members simultaneously, we found high-order (beyond-pairwise) inter-brain correlations, suggesting that these neurons track information about multiple individuals simultaneously. Finally, presenting the subjects with odor stimuli that represent individual mice in a highly controlled, pseudo-randomized design demonstrates directly that these neurons represent the familiarity, social rank, and identity of the other group members per se. To elucidate these neurons' causal role in orchestrating complex

group dynamics, we have begun wirelessly silencing mPFC neurons and their inputs from the mediodorsal thalamus during these behavioral paradigms. Taken together, this study enhances our understanding of how mPFC neurons represent both the identity and behavior of conspecifics in a semi-naturalistic group setting.

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Poster

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Program #/Poster #: PSTR346.14/BB4

Topic: G.01. Neuroethology

Support: NIH Grant P20GM125508
NIH Grant R01GM148960

Title: Positive Influence of Community Microorganisms on Social Interactions in a Naturally Autism-Like Fish Species

Authors: *X. BARRAGAN¹, M. IWASHITA², M. YOSHIKAWA²;

¹Sch. of Life Sci., Univ. of Hawaii at Manoa, Honolulu, HI; ²Sch. of Life Sci., Univ. of Hawaii at Manoa, Honolulu, HI

Abstract: The gut microbiota is reported to play a crucial role in regulating host behaviors, including social interactions, from insects to mammals. Commensal microbiome is known to be heritable to offspring and serves as an 'overlooked' heritable factor. For example, the heritability of autism spectrum disorders (ASD) was estimated to be 50% to 90%, and patients' gut microbiota is thought to contribute largely to these heritability estimates. However, the relationship between the host genetics and gut microbiota in terms of the host expression of asociality and stereotypic repetitive behavior is largely unknown. In the naturalistic genetic model, the Mexican tetra (*Astyanax mexicanus*), its cave-dwelling populations exhibit parallel behavioral symptoms (e.g., asociality, repetitive behavior), as well as gene dysregulation in the brain (>58.5% of 3,152 orthologs in the same directional up- or down-regulation) similar to those observed in patients with ASD. This study investigates whether microbiota transfer during cohousing has a positive impact on social behavior in the asocial cave population. Three experimental setups were used: (1) control - antibiotics (ciprofloxacin)-treated cavefish alone, (2) antibiotics-treated surface fish cohoused with antibiotics-treated cavefish, and (3) intact surface fish cohoused with antibiotic-treated cavefish. One by one, the duration of nearby interactions was used as the score for social behavior. The gut microbiome was analyzed through the 16S rRNA gene V4 region, and visualized using Topological Data Analysis (TDA) and identify the associated gut microbes and pathways via Sparse Modeling. TDA visualized the manifold structures of normalized microbial count data. Sparse Modeling using LO and L1 norm penalties

regressed pathway enrichment scores (estimated from gut bacteria) against social or repetitive behavior scores. As a result, cavefish cohousing with intact surface fish significantly increased their social interactions. We are currently identifying the microbes and enriched pathways associated with social and repetitive behavior scores. These findings offer new mechanistic insights into the heritable microbial components of asociality and repetitive behavior in an ASD-like condition.

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Poster

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Topic: G.01. Neuroethology

Support: NIH R01 DC017466-01
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Title: Preadaptation in allopatry as a foundation for reinforcement in sympatry: Insights from auditory midbrain single-unit recordings across populations of chorus frogs

Authors: *A. MUKHOPADHYAY¹, V. G. KAMATH², J. MCDOWELL⁴, A. S. ACORD³, R. K. ALLURI¹, E. LEMMON⁵, G. J. ROSE¹;

¹Sch. of Biol. Sci., ³Neurosci., ²Univ. of Utah, Salt Lake City, UT; ⁴Neurobiology/ Psychology, UCLA, Los Angeles, CA; ⁵Biol. Sci., Florida State Univ., Tallahassee, FL

Abstract: Diversification of reproductive communication behaviors can occur within a species when different populations interact with different heterospecifics, generating a mosaic of divergent selective pressures across geography. To avoid maladaptive hybridization, unique male call properties and female mating preferences may evolve within (sympatry) compared to outside of (allopatry) these species contact zones, a process called Reinforcement. Chorus frogs, *Pseudacris feriarum*, show a divergence in call traits (pulse number and rate) across geographical locations; for example, advertisement (Adv) calls of frogs from populations in Georgia (GA; sympatric with *P. nigrita*) and South Carolina (SC; sympatric with *P. nigrita* and *P. brimleyi*), have faster pulse rates (PRs) and/or more pulses, respectively, relative to allopatric populations. To identify the neural correlates of divergence in call preference, we made extracellular recordings in vivo from auditory midbrain neurons (AMNs) while presenting stimuli with pulse rates and numbers representative of heterospecific advertisement (Adv), conspecific Adv, and aggressive (Agg; fast PR) calls. Studies in other anurans have suggested that a class of AMNs, Interval Counting Neurons (ICNs), play a crucial role in species recognition; they show selectivity to fast PRs and respond only after a threshold number of pulses have occurred. In allopatric populations, we observed that ICNs were selective to a wide range of PRs spanning both Adv and Agg calls while those recorded from the sympatric GA

population were mostly selective to local Adv call PRs. While some ICNs recorded from the SC population showed tuning to local Adv call PRs, other specialized ICNs showed pulse-number-dependent tuning to PR; PR tuning was broad for stimuli with few pulses but narrowly centered on PRs characteristic of their local Adv call when presented with high pulse- number stimuli. Our results suggest that allopatric populations are preadapted for rapid divergence in cases of contact with heterospecific populations, i.e., conditions of sympatry. Preexisting variation in PR selectivity and plasticity of PR tuning mechanisms allow for relatively rapid increase in the proportions of ICNs selective to PRs distinct from the heterospecific Adv calls. In the case of SC sympatric populations, employing an apparently ‘inefficient’ mechanism of PR retuning has enabled a parallel increase in pulse-number thresholds of ICNs, thereby permitting divergence in call PR and pulse number.

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Poster

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Title: Dopaminergic gating of auditory cortical plasticity links early social experience to memory encoding in the zebra finch

Authors: *S. YANAGIHARA¹, M. IKEBUCHI¹, C. MORI², K. J. HOMMA², K. OKANOYA¹;
¹Advanced Comprehensive Res. Organization, Div. of Complex Syst. and Cognitive Sci., Teikyo Univ., Tokyo, Japan; ²Dept. of Mol. Biol., Fac. of Pharmaceut. Sci., Teikyo Univ., Tokyo, Japan

Abstract: Social interaction powerfully drives vocal learning, yet the underlying neural mechanisms are not fully understood. Juvenile zebra finches learn the tutor’s song better when the adult sings face-to-face than when the same song is played through a speaker. Although dopaminergic control of sensorimotor learning and vocal plasticity is well documented, its role in sensory memory encoding is unclear. We thus hypothesized that the tutor’s presence serves as a social reward that activates midbrain dopamine (DA) neurons and drives auditory cortical plasticity to encode tutor song memory. To test this, we first recorded single-unit activity from

the ventral tegmental area/substantia nigra pars compacta (VTA/SNc) of freely behaving juveniles while the tutor song was delivered either by speaker alone or by speaker with a live tutor. A subset of putative DA neurons was auditory-responsive and responded more to the playback of a tutor song when the tutor was present. Comparable enhancement occurred when the live tutor sang to the juvenile, highlighting a social effect beyond the auditory stimulus alone. Next, we asked whether this DA signal shapes auditory circuits critical for song memory. We recorded single-unit activity from the caudomedial nidopallium (NCM; avian higher auditory cortex) and tested whether the tutor song response can be modulated by dopaminergic agents. Local DA injection or systemic D1 agonist SKF-38393 enhanced responses to the tutor song, whereas saline had no effect. Conversely, D1 antagonist SCH-23390 abolished the response enhancement induced by tutor presence. These findings indicate that dopamine signaling in the NCM is required for the tutor-evoked enhancement and likely facilitates the encoding of tutor song memory during live tutoring. Building on these results, we next asked whether artificially elevating dopamine in the NCM, mimicking the surge evoked by a live tutor song, could induce plasticity and encode a song-specific neural representation. As a substitute for live tutoring, we paired song playback with a dopamine agonist (D1 agonist SKF-38393 or D2 agonist quinpirole) or with a control injection (saline) in tutor-naïve juveniles. Juveniles received systemic SKF-38393 paired with repeated playback of a single adult song. One hour later, a subset of NCM neurons showed sharp tuning to the specific song, paired with D1 agonist. In contrast, D2 agonist or saline controls did not enhance the response selectivity. Together, these findings suggest that socially triggered DA release gates D1-dependent plasticity, transforming a live tutor experience into a song-specific neural representation.

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Poster

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Topic: G.01. Neuroethology

Title: Noradrenergic Neuronal Modulation in the Higher Auditory Cortex regulate Zebra Finch Song Learning

Authors: *K. BENSALAH¹, Y. MOROHASHI², Y. YAZAKI-SUGIYAMA³;

¹Neuronal Mechanism for Critical Period Unit, Okinawa Inst. of Sci. and Technol., Onna-son, Okinawa ken, Japan; ²Neuronal Mechanism of Critical Period Unit, Okinawa Inst. of Sci. and Technol., Kunigami, Okinawa, Japan; ³Okinawa Inst. of Sci. and Technol. (OIST) Grad. Univ., Okinawa, Japan

Abstract: Zebra finches, the most commonly used model of songbirds, learn to sing a song from a tutor (normally their father) much more effectively through social communications than from

passive song playbacks. In zebra finches, only males learn to sing by memorizing a tutor's song (sensory phase) and then vocally matching to it (sensory-motor phase) during developmental critical period. It has been suggested that the caudomedial nidopallium (NCM), a brain area analogous to the mammalian higher auditory cortex, is the neural substrate of tutor's song memories. Recently we reported that inactivating the inputs from the locus coeruleus (LC), the noradrenergic command center of the brain, in NCM of male juveniles during vocal communication with a tutor impaired song learning, suggesting involvement of noradrenergic signals in song learning (Katic et al., 2022). To further investigate this, we examined noradrenergic signals in the NCM of zebra finch male juveniles during vocally communication with a tutor by using the fluorescence noradrenaline sensor (GRAB_{NE}) with fiber photometry. We found a rise of fluorescence signal at most of the time when the tutor sung (90% (n=2)) on the first day of tutoring. However, the response decreased as learning progressed (2nd day: 72% (n=2); ~5th day: 62% (n=2); ~15th day: 36% (n=2); ~25th day: 16% (n=1)). We also examined whether social isolation from a tutor, known to delay the closure of the sensory phase, alters the development of adrenergic receptor expression levels in the NCM by using in situ hybridization. We found higher expression level of β_2 and α_1A adrenergic receptors in NCM in the isolated juveniles (~60 days post hatch (DPH)) comparing to the normally reared juveniles. Whereas comparative level of α_2B receptor expressions were observed between the isolated and normally raised juveniles. Together, these results suggest that noradrenergic signals in the NCM is involved in zebra finch song learning. Noradrenergic signals and its developmental time course may further shape the time window of song learning critical period.

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Poster

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Topic: G.01. Neuroethology

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Title: Auditory responses to offspring begging calls change in sex-specific ways in zebra finch parents

Authors: *K. O. SMILEY¹, F. CINI², L. REMAGE-HEALEY³;

¹Psychology, Univ. of Michigan, Ann Arbor, MI; ²Neurosci. and Behavior, Univ. of Massachusetts, Amherst, MA; ³Neurosci. and Behavior, Univ. of Massachusetts, Amherst, MA

Abstract: Parental care is critical for offspring survival. For many species, including humans, auditory cues unique to dependent offspring, such as baby cries, elicit the necessary behavior

from parents to care for young. Despite this, we know little about how the brain encodes auditory cues specific to offspring. Zebra finches are an excellent model to study this. Like humans, male and female zebra finch pairs raise the young together and rely on auditory cues (begging calls) to elicit chick feeding responses (parental behavior). It is well established that the caudomedial nidopallium (NCM), a higher-order region of secondary pallial cortex (analogous to the mammalian secondary auditory cortex), is involved in the perception of complex auditory signals. It is unknown, however, if/how NCM responds to offspring begging calls. To begin testing this, we recorded NCM activity using *in vivo* extracellular single-unit electrophysiology in adult zebra finches during an auditory playback experiment. Subjects were either parenting or non-parenting adult birds. Subjects were exposed to playbacks of their own and novel begging calls, male songs, and pure tone controls. Preliminary analyses indicate parenting females respond stronger (i.e., higher evoked firing rates) to both their own and novel begging calls relative to non-parenting females. Furthermore, females show more selectivity to their own chick begging calls, whereas males do not show similar levels of selectivity. These studies will lay the essential groundwork for future studies which will aim to test how auditory responses can elicit parental behavior and how these responses may change over the chick-rearing period.

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Poster

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Topic: G.01. Neuroethology

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Title: Perineuronal nets in the vocal motor nucleus HVC regulate song development in juvenile zebra finches

Authors: *X. WAN¹, M. I. PESCARU², J. T. SAKATA²;

¹Integrated Program in Neurosci., McGill Univ., Montréal, QC, Canada; ²Biol., McGill Univ., Montreal, QC, Canada

Abstract: Communicative behaviors such as human speech and birdsong are important for survival and reproduction and are learned through practice. Consequently, it is important to reveal the neural mechanisms that shape the acquisition and development of communication. Perineuronal nets (PNNs) are extracellular matrices that surround neurons important for plasticity and are enriched in sensorimotor circuitry regulating vocal motor learning and performance (“song system”). Perineuronal nets are known to increase in the song system over

development, and removal of PNNs in the vocal motor nucleus HVC affects the adult performance of learned songs. However, little is known about how manipulations of PNNs during development affect the trajectory of song development. Here we investigated the degree to which bilaterally degrading PNNs in the HVC of juvenile male zebra finches (45-57 days post-hatch) affected the development and performance (e.g., stereotypy) of song. Because PNNs have been found to restrict plasticity and consolidate circuit mechanisms, we hypothesized that degrading PNNs in HVC would increase the rate or extent of developmental song plasticity, increase the similarity of the pupil's song to its tutor's song, and decrease vocal stereotypy. We found that songs changed significantly more quickly following PNN degradation, though not necessarily towards the tutor song. Specifically, PNN degradation allowed song to develop away from baseline at a faster rate for 2-3 weeks following surgery. Degrading PNNs in HVC did not, however, affect the stereotypy of song performance. These data indicate a causal contribution of PNNs in motor circuitry to motor learning and performance, motivate further experiments into the role of PNNs in song learning, and provide greater insight in species and developmental variation in PNN expression.

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Poster

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.20/BB10

Topic: G.01. Neuroethology

Title: Extrinsic inputs from HVC shape auditory responses to tutor song in Area X during song learning

Authors: *A. AGARWAL¹, C. SCHARFF², D. VALLENTIN³;

¹Max Planck Inst. for Biol. Intelligence, Starnberg, Germany; ²Freie Univ. Berlin, Berlin, Germany; ³MPI for Biol. Intelligence, Seewiesen, Germany

Abstract: In songbirds, the basal ganglia, particularly Area X, is essential for vocal learning, contributing to sensory template formation and reinforcement learning. Lesions of Area X during the early critical period (before day 46) severely impair tutor song copying, whereas later lesions (after day 61) have minimal impact. This suggests a time-dependent shift in Area X's role. To investigate the underlying neural dynamics, we intracellularly recorded from Area X neurons in juvenile zebra finches exposed to a tutor song during both early and late critical periods. We observed precise, onset-locked spiking responses to tutor song playback during the early critical period. However, in the late critical period, tutor song failed to evoke spiking activity, instead eliciting precise subthreshold responses spanning the song's duration. This developmental switch prompted us to investigate whether it reflects intrinsic changes in Area X neural dynamics or altered extrinsic input. We hypothesized that changes in the input drive from HVC to Area X neurons might be responsible. To test this, we pharmacologically silenced HVC while

simultaneously recording from Area X neurons during tutor song presentation. This significantly diminished tutor song-evoked responses in Area X, confirming HVC as a primary driver of auditory input. We then explored whether disinhibiting HVC could restore spiking responses in Area X. By pharmacologically lifting local circuit inhibition in HVC using gabazine, we observed that Area X neurons exhibited spiking responses to tutor song playback similar to those seen during the early critical period. These findings indicate that HVC is the main driver of auditory responses in Area X, and critically, local inhibition within the HVC circuit governs the strength of these responses, providing a mechanistic explanation for the time-dependent changes in Area X's role during vocal learning. This HVC-gated mechanism for auditory input to Area X underlies the developmental plasticity crucial for song acquisition.

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Poster

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Title: Dopamine action in a cortical nucleus crucial for socially-guided learning

Authors: *A. A. MERCER¹, A. CHEN¹, R. D. MOONEY²;

¹Duke Univ., Durham, NC; ²Med. Ctr., Durham, NC

Abstract: Learning by imitation is central to speech, language, other key behaviors. Juvenile male zebra finches selectively memorize and copy songs produced by a conspecific adult tutor, which involves a learning mechanism that integrates social and auditory cues. The forebrain sensorimotor nucleus HVC is a crucial site where auditory and social cues are integrated to drive the early stages of song learning. The midbrain (A11) dopamine (DA) input to HVC signals the presence of a suitable vocal model. We sought to understand DA receptivity in various HVC cell types, beginning with inhibitory interneurons (HVC_{INT}), which are involved in shaping HVC projection neuron (PN) activity throughout development. We measured D1 receptor (D1R) and D2 receptor (D2R) mRNA colocalization with VGAT mRNA in juvenile males and found that most HVC_{INT} (60%) do not express either receptor, but many express D2R only (40%). Further categorizing HVC_{INT} based on somatostatin and parvalbumin expression did not show any differences in the proportion of cells expressing D2R. We also measured DA receptivity in RA-projecting HVC cells (HVC_{RA}) and Area X-projecting HVC (HVC_X) and found that in contrast to HVC_{INT}, most HVC_{RA} and HVC_X cells (55% and 65% respectively) express both D1R and D2R while some express D1R alone (25%, 16%). The remainder were negative for both D1R and D2R (18%, 16%). We next tested whether DA suppresses HVC_{INT} by recording spontaneous

IPSCs (sIPSCs) from HVC_{RA} in acute slices from juvenile males. Bath-application of DA or the D2R agonist Quinpirole significantly reduced sIPSC frequency but not amplitude, while the D1R agonist SKF-38393 had no effect, indicating that DA reduces HVC_{INT} activity. We tested the overall physiological effect of DA application on HVC PNs in slices by measuring sEPSCs from HVC_{RA} and found that DA increased sEPSC frequency but not amplitude. These experiments were conducted in the presence of a GABA_A receptor blocker, so the effect of DA on sEPSCs is independent of its effect on local inhibition. Taken together, our results show that DA has a net excitatory effect on the HVC microcircuit, through disinhibition of D2R-expressing HVC_{INT} and excitation of HVC PNs. Additionally, in vivo fiber photometric measurements of pan-neuronally-expressed GRAB DA fluorescence reveals that DA is released in HVC when a juvenile male is listening to a live tutor and even more reliably and potently when the juvenile sings. One intriguing possibility is that DA release is dynamically modulated across these two different contexts to preferentially activate or suppress different types of HVC neurons based on their expression of high affinity (D2) and low affinity (D1) DA receptors.

Disclosures: A.A. Mercer: None. A. Chen: None. R.D. Mooney: None.

Poster

PSTR346: Vocal/Social Communication

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.22/BB12

Topic: G.01. Neuroethology

Support: ANR CE37 AAPG2020-02

Title: Role of offline periods in sensorimotor learning in adult zebra finches

Authors: *N. SMITH;
Neurosci. Inst. of Paris-Saclay, Saclay, France

Abstract: Specific neuronal processing occurs during offline periods when individuals, engaged in a learning task, are resting or sleeping. This is considered as playing a key role in memory consolidation. Here, we aim at determining the contribution of the offline spontaneous neuronal activity in sensorimotor vocal learning performance of the zebra finch, a songbird species. Zebra finches learn to sing when juveniles and to maintain their song when adults, using a specialized neural circuit, homologous to mammalian basal ganglia-thalamo-cortical loop, known as the anterior forebrain pathway (AFP). In particular, the lateral magnocellular nucleus of the anterior nidopallium (LMAN), the output of the AFP loop, drives vocal variability required for song learning and plasticity. Adults, once learned song is stereotyped, are still able to adapt song features, e.g. the fundamental frequency (pitch) in a reinforcement-learning task that disrupts auditory feedback. Pitch changes are initially driven by LMAN. However, neurons in LMAN are known to be insensitive to auditory feedback disruption during singing, suggesting the contribution of offline periods to pitch changes. To address this issue, we exposed birds to a

reinforcement learning task while disrupting neuronal activity by stimulating bilaterally LMAN, when the bird was not singing, i.e. during rest and sleep periods. During the learning task, we continuously monitored songs and controlled that electrical stimulation of LMAN produced transient changes in song features. Preliminary results indicate disturbed spontaneous neuronal activity in LMAN during offline periods alter the learning course. Further investigations about the nature of offline spontaneous neuronal activity changes should be conducted to understand mechanisms underlying its contribution to sensorimotor learning.

Disclosures: N. Smith: None.

Poster

PSTR346: Vocal/Social Communication

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.23/BB13

Topic: G.01. Neuroethology

Support: NSF Grant 2046550

Title: Specialized enhancer activity associated with convergent evolution of vocal learning

Authors: *R. GANESAN¹, A. WANG¹, B. N. PHAN², M. LEONE², H. H. SESTILI², A. R. PFENNING³;

²Computat. Biol., ³Computat. Biol. Dept., ¹Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Vocal production learning, or vocal learning, is the ability to imitate sounds through social exposure. The rare trait has been observed in humans, bats, cetaceans, pinnipeds, songbirds, parrots, hummingbirds, and is believed to have independently evolved three times in birds and five times in mammals, representing a prime example of convergent behavioral evolution. All studied vocal learning species have evolved a specialized forebrain sensorimotor learning circuit, between the cortex, thalamus, and striatum, that is either absent or rudimentary in their closer, vocal non-learning relatives. Previous work within the lab has found that the “regulatory code” linking genome sequence to cell-type-specific function is highly conserved across mammals. In this study, we leverage this principle to identify candidate enhancers from publicly available Zoonomia datasets, and trained machine learning models predicting open and closed chromatin across all 240 mammalian motor cortex regions. We screened for differences in regulation between vocal learning and non-learning species by applying the Tissue-Aware Conservation Inference Toolkit, a machine learning approach to study how enhancer activity conservation relates to phenotype evolution across a large number of species at a cellular level. These machine learning models can learn sequence patterns of enhancers that robustly predict whether or not they have conserved activity across evolutionary distances, allowing us to test whether enhancer conservation patterns are associated with the evolution of vocal learning. Our results revealed lineage-specific gains and losses of regulatory elements, and we show that L6 corticothalamic neurons and oligodendrocytes had the strongest enrichment with the vocal

learning phenotype, suggesting key roles in vocalization-related traits through motor learning and synaptic plasticity. Additional enrichment in PV, SST, and VIP interneurons highlights modulation of cortical circuits involved in vocal learning. Future studies will include single cell integration across human, mouse, rat, macaque and the zebra finch to identify orthologous cell populations, and pathway analysis of the nearest genes of differentially expressed peaks, to better understand the molecular mechanisms associated with the convergent evolution of vocal learning.

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Poster

PSTR346: Vocal/Social Communication

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.24/BB14

Topic: G.01. Neuroethology

Support: Picker ISI major grant (colgate)

Title: Moving to the rhythm: Exploring song-entangled beat gesture in zebra finches

Authors: E. SLUPIK, E. OUYANG, A. ROMERO, I. JAFFE, C. OKAFOR, G. CIARAVINO, E. JOFFREY, Z. LIAO, S. KELLY, *W.-C. LIU;
Psychological and Brain Sci., Colgate Univ., Hamilton, NY

Abstract: When we speak, sing, or listen to music, we learn to move our hands, head, or body to the beat. Beat gestures often rhythmically align with particular tones, words, or phrases to emphasize prominence, or to enhance coordinated ensemble performance. Such highly dynamic sensorimotor coordination is hypothesized to be under the control of higher cortical regions and may have evolved only in vocal learning species. Songbirds have complex, learned vocalizations that are acquired in a process seeming to mirror human language development. The neural circuits for birdsong and human language learning also possess astounding similarities. We hypothesize that, like humans, vocal learning songbirds have evolved beat gestures that tightly align with their learned song. Using BORIS, a behavioral coding software, we quantified the gestures of 20 adult male zebra finches and analyzed corresponding acoustic features of each bird's song syllables. When singing a song motif, male finches produce non-random, rhythmic, and idiosyncratic lateral head movements that typically align with specific, more complex, syllables. These syllable affiliates are generally characterized by longer duration with higher variance of acoustic features. The song-entangled beats intensify or change during female directed song, accompanied by the courtship display (beak wipe, hops). The beat gestures are further manipulated by unilateral lesioning of a premotor nucleus, the HVC, which induced significant motif and syllable changes, quickly followed by rhythmic changes to beat gestures. The dynamic nature of co-song beat gestures is also supported by preliminary results of

developmental and video playback experiments. Our study provides the evidence that vocal learning songbirds perform subtle and dynamic co-song beat gestures that may convey robust and idiosyncratic signals for complex social communication.

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Poster

PSTR346: Vocal/Social Communication

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Program #/Poster #: PSTR346.25/BB15

Topic: G.01. Neuroethology

Support: NSF2154646

Title: Synaptic currents, neuronal excitability and energy efficiency in a songbird motor nucleus

Authors: *L. E. TAVARES¹, A. A. DAGOSTIN¹, C. V. MELLO², H. P. VON GERSDORFF¹;
¹Vollum Inst., ²Dept. of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Songbirds rely on fine motor control to produce learned complex song patterns. This is possible in part due to the unique properties of a specialized cortical circuit that dictates moment-by-moment the spectrotemporal features of the song. Nucleus RA sits at the final stage of song production in the brain and must transform sparse bursts from nucleus HVC into a denser but highly precise burst-pause spiking pattern. The principal neurons in RA are analogous to Layer 5 pyramidal neurons in M1 motor cortex and show a number of common features to Betz cells. The intrinsic excitable properties of RA neurons have been previously characterized but less is known about the short-term plasticity and biophysics of the synaptic inputs that RA projection neurons receive and how these are involved in song production. Using whole-cell patch clamp electrophysiology and afferent fiber stimulation, we characterize the heterogeneity in these inputs. We use these data to enable a detailed computational model to reproduce the bursting patterns of RA neurons during singing. With the help of the model, we make predictions relating ATP consumption and efficiency, as well as temperature dependence, excitability, the readily releasable pool of synaptic vesicles and short-term plasticity. Our results produce insights into the advantages avian brains attain by functioning at higher temperatures (40 degrees celsius and above).

Disclosures: L.E. Tavares: None. A.A. Dagostin: None. C.V. Mello: None. H.P. von Gersdorff: None.

Poster

PSTR346: Vocal/Social Communication

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR346.26/CC1

Topic: G.01. Neuroethology

Support: Simons Collaboration on the Global Brain

Title: A hierarchical neural circuit for flexible vocal behavior in the parrot brain

Authors: *A. BAHLE, Z. YANG, M. A. LONG;
Neurosci. Inst., New York Univ. Langone Med. Ctr., New York, NY

Abstract: Humans possess the remarkable ability to produce highly reproducible utterances, such as learned words or phrases, while retaining the capacity to generate completely novel sentences. This dual capability raises a fundamental question: how do neural circuits balance the stability of learned motor programs with the flexibility required for improvisation? We investigate this question in the budgerigar, a small species of parrot with the capacity for versatile, context-dependent vocal behaviors, including the mimicry of human words and phrases. In previous work, we showed that the primary vocal motor output region (the central nucleus of the anterior arcopallium (AAC)) contains a rich functional map of acoustic features, reminiscent of human cortical speech motor areas. Here we ask how this vocal acoustic map is controlled by upstream regions by recording from neurons in an area providing a major source of input to AAC: the central nucleus of the lateral nidopallium (NLC). In contrast to AAC, we find that neurons in NLC do not encode vocal acoustic features directly. Instead, NLC ensembles generate stereotyped sequences of bursting activity. This discrete set of, temporally structured neural-acoustic motifs that recur throughout vocalizations are reminiscent of words. Our findings reveal a hierarchical model of vocal motor control in which upstream areas like NLC organize behavior through the structured sequencing of motor primitives. This architecture may underlie the integration of stability and improvisation in complex learned behaviors, including human speech and music.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

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Topic: G.06. Autonomic Regulation

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System (TAME-AD)
Taub Institute Grants for Emerging Research (TIGER)

Title: Decoding the molecular, cellular, and functional heterogeneity of zebrafish intracardiac nervous system

Authors: *K. KOUTSOGIANNIS¹, A. PEDRONI¹, E. YILMAZ², L. DEL VECCHIO¹, P. BHATTARAI², I. TALAYA VIDAL¹, Y.-W. DAI¹, C. KIZIL², K. AMPATZIS¹;

¹Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; ²Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: The proper functioning of the heart relies on the intricate interplay between the central nervous system and the local neuronal networks within the heart itself. While the central innervation of the heart has been extensively studied, the organization and functionality of the intracardiac nervous system (IcNS) remain largely unexplored. Here, we present a comprehensive taxonomy of the IcNS, utilizing single-cell RNA sequencing, anatomical studies, and electrophysiological techniques. Our findings reveal a diverse array of neuronal types within the IcNS, exceeding previous expectations. We identify a subset of neurons exhibiting characteristics akin to pacemaker/rhythmogenic neurons similar to those found in Central Pattern Generator networks of the central nervous system. Our results underscore the heterogeneity within the IcNS and its key role in regulating the heart's rhythmic functionality. The classification and characterization of the IcNS presented here serve as a valuable resource for further exploration into the mechanisms underlying heart functionality and the pathophysiology of associated cardiac disorders.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Program #/Poster #: PSTR347.02/CC3

Topic: G.06. Autonomic Regulation

Support: BHF Fellowship FS/IBSRF/21/25060
Rosetrees Trust Grant M153-F2

Title: Neurohumoral regulation of coronary blood flow in cardioprotection: role of GLP-1R and K_{ATP} channels in cardiac pericytes

Authors: *S. MASTITSKAYA¹, D. ATTWELL²;

¹Univ. of Bristol, Bristol, United Kingdom; ²Univ. Col. London, London, United Kingdom

Abstract: Following reopening of a blocked coronary artery after MI, up to 50% of patients experience 'no-reflow,' a failure of microvascular reperfusion linked to poorer outcome. We previously showed this is caused by cardiac pericytes constricting capillaries, and that relaxing them reduces no-reflow. Remote ischaemic preconditioning (RPc) protects the heart via activation of the vagus nerve and release of the gut hormone GLP-1. Here, we used an *in vivo* ischaemia/reperfusion (IR) model in rats and mice, and *ex vivo* cardiac live tissue imaging in mice to define the molecular mechanisms downstream of GLP-1R activation involved in no-reflow prevention. RPc reduced pericyte-mediated coronary capillary constriction and no-reflow (11.4±0.4% coronary perfusion loss for IR+RPc vs 40.8±0.5% in IR; p=0.004, Sidak-corrected one-way Anova). This effect required GLP-1R activation: RPc-mediated pericyte relaxation was blocked by Exendin(9-39) (50 µg/kg), with 28.7±0.8% perfusion loss in IR+RPc+Ex9 vs 11.4±0.4% in IR+RPc; p=0.013. In pressurised right ventricular wall pieces, oxygen and glucose deprivation (OGD) for 25 min evoked pericyte contraction and capillary constriction (15.9±1.7% diameter reduction at n=21 pericytes in 6 hearts). Activating GLP-1R with Exendin-4 (100nM) during continued OGD relaxed pericytes within 25 min (constriction reduced to 5.0±1.1%, n=25 in 7 hearts; p<0.0001). GLP-1R-mediated pericyte relaxation in ischaemia required K_{ATP} channel opening: Kir6.1 deletion in pericytes abolished this effect (21.4±1.1% constriction, n=9 in 3 KO hearts vs 5.0±1.1%, n=25 in 7 WT hearts, p<0.0001). Consistently, RPc failed to protect Kir6.1 KO mice from IR injury (infarct size 29.0±2.8% of area at risk in RPc KO vs 10.0±1.0% in RPc WT; p=0.0002). GLP-1R activation also failed to relax pericytes in OGD when AMPK inhibitor compound C (CC, 5µM), L-NAME (100µM) or atropine (1µM) were present, and CC (10mg/kg) caused K_{ATP} channel internalisation during IR *in vivo*. These findings suggest that RPc-induced coronary flow enhancement is modulated by agents altering trafficking of K_{ATP} channels, including AMPK, NO and ACh, and define a brain-gut-heart pathway with therapeutic potential to prevent no-reflow and improve post-infarct recovery.

Disclosures: S. Mastitskaya: None. D. Attwell: None.

Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR347.03/CC4

Topic: G.06. Autonomic Regulation

Title: Heart rate changes related to unsure selections and outcomes in rat gambling

Authors: *F. FUKUSHIMA¹, A. TAMURA², N. KUGA^{1,3}, T. SASAKI^{1,4};

¹Dept. of Pharmacology, Grad. Sch. of Pharmaceut. Sciences, Tohoku Univ., Sendai, Japan;

²Med. Physics and Engin., Osaka Univ. Grad. Sch. of Med., Osaka, Japan; ³Creative

Interdisciplinary Res. Division, Frontier Res. Inst. for Interdisciplinary Sciences, Tohoku Univ., Sendai, Japan; ⁴Dept. of Neuropharmacology, Tohoku Univ. Sch. of Med., Sendai, Japan

Abstract: Risk-taking behavior involves uncertain outcomes and potential losses, and maintaining an appropriate balance of risk-taking is essential to maximize gains and minimize losses. One disorder characterized by impaired risk-taking balance is gambling disorder, a psychiatric condition in which pathological gambling severely disrupts daily life. Due to the limited availability of effective treatments, there is a pressing need to develop novel therapeutic approaches. Previous studies have reported that risk-taking behavior is influenced by physiological states. In this study, we specifically focused on the relationship between risk-taking behavior and heart rate. Our previous research has shown that heart rate decreases just before selecting the "Unsure option". In the present study, we modified our previously developed gambling task by adjusting the reward amount and probabilities to stabilize the choice rate. In this task, we used three nose-poke detectors. The left and right detectors were designated as the "sure option" (providing 1 drop of water) and the "Unsure option" (providing 4 drops of water with a 25% probability), respectively. After poking the central detector with their nose, the rats could obtain water by choosing either the left or right detector. Once the rats had learned the task, electrocardiogram electrodes were implanted, followed by a 3-day recovery period. The modified gambling task successfully stabilized the choice rate compared to previous versions. Furthermore, similar to our previous findings, heart rate significantly decreased just before selecting the unsure option. Additionally, heart rate varied depending on the number of water drops received. These results suggest a relationship between risk-taking behavior and heart rate. Information on heart rate is thought to be transmitted through the vagus nerve. Intervening in this pathway may influence the transmission of information between the periphery and the central nervous system, potentially affecting risk-taking behavior. Our preliminary experiments showed that vagus nerve stimulation increased the tendency for rats to select the unsure option again following an unrewarded trial. This result suggests that vagus nerve stimulation facilitates persistent risk-taking behavior. The progress of this study might establish a foundation for novel treatment strategies for psychiatric disorders that include impairment of decision-making.

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Poster

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Program #/Poster #: PSTR347.04/CC5

Topic: G.06. Autonomic Regulation

Support: NIH R01 HL162717

Title: Plasticity of stellate ganglion neurons marked by hypertrophy, increased neuronal excitability, and altered ion channel expression contributes to augmentation of sympathoexcitation after myocardial infarction

Authors: *J. D. TOMPKINS¹, A. NOSSEIR¹, V. OMILABU¹, S. SHARMA¹, C. R. MICCILE¹, C. CHEN¹, S. IKEDA¹, Y. ZHAO², X. YANG², O. A. AJIJOLA¹;
¹Dept. of Med. - Cardiol., ²Dept. of Integrative Biol. & Physiol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Enhanced cardiac sympathetic nerve activity (CSNA) increases risk for arrhythmias and sudden cardiac death (SCD) after myocardial infarction (MI). Mechanisms underlying the increased CSNA remain unclear. We hypothesized that changes in the firing properties of the postganglionic cardiac-projecting sympathetic neurons within stellate ganglia contribute to the increased CSNA after MI. We measured membrane properties of neurons from right (RSG; n=16) and left stellate ganglia (LSG; n=17) in mice with either chronic LAD ligation (4 weeks) or sham operation. Compared to sham animals, MI animals had significantly reduced left ventricular ejection fraction ($52.4 \pm 2.1\%$ in sham vs $23.63 \pm 4.0\%$ in MI, $p < 0.001$). Stellate ganglion neurons (SGNs) were randomly sampled around the origin of the inferior cardiac nerve, an area known to be enriched in cardiac-projecting neurons. Membrane excitability was tested in response to depolarizing currents and showed a greater number of action potentials in response to step increases in depolarizing current in the LSG of MI vs sham SGNs ($F(1, 554) = 13.12$, $p = 0.0003$). Rheobase, the minimum current needed to elicit an action potential, trended lower in the RSG after MI (198pA vs 150pA, $p = 0.07$), suggesting these neurons exhibit a lower excitability threshold. After-hyperpolarization amplitudes were smaller in LSG neurons after MI (-15 ± 2.7 mV vs -13 ± 2.6 mV, $p = 0.05$). These observations align with the hypothesis that neuronal excitability increases following MI, though differences are observed between the right and left sided ganglia which have unique patterns of cardiac innervation. Analysis of the cross-sectional area of RSG neurons in fixed ganglion whole-mount images revealed a significant increase in cell size in MI compared to sham neurons in both the LSG and the RSG ($p < 0.0001$). Consistent with these findings, single nucleus RNAseq of the SGNs identified significant changes in mRNA expression of sodium channels (NaV 1.7, NaV 1.3, & NaV 1.2) and minimal changes in potassium channels and calcium handling proteins. The results demonstrate significant hypertrophic changes and remodeling of conductances regulating neuronal excitability in SGNs post-MI.

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Poster

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Program #/Poster #: PSTR347.05/CC6

Topic: G.06. Autonomic Regulation

Support: NIH Grant R01HL141560

Title: High-frequency double-pulse VNS enhances NTS activation in heart failure

Authors: *M. OWENS¹, A. RADOVIC¹, E. BEAUMONT²;

²Biomed. Sci., ¹East Tennessee State Univ., Johnson City, TN

Abstract: Heart failure (HF) affects over 6 million adults in the United States alone and remains a leading cause of hospitalization and mortality worldwide. Excessive sympathetic and diminished parasympathetic tone contribute to disease progression and poor outcomes. The nucleus of the solitary tract (NTS), the first central site receiving vagal afferent input, plays a pivotal role in autonomic regulation by integrating peripheral signals and coordinating downstream responses. Vagus nerve stimulation (VNS) is a promising therapy designed to restore autonomic balance by increasing NTS excitability, thereby enhancing parasympathetic tone. However, the stimulation parameters that most effectively engage central autonomic circuits remain poorly defined. Importantly, only vagal shocks that successfully elicit action potentials in NTS neurons can influence physiological outcomes, yet no consensus exists on optimal VNS parameters, limiting clinical translation. In this study, we recorded single-neuron activity in the NTS of anesthetized male Sprague-Dawley rats with pressure-overload (PO) heart failure or controls using in vivo extracellular recordings. Baseline neuronal firing was first established, followed by six VNS paradigms (1-350Hz), including clinically relevant low-frequency standard-pattern stimulation (1-20Hz) and higher-frequency bursting patterns designed to enhance synaptic recruitment through temporal summation. This allowed us to evaluate how different stimulation strategies influence NTS activity in both healthy and diseased states. To further assess synaptic responsiveness, we applied single- and paired-pulse stimulation and quantified the total number of evoked action potentials within a 75ms window across 10 trials. This revealed a significantly greater proportion of negative responders (>15% reduced firing) in PO animals following single-pulse stimulation ($p = 0.005$). Conversely, high-frequency double pulses (350Hz) elicited a greater proportion of positive responders (>15% increased firing) in PO rats compared to controls ($p = 0.046$), while controls showed a significantly higher proportion of negative responders at this frequency ($p = 0.035$). These findings reveal frequency- and disease-specific differences in vagal afferent processing at the level of NTS and underscores the importance of optimizing VNS parameters to improve central autonomic engagement in HF. While single pulses increased negative responders in PO animals, high-frequency double pulses reversed this effect, highlighting the therapeutic potential of temporally patterned stimulation to restore vagal signaling.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Program #/Poster #: PSTR347.06/CC7

Topic: G.06. Autonomic Regulation

Support: NIH 5UG3MH126861

Title: A prefrontal cortex-hypothalamus circuit for heart rate control in non-human primates

Authors: *K. XU¹, T. OGASAWARA², J. YUAN¹, M. YUN², S. P. ERRINGTON², A. STARK¹, T. MINAMIMOTO³, K.-I. INOUE⁴, H. CHEN¹, I. E. MONOSOV²;

¹Biomed. Engin., Washington Univ. in St. Louis, Saint Louis, MO; ²Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO; ³Functional Brain Imaging, Natl. Inst. Quantum Sci. and Technol., Chiba, Japan; ⁴Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Japan

Abstract: Humans experience heart rate changes accompanying various emotional and cognitive events. This cognition-heart connection also becomes maladaptive in many psychiatric disorders (Ahmari & Rauch, 2022; Olbrich et al., 2022). However, how cognition is linked to heart function is poorly understood, particularly in primates. A candidate brain area that may link cognition with heart function is the ventral lateral prefrontal cortical area 47/12o. 12o is involved in many decision-making processes (Rudebeck et al., 2017; Jezzi et al., 2021) and sends strong projections to the lateral hypothalamus (LHA) - an area capable of heart rate regulation (Ongur et al., 1998; Wang et al., 1940; Zeng et al., 2018). The function of 12o→LHA remains unknown; therefore, we sought to uncover the role of 12o and LHA for heart rate regulation in monkeys. Electrical activation of a small ventral region in 12o evoked a rapid decrease in heart rate - akin to the heart skipping a beat. In contrast, nearby prefrontal areas elicited no or weaker heart rate effects. Anterograde tracer injection in 12o revealed dense projections to a region in LHA bordering the optic track. Like 12o, electrical activation of LHA, specifically in the region of 12o inputs, evoked a rapid decrease in heart rate. The decrease in heart rate evoked by 12o activation was more state dependent and required higher current compared with LHA activation. Unlike activation, both chemogenetic inactivation of 12o and muscimol inactivation of LHA caused heart rate increase. We then tested whether 12o→LHA regulates heart rate by using chemogenetics to dampen 12o inputs specifically to LHA. Dampening these inputs similarly increased heart rate, revealing 12o→LHA as a potent pathway for prefrontal heart regulation. Finally, we designed a method to modulate 12o in a cell-type specific, temporally precise, and minimally invasive manner that can facilitate closed-loop systems to treat cognitive diseases linked to cognition-heart dysfunction. We developed TRPV1 sonogenetics, which uses ultrasound-induced heating to activate many CaMKII+ 12o neurons engineered to express thermosensitive TRPV1. Sonogenetic stimulation of 12o evoked increased heart rate variability due to both heart rate decreases and increases that occurred on distinct timescales, roughly mirroring electrical activation responses, albeit with weaker intensity. Muscimol inactivation of LHA reduced sonogenetic-evoked heart rate responses, verifying that 12o→LHA is critical for heart rate modulation. Overall, 12o→LHA is a key prefrontal circuit enabling the cognition-heart connection, revealing a promising target for cognitive disease intervention.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR347.07/Web Only

Topic: G.06. Autonomic Regulation

Title: Effects of stochastic resonance on the characteristics of heartbeat evoked potentials and their relationship to cardiac cerebral synchronization

Authors: *V. RAMOS SCALABRIN^{1,2}, A. AVALOS SALGADO², K. DIAZ LOZANO², H. GALVAN GUERRERO², L. P. OSUNA CARRASCO², M. TREVINO VILLEGAS³, B. DE LA TORRE²;

¹Univ. de Guadalajara, Zapopan, Mexico; ²Bioingeniera Tanslacional - Lab. de Electrofisiologia, Univ. de Guadalajara, Guadalajara, Mexico; ³Mind/Brain Inst., Inst. De Neurociencias Udeg, Guadalajara, Mexico

Abstract: The Heartbeat Evoked Potential (HEP) is a neurophysiological signal that reflects cortical activity synchronized with events of the cardiac cycle and is considered an objective marker of the conscious perception of internal bodily signals (Park & Blanke, 2019). Time-locked to the R-wave of the ECG, the HEP exhibits a low amplitude (1-5 μ V) and is typically localized in frontocentral and centroparietal regions. Its amplitude and latency have been associated with processes such as interoceptive attention, bodily awareness, and stress, being more pronounced during states of calm and internal focus (Pollatos et al., 2005; Fukushima et al., 2011). Heart Rate Variability (HRV), as an indicator of autonomic flexibility, shows a positive relationship with HEP amplitude and is associated with enhanced self-regulatory capacity. Likewise, cardiac coherence defined as a harmonic oscillation in HRV, is associated with enhanced functional coupling between heart and brain, as reflected in increased activity in the alpha (8-13 Hz) and theta (4-8 Hz) bands both associated with sustained attention and psychophysiological balance (Gallo Villegas et al., 1999; McCraty & Shaffer, 2015). As part of interoceptive attention, respiration is incorporated as a key modulator, since cardiorespiratory synchronization measured by spectral coherence and cross-correlation with the ECG acts as an index of autonomic coupling and enhances cortical activity related to HEPs. Notably, spectral coherence between heart rate and respiration reveals characteristic harmonic patterns during interoceptive tasks, with significant peaks in low-frequency bands (~ 0.1 Hz), associated with increased alpha and theta activity, typical of internal concentration and emotional regulation states (McCraty et al., 2009; Kim et al., 2019). As part of the methodological approach, the stochastic resonance protocol is incorporated using subthreshold Brownian noise stimulation, aiming to induce more efficient HEPs in a time window. This approach is based on the principle that adding an optimal level of noise can improve neuronal detection of weak internal signals, modulating both amplitude and latency of the HEP. A significant increase in cardiorespiratory spectral coherence was observed during interoceptive attention compared to rest. Likewise, stochastic resonance stimulation produced a statistically significant increase in HEP amplitude ($p < 0.05$), as well as a reduction in latency, supporting its effectiveness in enhancing interoceptive processing under controlled experimental conditions.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Program #/Poster #: PSTR347.08/CC8

Topic: G.06. Autonomic Regulation

Support: National Council for Scientific and Technological Development (CNPq) - Brazil (Grant 444303/2024-3)

Title: Is autonomic imbalance a target to attenuate physical and cognitive disabilities in post-sepsis?

Authors: A. BETAT¹, G. AGUIAR², M. GOMES BIALESKI², ***R. M. LATARO**²;

¹Dept. of Pharmacol., ²Dept. of Physiological Sci., Federal Univ. of Santa Catarina, Florianopolis, Brazil

Abstract: Sepsis survivors exhibit a higher risk of rehospitalization and high mortality rates. The causes of the long-term post-sepsis ill-health are not well understood, and there is an urgent need for potential treatment targets. Individuals who recover from a sepsis episode develop physical and cognitive disabilities and also show symptoms of autonomic imbalance characterized by sympathetic hyperactivity. Further, sympathetic hyperactivity has been associated with cardiovascular and cognitive dysfunction, and it was demonstrated that the inhibition of the sympathetic nervous activity using beta-blockers exerts cardiovascular and neural protective effects. Hence, we hypothesized that sympathetic activity inhibition using a beta-blocker could prevent the physical and cognitive decline related to post-sepsis in rats. To test this hypothesis, we assessed physical and memory performance in rats chronically treated or not with carvedilol for 8 weeks. Carvedilol reduces sympathetic nervous activity, blocking both β_1 - and β_2 -adrenergic and α_1 -adrenergic receptors. Sepsis was induced in adult male Wistar rats (300 g, 90 days old) by the model of Cecal Slurry. The administration of carvedilol (60 mg/kg/day, drink water) or vehicle (carboxymethyl cellulose 0.2%, drink water) started 96h after sepsis induction and lasted 8 weeks. The results are presented as mean $\pm$ SD; vehicle (n=2) vs. carvedilol (n=3). The unpaired t-test was used to compare the groups. Differences were considered when $p < 0.05$. The exercise tolerance test on the treadmill was performed to analyze physical performance. In this test, running time and running distance were evaluated. The carvedilol treatment, compared to the vehicle, did not improve exercise tolerance in the post-sepsis rats (time: 77 $\pm$ 4 vs. 211 $\pm$ 198 s; $p=0.43$, and distance: 24 $\pm$ 2.8 vs. 74 $\pm$ 83 m; $p=0.4$). Recognition and work memory were assessed using the novel object recognition and Y-maze tests, respectively. In the novel object recognition task, rats treated or not with carvedilol exhibited similar new object discrimination index (0.6 $\pm$ 0.4 vs. 0.17 $\pm$ 1, $p=0.6$) and new object exploration time (230 $\pm$ 28 vs. 290 $\pm$ 147 s;

p=0.62), as well as, similar values of spontaneous alternation in the Y-maze test (331 ± 114 vs. 334 ± 264 s; p=0.99). These preliminary results suggest that the chronic administration of carvedilol does not affect physical or cognitive performance in post-sepsis rats.

Disclosures: A. Betat: None. G. Aguiar: None. M. Gomes Bialeski: None. R.M. Lataro: None.

Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Program #/Poster #: PSTR347.09/CC9

Topic: G.06. Autonomic Regulation

Support: NCCIH 1U54AT012307

Title: The REVEAL study: Unraveling Multisystem Responses to Vagus Nerve Stimulation Through Multimodal Physiological Modeling

Authors: *M. ZEBARJADI¹, A. ORGAN², H. H. LIM³;

¹Electrical and Computer Engin., Univ. of Minnesota, Twin Cities, Minneapolis, MN; ²Univ. of Minnesota, Forest Lake, MN; ³Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: The REVEAL (Research Evaluating Vagal Excitation and Anatomical Linkages) project investigates, for the first time, the effects of implanted cervical VNS on multiple physiological systems simultaneously to enable a whole-health approach towards treatment. While VNS implants are widely used to treat epilepsy and depression, they are emerging as potential therapies for conditions like rheumatoid arthritis, inflammatory bowel disease, obesity, heart failure, pulmonary hypertension, stroke recovery, and tinnitus. However, their effectiveness is limited by a lack of understanding of vagus nerve physiology, especially in how this complex vagus nerve modulates multiple organ systems across the body that maintains or can improve health outcomes. Most prior studies have focused on isolated effects of VNS, such as changes in heart rate or immune biomarkers, without considering interactions across systems. To address this gap, REVEAL collects a first-of-its-kind, extensive dataset from cardiovascular, immune, metabolic, and nervous systems. 144 participants with drug-resistant epilepsy and depression undergo two visits, 12 weeks apart, with three of six randomized VNS settings applied per visit. ECG, heart rate (HR), blood pressure (BP), muscle sympathetic nerve activity (MSNA), and blood biomarkers are collected, along with 24-hour ambulatory BP and ECG monitoring. This dataset enables evaluation of both acute and chronic effects of VNS, offering insights into individualized responses to stimulation. To date, six participants have completed both visits. Analyses include temporal, frequency, and nonlinear metrics. Heart rate variability and its dynamics were assessed using Poincare plots and instantaneous heart rate derived from ECG. BP trends were examined on an hourly basis, and MSNA bursts were detected using a deep learning-based algorithm developed in this study. Preliminary results show varied HR and BP trends but

consistent MSNA reduction. REVEAL will next investigate associations between physiological systems, assessing whether VNS enhances inter-system correlations as markers of autonomic and systemic coordination. AI-based models will predict VNS effects on one system using data from others, plus demographic and clinical information. Clustering will explore whether patterns like varied HR and BP, but consistent MSNA reduction align with demographic or medical history. These analyses aim to uncover individualized VNS response signatures and support personalized neuromodulation.

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Poster

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Program #/Poster #: PSTR347.10/CC10

Topic: G.06. Autonomic Regulation

Support: R01HL157366 to CRB

Title: Characterization of GABA_ARs-containing delta subunit contribution in cardiac vagal motor neurons in the nucleus ambiguus

Authors: *Y. B. WANG, C. R. BOYCHUK;
Biomed. Sci., Univ. of Missouri, Columbia, MO

Abstract: GABAergic neurotransmission mediated by GABA_A receptors (GABA_AR) is critical in cardiac vagal output generated by cardiac vagal motor neurons (CVN). GABA_ARs-containing δ subunit (GABA_AR δ) mediates tonic GABAergic current of CVN in the dorsal motor nucleus of the vagus. However, the contribution of GABA_AR δ to CVN in the nucleus ambiguus (CVN^{NA}) remains unknown. Therefore, we aim to characterize GABA_AR δ activity in CVN^{NA} using a combination of retrograde cardiac tracing and whole-cell patch-clamp electrophysiology. Since CVN^{NA} receives both glycinergic and GABAergic inputs, we first quantified the GABAergic contribution in brain slices of C57BL6/J mice. Using a glycine receptor blocker, strychnine (1 μ M), we isolated GABAergic currents and found that GABA contributes 26.5% of fast inhibitory synaptic input to CVN^{NA} (n=5 cells) with a mean frequency of 0.84 ± 0.5 Hz, amplitude of 26.6 ± 3.0 pA, and decay time of 4.0 ± 0.7 ms. To determine GABA_AR δ activity in CVN^{NA}, we applied a GABA_AR agonist, THIP (3 μ M), that preferentially activates the δ subunit. However, there is no significant change in phasic components (n=5 cells) or holding current (n=3 cells) after THIP exposure in CVN^{NA}. Then, we utilized a mouse line in which GABA_AR δ is insensitive to picrotoxin (d*Ki) to further elucidate GABA_AR δ role. This model enables us to differentiate between non-GABA_AR δ and GABA_AR- δ -mediated currents by applying GABA_AR antagonists, picrotoxin (PTX, 100 μ M) and gabazine (GBZ, 100 μ M), respectively. CVN^{NA} of d*Ki mice receives 36.2% fast inhibitory GABAergic input with a mean frequency of 0.37 ± 0.21 Hz, similar to C57BL6/J (n=4 cells). Application of PTX followed by GBZ reduced tonic

GABA_AR current by 9.58 ± 6.7 pA and 24.62 ± 10.7 pA (n=4 cells), respectively. Both antagonists also eliminated all sIPSCs. This shows that in CVN^{NA} of d*Ki mice, GBZ-sensitive current mediated by GABA_AR δ contributes 61.2% to tonic GABA_AR current, while 38.8% is PTX-sensitive, mediated by non-GABA_AR δ . In summary, GABA_AR δ robustly contributes to tonic GABA_AR in CVN^{NA} and is likely to be maximally active in normal slice conditions, given no significant increase in THIP-induced current. Future studies will investigate the plasticity of GABA_AR δ in CVN^{NA} and its impact on cardiac vagal output in health and disease.

Disclosures: Y.B. Wang: None. C.R. Boychuk: None.

Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Program #/Poster #: PSTR347.11/CC11

Topic: G.06. Autonomic Regulation

Support: BHF (FS/IBSRF/21/25060)

Title: Recruitment of vagal mechanisms to prevent ventricular tachyarrhythmias

Authors: *D. FEDOTOV, R. ASCIONE, A. F. JAMES, S. MASTITSKAYA;
Univ. of Bristol, Bristol, United Kingdom

Abstract: Ventricular arrhythmias (VAs), such as ventricular tachycardia and ventricular fibrillation, remain one of the most severe complications of myocardial infarction causing sudden cardiac death. Despite advances in understanding of the molecular mechanisms of VAs, searching for novel treatment targets to prevent or alleviate VAs acutely remains a valuable objective. The vagus nerve provides primary innervation to the parasympathetic ganglia, which subsequently prevent arrhythmias through the release of acetylcholine (ACh), nitric oxide (NO) and vasoactive intestinal peptide. The use of glucagon-like peptide-1 (GLP-1) receptor agonists has surged over the past decade with multiple beneficial effects on cardiovascular function being reported. Antiarrhythmic effect of GLP-1R activation with Exendin-4 is dependent on muscarinic receptors and NO, suggesting that GLP-1 acts on vagal post-ganglionic neurones in the heart, which release ACh and NO, and on microvasculature to activate endothelial NO synthase (Ang et al., 2018). GLP-1R-mediated prolongation of cardiac action potential duration in rat models was negated by atropine, suggesting a parasympathetic-mediated mechanism. Left ventricular electrophysiological responses to sympathetic and parasympathetic agonists were characterised in a mouse isolated Langendorff perfused heart preparation. The effective refractory period (ERP) was measured at five cycle lengths (CL): 40 ms, 60 ms, 80 ms, 120 ms and 160 ms, using a standard S1-S2 protocol (Hennis et al., 2022). Unexpectedly, we did not observe rate-dependent ERP shortening. The average ERP over all CLs was 35.0 ± 11.4 ms in control and was shortened in the presence of isoprenaline (ISO, 1 μ M: 28.4 ± 5.0 ms; $p < 0.05$) and extended in the presence of carbachol (1 μ M: 40.8 ± 11.2 ms; $p < 0.05$). We hypothesised that the

opening of K_{ATP} channels contributed to the ERP shortening upon sympathetic stimulation (Kim et al., 2012). The effects of ISO were completely abolished in the presence of K_{ATP} channel blocker glibenclamide (10 μ M). It has been previously shown that Exendin-4 also prolongs ERP in ISO-induced proarrhythmogenic conditions. That might indicate K_{ATP} -channels blockade as the underlying mechanism of GLP-1R-mediated antiarrhythmic effect. Further research on the modulation of VAs susceptibility by GLP-1R agonists and downstream signalling mechanisms in normoxic and ischaemic conditions could ultimately lead to the development of new therapeutic approaches for the treatment and prevention of ventricular tachyarrhythmias.

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Poster

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Program #/Poster #: PSTR347.12/CC12

Topic: G.06. Autonomic Regulation

Support: NHLBI R15HL145645-01
NIH NHLBI 1R15HL147286-01

Title: Characterizing the Role of the Medullary Raphe Nucleus in the Cardiogenic Sympathetic Afferent Reflex in Sprague-Dawley rats

Authors: *M. R. ZAHNER¹, M. J. CHANDLEY²;
²Biomed. Sci., ¹East Tennessee State Univ., Johnson City, TN

Abstract: Myocardial ischemia activates cardiac sensory afferents, triggering a sympathoexcitatory reflex known as the cardiogenic sympathetic afferent reflex. We have previously shown that the sympathetic activity derived from the paraventricular hypothalamic nucleus and the rostroventrolateral medulla plays an essential role in this reflex. However, the roles of other central nuclei are not fully understood. While the arterial pressure and renal sympathetic nerve activity (RSNA) response to epicardial bradykinin application are mediated by ionotropic glutamate receptors within the rostroventrolateral medulla, the reflex tachycardia involves other sympathetic supraspinal nuclei. The medullary raphe nucleus is well characterized in the sympathetic cardiovascular response to stress and metabolic activity; however, its role in the sympathetic cardiovascular response to noxious stimulation of visceral sensory afferents is not known. In this study, we tested the hypothesis that sympathetic activity from the medullary raphe nucleus plays an important role in the cardiogenic sympathetic afferent reflex. To do this, we measured arterial pressure, heart rate, and RSNA in anesthetized Sprague-Dawley rats after epicardial application of bradykinin (10 μ g/ml) before and after microinjection (100 nl) of either vehicle, the GABAA receptor agonist muscimol (1.0 nmol), or the ionotropic glutamatergic receptor antagonist kynurenic acid (10.0 nmol) into the medullary raphe. Vehicle microinjection (n=9) did not affect basal RSNA, arterial pressure, or heart rate, and epicardial application of

bradykinin elicited significant ($P < 0.05$) reflex responses. Muscimol inhibition of the raphe significantly reduced basal arterial pressure from 99.2 ± 4.6 to 86.7 ± 9.0 mmHg and heart rate from 346.8 ± 9.9 to 334.6 ± 8.7 bpm and attenuated the bradykinin-induced reflex responses in arterial pressure (from 36.5 ± 3.6 to 16.0 ± 4.7 mmHg), heart rate (from 13.1 ± 1.9 to 8.0 ± 1.9 bpm), and RSNA (from 133.6 ± 6.2 to 79.8 ± 12.8 % baseline). In contrast, microinjection of kynurenic acid ($n=9$) did not significantly change basal RSNA, arterial pressure, or heart rate, nor the reflex response elicited by epicardial application of bradykinin ($P > 0.05$). Collectively, these results suggest that sympathetic activity derived from the medullary raphe is important for the full manifestation of the cardiogenic sympathoexcitatory response. However, ionotropic glutamate receptors in the raphe are not directly involved.

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Poster

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Topic: G.06. Autonomic Regulation

Support: NIH Grant HL153916
American Diabetes Association (ADA) Pathway to Stop Diabetes, 1-18-
INI-14

Title: Molecular and functional taxonomy of cardiovagal neurons in the mouse medulla

Authors: *M. K. MACMILLAN¹, M. JALIL², M. CROOK², Y. WANG³, C. BOYCHUK³, J. CAMPBELL²;

¹Biomed. Sci., ²Biol., Univ. of Virginia, Charlottesville, VA; ³Univ. of Missouri, Columbia, MO

Abstract: Heart rate (HR) is one of the most widely used physiological measure of health, yet little is known about the molecular identity of upstream neurons that regulate the heart's activity. Parasympathetic neurons in the dorsal and ventral medulla project through the vagus nerve to innervate the heart and slow its rate at rest and during vital homeostatic reflexes - signaling which is impaired in many cardiovascular diseases and linked to their morbidity. Previous research identified cardiovagal neurons in two medullary regions, the nucleus ambiguus (NAmb) and the dorsal motor nucleus of the vagus (DMV). However, the molecular identity of these neurons was not known, limiting their further investigation. To identify these neurons, we performed single-cell transcriptomics on 1,245 and 2,200 cells of the NAmb and DMV, identifying 6 and 13 molecularly distinct subtypes, respectively. We then identified molecular markers for the cardiovagal neurons in each population, NAmb^{CVN} and DMV^{CVN}, using retrograde tracing from the cardiac fat pad in combination with RNA fluorescence in situ hybridization for candidate marker genes. Fluorescently labeling their axons with a genetically targeted tdTomato and tracing them to cardiac ganglia confirmed that the NAmb^{CVN} and

DMV^{CVN} subtypes both innervate the heart. We then implanted a wireless probe to record HR, heart-rate variability (HRV), and electrocardiogram (ECG) waveform metrics (ETA-F10, Data Sciences International) while activating NAmb^{CVN} and DMV^{CVN} with an intersectionally-targeted chemogenetic actuator, hM3Dq, and its ligand, deschloroclozapine (DCZ). Activating either NAmb^{CVN} (n=4 mice) or DMV^{CVN} (n=3 mice) in vivo with DCZ significantly decreased heart rate relative to vehicle treatment, though to a greater extent when activating NAmb^{CVN} neurons (vs. vehicle treatment, NAmb DCZ -205.33 +/- 61.75 bpm; DMV -144.22 +/- 27.19 bpm, mean +/- S.D.). Activation of both NAmb^{CVN} and DMV^{CVN} led to increased heart rate variability. These results paralleled a positive control experiment in which we activated all vagal efferent neurons in either NAmb or DMV. Interestingly, we also saw an increase in PR interval when activating NAmb^{CVN} but not DMV^{CVN}, pointing to potential changes in atrio-ventricular (AV) node conductivity specifically during NAmb^{CVN} activation. Together, these preliminary results molecularly, anatomically, and functionally identify NAmb^{CVN} and DMV^{CVN} and suggest they differentially alter heart rate, heart rate variability, and AV node conduction.

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Poster

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Topic: G.06. Autonomic Regulation

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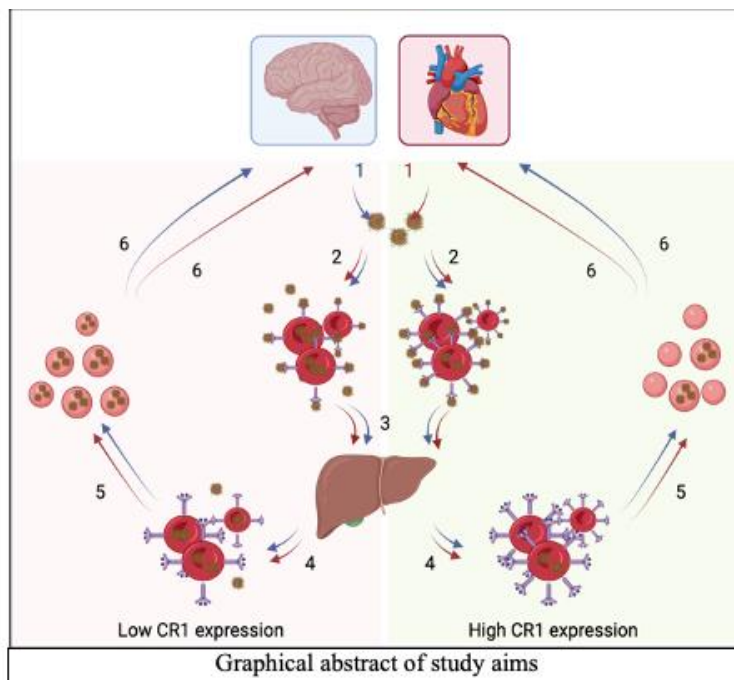
Title: Red Blood Cells shuttle β -amyloid between brain and heart: implications for the pathogenesis and the progression of Alzheimer's and Cardiomyopathy

Authors: C. BACCHIN¹, I. GHIRAN², *F. DEL MONTE³;

¹Univ. of Padua, Padova, Italy; ²Beth Israel Deaconess Med. Ctr., Boston, MA; ³Med. Univ. of South Carolina, Charleston, SC

Abstract: Alzheimer's cardiomyopathy (AC) has emerged as a result of an increasing understanding of the pathophysiological similarities between Heart Failure (HF) and Alzheimer's Disease (AD), including sharing AD biomarkers (amyloid-beta (A β) and tau aggregates). However, is still unknown if the pathology originates in one organ and then spreads to others, or if it occurs as a systemic manifestation. Soluble pre-amyloid oligomers (PAO) can migrate and impact nearby cells *in vitro*. *In vivo*, their diffusion in the circulatory system may provide an effective pathway for seeding pathogenic processes remotely. However, reliable quantification of circulating PAO as diagnostic biomarkers has proven difficult, implying that these species either circulate at undetectable concentrations or in a bound state, which prevents accurate

measurement by conventional techniques. Extracellular vesicles (EVs) are nanosized membrane enclosed vesicles that have emerged as major mediators of intercellular communication. Complement components can capture A β and bind to CR1 on red blood cells (RBC), allowing the delivery of molecular cargo for clearance. **Thus, we tested** the possibility that RBC-EVs can transport A β PAO, contributing to the spread of AD pathology. We hypothesized that reduced complement receptor 1 expression, due to polymorphism, impairs A β clearance while increasing its incorporation in RBC EVs for intercellular transfer. We generated RBC-EVs +/- exogenous A β PAO to test A β uptake *in vitro* in isolated adult cardiomyocytes and *in vivo* by injecting RBC-EVs +/- A β in wild-type mice, followed by testing cognitive and cardiac function by echocardiography after 3 months. Cardiomyocytes internalized A β laden EVs *in vitro* and *in vivo*, resulting in impairment in episodic and working memory and significant reductions in systolic and diastolic cardiac function. Together, these findings indicate that A β PAO can spread throughout the body via RBC-EVs, potentially contributing to the pathogenesis of AD and AC. This mechanism may operate alongside, or as an alternative to, CR1 mediated A β clearance.



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Poster

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Program #/Poster #: PSTR347.15/CC15

Topic: G.06. Autonomic Regulation

Title: Volitional regulation of heart rate modulates functional brain-autonomic coupling in healthy adults

Authors: *R. KANAI¹, J. USHIBA², M. TAKEMI³;

¹Grad. Sch. of Sci. and Technology, Keio Univ., Tsuzuki-ku, Yokohama-shi, Kanagawa, Japan;

²Fac. of Sci. and Technol., Keio Univ., Kanagawa, Japan; ³Fac. of Advanced Sci. and Engin., Hiroshima Univ., Higashihiroshima, Japan

Abstract: Humans unconsciously regulate heart rate (HR) and body temperature to maintain physiological homeostasis. The autonomic nervous system (ANS), central to this regulation, has traditionally been considered involuntary. However, recent animal studies have demonstrated that ANS activity can be voluntarily modulated (Yoshimoto et al., 2024), suggesting the involvement of cortical regions anatomically connected to the ANS (Levinthal et al., 2020). The present study aimed to examine whether humans can volitionally regulate HR, and how such voluntary modulation affects interactions between autonomic and cortical activity. Fifteen healthy adult males attempted to either increase (HR-Up) or decrease (HR-Down) their HR during 20-minute biofeedback sessions conducted on the same day. Electrocardiogram (ECG) and electroencephalogram (EEG) were simultaneously recorded. HR values during biofeedback were normalized to those obtained during a resting baseline without feedback. Heart rate variability (HRV), an index of sympathovagal balance, and EEG power in multiple frequency bands were evaluated. Results showed a significant HR increase during HR-Up ($t(29) = 2.16$, $p = 0.02$, one-tailed) and decrease during HR-Down ($t(29) = -2.80$, $p = 0.004$, one-tailed) relative to the baseline. The ratio between low and high frequency components of HRV was significantly higher during HR-Up than HR-Down ($F(1, 14) = 15.68$, $p = 0.001$), and frontal theta EEG power was also significantly higher during HR-Up compared to HR-Down ($F(1, 14) = 4.71$, $p = 0.048$). These results indicate that humans can volitionally control HR, accompanied by systematic modulation of autonomic and frontal cortical activity. To further examine directional brain-heart interactions, Brain-Heart Interaction (BHI) analysis was conducted (Catrambone et al., 2019), revealing that ascending cardiac signals modulated frontal cortical oscillations in a state- and frequency-specific manner: coupling between low-frequency HRV and frontal alpha-band activity was greater in HR-Up compared to HR-Down, whereas coupling between low-frequency HRV and frontal theta-band activity was reduced in HR-Down compared to Rest. No significant modulation was observed in descending brain-to-heart pathways. These findings suggest that afferent autonomic inputs dynamically shape cortical states during volitional HR control. Future studies should investigate whether such autonomically driven cortical modulation also affects cognitive functions.

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Poster

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Program #/Poster #: PSTR347.16/DD1

Topic: G.06. Autonomic Regulation

Title: Aging through the cardio-autonomic lens: heart rate variability in middle-aged and older adults

Authors: ***B. BALAKRISHNAN**¹, **P. GONZALEZ-CABRERA**¹, **N. CONTRERAS**², **M. GANESAN**³;

¹Physical Therapy, Univ. of St. Augustine for Hlth. Sci., San Marcos, CA; ²Univ. of Texas, El Paso, TX; ³Physical Therapy, Univ. of St. Augustine for Hlth. Sciences, San Marcos, CA, San Marcos, CA

Abstract: This study aimed to compare time-domain and frequency-domain measures of heart rate variability (HRV) between late middle-aged and older adults to explore age-related changes in cardiac autonomic regulation. Twenty-seven active, community-dwelling individuals participated, including 12 middle-aged adults (55.41 ± 5.80 years) and 15 older adults (75.76 ± 8.03 years), recruited from community centers. Ten-minute lead II ECG recordings were collected following standard procedures, and artifact-free 5-minute segments were analyzed using AD Instruments LabChart 8 software. HRV parameters included time-domain metrics—coefficient of variation of NN intervals (CVNN) and standard deviation of heart rate (HRSD)—and frequency-domain indices such as total power (ms²), low-frequency (LF) power (ms²), LF percentage (LF%), and high-frequency HF (ms²). Statistical tests were chosen based on data distribution as determined by the Shapiro-Wilk test. Results showed that middle-aged adults had significantly higher CVNN ($p = 0.013$), HRSD ($p = 0.007$), total power ($p = 0.019$), LF power ($p = 0.019$), and LF% ($p = 0.021$) compared to older adults. However, no significant difference was observed in sympathovagal balance, as indicated by the HF/LF ratio ($p = 0.157$). These findings suggest a reduction in sympathetic, parasympathetic, and baroreceptor activity in older adults, as reflected by lower LF values, indicating diminished autonomic modulation and flexibility with aging. The study underscores the value of heart rate variability (HRV) analysis in assessing cardiovascular autonomic function and highlights the physiological changes that accompany the aging process.

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Poster

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Program #/Poster #: PSTR347.17/DD2

Topic: G.06. Autonomic Regulation

Support: Medical Research Council Grant MR/Z504555/1

Title: Selective vagus nerve stimulation to target cardiac efferent activity in humans for the treatment of heart disease

Authors: *N. THOMPSON¹, E. RUTKOVSKIS¹, M. GUTIERREZ¹, I. JONES¹, E. RAVAGLI¹, A. MISEROCCHI², A. MCEVOY², D. HOLDER³, K. ARISTOVICH¹;
¹Univ. Col. London, London, United Kingdom; ²Natl. Hosp. for Neurol. and Neurosurg., London, United Kingdom; ³UCL, London, United Kingdom

Abstract: Introduction Spatially selective vagus nerve stimulation (sVNS) offers a promising solution to mitigate the off-target effects associated with traditional VNS in which the whole nerve is stimulated. This could serve as a precise method to address chronic heart failure by specifically targeting efferent cardiac fibres and thereby enhance therapeutic outcomes by avoiding off-target effects and eliminating the need for titration. However, the spatial organisation of afferent and efferent cardiac-related fibres within the vagus nerve remains unknown. To address this, we have determined the functional organisation of the cardiac afferent and efferent projections within the cervical vagus nerve of pigs and have initiated translational work in humans. **Methods** A multi-electrode sVNS cuff (14-electrode pair array) was placed at the mid-cervical level of the exposed left vagus nerve in pigs and in consenting epilepsy patients already undergoing surgery for, but before implantation of, the FDA approved device (NCT05664854). Current was applied sequentially to each pair of electrodes in the same radial (o'clock) position on the 2 rings while recording evoked cardiac, pulmonary and laryngeal responses. This was repeated at different parameters optimised for the respective functional responses and repeated after vagotomy in pigs. **Results** In pigs, there were 2.0 ± 1.7 most effective electrode pairs eliciting HR decrease of $-7.8 \pm 3.4\%$ associated with cardiac efferent activation. A heart rate increase of $10.2 \pm 5.3\%$ ($n=6$) was induced and sustained post-vagotomy, associated with cardiac afferent activation. There was significant spatial separation of all four regions but specifically, cardiac afferent and efferent CoMs ($200 \pm 137^\circ$, $p < 0.05$, $\text{mean} \pm 1\text{SD}$) at the mid-cervical level. Preliminary results in humans ($n=1$) shows a 5% tachycardia with full VNS. During sVNS, this was sustained with 3-4% tachycardia for 11/14 electrode pairs (cardiac afferent). On 3 electrodes, a 4, 5, and 7.5% bradycardia was achieved, respectively. This displayed selective activation of cardiac efferent projections. **Conclusions** Significant separation between afferent and efferent cardiac-related regions occurred in all pigs, localised to roughly the opposite sides of the nerve, in close proximity to the pulmonary and laryngeal regions, respectively. For the first time, we have shown this within the vagus nerve in humans. This would allow the ability to selectively elicit therapeutic-related efferent effects without affecting afferent-related reflexes. This paves the way for more targeted neuromodulation to enhance precision and efficacy of VNS therapy in treating heart failure.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR347.18/DD3

Topic: G.06. Autonomic Regulation

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PEDECIBA

Title: Autonomic Flexibility in the Stress Response in Teachers Trained in Mindful Self-Compassion

Authors: T. LIBERMAN¹, M. BIDEGAIN², A. BERRIEL³, F. ROMERO⁴, V. CONSONNI⁶, R. SANZ⁷, *M. E. CASTELLO⁵;

¹Dept. de Neurociencia y Aprendizaje, Facultad de Ciencias de la Salud, ³Facultad de Ciencias de la Salud, ²Univ. Católica del Uruguay, Montevideo, Uruguay; ⁴Dept. Neurociencias Integrativas y Computacionales, ⁵Neurociencias Integrativas y Computacionales, Inst. de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; ⁶Escuela de Tecnología Médica, Facultad de Medicina, Montevideo, Uruguay; ⁷Facultad de Humanidades y Ciencias de la Educación, Udelar, Montevideo, Uruguay

Abstract: Introduction: The stress response is an adaptive physiological mechanism that enables individuals to cope with threatening stimuli. However, prolonged activation of this response disrupts homeostasis, making efficient autonomic recovery after the stressful event essential. The Covid-19 pandemic exacerbated the already high levels of occupational stress experienced by teachers. This study aimed to evaluate whether a 9-week virtual training in Mindful Self-Compassion (MSC), compared to Kundalini Yoga (KY) as an active control, improves post-stress autonomic recovery in female primary school teachers. To this end, Heart rate variability (HRV) was assessed during a psychosocial stress test (Trier Social Stress Test, TSST).

Hypothesis: Participants trained in MSC would exhibit a more adaptive stress response to the TSST. This would be reflected in higher HRV compared to the control group.

Materials and methods: Uruguayan primary school teachers participated in a 9-week virtual training in either MSC or KY. They then completed the TSST while their electrocardiogram was recorded for heart rate and HRV analysis.

Results: Analysis of HRV (time domain, frequency domain and non-linear measures) revealed an increase in the stress response in both groups, followed by faster and more sustained recovery in the MSC group. This was evident through higher levels of vagally mediated HRV.

Discussion: MSC training promotes faster and more flexible autonomic recovery following stress, indicating greater ability in teachers who received the training to adapt to emotional demands.

Conclusion: These findings support the use of MSC training to promote psychophysiological health in teachers.

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Poster

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Program #/Poster #: PSTR347.19/DD4

Topic: G.06. Autonomic Regulation

Title: Comparative analysis of autonomic dysfunction in type 2 diabetics among highlander and lowlander populations of Nepal

Authors: *S. K. DEO^{1,2}, K. AGRAWAL¹, S. KUMAR²;

¹Birat Med. Col. & Teaching Hosp., Biratnagar, Nepal; ²Sikkim Manipal Inst. of Med. Sci., Gangtok, India

Abstract: Background: Autonomic dysfunction is a critical aspect of cardiovascular health, particularly in populations exposed to chronic high-altitude environments. This study is aimed to compare the autonomic function between diabetic highlanders and lowlanders using a series of cardiac autonomic function tests. **Methods:** Autonomic function was assessed in 60 diabetic highlanders and 60 diabetic lowlanders using the Deep Breathing Test, Heart Rate Standing Test, Valsalva Maneuver, Postural Hypotension Test, Sustained Handgrip Test, and Ewings Classification. Parasympathetic and sympathetic dysfunction was also evaluated. Statistical significance was determined using chi-square tests. **Results:** Diabetic highlanders exhibited significantly higher rates of autonomic dysfunction compared to Lowlanders. Key findings included: 1) **Sustained Handgrip Test:** 93.33% abnormal in Highlanders vs. 61.67% in Lowlanders ($p = 0.0002$). 2) **Parasympathetic Dysfunction:** Highlanders had more severe dysfunction (13.33% severe vs. 11.67% in Lowlanders, $p = 0.0037$). 3) **Sympathetic Dysfunction:** Highlanders showed higher rates of moderate to severe dysfunction (66.67% moderate, 13.33% severe vs. 51.67% moderate, 8.33% severe in Lowlanders, $p = 0.000006$). 4) **Ewings Classification:** Highlanders had a significantly higher prevalence of severe autonomic dysfunction ($p = 0.0000157$).

No significant differences were observed in the Heart rate standing test, Valsalva maneuver test, and Postural hypotension test. **Conclusion:** Diabetic highlanders demonstrate a higher prevalence of autonomic dysfunction, particularly in sympathetic domains, compared to diabetic lowlanders. These findings may reflect physiological adaptations to chronic high-altitude exposure and highlight potential cardiovascular risks in this population. Further research is needed to elucidate the underlying mechanisms and clinical implications of these differences.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Topic: G.03. Stress and the Brain

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Title: Experience- and region-specific parvalbumin interneuron plasticity reveals KAT6A dependent epigenetic mechanisms underlying stress-induced behavioral change

Authors: *Y. GUO¹, Y. GAO¹, S. KNAACK¹, Z. XU², M. SHEN¹, X. ZHAO¹;
¹Neurosci., Univ. of Wisconsin-Madison, Madison, WI; ²Cell. and Mol. Biol., Univ. of Wisconsin, Madison, Madison, WI

Abstract: Environmental inputs and life experiences profoundly influence brain development. Parvalbumin-expressing interneurons (PVIs) are sparsely distributed, fast-spiking cells that provide feedback and feedforward inhibition to principal neurons, which has critical roles in experience-driven brain plasticity. However, how PVIs respond to different environmental stimuli remains poorly understood. Here, using PVI-specific translational profiling (RiboTag-seq), we examined the gene expression networks in PVIs in frontal cortex (FC), dorsal hippocampus (dHip), and ventral hippocampus (vHip) in response to positive (voluntary running) and negative (chronic stress) experiences. We found that PVIs exhibit both brain region-specific responses to the same stimulus and experience-specific responses within the same brain region. We further investigated histone acetyltransferase KAT6A which exhibited elevated levels in the FC of stressed mice. We show that AAV-mediated KAT6A overexpression in the FC led to behavioral deficits and histone acetylation changes. Together, these findings reveal brain region- and experience-specific PVI plasticity and identify an epigenetic mechanism mediating stress-induced behavioral changes.

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Poster

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Program #/Poster #: PSTR347.21/DD6

Topic: G.03. Stress and the Brain

Support: College of Arts and Sciences, Quinnipiac University

Title: Chronic Unpredictable Stress, NF- κ B, and the Estrous Cycle: Behavioral and Metabolic Signatures

Authors: *N. SYME¹, P. SACK², S. AZERRAD³, T. PANTALENA⁴, J. MATHEWS⁵, A. J. BETZ⁴;

¹Quinnipiac Univ., North Andover, MA; ²Quinnipiac Univ., Plainville, NY; ³Biomed. Sci.,

⁴Psychology, ⁵Quinnipiac Univ., Hamden, CT

Abstract: Female rodents remain underrepresented in preclinical stress research, although stress-related disorders disproportionately affect women. This study investigated the effects of chronic unpredictable stress (CUS) on behavior and immune-related protein signaling in adult female rats, with a particular focus on the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). NF- κ B regulates the immune system, inflammation, and stress responses. Animals were subjected to a 26-day CUS protocol involving two randomized stressors per day, including a modified component for social isolation. A battery of behavioral tests was administered throughout the 26 days. No significant differences were observed in anxiety-like behavior on the elevated plus maze; however, some locomotor differences emerged. Like previous male CUS studies in our lab, CUS substantially reduced daily weight gain, suggesting metabolic alterations. We observed alterations in triglyceride quantities in fecal extractions. NF- κ B protein expression was measured via Western blot analysis of vaginal swab samples, with estrous cycle phases determined through vaginal cytology. Notably, alterations in NF- κ B expression across the estrous cycle were observed in stressed animals. These findings contribute to a deeper understanding of how chronic stress interacts with biological sex, metabolism, and immune pathways, with relevance to disorders such as depression and autoimmune disease.

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Poster

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Program #/Poster #: PSTR347.22/DD7

Topic: G.03. Stress and the Brain

Title: Nucleus accumbens CDKN1A/p21 escalates sugar consumption and oxidative stress in a mouse model of chronic electric shock stress

Authors: *B. KIM¹, H.-I. IM²;

¹Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; ²Ctr. For Brain Disorders, Korea Inst. of Sci. & Technol., Seoul, Korea, Republic of

Abstract: Excessive sugar consumption threatens the global health by increasing the risk of metabolic disorders and cardiovascular diseases. Chronic stress is a central driver of increased sugar consumption in animals. However, the biological mechanism underlying stress-induced sugar consumption has been undiscovered to date, mainly due to the lack of animal model that recapitulates stress-induced sugar consumption in humans. In this study, we demonstrate that a chronic stress regimen by mild, inescapable, continuous electric shock stress induced a depression-like phenotype characterized by heightened sugar consumption in mice. The chronic electric shock stress (SS) effectively caused an aberrant increase in oxidative stress response and its intermediary regulator CDKN1A/p21 without affecting cellular senescence in the nucleus accumbens. In addition, overexpression of nucleus accumbens CDKN1A/p21 was sufficient to escalate sugar consumption behavior. Importantly, SS impaired nucleus accumbens neural population activity and spike waveform, which were rescued by *in vivo* administration of the small-molecule CDKN1A/p21 inhibitor UC2288. In accordance, UC2288 effectively alleviated the stress-induced sugar consumption. Collectively, these results indicate that the chronic electric shock stress regimen can induce excessive sugar consumption in mice, and that nucleus accumbens CDKN1A/p21 is a potential therapeutic target of stress-induced sugar consumption.

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Poster

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Topic: G.04. Neuroimmunology and Neurovirology

Support: H.H.M.I. Hanna H. Gray Fellowship GT15986
Simons Society of Fellows - Simons Foundation 718232
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Title: Gut bacteria-derived succinate induces enteric nervous system regeneration

Authors: *B. AYDIN;
The Rockefeller Univ., New York, NY

Abstract: *Enteric neurons control gut physiology by regulating peristalsis, nutrient absorption, and secretion. Disruptions in microbial communities caused by antibiotics or enteric infections result in the loss of enteric neurons and long-term motility disorders. However, the signals and underlying mechanisms of this microbiota-neuron communication are largely unknown. We studied the effects of microbiota on the recovery of the enteric nervous system after microbial dysbiosis caused by antibiotics. We found that both enteric neurons and glia are lost after antibiotic exposure, but recover when the pre-treatment microbiota is restored. Using murine gnotobiotic models and fecal metabolomics, we identified neurogenic bacterial species and their derived metabolite succinate as sufficient to rescue enteric neurons and glia. Unbiased single-*

nuclei RNA-seq analysis uncovered a novel neural precursor-like population marked by the expression of the neuronal gene Nav2. Genetic fate-mapping showed that Plp1+ enteric glia differentiate into neurons following antibiotic exposure. In contrast, Nav2+ neurons expand upon succinate treatment and indicate an alternative mode of neuronal regeneration under recovery conditions. Our findings highlight specific microbial species, metabolites, and the underlying cellular mechanisms involved in neuronal regeneration, with potential therapeutic implications for peripheral neuropathies.

Disclosures: B. Aydin: None.

Poster

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Program #/Poster #: PSTR347.24/DD9

Topic: G.03. Stress and the Brain

Support: NIH R15ES036710

Title: Effects of short-term sublethal microcystin ingestion on the gut-brain axis of male mice

Authors: *S. GHAJU¹, R. S. C. TAKESHITA², X. MOU³, W. C. J. CHUNG³;

¹Sch. of Biomed. Sci., ²Anthrop., ³Biol. Sci., Kent State Univ., Kent, OH

Abstract: Blue-green algae, which make up the majority of cHABs (cyanobacterial harmful algal blooms), produce various cyanotoxins. This includes microcystin leucine arginine (MC-LR), which can have detrimental effects on the central nervous system. Humans can be exposed to MC-LR through drinking water, the food chain, or skin contact. Previous studies found that acute intraperitoneal exposure to MC-LR disrupts not only the gut microbiome but also hypothalamus-pituitary-adrenal (HPA) control of the stress response. Disrupted brain-gut axis function may contribute to mental illnesses, such as depression or anxiety. Here, we hypothesized that short-term ingestion of sublethal concentrations of MC-LR disrupts HPA function and gut integrity. To test our hypothesis, we gavaged young adult male C57/BL6 mice every two days for 21 days with water (vehicle) or MC-LR (50 µg/kg). We found a significant increase in plasma corticosterone levels as well as in hypothalamic corticotropin-releasing hormone and arginine vasopressin mRNA expression in MC-LR-treated mice. Furthermore, ΔFOSB was elevated in the paraventricular nucleus of the hypothalamus, dentate gyrus of the hippocampus, and amygdala in MC-LR-treated mice. In contrast, hypothalamic glucocorticoid receptor mRNA expression was decreased in MC-LR-treated mice. Moreover, our analysis of gut microbial composition and function using 16S RNA sequencing revealed significant changes in the function of the gut microbiome processes, which negatively impact the metabolism and cellular processes in MC-LR-treated mice. For example, MC-LR-treated animals had significantly reduced pathways like L-glutamate degradation V pathway, salicylate degradation pathways, and toluene degradation IV, which are vital for energy metabolism and production of

important biological compounds. Together, these data support the conclusion that short-term ingestion of sublethal levels of MC-LR disrupted HPA function and gut microbiome, which may negatively impact the gut-brain axis.

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Poster

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Program #/Poster #: PSTR347.25/DD10

Topic: G.03. Stress and the Brain

Support: GLYCORE

Title: Role of AGE/RAGE Signaling as a Pathological Driver in the Brain

Authors: *S. HULUGALLA;

Dept. of BioMolecular Sci., Dept. of BioMolecular Sci. The Univ. of Mississippi, Oxford, MS

Abstract: The accumulation of advanced glycation end products (AGEs) contributes a significant impact on the increased risk of cognitive and neurological impairments in the brain due to neuroinflammation. When AGEs bind to the Receptor of Advanced Glycation End Products (RAGE), it accelerates normal aging with the increased risk of diseases such as onset of dementia, diabetes, muscle degeneration, and vascular calcification. Previous studies indicate that reducing RAGE expression improves cardiovascular and cognitive function in young diabetic RAGE knockout (RKO) mice. However, our past studies using 3-4-month-old RAGE Knockout (RKO) and RAGE wildtype (RWT) mice showed learning and memory impairments in the RKO mice, which demonstrated an opposite effect from the literature. Therefore, further research is needed to understand the effects of AGE/RAGE signaling in the brain. The goal of this study was to investigate the behavioral changes in RKO and RWT mice to assess differences in neurological function to determine the effects of AGE/RAGE signaling in the brain in response to inflammation. The current study utilized a larger cohort of male and female RKO and RWT mice that were 6 months old. Additionally, inflammation was induced using lipopolysaccharide (LPS, 1 mg/kg) prior to behavioral assays, such as Elevated Plus Maze, Radial Arm Water Maze, and Light and Dark Box trials. Our data revealed RKO have increased anxiety, decreased memory and spatial learning, and decreased light sensitivity when compared to RWT mice. In addition to this, male RKO mice had more elevated levels of anxiety compared to the female RKO mice. LPS treatment also exacerbated anxiety, and heightened light sensitivity in RKO mice compared to WT mice. This study reveals that the lack of RAGE in 6-month-old knockout mice, exacerbated by LPS-induced inflammation leads to worsened behavioral changes underscoring the significant impact of AGE/RAGE signaling on neurological function.

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Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

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Topic: G.03. Stress and the Brain

Support: VR 2023-02000
VR 2020-01485
FoUI-985353
FoUI-955054

Title: Kynurenine Pathway Activation and Neuroinflammation: A Cross-Species Perspective on Post-Operative Cognitive Decline

Authors: *M. GOMEZ-GALAN^{1,2}, D. SCHREY², A. EBBERYD², L. ERIKSSON², S. ERHARDT²;

¹Dept of Physiol. & Pharmacol., Karolinska Inst., Stockholm, Sweden; ²Dept of Physiol. & Pharmacol., Karolinska Institutet, Stockholm, Sweden

Abstract: Post-operative neurocognitive disorders commonly affect older adults, impacting independence, dementia risk, and mortality. While its causes remain unclear, elevated inflammatory and neurodegenerative markers in blood and CSF, along with post-surgical brain inflammation, have been linked to long-term cognitive decline (Danielson et al., 2020; 2021). The kynurenine pathway (KP) of tryptophan degradation has emerged as a potential link between inflammation and cognitive function. Metabolites of the KP modulates glutamate transmission and have been implicated in psychiatric disorders with cognitive impairments, such as schizophrenia and bipolar disorder (Erhardt et al., 2001; Schwarcz et al., 2001; Sellgren et al., 2016). Here we used mass spectrometry (UPLC-MS/MS) to profile key KP metabolites in serum and CSF from 24 patients undergoing elective hip or knee surgery, collected pre-operatively and up to 48 hours post-surgery (4-, 8-, 24-, 32- and 48 hours). These data were compared with previously reported pre- and post-inflammatory and cognitive profiles from the same cohort (Danielson et al., 2020). Data show early activation of the brain KP, marked by a transient rise in CSF kynurenine at 4- and 8-hours post-surgery, followed by a sustained increase in CSF kynurenic acid (KYNA) lasting up to 48 hours. Notably, KYNA levels associate closely with the pro-inflammatory cytokine interleukin-6, whose elevated 48-hour levels correlate with cognitive impairment at 3-5 days and 3 months post-surgery. To investigate the molecular mechanisms underlying surgery-induced KP activation, we examined peripheral and striatal KP metabolites, as well as gene expression of key enzymes in the striatum and liver, using our established mouse model of post-surgical cognitive decline (Terrando et al., 2011; Femenía et al., 2018). Although temporal KP dynamics differ between humans and mice, we observed early brain KP activation at 6 hours post-surgery, along with increased expression of the rate-limiting enzyme Tryptophan dioxygenase 2 (TDO2) and the kynurenine aminotransferase isoform (KAT III). Together, these findings highlight a conserved early activation of the KP across species following surgery, likely

driven by neuroinflammation. The sustained elevation of KYNA, potentially through increased TDO2 and KAT III expression, may disrupt neurotransmission and contribute to cognitive decline, underscoring the KP as a potential therapeutic target for post-operative neurocognitive disorders.

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Poster

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Topic: G.03. Stress and the Brain

Support: NIH Grant K08-MH130802
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Title: Foxp2 regulates fear learning in the intercalated cells of the mouse amygdala

Authors: *O. PONOMAREVA¹, L. T. SEABROOK¹, C. KLENGEL¹, S. KINI², E. CATT², J. ZION², P. MARTINEZ GONZALEZ¹, Q. FLANAGAN-BURT¹, J. SUH¹, W. A. CARLEZON, Jr.³, K. J. RESSLER⁴;

¹McLean Hosp., Belmont, MA; ²Northeastern Univ., Boston, MA; ³Dept Psychiat, Harvard Med. Sch./McLean Hosp., Belmont, MA; ⁴Harvard Med. Sch., McLean Hosp. / Harvard Med. Sch., Belmont, MA

Abstract: Post-Traumatic Stress Disorder (PTSD) is a common and debilitating mental illness with limited available personalized treatments. Recent large-scale Genome Wide Association Studies (GWAS) have implicated the role of transcription factor Forkhead box P2 (FOXP2) in this disease. *Foxp2* mRNA is expressed in clusters of Intercalated Cells (ITCs) surrounding the basolateral amygdala. ITCs are functionally important for conditioned fear response and fear extinction; however, the role of the Foxp2 transcription factor in modulating fear-related behaviors in the amygdala remains unknown. Recent evidence suggests that Foxp2 is regulated by multiple components of the Wnt signaling pathway and, in turn, acts in a feedback loop to modulate regulation of Wnt pathway members. Using an adult mouse model, the current study tests the hypothesis that Foxp2 regulates specific components of fear learning through its interaction with the Wnt pathway. We show that *Foxp2* mRNA is dynamically regulated after fear learning in male mice and a Wnt agonist, Lithium, increases *Foxp2* levels in the amygdala. Following RNAi-mediated knockdown (KD) of Foxp2 in the ITCs, mice have reduced expression of fear learning and fear extinction. We find no difference in time ambulating, distance travelled in an open field task, time spent in open vs closed arms of an elevated plus maze, time exploring a novel object, or acoustic startle response, suggesting that the observed learning phenotype is specific to expression of fear. Recording from individual ITCs, we find

that Foxp2 KD increases ITC excitability, consistent with a model of increased inhibition to the central amygdala and subsequent reduction in behavioral output. Using bulk RNAseq from amygdala punches of animals with Foxp2 KD, we find a decrease in genes involved in previously characterized pathways involved in fear learning, including CRH, neurokinin, Wnt, and thyroid signaling. Gene ontology reveals an enrichment in processes at the synapse, with specific regulation of trans-synaptic signaling, regulation of pre- and post-synaptic membrane potential, and synaptic vesicle cycle. Intriguingly, we find that downregulated targets with the highest significance are enriched in genes identified as Tier 1 putative PTSD causal genes in a recent GWAS. Overall, this is the first study to describe the functional role of Foxp2 and its modulators in processes mediating fear learning in the amygdala and suggests that Foxp2 is an upstream hub gene for transcriptional regulation of multiple pathways previously identified in fear learning.

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Poster

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Topic: G.03. Stress and the Brain

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KQTD20200820113040070
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Title: Cyp450 inhibitor skf525a unexpectedly accelerates emergence from propofol anesthesia

Authors: *H. WANG, Z. ZHU, J. HU, H. YAO, B. CAO, X.-J. SONG;
Dept. of Med. Neuroscience, SUSTech Ctr. for Pain Med. and Med. Sch., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Although the notion that the return of consciousness from anesthesia is merely a passive byproduct of anesthetic metabolism is now considered outdated, pharmacological inhibition of the enzymes responsible for metabolizing anesthetics typically delays the recovery of consciousness. Here, we report that intraperitoneal injection (i.p.) of SKF525A—an inhibitor of the CYP450 enzyme responsible for propofol metabolism—significantly inhibited propofol metabolism without altering propofol-induced anesthesia. In contrast, intracerebroventricular (i.c.v.) administration of SKF525A accelerated recovery of consciousness from propofol anesthesia without affecting propofol metabolism. Our results showed that SKF525A, i.p. or i.c.v., activated noradrenergic neurons in the locus coeruleus (LC) as shown by c-Fos

immunostaining, fiber photometry and electrophysiology recordings. Chemogenetic inhibition of LC noradrenergic neurons abolished the arousal-promoting effect of SKF525A. Patch-clamp recordings from noradrenergic neurons in the locus coeruleus (LC) revealed that SKF525A treatment reduced the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) without altering their amplitude. In contrast, neither the frequency nor the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) in LC noradrenergic neurons was affected by SKF525A. These findings demonstrate that SKF525A activates LC noradrenergic neurons by reducing their inhibitory presynaptic inputs, suggesting that SKF525A induces disinhibition of postsynaptic noradrenergic neurons through the suppression of LC GABAergic neurons. Our patch-clamp recordings further showed that the firing rate of LC GABAergic neurons was significantly inhibited by SKF525A treatment. Chemogenetic activation of LC GABAergic neurons eliminated the arousal-promoting effect of SKF525A. As a control, i.p. carveol, another CYP450 inhibitor, effectively suppressed propofol metabolism and prolonged the emergence time from propofol anesthesia, as anticipated. In contrast, intracerebroventricular (i.c.v.) administration of carveol had no significant effect on the recovery of consciousness from anesthesia. The present study reveals an unexpected effect of the CYP450 enzyme inhibitor SKF525A in promoting emergence of consciousness from propofol anesthesia by activating the noradrenergic neurons through inhibiting the GABAergic neurons in the LC.

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Poster

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Topic: G.03. Stress and the Brain

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2023M741538

Title: Ubiquitin-proteasomal degradation of KCC2 in the ventral posteromedial nucleus of the thalamus is critical for the emergence of consciousness from anesthesia

Authors: *C. PENG¹, J. HU², Y. LIU², B. CAO², H. YAO², X.-J. SONG²;

¹Southern Univ. of Sci. and technology, Shenzhen, China; ²Dept. of Med. Neuroscience, SUSTech Ctr. for Pain Med. and Med. Sch., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Consciousness is suppressed when the brain is exposed to anesthetics. The mechanisms by which the brain loses and regains consciousness have long been subjects of

research and debate—yet they remain poorly understood. Emergence of consciousness from anesthesia has recently been reconsidered as an active and controllable process, rather than a purely passive process dependent on the elimination of anesthetics from the body. We have recently revealed that, when the brain is forced into the minimum responsive state by an anesthetic, a rapid and consistent downregulation of KCC2 in the ventral posteromedial nucleus of the thalamus (VPM) serves as a common molecular mechanism to resist the sharply decreased level of consciousness and to trigger and accelerate the process of recovery from anesthesia (Hu et al., Nat Neurosci 2023). It is demonstrated that the ubiquitin proteasomal degradation is responsible for the quick downregulation of KCC2 in anesthesia driven by multi-subunit E3 ubiquitin ligase cullin1^{Fbx14/Skp1}. The E3 ubiquitin ligase cullin1^{Fbx14/Skp1} catalyzes the transfer of the ubiquitin to KCC2. How then the ubiquitinated KCC2 is delivered to the ubiquitin proteasome for degradation remains unknown. We have recently provided an answer to this important question (data partly published, Chen et al., PNAS 2025). We report in cultured neuro-2a cells that the valosin-containing protein (VCP) transported ubiquitinated KCC2 to the proteasome and in mouse in vivo experiments that inhibition of VCP restored KCC2 expression in the VPM and enhanced the effects of anesthesia. In cultured neuro-2a cells, propofol-induced degradation of KCC2 was inhibited by VCP inhibitor DBeQ and VCP knockout plasmid sgRNA(VCP). Propofol-induced enhanced interaction between VCP and KCC2 was inhibited by knockout of Fbx14 or Fas-associated factor 1 (FAF1). In vivo studies, pharmacological or genetic inhibition of VCP in the VPM significantly prevented KCC2 degradation and enhanced propofol anesthesia; these effects were abrogated by a KCC2 antagonist VU0463271. These results demonstrate that the VCP controls ubiquitin-proteasomal degradation of KCC2 dependent on FAF1 recruitment and serves as a mechanism for the ubiquitin-proteasomal degradation of KCC2, which is responsible for the subsequent emergence from anesthesia. This ubiquitin degradation of KCC2 in anesthesia is distinct from the NMDAR-Ca²⁺-calpain- or BDNF-TrkB activation-mediated KCC2 degradation in physiological and pathological conditions.

Disclosures: C. Peng: None. J. Hu: None. Y. Liu: None. B. Cao: None. H. Yao: None. X. Song: None.

Poster

PSTR347: Cardiovascular Regulation and Stress Physiology

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR347.30/DD15

Topic: G.02. Neuroendocrine Processes and Behavior

Support: FAPESP grant # 2022/06260-3 and 2020/11827-7
CNPq grant # 304484/2022-9

Title: Corticotropin-releasing factor neurotransmission in the medial prefrontal cortex controls contextual conditioned responses in rats

Authors: *C. C. CRESTANI¹, L. GOMES-DE-SOUZA³, C. BUSNARDO², R. L. NUNES-DE-SOUZA⁴;

¹São Paulo State Univ. (UNESP), Araraquara, Brazil; ²São Paulo State Univ. (UNESP), ARARAQUARA, Brazil; ³Lab. of Pharmacology, Sch. of Pharmaceut. Sci., São Paulo State Univ., Araraquara, Brazil; ⁴Pharmacol., Sao Paulo State Univ., Araraquara, Brazil

Abstract: INTRODUCTION: The medial prefrontal cortex (mPFC) is a limbic structure involved in the expression of conditioned responses, including those related to contextual fear conditioning (CFC). However, the local neurochemical mechanisms involved in this control are not completely understood. In this sense, previous studies identified the presence of terminals within the mPFC containing corticotropin-releasing factor (CRF) and urocortin 1, as well as the expression of CRF₁ and CRF₂ receptors. Nevertheless, although evidence that stressful stimuli increase the release of CRF within the mPFC, a role of this neurochemical mechanism in contextual conditioned responses was never reported. **OBJECTIVE:** To investigate the role of CRF neurotransmission present within the mPFC in contextual conditioned responses in rats. **METHODS:** Male Wistar rats had cannula-guide implanted bilaterally in the mPFC. For the conditioning, each animal was placed individually in the conditioning chamber and received six shocks (1,5mA,3s). Twenty-four hours after the conditioning session, the animals were submitted to femoral artery cannulation surgery for cardiovascular recording. The fear retrieval test was performed 48 hours after the conditioning session. Independent set of animals received bilateral microinjections into the mPFC 10 min before the fear retrieval test of either vehicle (saline, 100nL), the selective CRF₁receptor antagonist CP376395 (5nmol/100nL) or the selective CRF₂ receptor antagonist antisauvagine-30 (5nmol/100nL). Blood pressure (BP) and heart rate (HR) increases and freezing behavior were evaluated during the fear retrieval test. **RESULTS:** Bilateral microinjection of the CRF₂ receptor antagonist enhanced the freezing ($F_{(2,17)}=4.7$; $P=0.0453$), but without affecting the cardiovascular changes (BP: $F_{(1,16)}=0.12$, $P=0.7298$; HR: $F_{(1,16)}=0.15$, $P=0.7067$). Conversely, bilateral pharmacological treatment of the mPFC with the CRF₁ receptor antagonist reduced the pressor effect during contextual fear retrieval ($F_{(1,16)}=5.6$; $P=0.0316$), but without changing the tachycardiac ($F_{(1,16)}=0.15$, $P=0.7067$) and freezing ($F_{(1,16)}=1.153$, $P=0.2980$) responses. **CONCLUSIONS:** These results indicate that CRF neurotransmission present within the mPFC modulates expression of contextual conditioned responses. However, data indicate that control of freezing and cardiovascular responses by CRF mechanisms within the mPFC during fear retrieval test is segregated, being mediated by different CRF receptors.

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Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.01/DD16

Topic: H.02. Reward and Appetitive Learning and Memory

Support: PAPIIT-DGAPA IN203124

Title: Alterations in dopamine and acetylcholine volume in the nucleus accumbens and prefrontal cortex induced by long-term sugar consumption

Authors: *M.-I. I. MIRANDA;
Inst. Neurobiologia-UNAM, Queretaro, Qro., Mexico

Abstract: Eating sweet foods activates memory and reward circuits, allowing us to decide which foods to seek out and consume in the future. Previous work in rodents has shown that long-term sugar consumption produces changes in appetitive relearning, which could lead to an escalation of intake due to a disability in learning new negative consequences associated with the same taste. The objective of this study was to evaluate the extracellular volume of dopamine and acetylcholine in the nucleus accumbens and prefrontal cortex of adult male Wistar rats during novel and familiar sugar consumption (2 days) and after prolonged sugar consumption (14 days). Rats were exposed *ad libitum* for 14 days to 10% sugar solution (long-term and familiar groups) or water (novel and familiar groups). During the first 14 days, rat weight, food intake, and fluid intake were measured, and the guide cannula was implanted under anesthesia. Subsequently, on days 15 to 17, rats were habituated to the microdialysis chamber. After 18 hours of total fluid deprivation, on days 18 (novel and long-term groups) and 19 (familiar group), microdialysis was performed with a single waterer presented for 15 minutes. HPLC-EC microdialysis analyses revealed significant differences in the extracellular volumes of dopamine and acetylcholine in both the NAc shell and PFC in rats exposed to sugar for a prolonged period, compared with those familiarized with sugar for 3 days or those exposed to acute (novel) sugar consumption. These data indicate a shift in cholinergic and dopaminergic signaling that correlates with increased familiarity with a highly reinforcing stimulus such as sugar.

Disclosures: M.I. Miranda: None.

Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.02/EE1

Topic: H.02. Reward and Appetitive Learning and Memory

Title: Semaglutide Selectively Suppresses Neuronal Activities in the Reward System Associated with Consuming a High Fat Diet.

Authors: *T. TAKEDA^{1,2}, K. KONDOH^{3,2}, K. ASAI¹, T. IDE¹, L. FU⁴, M. KUNO¹, M. SUGIYAMA¹, H. TAKAGI⁵, R. BANNO^{1,4}, H. ARIMA¹, Y. MINOKOSHI^{6,2};

¹Dept. of Endocrinol. and Diabetes, Nagoya Univ. Grad. Sch. of Med., Nagoya, Aichi, Japan;

²Div. of Endocrinol. and Metabolism, Natl. Inst. for Physiological Sci., Okazaki, Aichi, Japan;

³Dept. of Physiology, Fac. of Med., Tottori Univ., Yonago, Tottori, Japan; ⁴Dept. of Sports Med., Nagoya Univ., Nagoya, Aichi, Japan; ⁵Dept. of Endocrinol. and Diabetology, Nagoya City Univ.

East Med. Ctr., Nagoya, Aichi, Japan; ⁶Dept. of Human Nutr., Sugiyama Jogakuen Univ., Nagoya, Aichi, Japan

Abstract: Highly palatable foods, such as high-fat diets (HFD), promote hedonic eating regardless of homeostatic energy requirements and contribute to eating disorders like binge eating. This behavior is regulated by the mesolimbic reward system, specifically dopamine (DA) neurons in the ventral tegmental area (VTA), which project to the nucleus accumbens (NAc). Semaglutide, a glucagon-like peptide-1 receptor agonist, is known to reduce cravings for high-calorie foods by enhancing satiation and fullness. Recent rodent studies have shown that neuronal activity in various brain areas, including VTA DA neurons, may be influenced by semaglutide treatment. However, it remains unclear how hedonic HFD feeding affects the reward system and whether semaglutide influences the system associated with hedonic eating. To investigate this, we analyzed the neural activity of VTA DA neurons and the extracellular DA levels in the NAc during HFD feeding in freely moving mice, with or without semaglutide administration. To this end, we expressed a calcium sensor, GCaMP7s, in VTA DA neurons or a dopamine sensor, GRAB_DA2m, in neurons of the NAc in mice, conditioning them to learn the palatability of HFD. We then measured the activity of DA neurons or the levels of extracellular DA using fiber photometry when the mice were provided HFD with or without semaglutide treatment. The activities of DA neurons and extracellular DA levels in the NAc rapidly increased when the mice consumed HFD, correlating with both the amount and duration of HFD intake. A single administration of semaglutide decreased the activity of VTA DA neurons and DA levels in the NAc while reducing HFD intake. Repeated administration of semaglutide transiently reduced lab chow intake in mice, leading to sustained weight loss. We compared the DA response in the NAc of control mice to a pair-fed group that had equivalent food intake and body weight reduction as the semaglutide-treated group. When HFD was provided, the increase in DA levels in the NAc and HFD intake significantly rose in the pair-fed group. However, semaglutide significantly suppressed the increases in both HFD intake and DA levels compared to the pair-fed group. Semaglutide did not influence the DA response when the mice were presented with HFD but had not yet been fed. Furthermore, semaglutide did not affect the activation of VTA DA neurons or extracellular DA levels in the NAc when the mice were exposed to novel objects made of wood and cork, respectively. These findings suggest that semaglutide specifically suppresses the reward response to highly palatable foods. This mechanism could account for the clinical efficacy of semaglutide in weight management.

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Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.03/EE2

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIMH Grant R21MH132052
BBRF YI Award 274780

Title: The effect of reward palatability on dorsal striatal GABA release

Authors: *N. WENGER¹, O. DINCKOL², M. G. KUTLU²;
¹Ctr. for Substance Abuse Res. (CSAR) Temple Univ. - Lewis Katz Sch. of Med., Philadelphia, PA; ²Dept. of Neural Sci. / Ctr. for Substance Abuse Res., Temple Univ. - Lewis Katz Sch. of Med., Philadelphia, PA

Abstract: The striatum is a significant input region of the basal ganglia and has a variety of functions that include motor behavior and action initiation, reward processing, classical conditioning in the ventral striatum, and operant conditioning in the dorsal striatum. GABA is the primary inhibitory neurotransmitter of the nervous system, which is released throughout the striatum by GABAergic interneurons and GABAergic projections from distal sources. Studies have shown that GABA_B receptor activation has an inhibitory effect on the reinforcement of reward, and the dorsal striatum has been implicated in the development and maintenance phases of drug-seeking behavior. To better understand the role of GABA release in the dorsal striatum, we examined the effects of sucrose consumption on GABA release patterns in the dorsolateral (DLS) and dorsomedial (DMS) striatum using fiber photometry with the genetically encoded GABA sensor iGABA.Sn.FR2. Male and female C57BL/6J mice (n=16) were given access to sucrose solutions via a retractable sipper in an operant chamber for 30 minutes. We found that increasing the palatability of the reward by increasing the concentration of sucrose from 10% to 30% resulted in increased DLS GABA release during consumption. Conversely, we did not observe the same effect in the DMS, suggesting that the role of GABA in reward processing differs within the different sub-regions of the striatum. Overall, these results indicate that the DLS GABA release may signal the palatability of foods. In the future, we plan to record GABA levels in the dorsal striatum during the seeking of food rewards with increased value, following periods of food deprivation.

Disclosures: N. Wenger: A. Employment/Salary (full or part-time): Full-time, Temple University. O. Dinckol: A. Employment/Salary (full or part-time): Full-time, Temple University. M.G. Kutlu: A. Employment/Salary (full or part-time): Full-time, Temple University.

Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.04/EE3

Topic: H.03. Motivation

Support: NIH Grant R01AA027065
NIH Grant R01AR077183
NIH Grant R01DK103901

Title: Thermal Regulation of Feeding Through a Hypothalamic-Midbrain Circuit

Authors: *X. YANG¹, X. HU¹, Z. XIE², F. GAO¹, N. ABE¹, K. ZANG⁴, H. HU³;

¹Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Anesthesiol., Icahn Sch. of Med. at Mount Sinai, Forest Hills, NY; ³Anesthesiol., Icahn Sch. of Med. at Mount Sinai, Port Washington, NY;

⁴Washington Univ. in St. Louis, saint louis, MO

Abstract: Hot ambient temperatures (HAT) are potent anorexigenic cues, yet the central mechanisms linking thermal sensing to feeding inhibition remain poorly defined. Here, we identify a discrete population of lateral hypothalamic neurons expressing tachykinin receptor 3 (LH^{Tacr3}) as key mediators of HAT-induced feeding inhibition. Whole-brain c-Fos mapping and *in vivo* calcium imaging reveal selective activation of LH^{Tacr3} neurons under HAT exposure. Chemogenetic activation of these neurons suppresses food intake in both fed and fasted states, whereas their genetic ablation reverses HAT-induced feeding inhibition. Circuit mapping shows that LH^{Tacr3} neurons receive direct glutamatergic input from the ventromedial preoptic area (VMPO), a canonical thermoregulatory center, and project to feeding-regulatory nuclei in the parabrachial nucleus (PBN) and ventrolateral periaqueductal gray (vlPAG). These findings delineate a thermosensory-feeding circuit through which the CNS couples external thermal stress to behavioral energy conservation, uncovering a key neural substrate for temperature-dependent modulation of energy homeostasis.

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Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

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Program #/Poster #: PSTR348.05/EE4

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH T32 #2T32DA-7281-26A1
NIH T32 #5T32DA72-8127-27
E. Matilda Ziegler Foundation
NIH R01DK129366

Title: Metabolic and motivational state-dependent processing in visual association cortex

Authors: *A. CHIU^{1,2}, E. FRANKOWITZ¹, C. BURGESS^{1,3};

¹Michigan Neurosci. Inst., ²Neurosci. Grad. Program, ³Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: How we form associations between environmental stimuli and their associated outcomes is crucial for how we interact with our dynamic environment. Neural and behavioral responses to cues can be guided by internal states, for example hunger state can impact how food

cues are perceived. In visual association cortex (VisCtx), salient cues promote an enhanced response, supporting the integration of visual stimuli and predicted outcome. Even in the absence of learning, basic visual processing of stimuli in V1 adaptively changes in response to food-restriction in mice. We hypothesized that modulating metabolic and motivational state would induce adaptive changes to visual processing in the VisCtx. We imaged neural activity in VisCtx using chronic in vivo two-photon microscopy, while mice transitioned between different states of hunger and learned associations between visual stimuli and associated food rewards. We show a decrease in orientation selectivity in VisCtx during food-restriction which can be reversed by re-feeding, independent of any learning of food-related cues, as had been previously demonstrated in V1. As mice learn to associate cues with the availability of food, we show that the salience is reflected as an enhanced response in a subset of VisCtx neurons. We next aimed to identify possible subcortical circuitry that could relay metabolic or motivational signals to sensory cortices. Melanin concentrating hormone (MCH) expressing neurons in the lateral hypothalamus promote food intake and are implicated in behavioral responses to food cues. MCH neurons also project to areas involved in motivation and sensory processing, including to VisCtx. We pharmacologically inhibited MCH receptors and observed a decrease in the number of VisCtx neurons responding to the food-reward cue, previously enhanced through learning, but no decrease in neurons responsive to a neutral cue. Understanding how metabolic and motivational states directly or indirectly influence visual processing will help identify therapeutic targets for eating disorders.

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Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.06/EE5

Topic: H.02. Reward and Appetitive Learning and Memory

Support: DK134804
R25NS105143

Title: Isocaloric high-fat diet decreases motivation in the absence of obesity.

Authors: *K. ARBUCKLE¹, B. A. MATIKAINEN-ANKNEY²;
¹BSN, Psychology, Rutgers, Piscataway, NJ; ²Psychology, Rutgers Univ. Behavioral and Systems Neurosci., Highland Park, NJ

Abstract: Previous work has shown that obesogenic diets induce persistent changes in physical activity and motivation in rodents and people. It remains unclear whether these behavioral changes are driven by weight gain or exposure to obesogenic diets themselves. We investigated how exposure to a high-fat diet (HFD) in the absence of obesity affected physical activity, food motivation, and 24-hour activity cycles in mice. We gave C57Bl6J mice limited access to HFD

in a 6-week paradigm: after a baseline week in which all mice received ad libitum standard chow, one group continued to receive chow for 5 weeks (control, “con,” n = 8), and one group received 80% of their daily calories from an HFD in addition to ad libitum chow for 5 weeks (isocaloric, “iso,” n = 9). An isocaloric HFD did not significantly increase weight or change physical activity levels. Operant behavior assays were measured to determine how isocaloric HFD affected behavior and physiology. We trained the iso and con mice to perform a two-choice nose-poking task wherein a correct poke yielded a 20-mg sucrose pellet. Mice performed a 24-h fixed ratio task in which each trial required one nose poke to gain one pellet. Groups made similar numbers of pokes and exhibited similar poke accuracy, suggesting that iso mice did not display learning deficits relative to con. Mice then performed a 24-h progressive ratio assay, wherein subsequent trials required an increasing number of correct nose pokes to achieve a pellet. Iso mice made significantly fewer correct pokes than con mice throughout the assay and performed fewer overall trials than con mice, with no significant difference in accuracy. One interpretation of a decrease in performance on a nose-poking task for sucrose pellets is a decrease in motivation for palatable food. Therefore, we asked whether the decrease in sucrose demand observed in iso mice would correlate with future weight gain with ad libitum exposure to an HFD. In order to test this, we exposed the same cohort of mice to 3 weeks of an ad libitum HFD in the home cage and monitored weight and home-cage physical activity levels. Although both groups gained significant amounts of weight, there was no significant difference in overall weight gain between con and iso mice. We conclude that isocaloric preexposure to an HFD decreased motivation to work for food, but did not alter rate of weight gain with a subsequent ad libitum HFD. This suggests that exposure to an obesogenic diet, even in the absence of significant weight gain, can induce behavioral changes associated with obesity.

Disclosures: K. Arbuckle: None. B.A. Matikainen-Ankney: None.

Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.07/EE6

Topic: G.03. Stress and the Brain

Title: Effect of food deprivation of a palatable diet on behavior and food intake in female rats

Authors: *P. ROMERO¹, S.-M. ARACELI², E. BARRIOS DE TOMASI²;

¹Lab. de farmacología y conducta, Univ. de Guadalajara, Zapopan, Mexico; ²INSTITUTO DE NEUROCIENCIAS, Univ. de Guadalajara, Guadalajara, Mexico

Abstract: INTRODUCTION: The consumption of sugar-sweetened beverages and ultra-processed foods has been linked to obesity and food addiction. Stress increases intake of these foods, contributing to binge eating and weight gain. Female rats exposed for 20 weeks to a cafeteria diet show increases in perigonadal and retroperitoneal fat. This study examines how early exposure to different diets, followed by restriction in adulthood, affects anxiety-like

behaviors, caloric efficiency, body weight and food intake in female Sprague-Dawley rats. **METHODOLOGY:** Forty-five female rats were assigned from day 28 to 70 (pre-restriction phase), to five groups: high-carbohydrate (DHC), high-lipid (DL), compound (DCOM), cafeteria-type (DCAF), and control restriction (DCONR). From day 70 to 75 (restriction phase), access to the palatable food was withdrawn, and standard pellets were limited to 80% of the calories intake in the previous phase. From days 75 to 80 (post-restriction phase), original diets were reintroduced to evaluate rebound effects. On day 73, during the restriction phase, we performed the elevated T-maze. **PRELIMINARY RESULTS:** All groups increased intake in post-restriction. DHC showed the highest caloric efficiency and intake, compared to DCAF. DL and DHC also exhibited the greatest weight gain and rebound, if we compared the pre against the post restriction phase. However all groups gain weight in the post-restriction phase. In the elevated T-maze, DHC rats exhibited the lowest levels of anxiety-like behavior compared to all other groups. **CONCLUSIONS:** Early exposure to a high-carbohydrate diet may attenuate anxiety-like behaviors, enhance metabolic adaptation, and facilitate greater weight gain and rebound following periods of food deprivation. The macronutrient composition of palatable diets plays a critical role in shaping both behavioral and physiological responses after dietary restriction, with potential implications for relapse risk in dietary interventions.

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Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.08/EE7

Topic: H.02. Reward and Appetitive Learning and Memory

Title: Striatal insulin sensitivity is augmented by semaglutide in obese mice

Authors: *J. MULDOWNNEY¹, M. BOCARSLY²;

¹Rutgers Univ., Newark, NJ; ²Pharmacology, Physiol. and Neurosci., Rutgers NJMS, Newark, NJ

Abstract: Obesity affects one third of American adults and is associated with peripheral and central insulin resistance. Insulin has a variety of targets in the brain, including in the striatum, a brain region implicated in reward and motivation. Insulin receptors are expressed on striatal cholinergic interneurons (ChIs), inducing dopamine transmission augmentation to regulate feeding behaviors. Glucagon-like peptide-1 (GLP-1) receptor agonists, particularly semaglutide, have gained tremendous popularity for weight loss and correction of dysfunctional insulin secretion and signaling. Peripheral insulin resistance and impulsive feeding behavior are ameliorated in obese patients treated with semaglutide. In rodents, semaglutide exhibits very limited CNS access and does not cross the blood brain barrier; therefore, the mechanism by which the drug is generating its effects is unknown. Semaglutide's effect on insulin sensitivity in the dopaminergic pathway is yet to be investigated. The long-term goal of this research is to

identify the significant molecular regulators of dopaminergic signaling alteration in obesity that are remediated by semaglutide treatment. Our preliminary data suggests a role for insulin action on dopamine transmission augmentation in mice subjected to a high fat diet (HFD) and treated with semaglutide. Through fast-scan cyclic voltammetry, we demonstrate that mice fed a HFD and administered semaglutide have increased evoked dopamine concentrations in the dorsomedial striatum. Striatal brain slices of these treated mice also present a significant increase in dopamine release when washed with insulin, which is not observed in HFD-fed, vehicle-treated mice. This data provides the basis for the hypothesis that semaglutide rescues insulin sensitivity in ChIs of the striatum, generating higher evoked dopamine concentrations, and ultimately decreasing feeding behaviors in obese mice. The study results provide insight to central insulin sensitivity changes by semaglutide leading to stronger regulation of dopamine signaling within brain areas associated with feeding control.

Disclosures: J. Muldowney: None. M. Bocarsly: None.

Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.09/EE8

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R00AA030670

Title: Influence of adolescent alcohol drinking on appetitive and consummatory behaviors in male and female rats

Authors: *H. S. PROUTY¹, A. M. MCCANE²;

¹Neurosci., Oregon Hlth. & Sci. Univ., Beaverton, OR; ²Neurosci., Oregon Natl. Primate Res. Ctr. (ONPRC)/OHSU, Beaverton, OR

Abstract: Adolescence is a key developmental period during which exposure to rewarding substances such as alcohol may have lasting effects on brain function and behavior. Preclinical models stimulating voluntary intake and subsequent operant behaviors offer valuable insight into how early alcohol exposure impacts motivation and may reveal risk factors for long-term substance use. This experiment investigated the effects of adolescent alcohol exposure on appetitive and consummatory behaviors using a structured operant protocol. Male and female rats were first exposed to 20% ethanol mixed in 5% sucrose or sucrose (5%) via a home cage two-bottle choice procedure from postnatal day (PND) 30 to 34. After, rats remained in their home cages until PND 60 at which time operant training began. Training started on a fixed ratio (FR1) schedule, with responses reinforced by 30 seconds of sucrose access. Next, reward duration was gradually reduced to 5 seconds while the schedule increased to FR4. Following acquisition, rats advanced to response requirement (RR) schedules, progressing from RR4 to RR20 over several weeks, where completing the required responses granted 20 minutes of

sucrose access. After stable responding at an RR20, consummatory and appetitive behaviors were tested. To test consummatory behaviors, rats experienced RR1 schedule, with intake measured over 20 minutes. To test appetitive behaviors, rats experienced an extinction session during which responses were recorded but not reinforced. Following both consummatory and appetitive testing, rats experienced an escalating RR schedule to restore responding over several days. As expected, rats successfully increased their responses over reinforcement schedules, however, interestingly, intake increased as response requirement schedules increased. Pronounced sex differences in intake were also observed where females exposed to both ethanol and sucrose consumed more sucrose than males in both groups. Sucrose exposed females also consumed more sucrose during the consummatory test than ethanol exposed females. In contrast ethanol exposed females made more sucrose seeking responses compared to sucrose exposed females. This data suggests that adolescent alcohol exposure exerts sex-dependent and differential effects on consummatory and appetitive behaviors. Future work exploring the neural mechanisms by which alcohol drinking in adolescence alters neural development and subsequent motivated behaviors in adulthood are warranted. Importantly, these translational results enhance our mechanistic understanding of how adolescent alcohol exposure influences future reward seeking and taking behaviors.

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Poster

PSTR348: Bidirectional Regulation of Feeding, Metabolism, Motivation, and the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR348.10/FF1

Topic: H.02. Reward and Appetitive Learning and Memory

Title: Enhanced Prefrontal Inhibitory Tone Induced by the Incubation of High-Fat Food Craving is Reversed by Exercise

Authors: *L. G. BAILEY¹, K. MEYERMANN², T. E. BROWN²;

¹Integrative Physiol. and Neurosci., Washington State Univ. Grad. IPN, Pullman, WA;

²Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: As obesity becomes increasingly prevalent, many dieting strategies remain ineffective due to their inability to curb palatable food cravings. The phenomenon known as "incubation of craving" refers to the progressive intensification of cravings for rewarding stimuli, such as food or drugs, after periods of abstinence. Our laboratory has previously shown that forced, high-intensity exercise can reduce the incubation of craving for high-fat foods in male rats. However, the underlying mechanisms driving this process remain unclear, and it is not yet known whether voluntary exercise can produce similar effects. One potential mechanism involves hypofrontality, a state characterized by reduced activity or function in the prefrontal cortex (PFC). Hypofrontality has been implicated in maladaptive food seeking behaviors, where diminished top-down control from the PFC may lead to compulsive overeating and impaired regulation of

cravings for highly palatable foods. In this study, we tested the hypothesis that prolonged abstinence (30 days) from high-fat food would increase inhibitory tone onto pyramidal neurons in the PFC, and that voluntary exercise would restore this balance. We show for the first time that in male Sprague-Dawley rats, voluntary wheel running throughout the 30 days of abstinence blocks incubation of craving for high-fat diet (incubation scores, incubation group: 19.9 ± 5.35 , exercise group: 6.5 ± 4.70). Subsequently, using whole-cell patch clamp electrophysiology in layer V of the prelimbic PFC, we show that animals exhibiting incubation of craving for high-fat diet show increased inhibitory tone (frequency of spontaneous inhibitory events) onto pyramidal neurons, which was reduced with voluntary exercise (interevent interval, incubation group: 259.67 ± 15.48 , exercise group: 574.73 ± 44.78). Furthermore, the excitability of inhibitory fast-spiking interneurons (FSIs) is increased following incubation compared to animals exposed to voluntary exercise (excitability at 500pA injected current, incubation group: 37.46 ± 2.09 , exercise group: 32.21 ± 1.01). Overall, we show that incubation of craving leads to increased inhibitory tone onto pyramidal neurons, which coincides with increased FSI excitability that is restored with voluntary exercise during abstinence. These findings highlight how voluntary exercise may prevent the neural adaptations underlying incubation of food craving, offering new insights into strategies for curbing compulsive eating behaviors associated with obesity.

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Poster

PSTR349: Circuit Regulation of Reward-Related Behavior

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Program #/Poster #: PSTR349.01/FF2

Topic: H.03. Motivation

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Title: Intrinsic circuitry between monoaminergic and cholinergic state-setting nuclei in the mid- and hindbrain

Authors: R. SOUZA¹, L. GONCALVES², *M. METZGER³;

¹Dept. of Physiol. & Biophysics, Univ. of Sao Paulo, USP, Sao Paulo, Brazil; ²Dept. of Human Anat., Federal Univ. of the Triangulo Mineiro, Uberaba, Brazil; ³Dept. of Physiol. & Biophysics, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: The ventral tegmental area (VTA) and dorsal raphe nucleus (DR) are key structures involved in mechanisms of reward/aversion/motivation and both are implicated in psychiatric disorders such as major depression, anxiety, and schizophrenia. Another major state-setting nucleus in the periventricular mesopontine tegmentum is the laterodorsal tegmental nucleus (LDTg), which is mainly implicated in reward and arousal. It is well known that DR and VTA, as well as LDTg and VTA, are reciprocally connected. Much less is known about the functional

role of the median raphe nucleus (MnR) and its connections with the VTA and DR. Moreover, it has become increasingly evident that nuclei traditionally considered dopaminergic (VTA), serotonergic (DR, MnR) or cholinergic (LDTg) are in fact heterogeneous, composed of neuronal populations with distinct neurochemical phenotypes, projections, and electrophysiological properties. Furthermore, there is evidence that specific subregions or populations of VTA, DR/MnR, and LDTg neurons participate in distinctive aspects of reward/punishment signaling, and/or play different roles in the regulation of anxiety, panic syndrome, and depression, respectively. Even so, the topographical/neurochemical organization of the circuits underlying the interactions between VTA, DR/MnR, and LDTg has hitherto not been systematically investigated with modern sensitive tracers. Thus, we placed small topic injections of the retrograde tracer cholera toxin subunit b (CTb) or the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the DR, MnR, LDTg and VTA of male Wistar rats (n = 42). In order to check the GABAergic component of inputs to the VTA we also performed CTb injections into the VTA of VGAT-reporter mice (n = 4). By analyzing retrograde and anterograde tracing in rats and mice, we found that both, DR and MnR send very robust connections to the VTA. In turn, VTA projections to the DR/MnR were relatively meager. Within the subregions of the DR, the lateral wings stood out as the major source of mainly GABAergic inputs to the VTA. DR and MnR were robustly interconnected with one-another, and again, the lateral wings of the DR were the primary source and target of the reciprocal connections between DR and MnR. Finally, the LDTg sends robust GABAergic and non-GABAergic inputs to the VTA, with VTA projections to the LDTg being relatively weak. Our anatomical findings indicate that there is a complex network of GABAergic and non-GABAergic interconnections between the state-setting nuclei VTA, DR/MnR, and LDTg. Furthermore, they reveal robust reciprocal connections between the DR and MnR that mainly involve the lateral wings of the DR.

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Poster

PSTR349: Circuit Regulation of Reward-Related Behavior

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Program #/Poster #: PSTR349.02/FF3

Topic: H.03. Motivation

Title: Functional dissociation of dopamine signaling in mouse medial prefrontal and lateral orbitofrontal cortex during cue-action-outcome association learning

Authors: *B. RIGHETTI, T. W. BERNKLAU, S. N. JACOB;
Translational Neurotechnology Laboratory, Dept. of Neurosurg., TUM Univ. Hospital, Tech. Univ. of Munich, Munich, Germany

Abstract: As a neurotransmitter with extensive neuromodulatory properties, dopamine plays a pivotal role in a wide range of distinct brain functions. Previous studies in rodents have shown

that midbrain dopamine neurons also strongly project to the medial prefrontal cortex (mPFC) and lateral orbitofrontal cortex (LOFC), regions that play a crucial role in reinforcement learning. However, it is not known how dopamine neurotransmission in the frontal cortex evolves across learning and how it may shape the development of associations between behaviorally relevant task variables. Here, we examined the time course of dopaminergic transients in mouse mPFC and LOFC during abstract associative learning to study how frontal dopaminergic signatures change with increasing task competency. We trained mice ($n = 23$) on an auditory decision-making task with implicit rules switches, requiring the animals to associate auditory cues with directed motor responses (licks) to obtain liquid rewards. Auditory stimuli varied along the dimensions of location and frequency. Only one feature dimension was relevant at a given time, depending on the currently applied rule. After acquiring an initial association, the reward contingencies were changed in the sense of extra- and intra-dimensional shifts (i.e., from location to frequency, and from frequency to frequency with reversed motor responses). We virally expressed the fluorescent dopamine sensor GRAB DA3h in the mPFC and LOFC in order to perform specific, sensitive and temporally highly resolved measurements of dopaminergic signals with fiber photometry over the course of several months while the animals learned the different tasks. Robust dopamine transients in frontal cortex were observed for all task-relevant variables. Dopamine activity in both mPFC and LOFC scaled according to the animals' level of expertise. In the mPFC, dopamine levels were linked to cue and outcome processing. In the LOFC, dopamine levels were mainly linked to outcome processing. In both regions, negative outcomes (reward absence) were associated with a marked reduction in dopamine levels. After rule switches, cue triggered-dopamine transients were indicative of reward anticipation in the mPFC, but only to a lesser extent in the LOFC. These findings suggest a functional dissociation in frontal dopamine signaling, with the mPFC supporting learning by associating cues, actions and outcomes, and the LOFC primarily evaluating received outcomes.

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Poster

PSTR349: Circuit Regulation of Reward-Related Behavior

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Topic: H.03. Motivation

Support: NIMH ZIA MH002951-09
NIMH ZIC MH002952-09

Title: A novel transgenic rat for investigating dopamine D₃ receptor function

Authors: ***A. BOURD**, M. H. LOWRIE, Y. CHUDASAMA;
Section on Behavioral Neurosci., Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The highest level of dopamine D₃ receptor (D₃R) mRNA is expressed in evolutionarily conserved structures including the olfactory tubercle, nucleus accumbens, and islands of Calleja. In these and other limbic structures, D₃R is thought to influence motivated behaviors, particularly those related to behavioral control. Pharmacological studies investigating D₃R function are confounded by high D₂R homology. Thus, by genetically targeting the D₃R in the rat, we can specifically investigate this receptor in both normal brain function and psychiatric conditions where cognitive control behaviors are compromised. While genetically modified mice are standard tools in neuroscience research, the rat is a preferable behavioral model due its large size, broad behavioral repertoire, and ability to quickly learn complex cognitive tasks. Accordingly, we characterized a novel D₃R Cre rat line generated with CRISPR/Cas9 on a Long-Evans background. We first validated Cre expression by crossing the transgenic D₃R-Cre rats with tdTomato and ZsGreen reporter rats. Brain-wide fluorescence within the D₃R-tdTomato and D₃R-ZsGreen brains was consistent with known D₃R expressing regions. We further confirmed that Cre was expressed specifically under the control of the D₃R gene by using RNA FISH in the Islands of Calleja and dorsal hippocampus. We then confirmed Cre availability for viral transfection by injecting Cre-dependent AAVs in D₃R-expressing regions. We found robust and specific neuronal labeling in various brain areas including the Islands of Calleja and dorsal hippocampus, allowing for circuit tracing and activity manipulation. Finally, D₃R-Cre expressing animals showed behavior comparable to their littermate controls in behavioral assays that measured motor and cognitive functions. Both groups showed intact motor ability as assessed by open field and gait scan. Since the D₃R is implicated in aspects of behavioral control, we examined cognitive flexibility in these animals by testing them on a simple discrimination and reversal task. Both groups successfully discriminated the visual stimuli and inhibited their response to the previously rewarded stimulus during reversal. Future studies will examine the functional anatomy of the islands of Calleja by using a combination of viral tract tracing and cell specific manipulations to better understand the role of the D₃R in motivated behavior.

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Poster

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Program #/Poster #: PSTR349.04/FF5

Topic: H.03. Motivation

Title: Optogenetic Excitation of VTA and PVN Alter Social Motivation in C57Bl/6J Mice

Authors: *C. B. WELLS¹, J. KITTRELL¹, I. JACOB², K. EVANS³, A. MARRISON², T. D. ROGERS¹;

¹Psychology, ²Middle Tennessee State Univ., Murfreesboro, TN; ³Middle Tennessee State university, Murfreesboro, TN

Abstract: Reduced social motivation is a common symptom in multiple neuropsychiatric disorders including schizophrenia, depression, and autism spectrum disorder (ASD). Reduced social motivation can in turn lead to social withdraw and higher rates of loneliness in these clinical populations. While oxytocin is currently being explored as a potential treatment for increasing social engagement in these disorders, results have been inconsistent. Our prior work suggests that while the administration of exogenous oxytocin increases sociability, it conversely decreases social motivation, social interactions which require effort to access the social partner. Additionally, oxytocin and dopamine have been shown to have reciprocal modulatory interactions which may influence social behavior and social motivation. The current study aims to identify the effects of optogenetic stimulation of the ventral tegmental area (VTA), where dopamine cell bodies of the mesolimbic pathway are located, and the paraventricular nucleus (PVN), a major source of oxytocin production, on social motivation in mice. Male and female C57BL/6J mice received bilateral injections of AAV1-hSyn-hChR2(H134R)-EYFP or AAV1-hSyn-EYFP into the VTA and were implanted with bilateral LED optic fibers. Following recovery, mice completed a behavioral battery assessing sociability (low-effort social behaviors) and social motivation (high-effort social behaviors). The three-chamber test and the free dyadic social interaction (FDSI) test were used to measure sociability. Novel measures of social motivation recently developed by our lab, the weighted door task and the ladder task were used to measure social motivation. Additional behavioral tests were conducted to measure confounds including social dominance, motor control, and anxiety. Preliminary results suggest that optogenetic stimulation of the VTA increases sociability and social motivation. We are currently investigating the effects of optogenetic stimulation of the PVN, and we hypothesize that it will increase sociability but decrease social motivation. The current study aims to examine the roles of the VTA and PVN in sociability and social motivation while extending our previous findings that oxytocin inversely affects these behaviors in mice. These results may provide insights for the role of oxytocin in social motivation and better inform the search for novel therapeutics for disorders characterized by social withdrawal and reduced social motivation.

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Poster

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Program #/Poster #: PSTR349.05/FF6

Topic: H.03. Motivation

Support: National Institute of General Medical Sciences: T34GM145384

Title: The Effects of Environmental Enrichment on Aberrant Choice Behavior Induced by a High Fat Diet

Authors: *K. BANDUCCI¹, B. REED¹, K. ROMERO², A. MELTON², M. AGABAO², V. DASANAYAKE², C. L. MACHUCA², C. J. HORKY², I. C. SUMAYA², A. M. GANCARZ-KAUSCH²;

²Dept. of Psychology, ¹California State Univ. Bakersfield, Bakersfield, CA

Abstract: Chronic consumption of a diet high in fat and sugar is known to be associated with obesity, cardiovascular disease, and diabetes, among other negative health effects. It has been shown that individuals who overconsume food share similar genetic vulnerabilities with substance use disorder (SUD) in genes regulating impulsivity and dopamine release. Yet, little is understood about the effects of a high fat diet on reward-seeking and choice behavior. We previously discovered that exposure to a high fat, Western diet (45% fat, 6% sucrose) in male Sprague Dawley rats caused a significant decrease in reward maximization compared to controls. Here, we investigated whether environmental enrichment (EE) would ameliorate deleterious effects induced by a high fat diet. EE provides a larger housing space for social interaction, exploration, and physical activity and has previously been shown to decrease impulsive choice. To test this, we randomly assigned rodents to a high fat diet or standard chow for 60 days. While subject to this diet, rodents were also randomly assigned to standard pair-housing or an EE chamber containing a maze, tunnels, and running wheels that were changed weekly to ensure novelty. At the end of the diet period, rats were tested on a sequential patch depletion paradigm. During this task, rats were offered the simultaneous choice between two water “patches.” They could choose to stay in their current patch, which decreased in subsequent reward magnitudes, or leave for an alternative patch that reset to the maximum reward magnitude. Rats fed a high fat diet under enriched conditions spent significantly less time in the patch and made significantly more patch changes compared to control, standard housed rats. Thus, rats fed a high fat diet with environmental enrichment earned greater rewards and consumed more water in total per session compared to controls. This suggests that environmental enrichment may alleviate negative effects of a high fat diet and restore poor choice maximization. We also investigated locomotor response to novelty in rats fed a high fat diet under enriched conditions compared to controls. This measure has shown mixed results for novelty-seeking behavior for rats fed a high sucrose diet, but little is known about high fat. Preliminary data indicates rats exposed to EE traveled significantly greater distances compared to controls. However, no significant effects of diet were found, suggesting high fat may not be a critical mediator of novelty response. More research is needed to understand the effects of enrichment on exploratory and choice behavior.

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Poster

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Topic: H.03. Motivation

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National Institute on Drug Abuse (R01DA045783)

Title: Acetylcholine and Dopamine Dynamics in the Nucleus Accumbens: Relationships to Costs and Benefits during Spatial Foraging

Authors: *J. F. FIGUEROA¹, Y.-S. HWANG¹, S. CRATER¹, J. D. BERKE²;
¹Univ. of California - San Francisco, San Francisco, CA; ²Neurol., Univ. of California - San Francisco, Portola Valley, CA

Abstract: Motivated behavior can be modulated by both dopamine (DA) and acetylcholine (ACh) in the nucleus accumbens (NAc). Each neurotransmitter influences the other: stimulating ACh neurons can promote DA release via axonal nicotinic acetylcholine receptors, and DA can inhibit ACh release via D2 receptors. How such interactions contribute to motivation is not well understood. As animals approach rewards, NAc DA release ramps up in proportion to reward expectation. This motivation-related DA signal has been proposed to reflect local enhancement of DA release by ACh, rather than spiking of DA cell bodies. However, other studies suggest that the predominant role of ACh is to suppress DA release, or even that ACh has no influence over striatal DA dynamics. As an initial step towards distinguishing between these competing possibilities, we have been comparing NAc DA and ACh dynamics as rats forage in a complex maze (Krausz et al. 2023). This task features both variable expectation of reward ("benefit"; reward probability) and variable effort needed to obtain it ("cost"; run distance). We simultaneously recorded ACh and DA using dual-color fiber photometry after co-expression of both GRAB-ACh4h and GRAB-rDA3m sensors (Zhuo et al. 2023). In analyses of preliminary data (n = 4 rats, 26 sessions, 7886 trials) we replicated findings that DA approach ramps scale positively with reward prediction. Multiple regression also revealed a negative influence of distance cost on DA. For ACh we found the opposite: less ACh release when rats had higher expectation of reward, and when the run distance is shorter. These observations support the hypothesis that ACh primarily opposes NAc DA release during motivated behavior, in particular when rats must jointly weigh the cost and benefits of effortful behavior. Further studies will be required to determine the precise behavioral and circuit states determining the relationships between these two key neuromodulators.

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Poster

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KAKENHI21H04423

Title: Experience-dependent changes in temporal expectation and dopamine dynamics during a reward waiting task in rats

Authors: K. MUROYA¹, K. OKANO¹, R. ISHIDA¹, R. SAITO¹, N. KATAYAMA³, Y. KOMURA⁴, *R. KAJIWARA^{2,1};

¹Grad. Sch. of Sci. and Technol., ²Dept. of Electro. & Bioinfo., Meiji Univ., Kawasaki, Japan;

³Shokei Gakuin Univ., Natori, Japan; ⁴Grad. Sch. of Human and Envrn. Studies, Kyoto Univ., Kyoto, Japan

Abstract: Rewards act as reinforcers that shape future behavior, partly through the activation of dopaminergic neurons triggered by actual or expected outcomes. Dopamine contributes not only to evaluating reward value but also to predicting when a reward will be delivered (Soares et al., 2016). However, it remains unclear how temporal expectations formed through repeated experiences lead to behavioral changes when outcomes deviate from those predictions. In this study, we examined how prior experiences affect time perception and waiting behavior using a reward waiting task in which rats encountered varying waiting durations across trials.

We used a U-shaped maze where rats began each trial by entering one of two corners. To obtain a water reward at the opposite end, they needed to wait 9 seconds in the center until an LED turned on. We tested three trial sequences: (1) all trials fixed at 9 seconds, (2) three fixed 9-second trials followed by a fourth trial randomly set to 3, 9, or 15 seconds, and (3) all trials randomly set to 3, 9, or 15 seconds. Each cycle consisted of four trials, and rats completed 12-15 cycles (50-60 trials) in one hour. Sessions were repeated daily for two weeks.

Learning curves showed a significant improvement in success rate within each daily session, while the impact of the previous day's behavior was minimal. This suggests that rats learn mainly through repetition within the same day rather than retaining memory across days. The within-session learning curve resembled predictions from a Q-learning algorithm, suggesting that reinforcement learning processes may effectively model rat behavior. In the behavioral analysis, rats that experienced three consecutive 9-second trials showed a marked decrease in success rate when faced with a 15-second trial. Interestingly, analysis of failed trials revealed that rats often initiated movement not only around the 9-second mark but also as early as 7 seconds. This suggests that prior expectations influenced action timing but did not rigidly align with the experienced duration, indicating a broader distortion in temporal anticipation.

We recorded dopamine signals in the nucleus accumbens using fiber photometry with a genetically encoded GRAB_{DA} sensor. Dopamine levels showed a bimodal response after the 9-second LED cue: a rapid increase followed by a drop, then a second rise and fall as rats moved to the reward area. A gradual rise in dopamine was also observed before the LED, possibly reflecting internal timing and anticipation. Further analysis of this gradual component may help uncover how subjective time is represented during waiting.

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Poster

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Title: Defining NAc neuron heterogeneity across multiple modalities

Authors: *X. XU¹, J. LI¹, J. JING², Q. WANG¹, C. YAO¹, X. JIANG², L. LI¹;

¹The Univ. of Alabama at Birmingham, Birmingham, AL; ²Baylor Col. of Med., Houston, TX

Abstract: The nucleus accumbens (NAc) plays an important role in regulating drug reward, feeding, social interaction, pain, and instrumental learning, and its dysfunction has been linked to multiple neuropsychiatric disorders. Consistent with its functional diversity, NAc neurons are highly heterogeneous. Over 90% of NAc neurons are GABAergic D1R- and D2R-medium spiny neurons (MSNs), with a small percentage of interneurons. For decades, NAc neurons have been classified into subtypes by structural, electrophysiological, biochemical, or genetic data characteristics, and more recently by transcriptomic characteristics. As a central hub in the brain, the NAc not only receives a diverse range of inputs to process reward-related information but also generates outputs that drive motivated behaviors and learning. For input circuits, NAc neurons receive dopaminergic projections from the VTA and a large amount of glutamatergic monosynaptic inputs from a variety of limbic and cortical regions, some of the most notable being medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and ventral hippocampus (vHIPP). For outputs, D1R-MSNs project directly to the VTA and are thought to control reward-seeking behavior, while D2R-MSNs relay signals to the VTA via the ventral pallidum (VP) and are supposed to contribute to aversion. Accumulating evidence suggests that specific input/output circuits of the NAc are formed by distinct classes of neuronal subtypes and underlie different functions. A formidable challenge is to define NAc neuron subtypes by integrating anatomical information (morphology, soma location, inputs, and outputs), gene expression analysis (molecular markers, specific ion channels, and receptors), and biophysical properties (electrophysiological phenotypes) on a single cell level. Here, we first use Patch-seq recording to classify NAc neuron subtypes by integrating morphological, electrophysiological, and transcriptomic properties and soma location in *Drd1-Cre/Ai14*, *Drd2-Cre/Ai14*, and wildtype C57BL/6J lines. Then, combining anterograde and retrograde virus tagging techniques and Patch-seq recording, we probe and characterize these subtypes in the NAc that receive synaptic inputs from the VTA, mPFC, BLA, and vHIPP and project to VP and VTA. Our study represents a unified definition of MSN subtypes across multiple modalities in the NAc and provides a

foundation for further understanding of how different neuron subtypes of the NAc are involved in various physiological and pathological conditions.

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Poster

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Topic: H.03. Motivation

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Title: Perinatal interference with the serotonergic system affects VTA function in the adult and leads to motivation deficits

Authors: V. WALKER¹, F. FRANCISCA DE ABREU², *C. M. TEIXEIRA²;

¹Nathan Kline Inst., New York, NY; ²NYU - NKI, New York, NY

Abstract: The interaction between the serotonergic and the dopaminergic systems during development and the impact of that interaction on subsequent behavior later in life is unknown. Multiple environmental factors may affect serotonin levels during development including exposure to Selective Serotonin Reuptake Inhibitors (SSRIs). Altered levels of serotonin during development are leading to behavioral deficits in adult mice such as anhedonia and anxiety-like behavior. We hypothesize that early-life exposure to altered serotonin levels causes alterations in the dopaminergic system and long-lasting dopamine-dependent behavioral changes. To test whether changes in serotonergic levels in early-life affect dopamine dependent behaviors we administered the SSRI Fluoxetine (FLX) to mice, from postnatal day (P)2 to P11 (PN-FLX). Once the mice reached adulthood, we evaluated their behavior. In previous studies, we found that there is VGLUT3 expression in serotonergic neurons, suggesting a potential interaction between the two systems. Activation of serotonergic terminals in the VTA led to the activation of dopaminergic cells through glutamate signaling. We found that postnatal exposure to FLX (PN-FLX) caused deficits in glutamatergic transmission, similar to the effects observed with ablation of VGLUT3 in serotonergic cells. Also, in both manipulations we observed deficits in exploration. Furthermore, we found a decrease in motivation in PN-FLX. As motivation is influenced by dopaminergic function, we tested whether PN-FLX mice had dopaminergic deficits. Interestingly, we found a significant decrease of dopaminergic function in brains of mice that were exposed to SSRI during the development using fiber photometry and microdialysis. To test whether manipulating the dopaminergic system could rescue the motivation deficits observed in PN-FLX mice we used bupropion. Adult administration of bupropion, a compound that modulated the dopamine and norepinephrine systems, rescued the motivation deficits seen in PN-FLX mice. However, administration of FLX did not improve the

lack of motivation. Pilot work has also shown deficits in the novelty suppressed feeding test in PN-FLX. This deficit was successfully ameliorated by administering bupropion in PN-FLX mice, while the administration of FLX did not yield similar results. Currently we are testing whether directly stimulating the dopaminergic system improves the deficits in behavioral phenotypes caused by early-life exposure to SSRIs.

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Topic: H.03. Motivation

Title: Modulations in ECoG activity correlate with the control of force in hand grasping

Authors: *S. AOYAMA, M. SAWADA, T. KIKUCHI, Y. ARAKAWA;
Dept. of Neurosurgery, Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan

Abstract: Motivation plays a pivotal role to enhance motor output. It is generally accepted that orbitofrontal cortex (OFC) processes the motivational control of goal-directed behavior. Further, Nucleus accumbens (NAc) which is closely modulated by OFC functionally connected to sensory motor cortex (SMC). Thus, functional connection between OFC and M1 mediated by NAc is suggested. However, little is known about the relationship between the intensity of the motivation and the magnitude of force in high temporal resolution in humans. To clarify the neural mechanism underlying that motivation facilitates motor function in high temporal resolution, we analyzed the electrocorticogram (ECoG) during the grip task with monetary incentive and analyzed the information flow from OFC to SMC by using granger causality (GC). We conducted a study with patients undergoing awake craniotomy for glioma. In the awake period, the patients immediately had their maximum grip strength measured. Cues indicating 15%, 30%, and 60% of the maximum grip strength were displayed on a monitor in front of the patients. They were instructed to squeeze until the grip strength reached the power indicated by the cue while receiving visual feedback. ECoGs were measured using 16-channel cortical electrode placed across the central sulcus identified during the surgery, and EMG was simultaneously recorded. Event-related desynchronization/synchronization (ERD/ERS) was calculated using the EMG as a trigger. During the high reward task which demands the strong grip force, SMC was widely and strongly activated as compare to the low reward-weak grip task. In addition, information flow from OFC to SMC was more intense during the strong grip task than weak grip task. At the beginning of grip, GC of hi-gamma frequency from OFC to M1 was observed and the causality varied in response to the gripping motion. These results suggest that the OFC which is center of motivation modulated the intensity of grip force in high temporal resolution.

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Title: A Velocity-Driven Paradigm to Investigate Effort-Based Decision-Making in Rats

Authors: *M. C. VELASQUEZ-MARTINEZ^{1,2}, P. A. FELICIANO³, K. LABOY^{4,2};
¹Ciencias Basicas, Univ. Industrial De Santander, Fac Salud, Bucaramanga, Colombia; ²Univ. of Puerto Rico, Mol. Sci. Res. Ctr., San Juan, Puerto Rico; ³Picower Inst. for Learning and Memory, Massachusetts Inst. of Technol., Boston, MA; ⁴Univ. of Puerto Rico MSRC, San Juan, Puerto Rico

Abstract: Flexible regulation of effortful actions is essential for adaptive behavior, yet the neural mechanisms that support value-based decisions under changing physical cost conditions remain unclear. We introduce a novel, linear maze paradigm inspired in greyhound racing, where rats initiate trials by exiting a start box after a gate opens, and sprint across a 6-foot corridor to earn rewards scaled to their crossing velocity. Physical effort is manipulated by randomly interleaving flat-floor and ramped trials, with steeper inclines requiring greater effort. Crossing velocity requirements are enforced via a timeout penalty for slow runs. Critically however, rats can omit any trial without penalty by remaining in the start box for 10 s after the gate opens, enabling trial-by-trial effort regulation based on internal state (e.g., satiety, fatigue) and external effort demands (ramp vs. flat trials). Our data show that rats ($n = 3$) learn to initiate trials rapidly (< 2 s latency), engage in high-velocity runs, and modulate behavior according to effort. Across sessions, rats exhibited stable velocity profiles (1-5 s latencies; 22-99 m/min) and trial-by-trial adjustments in performance, demonstrating that velocity-scaled rewards are effective in driving high-velocity crossings. Omission rates were higher for high-effort ramp trials, indicating physical cost sensitivity. These behaviors emerged without extensive operant conditioning (< 4 weeks), highlighting the naturalistic structure of the task. Its ethological design and scalable effort-reward contingencies make it ideal for probing the neural circuits underlying cost-sensitive, goal-directed behavior across physiological and pathological states.

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Poster

PSTR349: Circuit Regulation of Reward-Related Behavior

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"Enhancing physical activity in a mouse model of type 2 diabetes by modulating the dopaminergic system"

Title: Motor slowness and reduced motivation in obesity - a cross-species investigation with impaired striatal dopamine transmission identified in mice

Authors: *F. SCHOUKROUN, Z. LI, M. O. RAY, K.-S. SPIGHT, J. ALBERT, T. HERSHEY, A. V. KRAVITZ;
Psychiatry, Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Reduced physical activity in obesity may result not only from weight-gain but also from neurobiological changes. Dopamine transmission in the striatum is critical in motor control and motivated behavior. Therefore, we propose that impaired dopamine transmission contributes to difficulties engaging in physical activity. We conducted parallel investigations of dopamine-related behavior in humans and mice. In humans, motor slowness was assessed by measuring inter-trial latency during a reward-based task in individuals with normal weight (n=20) and overweight/obesity (n=27). In mice, a similar task was used with food-pellets in chow- (n=35) and high-fat diet fed mice (n=28), the latter displaying significant weight gain and glucose intolerance. Motivated behavior was assessed through a progressive ratio (PR) task in mice, known to be sensitive to dopamine transmission. Spontaneous dopamine transmission was measured in the striatum of a subset of mice from the two groups with dopamine sensor grabDA and fiber photometry. Further, changes in spontaneous and evoked dopamine response were tracked across the development of obesity in transgenic DAT-cre mice (n=4). Group comparisons were performed with an unpaired t-test using Welch's correction while within-subject analysis was performed with paired t-test ($\alpha=0.05$). Consistent with our hypothesis, individuals with overweight/obesity exhibit a longer latency between task trials (2.7s vs. 3.4s, $p<0.05$), indicative of motor slowness. Similarly, HFD-fed mice displayed a longer latency to retrieve the pellet (0.8s vs. 1.5s, $p<0.001$) and a lower breakpoint (50.8 vs. 13.8, $p<0.001$) in the PR task, demonstrating both motor slowness and lower motivation. Interestingly, the motor slowness was correlated with high glycemia in humans ($\rho=0.312$, $p<0.05$) and mice ($\rho=0.430$, $p<0.001$). Photometry recordings revealed reduced spontaneous dopaminergic signal in the striatum of HFD-fed mice, with a lower transient rate (5/min vs. 4/min, $p<0.05$). Preliminary analysis of the evoked stimulation showed that the decay of the dopamine transient was longer in HFD-fed mice (0.8s vs 1.5s), suggestive of impaired dopamine clearance, potentially explaining the reduced spontaneous dopamine transmission. Future work will assess whether a similar motivational deficit can be observed in individuals with overweight or obesity using a progressive ratio (PR) task. This would further support our hypothesis of impaired dopamine

transmission. Follow-up experiments will address whether reduced dopamine transmission and the subsequent behavior can be attributed to the weight gain and/or the impaired glucose homeostasis.

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Poster

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Topic: H.03. Motivation

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Title: Oxytocin and vasopressin receptors mediate dopamine release in the nucleus accumbens

Authors: *R. C. CRENSHAW¹, L. J. YOUNG³, D. B. LESTER²;

¹Univ. of Memphis, Cordova, TN; ²Psychology Dept., Univ. of Memphis, Memphis, TN; ³Ctr. for Translational Social Neurosci., Emory Univ., Decatur, GA

Abstract: Oxytocin is being investigated as a treatment for substance use disorders due to its influence on mesolimbic dopamine signaling. Prior work in our lab demonstrated that oxytocin infusion into the nucleus accumbens (NAc) suppresses stimulation-evoked dopamine release in both male and female mice. To further assess the role of oxytocin receptors (OXTRs), we administered a selective OXTR agonist [Thr4, Gly7]-oxytocin (TGOT; 25 ng in 1 μ L over 1 min) directly into the NAc while measuring dopamine release using in vivo fixed potential amperometry in anesthetized C57BL/6J mice. In females, TGOT significantly reduced phasic dopamine release, replicating the effect of oxytocin and further indicating OXTR involvement. However, in males, TGOT had no effect on stimulation-evoked dopamine release, suggesting oxytocin's actions in males are mediated via non-OXTR mechanisms. Given that oxytocin can bind to vasopressin 1A receptors (V1ARs) which are also located in the NAc, we tested whether these receptors contribute to the observed oxytocin effect in males. When oxytocin was co-infused with a selective V1AR antagonist (SR49059; 250 ng in 1 μ L over 1 min), the suppression of dopamine release by oxytocin was blocked. Overall, these findings suggest that intra-NAc oxytocin alters dopamine release primarily through actions on OXTRs in female mice and V1ARs in males. Ongoing studies aim to further define how these neuropeptide mechanisms contribute to individual differences in reward processing and vulnerability to addiction.

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Title: A glucagon-like peptide-1 receptor (GLP-1R) agonist alters Pavlovian cue-evoked reward-seeking, dopamine release, and neural signaling in the nucleus accumbens

Authors: J. TAN¹, M. MCGROSKY¹, E. PATEL¹, E. HERRING¹, M. HAFENBREIDEL², M. M. TORREGROSSA², *S. E. MORRISON¹;

¹Neurosci., ²Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In recent years, a category of medications known as glucagon-like peptide-1 receptor (GLP-1R) agonists, including brands such as Ozempic and Wegovy, have exploded in popularity for the treatment of obesity. One way in which these drugs work is by directly acting upon the brain's reward system, including the ventral tegmental area (VTA) and nucleus accumbens (NAc), which is likely related to a reduction in food cravings. Overlapping neural mechanisms are involved in reward-seeking motivated by food and by drugs of abuse such as cocaine or alcohol, and recent preclinical studies have shown that GLP-1R agonists such as exendin-4 (exenatide) mitigate drug-seeking behavior as well as reinstatement after withdrawal. However, we have limited understanding of how GLP-1R agonists work to reduce drug-seeking; in particular, it is unclear how GLP-1R agonists affect behavioral and neural responses to reward cues, which are central to many facets of drug abuse.

To address this question, we examined the effects of systemic administration of exendin-4 on behavior in response to a Pavlovian reward cue, along with the concurrent activity of individual neurons in the NAc. We also measured cue- and reward-evoked dopamine release in the NAc using a fluorescent dopamine indicator (GRAB_{DA}) and fiber photometry. During Pavlovian conditioning, motivational value can be transferred from the reward to the cue, eliciting approach and interaction with the cue - a behavior called sign tracking. We found that exendin-4 dose-dependently reduced sign tracking while having a lesser impact on goal tracking (approach to the site of reward). Meanwhile, a substantial proportion of NAc neurons had cue- and/or reward-evoked activity that was altered by exendin-4. Most cue-evoked responses were attenuated, while reward responses showed mixed effects; a subset of neurons also showed changes in baseline firing rate. These changes may have been partially driven by the effects of exendin-4 on NAc dopamine: interestingly, the drug caused sign tracker individuals to exhibit a goal tracker-like profile of dopamine release. Overall, these findings provide evidence that GLP-1R agonists work, at least in part, by directly attenuating neural representations of the value of reward-associated cues.

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Title: Sex-specific structure and function of a CRH/GABAergic basolateral amygdala→nucleus accumbens projection governs the impact of early-life adversity on adult motivated behaviors

Authors: *L. TANIGUCHI^{1,2}, C. M. GOODPASTER⁴, M. T. BIRNIE³, G. DE CARVALHO², A. CHIANG², A. PAUL², Y. CHEN³, L. Y. CHEN², L. A. DENARDO⁴, T. Z. BARAM⁵; ²Anat. and Neurobio., ³Pediatrics, ¹Univ. of California, Irvine, Irvine, CA; ⁴Physiol., UCLA, Los Angeles, CA; ⁵Anat. and Neurobiology; Pediatrics, Univ. of California Irvine, Irvine, CA

Abstract: Rationale: Early-life adversity (ELA) is a risk for human affective disorders involving dysregulated reward behaviors. In our limited bedding and nesting mouse model, ELA induces anhedonia-like behaviors in adult male mice while increasing motivation for rewards such as palatable food and sex cues in adult females, indicating sex-dependent disruption of reward circuit operations. We identified a corticotropin-releasing hormone (CRH) expressing GABAergic projection from basolateral amygdala (BLA) to nucleus accumbens (NAc) that governs reward-seeking deficits in adult ELA males—but not females. Here, we investigate the function of this projection in female mice and the hypothesis that there are fundamental differences in the projection to explain sex or ELA-dependent differences in its function. Methods: To probe the sex-specific role of this projection in reward behaviors, adult male and female CRH-Cre mice raised in control or ELA conditions received excitatory or inhibitory Cre-dependent DREADDs in BLA, and then clozapine N-oxide or vehicle to NAc medial shell during reward behaviors. We determined the cell identity of the projection using immunostaining and electrophysiology. Using tissue clearing, light sheet fluorescence microscopy and deep learning pipelines, we mapped brain-wide BLA CRH+ axonal projections to uncover sex differences in innervation. Results: Chemogenetic manipulations in male mice demonstrated inhibitory effects of the CRH/GABA BLA→NAc projection on reward behaviors, whereas neither excitation nor inhibition influenced female behaviors. Molecular and electrophysiological cell-identities of the projection did not vary by sex. By contrast, comprehensive whole-brain mapping uncovered significant differences in NAc innervation patterns that were both sex and ELA-dependent, as well as selective changes of innervation of other brain regions. Conclusions: The CRH/GABA BLA→NAc projection that influences reward behaviors in males differs structurally and functionally in females, uncovering potential mechanisms for the profound sex-specific impacts of ELA on reward behaviors. Further investigation of this projection will give us a deeper understanding of intrinsic sex differences in the organization of the reward circuitry and the effects of ELA on reward behaviors that underlie many mood disorders.

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Poster

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Title: Intracranial Signatures of Effort Based Decision Making

Authors: *V. ANAND¹, D. KIM², C. MAY³, E. GLEICHGERRCHT⁶, N. G. LAXPATI⁷, C. ROZELL⁴, S. ALAGAPAN⁵;

¹Electrical and Computer Engin., ³Electrical Engin., ²Georgia Inst. of Technol., Atlanta, GA;

⁴Electrical and Computer Engin., Georgia Inst. of Technol., Decatur, GA; ⁵Georgia Inst. of Technol., Chamblee, GA; ⁶Neurol., Emory Univ., Atlanta, GA; ⁷Dept. of Neurolog. Surgery, Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Would you bike to work and incur a metabolic cost or take the bus and incur a monetary cost?

Effort Based decision-making (EBDM) paradigms can answer these questions by objectively quantifying how individuals weigh metabolic or cognitive costs against rewards via controlled and simplified decision-making tasks offering insights into motivation beyond subjective reports. We implemented a button pressing EBDM task in which participants chose between varying levels of button pressing effort and virtual currency rewards, while their neural activity was recorded using stereoelectroencephalography (SEEG), along with synchronous EKG, and eye-tracking. This approach enabled us to examine the millisecond-scale temporal dynamics of neural circuits implicated in EBDM.

Preliminary analysis of high-resolution SEEG data revealed that, during the button pressing task, the insula exhibited significant modulation between the decision and effort execution phases in the gamma and high beta bands ($p < 2e-3$, paired t-test with bonferroni correction) in all initial participants. Additionally, the gamma and high beta bands for the isthmus cingulate ($p < 4e-5$, paired t-test with bonferroni correction) and the high beta bands for orbitofrontal cortex ($p < 2e-5$, paired t-test with bonferroni correction) showed phase specific modulation in individual patients. These findings highlight the value of intracranial recordings for capturing rapid, phase specific neural dynamics in key regions involved in effort-based choices.

We plan to extend these analyses to additional participants and further characterize the temporal profiles of EBDM-related activity across brain regions. This work aims to advance our understanding of the fast neural computations underlying motivated behavior.

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Poster

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CONAHCYT-CVU 1312468

Title: Evaluation of subjective reward value of brain stimulation under cost and aversive conditions

Authors: ***B. L. PACHECO GÓMEZ**¹, A. TOSCANO ZAPIEN², W. ZEPEDA-RUIZ³, D. VELAZQUEZ-MARTINEZ⁴;

¹Facultad de Psicología, UNAM, Mexico City, Mexico; ²Facultad de Psicología, Univ. Nacional Autonoma de Mexico, Mexico, D.F., Mexico; ³Facultad de Psicología, Univ. Nacional Autonoma De Mexico, Mexico, Mexico; ⁴Facultad de Psicología, Univ. Nacional Autonoma De Mexico, Mexico, D.F., Mexico

Abstract: Electrical brain stimulation (EBS) of the medial forebrain bundle (MFB) has both reinforcing and discriminative properties, making it a suitable model for studying reward-based decision-making. Additionally, response cost or negative valence stimuli (such as footshocks) could influence the discrimination of reinforcing stimuli. To evaluate these effects, male Long Evans rats ($n = 8$) were implanted with an electrode in the MFB and trained to discriminate between high and low amplitudes of EBS. Correct responses, maintained for 1, 2, or 4 seconds (response cost), were reinforced with EBS. Generalization tests were conducted varying the pulse amplitude and response cost. Finally, response- or reinforcer-contingent shocks (0.25 - 0.63 mA) were introduced during pulse-amplitude generalization tests. A sigmoidal function was fitted to assess changes in the generalization gradients. Footshocks did not significantly alter the generalization gradients, whereas increased response cost shifted the pulse amplitude required to reach a discrimination index of 0.5. Moreover, higher response costs and intensities of footshocks increased missed trials and reduced earned reinforcers. These findings suggest that while footshocks may not affect the subjective perception of reward, animals integrate distinct types of costs in their performance.

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Poster

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Title: Prefrontostriatal terminal dynamics guide effort-based decision making

Authors: *A. E. KAO, A. Z. LIU, S. A. WILKE;
Psychiatry and Behavioral Sci., Univ. of California, Los Angeles (UCLA), Los Angeles, CA

Abstract: Evaluating effort-reward tradeoffs is critical for adaptive, goal-directed behavior and is disrupted in many psychiatric disorders. The anterior cingulate cortex (ACC) is thought to regulate effort-based decision making (EBD), in part via projections to the dorsomedial striatum (DMS). While the DMS is also implicated in goal-directed action selection, the specific role of ACC-DMS projecting neurons in EBD, or how their terminals encode effort and reward-related information, remains unclear. To investigate this, we used a classic barrier T-maze assay in which mice choose between an unimpeded low reward (LR) arm or a high reward (HR) arm blocked by an effortful barrier. Projection-targeted optogenetic inhibition revealed that ACC neurons projecting to the striatum are required for effortful action selection, particularly when animals switch from a low- to high-effort choice strategy. In contrast, inhibiting ACC neurons projecting to non-striatal targets did not reliably disrupt EBD. This suggests a role for ACC-DMS projections in updating effort-based choice strategies. To build on this, we developed and validated a fully automated version of the T-maze task to allow continuous recording of ACC-DMS terminal activity without handling-related artifacts. Using axon-targeted GCaMP and fiber photometry recordings, we observed that this pathway encodes both reward magnitude and effort cost. In a no effort condition, terminal activity was not strongly modulated during action selection. However, introduction of a barrier resulted in transient increased activity associated with high effort choices, suggesting a role in overcoming effort cost. Reward-aligned activity sharply decreased at the onset of reward consumption, with larger drops following HR outcomes. After mice learned the effort contingency associated with the barrier, choice-related signals diminished and drops in activity began to precede reward delivery - particularly for HR trials. Additional dynamics emerged during conditions in which effort was devalued or revalued. These analyses are ongoing, but our data so far suggest that ACC-DMS projection activity dynamically tracks relative effort and reward to support optimal allocation of effort during decision making.

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Title: Integration of effort, footshock and ICSS strength

Authors: *D. VELAZQUEZ-MARTINEZ¹, A. TOSCANO ZAPIEN³, B. L. PACHECO GÓMEZ², W. ZEPEDA-RUIZ², D. VELAZQUEZ-LOPEZ⁴;

¹Facultad de Psicología, ²Univ. Nacional Autonoma De Mexico, Mexico City, Mexico; ³Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; ⁴Univ. Nacional Autónoma De México., Mexico City, Mexico

Abstract: Particular values of positive and negative aspects of a response or its reward determine whether subjects may choose to engage in reward pursuit. Rats are eager to respond to obtain electrical brain stimulation (EBS) of the medial forebrain bundle. Time allocation (TA) to obtain a reinforcer is a joint function of effort and reinforcer strength. Reinforcer strength is an inverse function to the difficulties an organism may overcome to obtain it; to integrate a final decision, a common scale that integrates both positive and negative aspects should determine reward intensity in order to consider effort (or value) to determine how much TA is the reinforcer worth. To evaluate these interactions, male Wistar rats were implanted with an electrode in the MFB and trained to respond to obtain EBS. To manage the negative factor, we varied a low intensity footshock contingent to the response initiation or to the EBS delivery. We observed that decreasing EBS amplitude or increasing the required effort to obtain a reinforcer produced an orderly decrease in TA. Adding a shock coincident to response initiation or EBS delivery produced a further decrease in TA. This observation is consistent with a common scale that integrates both positive and negative aspects of a reinforcer. However, a larger decrement in TA was observed when footshock was coincident with the EBS than with the initiation of the response. This observation is consistent with the posited existence of various stages of integration for reward strength and reinforcer value.

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Poster

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Title: Prefrontal cortical dynamics underlying goal-directed behavior in freely moving common marmosets

Authors: ***M. TAKAJI**¹, T. OSUKA³, M. MURAYAMA³, M. KOMATSU²;
¹Inst. of Sci. Tokyo, Yokohama, Kanagawa, Japan; ²Inst. of Sci. Tokyo, Tokyo, Japan; ³Ctr. for Brain Sci., RIKEN, Wako, Japan

Abstract: Behaviors require animals to process sensory inputs, make decisions at the appropriate time, and modify their motions. In this way, neural dynamics mediating behaviors are thought to be coordinated among multiple brain regions. Many researches are focusing on a specific behavior, but there is little known about the difference between intended and contingent behaviors. To gain insights into neural dynamics of this issue, we recorded neural activity of free-moving common marmosets using a wireless electrocorticography (ECoG) system. We assigned a task to the common marmosets to keep touching a stimulus displayed on a touch screen for 1 seconds to get a reward. Using these data, we found that there was high-frequency neural activity in the motor and visual areas before touching the stimulus in both succeeded and failed trials. We also found that there was high-frequency neural activity in the dorsolateral prefrontal areas followed by high-frequency neural activity in the ventrolateral prefrontal areas before stop touching the stimulus only in the succeeded trials. These observations have implications for our understanding of the neural dynamics about the conscious behavior.

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Title: Dorsal raphe serotonin neurons encode probability but not amount of future rewards during operant waiting for delayed rewards

Authors: K. W. MIYAZAKI¹, K. DOYA¹, *K. MIYAZAKI²;
¹Okinawa Inst. of Sci. and Technol. (OIST), Okinawa, Japan; ²Okinawa Inst. Sci. and Tech. (OIST), Okinawa, Japan

Abstract: Our previous research revealed a causal relationship between dorsal raphe serotonin neural activity and operant waiting for future rewards by showing optogenetic activation and inhibition of serotonin neurons prolonged and shortened waiting time for future rewards, respectively (Miyazaki et al., 2014, Taira et al., 2024). We also found that serotonin effect in promoting waiting is maximized by both high reward probability and high reward timing uncertainty (Miyazaki et al., 2018). We further proposed a Bayesian decision model of waiting which assumes serotonin activation increases the prior reward probability to reproduce the major features of the experimental results (Miyazaki et al., 2018, 2020). However, there was no direct evidence serotonin neural activity was modulated by reward probability. Tph2- GCaMP6 transgenic Mice (n = 7) in which GCaMP6 is selectively expressed in serotonin neurons were trained to perform a sequential tone-food waiting task (Miyazaki et al., 2014, 2018) that required them in one trial to wait for a delayed tone (tone delay: 0.3 s, tone duration: 0.5 s) at tone site and then to wait for a delayed food (reward delay: 3 s) at reward site. The sequential tone-food waiting task lasted until the mouse had completed 40 trials. An optical fiber (400 µm diameter) was implanted into the DRN and serotonergic neural activity was recorded by fiber photometry. We prepared four reward probability tests (100, 75, 50, and 25%) and associated with four tones (8 kHz, 2.1 kHz, white noise, and 4.5 kHz), respectively. In one test, the reward probability was constant. We focused on serotonergic neural activity during operant waiting for delayed reward. dF/F was the highest in the 100% test $23.9 \pm 2.1\%$ and gradually decreased $19.5 \pm 1.9\%$, $16.7 \pm 1.9\%$, and $14.2 \pm 1.8\%$ in the 75%, 50%, and 25% tests, respectively. When the reward amount presented in reward trial was changed in the 25% test, waiting time in the 25% three-pellets test (5.1 ± 0.1 s) was significantly longer than that in the 25% one-pellet test (4.8 ± 0.1 s). This result indicates that waiting behavior for future rewards is affected by reward amount. We found that there is no significant difference in serotonin neural activity during waiting between the 25% three-pellets test ($14.1 \pm 1.7\%$) and the 25% one-pellet test ($14.2 \pm 1.8\%$). These results show dorsal raphe serotonin neurons encode probability not value of future rewards and serotonin response would be used to support flexible behavior.

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Title: Identifying prefrontal neural signatures underlying motivated behaviors and effort-based decision making

Authors: *M. ELLENBERGER^{1,2}, S. BHATTACHARYYA^{1,3}, H. DEMPSEY^{1,2}, S. D. FISHER¹, J. NITHIANANTHARAJAH^{1,2};

¹The Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia; ²Univ. of Melbourne, Melbourne, Australia; ³Univ. of Melbourne, Melbourne, Australia

Abstract: A challenge for advancing effective treatments for complex mental health conditions like major depressive disorders is the need for greater understandings of the neurobiology underlying disrupted mood and motivational processing. Human neuroimaging studies in individuals with clinical depression have identified dysfunctional activity in key brain regions like the prefrontal cortex (PFC) and the lateral habenula (LHb). Yet, due to limitations in resolution, the mechanistic nature of cellular dysfunction in these regions and how this correlates to regulating motivation-based behaviors remains elusive. The use of preclinical animal models in advanced cognitive assays combined with *in-vivo* calcium imaging provides valuable opportunities to address this challenge. Here, we measured the activity of PFC neurons using UCLA Miniscopes while mice performed a range of touchscreen-based tests for motivational control and behavior. In the first experiment, we tracked the activity of PFC neurons that project to the LHb across changing task demands (fixed ratio, progressive ratio, reward omission and effort-related-choice). Instead of a uniform population activity, we found neurons with increased or decreased activity depending on the behavioral response. In line with this, we identified heterogeneous ensembles that show selective activity patterns depending on the behavioral response. Importantly, we also show that cells retain an encoding identity across days and when task demands change. These data suggest PFC-LHb projection neurons track and update information about reward-based outcomes to inform behavioral actions. In the second experiment, we tested mice lacking *Neurologin-1* (*Nlgn1*^{-/-}), a model of impaired motivated behaviors, and wildtype controls in the novel rearing-effort discounting task to specifically test effort-based decision making. We observed that with increasing effort demands, *Nlgn1*^{-/-} mice chose the low-effort option more than controls, while their general engagement in responding was unaffected. Additionally, we identified neural representations for effort, reward size and previous trial outcome, showing that PFC excitatory neurons contribute to effort-reward computations. The impact emerging psychoactive antidepressants, ketamine and psilocybin, may have on behavioural and neural responses is currently being investigated. Taken together, our findings contribute to better understanding the neurobiological processes that shape mood and motivational processing, towards developing improved treatments for psychiatric conditions where these processes are disrupted.

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Poster

PSTR349: Circuit Regulation of Reward-Related Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR349.23/GG12

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Title: Prefrontal outcome-related activity shapes ongoing effort-based choice strategies

Authors: *A. J. YANG¹, D. NASERELDIN¹, A. KAO², T. A. GUPTA³, S. WILKE¹;
¹UCLA, Los Angeles, CA; ²UCLA, Palo Alto, CA; ³Psychiatry, Santa Clara Univ., Los Angeles, CA

Abstract: Effort-based decision-making (EBD) involves evaluating effort-reward tradeoffs to flexibly guide adaptive choice strategies. The anterior cingulate cortex (ACC), a subregion of the prefrontal cortex, has been implicated in overcoming effort costs during decision making. The ACC also encodes positive and negative value signals relevant to decisions about whether to flexibly adjust versus persist with the current behavior. These features position the ACC to play a key role in shaping ongoing choice strategies. However, the precise mechanisms by which the ACC regulates decision making in response to changing effort-reward tradeoffs remains unclear. To address this gap, we developed and validated a full automated, computer-controlled T-maze apparatus for studying spatial EBD in mice. Prior work using a hand-operated version of this task demonstrated a role for ACC in effortful action selection, particularly when switching from a low- to a high-effort choice strategy. Complementary fiber photometry recordings showed that ACC activity is modulated by effort, particularly following reward consumption, where activity diverges most for high- vs. low-effort choices. To test the causal role of this post-reward activity, we expressed excitatory or inhibitory opsins in bilateral ACC and used spatiotemporally precise optogenetic manipulations immediately after high and low effort choice outcomes. These manipulations were performed during devaluation and revaluation of the effortful option, designed to promote flexible strategy updating. Although experiments are ongoing, preliminary results suggest that stimulating or inhibiting ACC during this post-reward window alters choice strategy in distinct, task-dependent ways. Together, these findings reveal a temporally specific role for ACC in integrating choice outcomes to guide ongoing effort-based decisions.

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Poster

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Topic: H.03. Motivation

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Title: Dysregulation of a nucleus accumbens shell hypothalamus pathway supports overeating

Authors: *J. HINZ¹, X. WANG¹, C. LUSCHER²;

²Basic Neurosci., ¹Univ. of Geneva, Geneva, Switzerland

Abstract: Compulsive overeating of calorie-dense, highly palatable food is known to cause many health condition, in some cases even leading to premature death. The circuit adaptations that transform controlled feeding into a pathological, treatment-resistant habit remain elusive. The nucleus accumbens (NAc) shell may be a key brain region involved: akin to addictive drugs that remodel glutamatergic and neuromodulatory signaling, modulating NAc shell activity may affect palatable food intake. We therefore asked whether chronic access to a sweet, energy-rich liquid diet (e.g. Ensure) remodels dopaminergic activity in the NAc and its projection to the lateral hypothalamus (LH), a circuit that gates palatable feeding. To test this hypothesis, we built an automated home-cage system that continuously tracks spontaneous foraging and licking while recording dopamine release (dLight1.3b) and calcium dynamics in LH-projecting D1-medium spiny neurons (MSNs) in the NAc via fiber photometry. We developed a paradigm that allows imaging for three weeks during which the mice had unrestricted access to Ensure. This setup models voluntary overconsumption without caloric restriction, task demands, or learning confounds. Despite prolonged exposure, lick-locked dopamine transients remained elevated, without signs of reward-signal inhibition, typically observed in operant conditioning tasks. In parallel, LH-projecting D1-MSNs were consistently suppressed during each feeding bout, in line with their inhibitory control over LH-mediated palatable intake. These findings point to a dopamine-driven, feed-forward NAc - LH loop that stabilizes hedonic overeating and may explain its clinical refractoriness. Projection-specific interventions that reverse this plasticity may offer a tractable path toward durable anti-obesity therapies.

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Poster

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Topic: H.03. Motivation

Support: ERC
Lemanic doctoral school
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Title: Synaptic plasticity on glutamate afferents onto NAc LH-projectors in diet induced overeating

Authors: *X. WANG¹, J. HINZ¹, F. CHAUDUN¹, M. LOUREIRO¹, C. LUSCHER^{1,2};
¹Univ. of Geneva, Geneva, Switzerland; ²Clin. Neurosciences, Geneva Univ. Hosp., Geneva, Switzerland

Abstract: With today's increased availability of palatable processed food, overeating promotes weight gain through unbalancing energy expenditure and intake, eventually leading to obesity in many individuals. Lifestyle interventions are the foundation of obesity management; however, maintaining weight constant is challenging. Thus, understanding the neural mechanism underlying the emergence of overeating is critical to develop durable interventions. Previous work has implicated the dopamine system and the lateral hypothalamus to be overactive in obese patients. In particular, D1-MSNs in the nucleus accumbens (NAc) projecting to the lateral hypothalamus (LH) authorizes feeding. Here, we hypothesize that synaptic plasticity of the glutamate projections targeting NAc LH-projectors could contribute to the long-term weight increase in overeating.

We observed weakened excitatory transmission (i.e. reduced AMPA/NMDA ratio) onto NAc LH-projectors after chronic exposure to highly palatable liquid food and concomitant weight gain. To identify the relevant inputs, we mapped NAc LH-projectors' inputs using a TRIO tracing approach and located the paraventricular thalamus, the basolateral amygdala (BLA), the medial prefrontal cortex, xyphoid body and the ventral hippocampus as fiber origin. Using a combination of retrograde tagging and optogenetics in patch clamp experiments, we found reduced AMPA/NMDA ratio on NAc LH-projectors receiving afferents from BLA. To parse the induction mechanism of this synaptic depotentiation *in vivo*, we monitored dopamine transients in the NAc during self-paced feeding on a highly palatable liquid in an automated home-cage system. Optogenetic manipulation of dopamine and BLA inputs to the NAc *in vivo* will test for a causal link of observed synaptic changes, overconsumption and weight gain.

We addressed the synaptic changes in dopaminergic systems that underlie hedonic overeating, to propose a rational design for circuit-based treatments of obesity.

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Poster

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Title: Temporal encoding of expectations in human orbitofrontal cortex

Authors: *S. ALNES¹, L. BELLIER¹, M. RUVALCABA¹, A. O. BLENKMANN^{5,7,6}, A.-K. SOLBAKK^{5,6,7,8}, T. ENDESTAD^{5,6,7,8}, S. VADERA⁹, K. D. LAXER¹⁰, J. T. WILLIE¹¹, P. BRUNNER¹¹, J. J. LIN¹², M. HSU², R. T. KNIGHT^{3,4}, D. MARCIANO^{3,13};

²Haas Sch. of Business, ³Helen Wills Neurosci. Inst., ⁴Departments of Psychology and Neurosci.,

¹Univ. of California Berkeley, Berkeley, CA; ⁵Dept. of Psychology, ⁶RITMO Ctr. for Interdisciplinary Studies in Rhythm, Time, and Motion, Univ. of Oslo, Oslo, Norway; ⁷Dept. of Neurosurg., Oslo Univ. Hosp., Oslo, Norway; ⁸Dept. of Neuropsychology, Helgeland Hosp., Mosjøen, Norway; ⁹Dept. of Neurosurg., Univ. of California Irvine, Orange, CA; ¹⁰Dept. of Neurol. and Neurosurg., California Pacific Med. Ctr., San Francisco, CA; ¹¹Dept. of Neurosurg., Washington Univ. Sch. of Med., St. Louis, MO; ¹²Dept. of Neurol. and Ctr. for Mind and Brain, Univ. of California Davis, Davis, CA; ¹³Dept. of Psychology and the Federmann Ctr. for the Study of Rationality, Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: A slot-machine near miss, when the final reel stops one symbol away from a jackpot, provokes an urge to keep playing despite the absence of reward. However, the rapid encoding of expected outcomes during the anticipatory wheel spinning phase and its influence on outcome evaluation remains poorly understood. Here, we recorded intracranial electroencephalography data from 25 patients with medically refractory epilepsy undergoing presurgical evaluation while they performed a slot-machine task. Participants chose a symbol on one reel and won when the second reel stopped on their choice (1/6 chance). Using time-resolved decoding, we found distributed neural encoding of expected wins and losses in limbic areas (including anterior cingulate cortex, orbitofrontal cortex (OFC), and hippocampus) during the 1500 msec before reel stop. We also found win-like encoding prior to reel stop on Near Miss trials, where the winning symbol passed the pay-line (-1210 to -430 ms; $F_{\text{thresh}} = 4.45$, $p < .05$) or stopped just before (-1250 to 40 ms; $F_{\text{thresh}} = 4.45$, $p < .05$). Mixed-effect models revealed that OFC tracked reward expectations as outcome-dependent activity in low-frequency delta band power (1-4 Hz; $p < .05$) and in high-frequency activity (70-150 Hz; HFA; $p < .05$). Given that OFC encoded outcome, we investigated how outcome-specific activity related to temporal variations in expectation of winning. Expectation data was obtained from a separate cohort of healthy individuals playing the same slot-machine game but with the option to switch back and forth between gambling on the spinning slot machine or receiving a guaranteed, smaller reward. After comparing behavior to a permuted distribution ($n = 1000$) of OFC trial labels, we found that the probability of gambling on the slot machine correlated with outcome-dependent OFC delta power and HFA on Win and Near Miss trials (all $p_{\text{corr}} < .05$). Finally, we investigated whether OFC activity predicted trial-by-trial reported willingness to continue playing and happiness. We found an interaction between delta band power and outcome on willingness to play again in the pre-spin stop period, indicating outcome-dependent OFC activity prior to spin stop related to the urge to continue gambling ($F_{\text{thresh}} = 2.6$, $p < .05$). OFC activity and outcome also interacted on participants' happiness with the outcome in the post-spin stop period ($F_{\text{thresh}} = 2.6$, $p < .05$). Together, our findings show that OFC encodes outcome expectations on a fine-grained temporal scale, which are linked not only to behavioral reports of expected outcomes but also valuation of the outcome and willingness to continue gambling.

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Poster

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Title: Opto-dialysis: illuminating neurocircuit- and cellular phenotype-specific neurochemical dynamics in awake behaving animals

Authors: *H. NEDELESCU¹, C. MILIANO³, S. AZUMA⁴, G. E. WAGNER⁵, F. WEISS², M. W. BUCZYNSKI⁶, N. SUTO⁷;

¹Scripps Res. Inst., San Diego, CA; ²Dept. of Neurosci., Scripps Res. Inst., La Jolla, CA; ³Virginia Polytechnic Inst., Blacksburg, VA; ⁴EICOM Corp., San Diego, CA; ⁵Mol. and Cell. Neurosci., The Scripps Res. Inst., La Jolla, CA; ⁶Sch. of Neurosci., Virginia Polytechnic Inst. and State University, Blacksburg, VA; ⁷Dept. of Mol. Pharmacol. & Exptl. Therapeut., Mayo Clin., Rochester, MN

Abstract: Opto-dialysis is an advanced technique that integrates optogenetics with microdialysis to monitor neurocircuit-specific or cellular phenotype-specific neurotransmitter release in vivo. A custom-designed probe, combining an optical fiber for light delivery with a microdialysis membrane for extracellular neurochemical sampling, is stereotactically implanted in the brain and paired with wireless stimulation of genetically tagged neurons within specific neurocircuits. The resulting neurochemical changes are then analyzed using established analytical methods. Using this technique, we determined extracellular neurotransmitter fluctuations in the nucleus accumbens (NAc) in response to optogenetic stimulation of afferent terminals from the basolateral amygdala (BLA) selectively reactive to morphine vs. naloxone (an opioid receptor agonist vs. antagonist). We used high performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) to qualitatively and quantitatively measure the following 13 neurotransmitters in each dialysate sample: acetylcholine, adenosine, aspartate, dopamine, GABA, glycine, glutamate, glutamine, histamine, norepinephrine, serine, serotonin and taurine. While BLA→NAc neurons are predominantly glutamatergic, optogenetic stimulation of morphine- vs. naloxone-reactive terminals each elicited distinct combinations of neurotransmitter fluctuations. Beyond opioids, this technique enables investigation of neurochemical responses to a wide range of compounds, including psychedelics, for which in vivo circuit-level neurochemical effects remain poorly understood. More broadly, opto-dialysis offers a versatile

platform for dissecting the neurocircuit- and cellular phenotype-specific neurochemical dynamics.

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Poster

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Title: A progressive ratio task with costly resets reveals adaptive effort-delay tradeoffs

Authors: *Z. M. RIVERA¹, M. CERVERA¹, K. GUERRERO LEON³, A. IZQUIERDO², A. M. WIKENHEISER²;

²Dept. of Psychology, ¹UCLA, Los Angeles, CA; ³California State University, San Bernardino, Highland, CA

Abstract: The Progressive Ratio (PR) schedule is a widely used method for quantifying the motivational value of a reinforcer, where subjects are required to exert an increasing amount of work to earn each successive reward. A limitation of PR is that, as the ratio requirement balloons over the length of the session, so does the time it takes to reach the subsequent ratio. Time to reward and effort are inextricably linked in the standard PR. Instead, the Progressive Ratio with Reset (PRR) task transforms PR into a problem that can be modeled similarly to existing patch-leaving foraging scenarios where time and effort costs can be discretely manipulated. In this task, rats engage with a standard PR1 schedule on one lever but have the option to press a second lever to reset the current ratio requirement back to its initial level. This reset action comes at the cost of a delay period, during which both levers are retracted, introducing an opportunity cost. We tested 24 Long-Evans rats (PND $\geq$ 96; 12 females), and found that they used the reset lever adaptively, with decisions sensitive to the imposed reset delay. We developed a normative model to compute the optimal bout length—the number of rewards rats should obtain before resetting to maximize the long-term rate of reward. Rats adjusted their behavior in ways that closely tracked these optimal strategies, suggesting a high degree of cost-benefit sensitivity. Ongoing studies include using inhibitory DREADDs virus (AAV8-CamKIIa-hM4Di-mCherry) in ventral

orbitofrontal cortex (VO) to study how VO represents relative cost during PRR. Previous research has shown that VO lesions bias rats towards smaller, sooner rewards (Mobini et al., 2002) and also decreased breakpoint ratio, possibly through decreasing the value of food reinforcers (Kheramin et al., 2005). Research from our lab has shown that VO lesioned rats fail to represent temporally based expected uncertainty, indicating that timing rather than effort may be perturbed (Stolyarova & Izquierdo, 2017). The PRR task offers a novel and more nuanced tool for evaluating motivational decision-making than previously explored. It extends traditional reinforcement learning frameworks into the domain of sequential decision-making and connects behavioral performance in laboratory settings to ecologically relevant foraging principles.

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Poster

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Topic: H.03. Motivation

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Title: Interrogating Ventral Pallidal Circuits as a Therapeutic Target for Obesity

Authors: ***J. G. WANG**¹, L. Z. FANG², C. L. MURRELL³, F. SCHOUKROUN³, M. BARRETT³, C. S. XU², M. CREED⁵, A. V. KRAVITZ⁴;

¹Psychiatry, Washington Univ. in St. Louis, St. Louis, MO; ²Anesthesiol., ³Psychiatry,

⁴Washington Univ. in St. Louis, Saint Louis, MO; ⁵Washington Univ. In St. Louis, Saint Louis, MO.

Abstract: It is hypothesized that obesity is caused by the widespread availability of highly palatable foods, which cause animals to eat beyond their caloric needs. However, it is not clear which the brain circuits drive feeding beyond caloric need. We hypothesized that GABAergic neurons in the ventral pallidum (VP^{GABA} neurons) can drive feeding beyond caloric need and tested this hypothesis using a combination of cell-type specific calcium recordings and optogenetic stimulation. First, we found that calcium activity of VP^{GABA} neurons is robustly increased during food consumption. Second, we found that optogenetic activation of VP^{GABA} neurons is sufficient to drive high-fat food consumption, even in sated animals. Interestingly, the same optogenetic stimulation had mild effects on consumption of low-fat laboratory chow, suggesting that stimulation of VP^{GABA} neurons selectively drives intake of palatable foods. In the head-fixed context, analysis of licking microstructure during VP^{GABA} optical stimulation showed that licking bout duration was extended, suggesting that these neurons play a role in mediating meal-termination. Next, we tested whether VP^{GABA} neurons were sensitive to changes in internal hunger state, by recording their calcium activity in the fasted vs. sated condition, and after

artificial induction of hunger via injection of the hormone ghrelin. Here, we found that VP^{GABA} neurons were insensitive to both internal hunger state and ghrelin, which contrasts with canonical hunger-promoting AGRP-expressing neurons in the arcuate nucleus (Arc^{AGRP} neurons) that are sensitive to both. Previous studies demonstrated that optogenetic activation of Arc^{AGRP} induced place avoidance, consistent with the induction of hunger being a negative state. In contrast, optogenetic activation of VP^{GABA} neurons did not induce a place avoidance, but instead induced place preference (as demonstrated in prior work by multiple other researchers), further demonstrating that VP^{GABA} neurons drive feeding without inducing hunger. To test whether manipulating VP^{GABA} neurons is therapeutic against high fat diet (HFD) induced weight gain, we used virally overexpressed Caspase3 ablation strategy and found that ablated animals were resistant to HFD induced weight gain, compared to mCherry injected controls. Taken together, these experiments indicate that VP^{GABA} neurons play a critical role in driving feeding behavior that is independent of hunger, highlighting their potential as a target for developing obesity therapeutics.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR350.01/HH4

Topic: H.04. Emotion

Support: NIDA Drug Supply Program
TCU/CSE SERC grant

Title: Recovery from frustrative nonreward: Role of the BLA-NAc pathway

Authors: J. SUAREZ, *M. R. PAPINI;
Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Frustration is an emotional state that can be induced by the unexpected reduction or omission of an appetitive reinforcer. We used consummatory successive negative contrast (cSNC) as an animal model to induce frustration caused by an unexpected 32-to-2% sucrose downshift. Animals respond to the downshift by initially rejecting the reward, followed by a recovery of behavior in subsequent sessions. The present research was designed to explore the role of the pathway connecting the basolateral amygdala (BLA) with the nucleus accumbens (NAc) in this recovery process. The BLA-NAc pathway plays a role in the development of active avoidance learning, in which the animal suppresses freezing to actively avoid shock in response to a signal. We hypothesized that a similar process may be involved in recovering consummatory behavior following the initial suppression after the reward downshift. We used a double-infection chemogenetic procedure to either activate or inhibit the BLA-NAc pathway during

downshifted sessions in the cSNC task. Cre-dependent viral vectors were infused in the departure area, BLA, to activate (pAAV-hSyn-DIO-hM3D(Gq)-mCherry) or inhibit (pAAV-hSyn-DIO-hM4D(Gi)-mCherry) this pathway. A Cre virus vector was infused in the destination area, NAc (pEN-AAV9-hSyn-HI-eGFP-WPRE-SV40). This Cre virus vector moved to the BLA via retrograde transport where it activated the corresponding DREADDs. Animals received access to 32% sucrose for ten 5-min preshift sessions followed by 4 postshift sessions of access to 2% sucrose. In addition to licking, we measured general activity during postshift sessions and in a single open field (OF) test to assess potential motor effects of the chemogenetic manipulations. Immunohistochemistry of c-Fos expression was performed to verify changes in BLA activation. An i.p. injection of clozapine N-oxide (CNO; 3 mg/kg) 30 min before each postshift session and before the OF test was used to activate or inhibit the BLA-NAc pathway. Activation of the BLA-NAc pathway enhanced recovery from reward loss, whereas inhibition of the pathway delayed such recovery. Pathway activation or inhibition had no detectable effects on activity during postshift sessions. A greater distance traveled in the periphery of the OF was observed after pathway activation, but no detectable motor effects in the OF after pathway inhibition. Additionally, a c-Fos expression in the BLA showed a tendency to increase after excitatory activation, but no detectable difference in expression after pathway inhibition. These results suggest that the BLA-NAc pathway plays a key role in the recovery process after frustration.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

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Program #/Poster #: PSTR350.02/HH5

Topic: H.04. Emotion

Support: National Natural Science Foundation of China (grant no. 32200801)

Title: A paraventricular hypothalamic to brainstem circuit mediates stress-induced feeding suppression

Authors: *D. DAI^{1,3}, J. NI^{2,3}, D. SHUMIN^{4,5};

¹Fudan Univ., Shanghai City, China; ²Fudan Univ., Shanghai, China; ³Inst. for Translational Brain Res., Shanghai, China; ⁴Inst. of Neurosci., Hangzhou, China; ⁵Sch. of Brain Sci. and Brain Med., Hangzhou, China

Abstract: Stress profoundly impacts feeding behavior. While neurons in the hypothalamic paraventricular nucleus (PVN) are known to regulate appetite via endocrine pathways, whether they directly control stress-induced changes in feeding through neural projections has remained unclear. Here, we identify a PVN-to-brainstem circuit in which neuropeptide Y1 receptor (NPY1R)-expressing neurons mediate the suppression of feeding under acute stress. Fiber photometry recordings show rapid activation of PVN NPY1R neurons in response to acute stress.

Activation of these neurons or their terminals in the dorsal vagal complex significantly suppresses food intake, whereas inhibition of this pathway reverses stress-induced feeding suppression. Anatomical analyses demonstrate that PVN NPY1R neurons form direct synaptic connections with cholinergic (ChAT) neurons in the dorsal motor nucleus of the vagus. Disruption of ChAT neuron activity partially rescues the feeding suppression caused by PVN NPY1R activation. Moreover, stimulation of the PVN-brainstem circuit evokes anxiety-like behaviors, indicating a coordinated modulation of feeding and emotional responses under stress. These findings uncover a previously uncharacterized hypothalamus-brainstem circuit that links acute stress to appetite suppression and highlight a potential target for the treatment of stress-related eating disorders.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

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Program #/Poster #: PSTR350.03/HH6

Topic: H.04. Emotion

Support: JST FOREST Program (JPMJFR214A)
JSPS KAKENHI (24KJ0492)

Title: Selective Modulation of Attack Bites by a Hypothalamic-Raphe Neural Pathway in Male Mice

Authors: ***K. MITSUI**¹, A. TAKAHASHI²;

¹Univ. of Tsukuba, Tsukuba, Ibaraki, Japan; ²Fac. of Human Sci., Univ. of Tsukuba, Tsukuba, Japan

Abstract: Aggressive behavior is essential for animals to survive and reproduce, but it requires appropriate regulation to prevent excessive or misdirected aggression. The dorsal raphe nucleus (DRN) plays a crucial role in aggression modulation and receives dense innervation from the lateral hypothalamus (LH). We previously showed that aggressive interactions lead to elevated c-Fos expression in LH neurons projecting to the DRN (LH-DRN projection) in mice. This study investigated the role of the LH-DRN projection in modulating aggressive behavior in male mice. Using optogenetic approaches, we injected retrograde Cre-expressing AAV into the DRN and Cre-dependent ChR2-expressing AAV into the LH, with optical fiber insertion into the DRN. Inter-male aggression was assessed using a 5-minute resident-intruder test. Photostimulation of the LH-DRN projection significantly increased attack bites during inter-male aggression while leaving threatening behavior unchanged, indicating selective enhancement of physical attacks without affecting indirect aggressive displays. Remarkably, LH-DRN activation also promoted male-to-female aggression. While some aggressive male mice showed aggression toward females during the first 5 minutes of a 10-minute test, attacks typically disappeared in the latter 5

minutes under control conditions. However, ChR2-expressing mice maintained aggressive behavior throughout the test period, demonstrating the circuit's capacity to drive maladaptive aggression. To validate the functional significance of this pathway, we employed optogenetic inhibition using halorhodopsin expressed in LH neurons, with optical fiber placement in the DRN. This suppression selectively attenuated attack bites during inter-male aggression, suggesting that the LH-DRN projection specifically contributes to the enhancement of attack bites. Preliminary fiber photometry recordings using GCaMP6s specifically expressed in LH-DRN projection neurons revealed increased neural activity during attack bite execution, providing evidence for the circuit's engagement in direct aggressive actions. In conclusion, our findings demonstrate that the LH-DRN projection serves as a neural substrate that selectively modulates direct physical aggression while preserving other components of the aggressive behavioral repertoire.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

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Program #/Poster #: PSTR350.04/HH7

Topic: H.04. Emotion

Support: KAKENHI 23K18257

Title: The critical role of the NTS-CeA pathway in the modulation of aggressive behavior through umami ingestion

Authors: D. MUSTIKA, M. SHINDO, Y. NISHIMURA, S. UENO, H. HIDA;
Nagoya City Univ. Grad Sch. Med. Sci., Nagoya, Japan

Abstract: The consumption of umami during developmental stages mitigates aggressive behavior via the vagus nerve. Nonetheless, further investigation is required to elucidate the neural pathways underlying this effect. Spontaneously hypertensive rats (SHR/Izm), which serve as a model for ADHD, along with ICR mice, were utilized to examine the impact of monosodium glutamate (MSG), an umami compound, on aggressive behavior. Following weaning at postnatal day 25 (P25), the animals were housed individually for a duration of five weeks to promote aggressive behavior. Monosodium glutamate (MSG) at a concentration of 60 mM was administered through the drinking water until the execution of a resident-intruder test. Aggressive behavior was assessed by quantifying the frequency, duration, and latency of anogenital sniffing, aggressive grooming, and attacks. c-Fos immunohistochemistry was performed to assess neural activation in the nucleus of the solitary tract (NTS) and aggression-related brain regions. Furthermore, a DREADD system utilizing a double-virus infection technique was employed to selectively inhibit the iNTS-CeA pathway, thereby corroborating its involvement in the reduction of MSG-mediated aggression. The ingestion of MSG significantly

decreased the frequency and duration of aggressive grooming and attack behaviors, while it increased the latency of attack behavior in comparison to the control group ($p < 0.05$). Immunohistochemical analysis conducted subsequent to the resident intruder test demonstrated an elevation in c-Fos expression within the intermediate nucleus of the solitary tract (iNTS), a terminal site for gastrointestinal sensory afferent fibers of the vagus nerve. In contrast, a decrease in c-Fos expression was noted in the central amygdala (CeA) of the MSG group when compared to the control group. Importantly, the acute intragastric administration of MSG, in the absence of prior post-weaning isolation and the resident-intruder test, resulted in a significant increase in c-Fos⁺ cells within both the iNTS and CeA. Furthermore, the transient inhibition of the iNTS-CeA pathway via the DREADD system eliminated the effects of MSG on aggression, thereby substantiating the pivotal role of the iNTS-CeA pathway ($p < 0.05$). These data suggest that the ingestion of MSG mitigated isolation-induced aggression through the stimulation of the vagus nerve and modulation of the iNTS-CeA neural circuit.

Disclosures: D. Mustika: None. M. Shindo: None. Y. Nishimura: None. S. Ueno: None. H. Hida: None.

Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.05/HH8

Topic: H.04. Emotion

Support: Grant Nos. ZIAMH002881 to ZL
Center on Compulsive Behaviors (CCB) of the National Institutes of Health (NIH) via the NIH Shared Resource Subcommittee to AAN

Title: Frustrative Non-Reward Reconfigures the Brain Network and Increases Aggression and Locomotion in Mice

Authors: *A. A. NAIK, X. MA, Z. LI;
NIH/NIMH, Bethesda, MD

Abstract: Irritability—a transdiagnostic symptom of psychiatric disorders including disruptive mood dysregulation disorder (DMDD) stems from maladaptive responses to frustrative non-reward (FNR), an evolutionarily conserved RDoC construct. To dissect FNR circuitry, we developed the APRO (Alternate Poking Reward Omission) task, a novel behavioral paradigm for mice. In sex-balanced cohorts, FNR exposure elicited aggression and hyperlocomotion without altering anxiety-like, depression-like, or non-aggressive social behaviors. Whole-brain c-Fos staining identified neural activation in 13 discrete regions including prefrontal/cingulate cortices, hippocampal subfields (CA1, dentate gyrus), dorsal thalamic nuclei, ventral pallidum, cuneiform nucleus, and pontine nuclei. Analysis of c-Fos data at the whole brain level revealed that FNR alters the brain network, causing reorganization of its modular structure and shift its processing

mode towards enhanced global integration (increased inter-modular connectivity) and reduced segregation. This network reconfiguration mirrors the functional connectivity alterations observed in irritable youth, linking our FNR model to human irritability. The APRO paradigm successfully recapitulates core clinical phenotypes of irritability, particularly temper outburst and hyperarousal observed in DMDD, demonstrating its validity for cross-species studies of irritability. This work establishes a robust preclinical model for mechanistic dissection of FNR and identification of novel targets for therapeutic intervention of irritability. The detected changes in behavior and brain activity following APRO suggest that FNR recruits specific neuronal ensembles across multiple anatomically connected brain regions to express frustration.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR350.06/HH9

Topic: H.04. Emotion

Support: R01DC013826-07
R01MH117778-05
ALTF 511-2020

Title: The Mouse Posterior Insular Cortex Encodes Expressive and Receptive Aspects of Courtship Vocalizations

Authors: *T. POMBERGER¹, R. D. MOONEY²;
¹Duke Univ., Durham, NC; ²Med. Ctr., Durham, NC

Abstract: Socially effective vocal communication requires brain regions that encode expressive and receptive aspects of vocal communication in a social context-dependent manner. Here, we combined a novel behavioral assay with microendoscopic calcium imaging to interrogate neuronal activity (ROIs) in the posterior insula (pIns) in socially interacting mice as they switched rapidly between states of vocal expression and reception. We found that largely distinct subsets of pIns ROIs were active during vocal expression and reception. Notably, pIns activity during vocal expression increased prior to vocal onset and was also detected in congenitally deaf mice, pointing to a motor signal. Furthermore, receptive pIns activity was modulated strongly by social context. Lastly, tracing experiments reveal that deep layer neurons in the pIns directly bridge the auditory thalamus to a midbrain vocal gating region. Therefore, the pIns is a site that encodes vocal expression and reception in a manner that depends on social context.

Disclosures: T. Pomberger: None. R.D. Mooney: None.

Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.07/HH10

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01 AA028879
NIH Grant P60 AA006420
NIH Grant P21 AA029498

Title: Trapping neural activity in anterior cingulate and nucleus accumbens of cycled vs. ad lib feeding mice

Authors: *Y. LOU^{1,2}, C. WHITE², C. FAVORETTO^{2,3}, A. NGUYEN², E. P. ZORRILLA²;
¹Scripps Res. Inst., La Jolla, CA; ²The Scripps Res. Institute, Dept. of Translational Med., La Jolla, CA; ³Univ. Federal de São Paulo, Dept. of Pharmacol., São Paulo, Brazil

Abstract: Binge eating is a widespread behavior and a diagnostic symptom of the most prevalent eating disorder in the United States. Palatable foods, including those high in sugar, may drive overconsumption and changes in neuronal activation patterns relevant to binge eating. This project used TRAP2-Ai9 activity-dependent reporter mice (n=26 adult females) to investigate the effects of feeding schedule on neuronal activation in the anterior cingulate cortex (Cg) and nucleus accumbens (NAc), regions implicated in impulse control, emotional regulation, reward sensitivity, and motivational salience. Mice were assigned to one of two feeding schedules: ad libitum or cyclic access to a high-fructose corn syrup-sweetened chow (5TUM). Ad libitum fed mice had unlimited choice access to a less preferred standard chow and a preferred HFCS-sweetened chow on all days. Cycled mice had unlimited daily access to the less preferred food on all days but only had access to the preferred, sweetened diet on selected days. We used Targeted Recombination in Active Populations (TRAP) in TRAP2-Ai9 mice to permanently tdTomato-label neurons that were active during the 1st ("Early TRAP") or 4th ("Late TRAP") week of cycling on the diet schedules. Animals were sacrificed after 4.5 weeks on the feeding schedule to quantify neurons in the Cg and NAc regions. Brains were collected, sectioned, and imaged using confocal fluorescence microscopy. TRAP+ (tdTomato+) neurons in the Cg and NAc were quantified across 2-8 bilateral sections per mouse using ImageJ (FIJI). Generalized linear models revealed no significant group differences based on feeding schedule or tagging time point on TRAP+ cell counts in either region ($p > 0.05$). These findings suggest that, under the current conditions, feeding schedule and timing of exposure did not significantly impact TRAP+ neuronal activation. This study demonstrates the feasibility of using TRAP2-Ai9 to assess activity-dependent neuronal engagement in a model of binge-like eating and highlights the need to consider additional behavioral or contextual factors. Ongoing work will examine correlations between TRAP+ cell counts and behavioral measures (e.g., food intake and body weight), as well as interregional relationships between NAc and Cg activation, to further investigate the neural underpinnings of binge-like eating behavior.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.08/HH11

Topic: I.03. Decision Making

Support: Howard Hughes Medical Institute

Title: Behavioral Dynamics of Freely-Moving Mice Hunting in a Complex Environment

Authors: *L.-Q. ZHANG, N. SHETTIGAR, J. TERAN, K. M. BRANSON, E. J. DENNIS;
Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Models of mouse decision-making typically focus on tasks designed to isolate a single cognitive process within a simplified environment. We are interested in studying computations within natural behavior, where decisions are self-directed by the animal and not restricted to stereotypical movements. Drawing inspiration from nature, we ask freely-moving mice to hunt for crickets, guided by sensory cues, in a complex environment. Our large hexagonal arena (~2x2 meters) consists of 144 modular hexagon tiles, each surrounded by felt walls, creating a repeated Y-maze structure. Crickets are hidden beneath trap doors under 16 of these tiles, with only one of them serving as the target at any given time. As the animal moves around, a real-time system tracks the mouse's location and plays a sound through the speaker from the target tile when the mouse remains still for more than 0.5 seconds. If the animal waits inside the target tile, a trap door opens and releases a live cricket reward. After each successful capture, a different cricket tile becomes the target, and the process repeats. Mice quickly learn this task, achieving peak performance of 4-7 captures per 1-hour session. To understand mouse behavior during hunting, we analyze their movement and poses obtained through keypoint tracking. We find systematic changes in their pose minutes before and after cricket capture, potentially reflecting different cognitive processes at various stages of the task. In addition, we develop probabilistic models of their search behavior, which reveal distinct periods of highly efficient, auditory-driven search strategy, as well as intervals of exploratory, less auditory-guided behavior. We are currently developing methods to integrate pose and movement information to gain deeper insights into these search strategies.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.09/HH12

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NSF CAREER 2143910 to MJS
R21MH126278 to MJS
BBRF 30637 to MJS
Sydney University School of Psychology ECR Pathways Funding to JL

Title: A dynamic shift in the role of the basolateral amygdala and lateral hypothalamus in the formation of fear memories following positive experience

Authors: *J. LEAKE¹, A. ABIERO², A. DAWSON³, M. SHARPE⁴;

¹The Univ. of Sydney, The University of Sydney, Australia; ²Fac. of Sci., The Univ. of Sydney, Camperdown, Australia; ³Sharpe Lab, Univ. of Sydney, The University of Sydney, Australia;

⁴Psychology, Dept. of Psychology, Univ. of Sydney, Australia, Sydney, Australia

Abstract: The basolateral amygdala has long been recognized as critical for the encoding and storage of fear memories. This is supported by extensive causal research in rodents demonstrating that BLA damage or inactivation disrupts the formation and recall of learned fear, which is supported by correlative studies in humans. However, the causal evidence garnered from studies in rodents has largely focused on experimentally naïve animals that have limited experience outside the fear protocol. We have recently shown that the lateral hypothalamus (LH) can also become important for encoding fear memories if rats have recently had a positive experience (Sharpe et al., 2021). That is, the LH is not required for formation of fear memories if rats are experimentally-naïve but becomes important if rats have recently had a positive experience. This is surprising as the LH is not considered part of the canonical fear circuit and is typically restricted to reward processing. The current experiments critically extend this work by examining (1) how the role of the LH in encoding fear memories after positive experience influences the well-established role of the BLA in fear, (2) whether the recruitment of LH for formation of fear memories changes with the passage of time between the positive experience and the formation of the fear memory and, (3) how positive experience shapes neural activity in the LH to support subsequent fear memories. To answer these questions, we are using a modern-suite of neuroscience techniques, including cell-type specific optogenetics and single-unit calcium imaging. Together, these experiments reveal how prior experiences regulates the neural circuitry of fear, highlighting the flexibility of the brain's memory systems.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.10/HH13

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NSF CAREER 2143910
R21MH126278
BBRF 30637

Title: Positive experience shifts the fear circuit away from the basolateral amygdala

Authors: *A. ABIERO¹, L. E. DIFAZIO², K. GANESAN¹, M. TAIRA¹, M. SHARPE^{1,2};
¹Dept. of Psychology, The Univ. of Sydney, Camperdown, Australia; ²Dept. of Psychology, Univ. of California, Los Angeles, CA

Abstract: One of the most replicable findings in the behavioural neuroscience literature is that lesions or pharmacological inactivation of the basolateral amygdala (BLA) abolishes the encoding and recall of fear memories. This foundational work in rodents has also been replicated in correlative studies in humans. Thus, the basolateral amygdala (BLA) is believed to be the brain's fear center, necessary for formation and storage of fear memories. Yet we previously showed that GABAergic neurons in the lateral hypothalamus (LH_{GABA}) can become critical for formation of fear memories if rats have had a recent positive experience. That is, LH_{GABA} neurons are not necessary to form fear memories in naïve rats. However, if rats have had a recent positive experience, LH_{GABA} neurons suddenly become necessary to encode the fear memory. In the current experiments, we first set out to determine whether the recruitment of LH_{GABA} neurons to encode fear memories after positive experience influences the well-established role of the BLA in formation of the fear memories. Using optogenetic and lesion manipulations of BLA activity, we first replicated the existing findings that BLA is necessary for the formation of fear memory in experimentally-naïve rats. However, if rats had a recent positive experience, the BLA was no longer necessary to form the fear memory. Our findings reveal that the recruitment of lateral hypothalamic circuits to encode fear memories shifts the fear memory away from BLA following positive experience. Next, we were interested in uncovering the physiological mechanisms that might drive the shift in the fear circuit from the BLA to the LH. A possible mechanism underlying the LH recruitment into the fear circuit is through the involvement of astrocytes. Astrocytes are found in close proximity to neurons and play important roles in the brain including the modulation of neurotransmission and construction of synaptic circuits. Thus, it may be that astrocytes modulate the excitability of LH neurons encoding the memory for the positive experience, which then allows this neuronal population to encode a new fear memory in the future. Accordingly, we used a variety of approaches including immunohistochemistry, chemogenetics, and optogenetics to reveal whether astrocytic function is critical for recruitment of the LH into the fear circuit. Together, this work demonstrates that prior experience changes the way we encode memories and suggests a more fluid approach to conceptualising mnemonic processing.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.11/HH14

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01 MH119089
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Friends of Semel Scholar Award

Title: A scalable internal code for threat imminence in prefrontal neural ensembles

Authors: *F. M. REIS¹, S. MAESTA PEREIRA², G. J. BLAIR³, B. MIRANDA¹, P. J. SCHUETTE¹, X. A. LIU¹, H. LEEDS¹, A. ADHIKARI¹;
¹Psychology, UCLA, Los Angeles, CA; ²Biomed. Engin., Columbia Univ., New York, NY; ³Ctr. for Neural Sci., NYU, New York, NY

Abstract: Medial prefrontal cortical (mPFC) neurons exhibit mixed selectivity during threat exposure, responding to multiple features simultaneously and supporting nuanced, high-dimensional representations. Likewise, adaptive responses to threat rely on the integration of behavior, kinematics, and experience-dependent perception. Therefore, mPFC threat encoding must reflect these external variables alongside a scalable internal code for perceived threat imminence. To examine ensemble-level coding in the mPFC, we developed the FUGA (Flight Upon Grid Approach) paradigm, where a shock grid advances toward the mouse, evoking spatially and dynamically modulated defensive responses. This context elicits both conditioned escape and freezing, allowing assessment of internal threat imminence through the dissociation of threat approach, reactive immobility, goal-directed flight, and threat proximity. Using miniaturized microscopes, we recorded calcium transients from glutamatergic mPFC neurons in the FUGA task to examine how mPFC activity dynamically integrates multimodal features during threat processing. Ensemble activity represented a wide range of kinematic features and defensive behaviors. However, interestingly, the encoding of features related to high threat imminence clustered together. One major ensemble was more active during both behavioral actions and kinematic measures indicative of higher perceived threat imminence, such as freezing and escape, and voluntary avoidance of the grid. The other major ensemble was inhibited during all these situations and was instead activated during exploration of the grid. Thus, the majority of the cells were not representing specific concrete behavioral and kinematic features, but rather encoding a dynamically varying and scalable measure of threat imminence. Furthermore, after conditioning, the ensemble representation of grid proximity became more similar to the aversive shock, revealing an experience-dependent learned neural association between the grid and the shock. Additionally, mPFC ensembles represented an anticipatory threat state that preceded learned flight, suggesting specific neural activity encodes escape preparation or intentionality rather than execution. Lastly, optogenetic inhibition during FUGA acquisition revealed that mPFC activity is necessary for the formation of avoidance and learned escape, but not freezing, underscoring its role in forming memory traces associated with high

threat imminence. These findings show mPFC dynamics encode a scalable internal representation of threat imminence enabling cognitive flexibility in defensive behavior selection.

Disclosures: F.M. Reis: None. S. Maesta Pereira: None. G.J. Blair: None. B. Miranda: None. P.J. Schuette: None. X.A. Liu: None. H. Leeds: None. A. Adhikari: None.

Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR350.12/HH15

Topic: H.02. Reward and Appetitive Learning and Memory

Support: F31 MH127943-01A1
R00 MH106649
R01 MH119089
R01DK139605
NSF-GRFP

Title: Dorsal premammillary nucleus supports acquisition of learned escape

Authors: *B. A. MIRANDA¹, A. TOROSSIAN², F. M. REIS³, E. HILGERT⁴, R. GUDIPATI⁴, A. ADHIKARI⁴;

¹Neurosci., UCLA Chapter, Los Angeles, CA; ²Neurosci., ³Dept. of Psychology, ⁴Psychology, UCLA, Los Angeles, CA

Abstract: When encountering threats, animals display a variety of flexible, experience-driven defensive behaviors such as learned escape in order to maximize their chances of survival. Despite its high adaptive value, flight has predominantly been studied in response to innate threats, leaving much of the mechanisms underlying learned escape unknown. To investigate learned escape, we therefore developed a novel paradigm where mice learn to evade a moving shock grid, mimicking aspects of naturalistic predatory chase. Mice quickly acquire the ability to evade the moving shock grid within a single session, demonstrating a behavior we term as ‘flight upon grid approach’ (FUGA). This conditioned flight response persists into the following fear retrieval day in the absence of shock. We hypothesized that neural circuits involved in both innate escape and conditioned fear are essential to this FUGA behavior. We have previously shown that the activity of cholecystokinin (cck)-expressing cells in the hypothalamic dorsal premammillary nucleus (PMd-cck neurons) is necessary and sufficient to drive escape from both innate and conditioned threats. We reasoned that the activity of PMd-cck neurons may support the acquisition of learned FUGA escapes. Here, we demonstrate that chemogenetic inhibition of PMd-cck neurons during FUGA acquisition disrupts learned escape at fear retrieval. This effect was specific to the moving threat paradigm, as inhibition during contextual and auditory cued fear conditioning had no effect on fear retrieval. Furthermore, PMd-cck neurons encoded the proximity of the moving grid and the speed of FUGA escapes but did not respond to fear

conditioned tones or freezing. Lastly, PMd-ckk neural activity was elevated during conditioned FUGA escapes compared to speed-matched ambulation, suggesting that PMd-ckk cells reflect learned escape rather than general locomotion. Taken together, these findings reveal an essential role of the PMd for retaining the memory of the grid-associated threat and support a growing body of evidence that posits the hypothalamus as a key contributor to learning adaptive, experience-dependent survival behaviors.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.13/II1

Topic: J.07. Data Analysis and Statistics

Title: Adapting FreeClimber for Falling Behavior in *Drosophila Melanogaster*

Authors: *J. M. VASU¹, W. CREEL¹, K. VU², R. E. HARTMAN¹;

¹Psychology, ²Loma Linda Univ., Loma Linda, CA

Abstract: Negative geotaxis, the natural inclination to climb in *Drosophila melanogaster*, is a standardized way to assess locomotor ability in fruit flies. Failed negative geotaxis (FNG), a behavioral event in which a fly begins to climb against gravity but falls, offers researchers a novel way to measure locomotor dysfunction in *Drosophila melanogaster*. FNG discriminates trauma specific deficits from amyloid pathology associated behavior. *Drosophila* subjected to a high impact trauma device exhibited higher instances of FNG compared to healthy controls ($M = -2.65$, $SE = 0.564$, $p < .001$) whereas transgenic flies that expressed amyloid-beta did not. FNG, however remains laborious to quantify as current methods require manual frame-by-frame scoring, which is slow and prone to observer bias. FreeClimber (Spierer et al., 2020), an open-source *Drosophila* motion tracking software, can track fly motion with a variety of video formats. We augmented FreeClimber with an automated FNG detector. A new detect_fng routine tracks fly's centroid and flags descents that exceed a user-defined displacement at any time during a fly's ascent. This new module writes time-stamped CSV files alongside existing slope and negative geotaxis outputs and can be called from the command-line. Scoring FNG by hand has been reduced from approximately 10 minutes per vial to less than one minute and initial tests on benchmarking data suggests high accordance with human scoring. By capturing both negative geotaxis (climbing) and FNG (falling) in a single pass, this extension converts a locomotor assay into a bidirectional screen for early motor deficits, enabling high-throughput evaluation of aging, traumatic brain injury, and/or neurodegeneration.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.14/II2

Topic: J.07. Data Analysis and Statistics

Title: Developing toolkits for end-to-end annotation of animal behaviors

Authors: *K. SHI¹, G. ZHANG², S. ZHANG¹, L. I. ZHANG¹, H. W. TAO³;

²Dept. of Neurosci. and Physiol., ¹USC, Los Angeles, CA; ³Physiol. and Neurosci., USC Keck Sch. Med., Los Angeles, CA

Abstract: Quantifying complex mouse behaviors often relies on time-consuming, subjective manual annotation. Existing automated tools for pose estimation tend to lose keypoints or exhibit drift when occlusion occurred, and an end-to-end video-based action-classification solution for mice is still lacking. To address these challenges, we try to develop an open-source toolkit integrating three modules: (1) real-time deep-learning-based pose estimation that infers occluded landmarks to prevent keypoint drift, (2) real-time tracking for multiple individuals, and (3) video-based behavior detection. The toolkits will include a modern web-based UI, providing a user-friendly platform for mouse behavior analysis. By centralizing all functions on a single server installation, the toolkits aims at eliminating the need for individual installations on each computer, significantly reducing manual annotation effort, and accelerating insights into neurobiological processes.

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Poster

PSTR350: Behavioral Responses and Frustrative Non-Rewards in Animal Models

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR350.15/II3

Topic: H.04. Emotion

Support: R01 HL1660317

Title: Investigating the function of central amygdala projections to the preBötzing complex in modulating breathing

Authors: *E. REILLY¹, J. ARTHURS¹, R. S. PHILLIPS¹, J. SEDANO¹, G. LOGINOV², N. A. BAERTSCH¹;

¹Seattle Children's Res. Inst., Seattle, WA; ²Harvard Univ., Boston, MA

Abstract: In epilepsy patients, electrical stimulation of the amygdala has been shown to produce apnea without distress, suggesting an inhibitory pathway to critical respiratory centers that may have important implications for sudden unexpected death in epilepsy (SUDEP). In our previous work mapping direct excitatory and inhibitory inputs to the respiratory-rhythm-generating preBötzinger complex (preBötC), we confirmed a well-defined inhibitory projection from the central amygdala (CeA). We hypothesized that, in mice, cell-type-specific stimulation of inhibitory CeA neurons or projection-specific activation of their axon terminals within the preBötC, would cause respiratory suppression, consistent with the model proposed in humans. To test this hypothesis, we injected the CeA of Vgat-Cre mice with a Cre-dependent channelrhodopsin virus and placed fibers above the CeA and the preBötC - targeting cell bodies of the entire Vgat⁺ CeA population or the axon terminals of the preBötC projecting subpopulation. Contrary to our prediction, stimulations of CeA cell bodies during awake whole-body plethysmography revealed a small, delayed increase in breathing. Video analysis suggested that this was most prominent when the animal was calm prior to stimulation, and that respiratory changes were typically secondary to a behavioral change. No effects on breathing were observed during stimulation of axon terminals in the preBötC, suggesting the behavioral effects and associated breathing changes are due to activation of CeA neurons that do not project to the preBötC. To test this, we ran open field assays and observed that the mice had reduced motion during cell body stimulation but not terminal stimulation. To further distinguish a possible direct effect on breathing vs. from the associated behavioral change, cell body and terminal stimulations were repeated while recording breathing under anesthesia. There was no change in breathing rate during either stimulation. However, during or immediately after stimulation we noticed an increase in the likelihood of sighs - a reconfiguration of inspiratory pattern that is often associated with changes in affect, arousal, and physiological state. Notably, this effect was observed with both cell body and terminal stimulation, suggesting an important role of this inhibitory CeA to preBötC pathway for sigh modulation, but not apnea or respiratory suppression. Ongoing experiments will expand on this work by mapping inputs to the preBötC that are vulnerable to opioids (express the mu opioid receptor) and determining how these pathways modulate breathing.

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Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.01/II4

Topic: H.05. Mood Disorders

Support: 5R21MH128574-02

Title: Glyoxalase I inhibitors induce fast-acting antidepressant effects through a novel mechanism in mice

Authors: *M. ULIVIERI¹, X. FAN², C. BUI², A.-J. LEE², H. ROSBERG², E. ALCANTARA², M. GENEVRIERE², T. ALEMAN², S. C. DULAWA²;
²Psychiatry, ¹Univ. of California - San Diego, La Jolla, CA

Abstract: Depression is a complex mood disorder affecting millions of people worldwide. Classical antidepressants require weeks to show efficacy, and fails in ~40% of patients. Ketamine, the only approved fast-acting antidepressant, mediates therapeutic effects within hours to days, and requires activity-dependent BDNF release. We previously showed that short-term treatment (5 days) with glyoxalase I (Glo1) inhibitors, including methyl-gerfelyn (MeGFN), induces fast-acting antidepressant effects in mice. Glo1 inhibitors reduce the catabolism of methylglyoxal, an endogenous GABA_A partial agonist and direct TrkB agonist. However, unlike ketamine, Glo1 inhibitors do not induce EEG cortical γ oscillations, a marker of activity-dependent BDNF release. Here, we tested the hypothesis that Glo1 inhibitors exerts fast antidepressant effects following acute injection and that these effects occur independently of activity-dependent BDNF release. Balb/cj animals (both sexes) received 0-12.5mg/kg MeGFN, or 0-15mg/kg ketamine following 6 weeks of chronic mild stress (CMS). Antidepressant-like behaviors were assessed via reward omission, social interaction, coat state, forced swim test (FST). Acute MeGFN improved sucrose-seeking within 1 hour and reversed coat deterioration, FST immobility, and social deficits within 24-48 hours, like ketamine (positive control). We then compared the antidepressant actions of ketamine and MeGFN in wild-type, heterozygous, and homozygous BDNF Val66Met mice on Balb/cj background, a mutation which impairs activity-dependent BDNF release, following 6 weeks of normal housing or CMS. MeGFN improved sucrose-seeking in stressed male wild-type and female homozygous mice; ketamine worked only in wild-type females. MeGFN improved coat state and FST immobility across all genotypes, while ketamine was effective only in wild-types (coat-state and FST) and heterozygous mice (FST). Both treatments improved sociability in wild-types, but only MeGFN worked in homozygous animals. The presence of Met/Met genotype determines the absence of activity-dependent BDNF release and depressed humans harboring this genotype show a poor response to ketamine's antidepressant effects. Our data shows that MeGFN exert fast-acting antidepressant effects and these effects are mediated by a novel mechanism independent of activity-dependent BDNF release. This suggests that Glo1 inhibitors may be a valuable novel fast-acting antidepressant drug that may be effective in individuals with impaired BDNF signaling, including the ~20% carrying the BDNF Val66Met polymorphism.

Disclosures: M. Ulivieri: None. X. Fan: None. C. Bui: None. A. Lee: None. H. Rosberg: None. E. Alcantara: None. M. Genevriere: None. T. Aleman: None. S.C. Dulawa: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR351.02/II5

Topic: H.05. Mood Disorders

Support: NIH Grant R01MH129277
Maryland Stem Cell Research Fund

Title: Mouse and human iPSC models harboring ultra-rare protein-truncating variants in AKAP11 reveal cell type-specific, activity-dependent mechanisms of bipolar disorder pathogenesis.

Authors: *A. X. LUO¹, L. DENG², J. LI², Y. LI², E. FEI², F. TANG², Z. LIAO², Y. SU³, X. ZHU², C. A. ROSS², P. P. LI²;
²Dept. of Psychiatry and Behavioral Sci., ¹Johns Hopkins Univ., Baltimore, MD; ³Univ. of Pennsylvania, Philadelphia, PA

Abstract: Bipolar disorder (BD) is a major neuropsychiatric disorder that impacts approximately 1-4% of the world population. Whole-exome sequencing identified *AKAP11*, which encodes an A-kinase anchoring protein, as a significant risk gene that presents ultra-rare protein-truncating variants (PTVs) in BD patients. However, the molecular and cellular mechanisms underlying BD pathogenesis in the central nervous system in the presence of *AKAP11* PTVs remain unclear. Here we generated transgenic mouse models harboring orthologous PTVs in *Akap11* identified in BD patients. Multi-omic analysis of these mouse models demonstrated loss of function of the Akap11 protein, which leads to alterations in protein kinase A (PKA) signaling and other pathways in a cell type-, and brain region-specific manner. At the cellular level, *Akap11* deficiency differentially impacts the dynamic range of cAMP-PKA signaling in distinct subpopulations of cultured cortical neurons. Glutamatergic neurons derived from human iPSC lines with knock-in of *AKAP11* PTVs further revealed changes in the composition of the PKA signaling complex. These results demonstrate that *AKAP11*-mediated BD pathogenesis involves complex cellular and network mechanisms in an activity-dependent manner, presenting potential targets for therapeutic intervention.

Disclosures: A.X. Luo: None. L. Deng: None. J. Li: None. Y. Li: None. E. Fei: None. F. Tang: None. Z. Liao: None. Y. Su: None. X. Zhu: None. C.A. Ross: None. P.P. Li: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR351.03/II6

Topic: H.05. Mood Disorders

Support: RS-2025-00520820

Title: Sex-specific metabolic reprogramming of arachidonic acid pathways to reverse stress-induced depression in female mice

Authors: *J. NAM;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: Depression is more prevalent in females than in males, yet the metabolic mechanisms contributing to this disparity remain poorly understood. Recently, the sub-chronic variable stress (SCVS) protocol has been proposed as a model to simulate female vulnerability to stress. In our previous study, we used this model to perform a non-targeted metabolomics comparison, revealing elevated levels of arachidonic acid (AA) in the plasma of stressed females (FS), whereas AA levels decreased in stressed males. To investigate whether increased AA levels directly contributed to female-specific depressive behaviors, we administered AA to female mice, but observed no behavioral changes. This suggests that the AA metabolic pathway, rather than AA itself, may play a critical role. Consequently, we reprogrammed AA metabolism by inhibiting key enzymes involved in its synthesis: fatty acid amide hydrolase (FAAH) with URB597 and phospholipase A2 (PLA2) with quinacrine. Remarkably, URB597 treatment during stress alleviated depressive behaviors in females, while quinacrine did not. Furthermore, stress-induced enhancement of spontaneous excitatory postsynaptic currents in the basolateral amygdala (BLA) was normalized following URB597 administration. This effect was abolished by AM251, a CB1 receptor antagonist, suggesting that URB597's actions are mediated by CB1 receptor activation due to increased anandamide (AEA) levels. These findings indicate that metabolic differences may underlie sex-specific depression and that metabolic reprogramming, specifically targeting the FAAH pathway, could offer a novel, female-specific therapeutic strategy for depressive disorders.

Disclosures: J. Nam: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR351.04/II7

Topic: H.05. Mood Disorders

Support: Mark Wallace Endowment

Title: Bipolar Disorder associated missense variants in RAB5A cause endosomal dysfunction leading to neurophysiologic abnormalities.

Authors: *J. HOLDER;
Baylor Col. of Med., Houston, TX

Abstract: Mood disorders including Bipolar Disorder, are known to have a significant genetic predisposition; however, the genes and genetic changes that can underlie Bipolar Disorder are poorly understood. This has led to a lack of fundamental knowledge of the molecular abnormalities that cause mood disorders such as Bipolar Disorder. We previously reported that overexpression of *SHANK3* through the *SHANK3* duplication syndrome is a rare cause of Bipolar Disorder. In discerning the molecular mechanisms through which overexpression of *SHANK3* leads to Bipolar Disorder, we identified the interacting protein network, protein-protein

interactome, of SHANK3. We then interrogated the literature and genetic databases for variants in genes encoding SHANK3 interactors that are associated with Bipolar Disorder. We discovered multiple missense variants in *RAB5A* in individuals with Bipolar Disorder. We hypothesized that these missense variants alter RAB5A function. To determine the impact of Bipolar Disorder associated *RAB5A* variants on its function, we first overexpressed wild-type and variant *RAB5A* (D136N, Q79E, Q44R) in *Drosophila* to determine if these variants alter its function. Overexpression of wild-type *RAB5A* had no impact on fruit fly survival, morphology or neurophysiology. In contrast, overexpressing D136N *RAB5A* led to reduced survival and abnormal wing morphology. Recording the frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs) from the larval neuromuscular junction revealed that the *RAB5A* Q44R and D136N flies had a significant increase in the frequency of mEPSCs. Finally, in mammalian cultured cells, overexpressing the *RAB5A* D136N variant significantly altered early endosomal morphology while overexpressing wild-type *RAB5A* had no impact. In conclusion, Bipolar Disorder associated *RAB5A* missense variants alter the protein's function in both fruit flies and mammalian cells. Further work is underway to determine how these variants drive dysfunction.

Disclosures: J. Holder: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR351.05/II8

Topic: H.05. Mood Disorders

Support: Oscar Stern Award/Eisenberg Family Depression Center University of Michigan to NCT
NIH NRSA F31-HD-114532 to KMS

Title: Regulation of stress and depression by oral contraceptives

Authors: *K. M. SCHUH, M. G. WOOCK, D. LUDMIR, N. C. TRONSON;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Oral contraceptives (OCs) are a critical part of healthcare, with broad health and economic benefits. For many users, OCs have beneficial mood effects, including decreased premenstrual mood changes and overall improved mood. Yet for 4-10% of people, OCs trigger adverse mood effects and increase the risk for depression. We have recently developed a mouse model of OC exposure that mimics key OC effects, including disrupted estrous cycles and a blunted acute stress response. Adult female mice were treated with ethinyl estradiol (EE, 0.02 µg) and levonorgestrel (LVNG, 0.75 µg) daily in 0.25 mL of 10% sucrose; control animals received 0.25 mL of 10% sucrose alone. All treatments were administered for at least two weeks prior to and throughout behavioral testing. At these doses, EE+LVNG suppresses the estrous

cycle, causes an anhedonia-like effect, and increases risk-assessment behavior. Here, we use our model to (1) identify the stress-related molecular mechanisms in the brain modified by OCs, and (2) determine how individual differences in stress responsivity contribute to risk or resilience to OC-triggered depression. To investigate how OC exposure modifies hypothalamic-pituitary-adrenal (HPA) axis responses to stress, we used C57Bl/6N mice. Our data suggest that OCs decrease the ratio of mineralocorticoid (MR) to glucocorticoid receptor (GR) expression within the CA3 region of the hippocampus and decrease GR expression in the ventral hippocampus at baseline. EE+LVNG increases FKBP5 and MR expression in the dorsal hippocampus but decreases FKBP5 expression in the PVN after acute stress, suggesting that EE+LVNG alters stress-regulatory mechanisms through region-specific modulation of MR and FKBP5 signaling. We then tested the hypothesis that higher stress responsivity—due to either heritable factors (strain) or prior stress exposure—may be protective against adverse OC-related mood effects. To do this, we compared C57Bl/6N and BALB/c mice using our OC model. EE+LVNG increased vulnerability to stress-induced depression-like behavior in C57Bl/6N mice but had a protective effect in BALB/c mice, suggesting individual differences in how OCs modulate mood. Together, these findings demonstrate that both baseline state and HPA axis modulation are key mechanisms for vulnerability or resilience to OC-triggered depression. This research will pave the way for a personalized approach to prescribing OCs to limit adverse effects and enhance benefits, improving precision medicine and advancing women's health.

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Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.06/II9

Topic: H.05. Mood Disorders

Support: DoD #PR230701
ABRC #RFGA2024-022-008

Title: Evaluating comorbidities of depression and cardiovascular dysfunction in socially defeated mice

Authors: *A. BARNEY, A. NGUYEN, H. J. CHO, H. LE, C.-W. LIU, R. J. GONZALES, T. HALE, W. H. LEE, D. FERGUSON, H.-D. KIM;
Basic Med. Sci., Univ. of Arizona Col. of Med. Phoenix, Phoenix, AZ

Abstract: Depression is a major cause of disability worldwide and significantly contributes to both the incidence of suicide and the broader socio-economic burden. It often coexists with cardiovascular diseases, which are similarly associated with chronic stress. Decades of research have demonstrated a strong relationship between cardiovascular function and brain activity,

suggesting a potential interplay between mental and heart health. Comorbidities between cardiovascular disease and psychiatric disorders, particularly depression, have been observed in clinical populations, however, the causal relationships remain unclear. In this study, we investigate the hypothesis that neurovascular dysfunction within the brain's reward circuitry contributes to both mood disorders and cardiovascular impairments. Using a well-validated preclinical model of depression, chronic social defeat stress, we identified structural and genetic alterations in the neurovascular components of the nucleus accumbens, a key region involved in reward processing, in socially defeated mice. Concurrently, these mice exhibited cardiovascular abnormalities, including elevated heart rate and aortic dysfunction. Notably, we observed sex-specific differences in both neurovascular and cardiovascular impairments. These findings suggest a potential link between neurovascular dysfunction in the brain's reward circuitry and the development of both mood disorders and cardiovascular impairments. This underscores the need for further research to elucidate the underlying mechanisms and to inform the development of more integrated, systems-level treatment strategies for mood and cardiovascular disorders associated with chronic stress.

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Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.07/II10

Topic: H.05. Mood Disorders

Support: NIMH R01 MH128192

Title: Molecular and Circuit Specific Mechanisms of Depression in the mPFC of Socially Defeated Mice

Authors: *H.-D. KIM, H. J. CHO, J. WEI, X. MA, A. NGUYEN, A. BARNEY, S. QIU, D. FERGUSON;
The Univ. of Arizona Col. of Med., Phoenix, AZ

Abstract: Depression is a severe and debilitating mental disorder that affects nearly one in five individuals worldwide over lifetimes. Although environmental factors, particularly chronic stress, are well recognized as key contributors to its development, the underlying neurobiological mechanisms, including the role of specific neural circuits in the pathogenesis of depression, remain incompletely understood. Chronic social defeat stress (cSDS) in mice is a well-established and translationally relevant model for investigating neural circuit mechanisms underlying stress-related disorders. cSDS has been shown to induce functional alterations across multiple brain regions, including the medial prefrontal cortex (mPFC), a heterogeneous structure

composed of diverse cell types and extensively connected with limbic areas such as the nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus, and amygdala. Using the FosTRAP2 neuronal activity reporter line, we found that acute (aSDS) and chronic (cSDS) social defeat stress engage distinct populations of projection neurons within the mPFC. In this study, we investigated dysregulated gene networks in mPFC neurons activated by chronic social defeat stress (cSDS). By combining the FosTRAP2 and RiboTag mouse models with cSDS, and conducting transcriptomic profiling of activated neurons via RNA sequencing and bioinformatics analysis, we identified distinct molecular signatures associated with stress susceptibility. These signatures include alterations in pathways related to energy metabolism and presynaptic function. Our findings highlight the therapeutic potential of targeting specific mPFC circuits and suggest that restoring synaptic homeostasis within these pathways may offer a novel strategy for preventing or treating depression. Future research should focus on validating these gene networks as therapeutic targets and exploring their role in other forms of stress-related disorders, potentially broadening the impact of our findings across various types of mental illnesses.

Disclosures: H. Kim: None. H.J. Cho: None. J. Wei: None. X. Ma: None. A. Nguyen: None. A. Barney: None. S. Qiu: None. D. Ferguson: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.08/II11

Topic: H.05. Mood Disorders

Support: NIMH R01 MH128192
DoD #PR230701

Title: Transcriptomic evaluation of chronic stress-induced changes in the nucleus accumbens

Authors: *H. J. CHO, H.-D. KIM, J. WEI, X. MA, A. BARNEY, A. NGUYEN, S. QIU, D. FERGUSON;

Basic Med. Sci., Univ. of Arizona Col. of Medicine-Phoenix, Phoenix, AZ

Abstract: Depression is a common and severely debilitating mental disorder that represents a leading cause of disability worldwide, imposing substantial health and economic burdens. While environmental factors such as stress are well-established contributors to its etiology, current treatments are often only partially effective. This limited efficacy is largely due to the complex, multifactorial pathophysiology of depression and an incomplete understanding of the cell type-specific molecular alterations that contribute to its development. To address this gap, there is a critical need for transcriptome-wide characterization of chronic social defeat stress (CSDS), a well-validated preclinical model of depression, to investigate the underlying brain circuit mechanisms. In particular, examining transcriptional changes in the nucleus accumbens (NAc), a key brain region implicated in depression pathophysiology, is essential. Previously, our group

identified circuit- and cell type-specific molecular targets of depression, including *Shisa6* and *Sirt1*, which modulate synaptic function in D1-type medium spiny neurons (MSNs) within the NAc following CSDS in a mouse model, using a RiboTag-based transcriptomic approach. In the present study, we employed single-cell RNA sequencing to unbiasedly examine differential gene expression across distinct NAc cell types in response to CSDS. Our findings revealed significant changes in cell population composition, including alterations in non-neuronal cell types such as glial and endothelial populations, alongside cell type-specific transcriptional changes within the NAc. Moreover, comprehensive bioinformatic analyses identified distinct molecular signatures that correlate with stress-induced behavioral phenotypes. These findings underscore the potential of targeting specific molecular pathways in the NAc and offer insight into transcriptome-guided interventions to restore synaptic homeostasis and alleviate symptoms of depression. Future studies should validate these gene networks as therapeutic targets and explore their relevance across other stress-related disorders and mental illnesses.

Disclosures: H.J. Cho: None. H. Kim: None. J. Wei: None. X. Ma: None. A. Barney: None. A. Nguyen: None. S. Qiu: None. D. Ferguson: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.09/II12

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Support: 5P20GM121310

Title: Sex-specific effects of amygdala activation following vicarious chronic social defeat stress in mice

Authors: D. JEAN, *Y. LI;
Univ. of Wyoming, Laramie, WY

Abstract: Stress-related psychiatric disorders such as post-traumatic stress disorder (PTSD) and major depressive disorder (MDD) exhibit marked sex differences in prevalence and symptoms. However, the neural mechanisms underlying these disparities remain poorly understood. In this study, we investigated the role of the amygdala in mediating sex-specific social withdrawal behavior following vicarious chronic social defeat stress (vCSDS) paradigm in mice. Male and female mice received bilateral amygdala injections of Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADD) and were administered either saline or a CNO analog during social interaction tests conducted before and after vCSDS exposure. Prior to vCSDS, 31% of females displayed social withdrawal upon amygdala activation, while males exhibited no behavioral changes. Post-vCSDS, amygdala stimulation induced social avoidance in 18% of males and 17% of females. Interestingly, after three weeks' incubation following vCSDS, 60% of females exhibited social withdrawal behavior whereas only 27% of males

displayed social withdrawal behavior, suggesting a prolonged and sex-specific effect of amygdala stimulation on females. These findings highlight a differential sensitivity to amygdala modulation in females and suggest a potential neural substrate for the increased vulnerability to stress-related disorders observed in women. Future work will explore additional brain regions contributing to this divergence.

Disclosures: D. Jean: None. Y. Li: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.10/II13

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Chronic Unpredictable Mild Stress Model as a Preclinical Paradigm of Depression: Behavioral, Neurobiological and Pharmacological Evaluation in male Balb/c mice.

Authors: *D. PUSHETT¹, E. ESNEAULT², C. FROGER-COLLEAUX⁴, A. HERNIER³;
¹Porsolt, Le Genest-Saint-Isle, France; ²Porsolt, Le Genest St Isle, France; ³Porsolt, le genest saint isle, France; ⁴Porsolt S.A.S., Le Genest-Saint-Isle, France

Abstract: Major depression is a multifactorial psychiatric disorder characterized by persistent low mood accompanied by cognitive, and physiological impairments that lead to maladaptive behavior. The high lifetime prevalence of this devastating and disabling disorder, coupled with limitations in existing medications, makes the development of improved therapeutics essential. The mild unpredictable chronic stress (CUMS) protocol is widely used to induce depressive phenotypes in rodents, mimicking many symptoms seen in humans. The aim of this study was to comprehensively evaluate the reliability and robustness of the CUMS model as a preclinical model of depression, by assessing both behavioural outcomes (body weight gain, fur condition, sucrose preference, and open-field exploration) and neurobiological markers (IL-6, IL-1 β , and TNF α for neuroinflammation; BDNF expression for neuroplasticity; and corticosterone levels for stress). In addition, the study aimed to examine the therapeutic effects of two antidepressant compounds, fluoxetine and ketamine, administered chronically starting after 4 weeks of CUMS exposure, to further validate the model's sensitivity to pharmacological intervention. Animals subjected to CUMS showed depressive-like behaviors as indicated by decreased body weight gain, deterioration of the coat condition and hyperactivity, as well as increased corticosterone level in plasma. Expression levels of pro-inflammatory cytokines were increased both in the hippocampus and the pre-frontal cortex, while no clear effects were observed on neuroplasticity. Treatment with fluoxetine, and to a lesser extent ketamine, partially restored stress-related physical declines including body weight, fur appearance and locomotor activity. Ketamine slightly decreased corticosterone levels and pro-inflammatory cytokines, whereas fluoxetine had no clear effects on these biomarkers. These findings support the validity and robustness of the CUMS model for studying depression-related phenotypes and highlight its utility in evaluating

novel antidepressant treatments. Moreover, the differential effects observed with fluoxetine and ketamine emphasize the importance of comparing time-dependent and mechanistically distinct pharmacotherapies within preclinical models of depression.

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Poster

PSTR351: Mood Disorders: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR351.12/II14

Topic: H.05. Mood Disorders

Title: Sex-specific effects of chronic fluoxetine on c-Fos expression in Murine brain regions

Authors: *B. HUERTA CAMPOS;
Univ. de Colima, Colima, Mexico

Abstract: Fluoxetine (FLX), a selective serotonin reuptake inhibitor, is widely prescribed for treating mood disorders. Despite its therapeutic benefits, the precise mechanisms and brain regions impacted by long-term FLX treatment remain understood. To explore this, we analyzed c-Fos immunoreactivity in brain regions associated with emotional regulation after 30-day oral FLX treatment in male and female mice. Our findings showed a significant reduction in c-Fos expression in FLX-treated groups compared to controls, suggesting decreased cellular activity in several brain regions. Furthermore, significant sex differences were observed as male mice exhibited higher c-Fos activity than females. Treatment interaction indicated FLX might reduce these sex disparities, except in the CA2 region of the hippocampus, where males maintained higher activity. Notably, FLX appeared to minimize the sex-based disparities in c-Fos expression in several regions, suggesting that FLX equalizes cellular activity across sexes in areas associated with cognitive and emotional functions. Altogether, these data indicate that FLX modulates cellular activity via a sex-dependent mechanism, which highlights the relevance of considering sex differences in the assessment of the neurobiological effects of FLX.

Disclosures: B. Huerta Campos: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR351.13/JJ1

Topic: H.05. Mood Disorders

Support: BD2

Title: Cell-type targeted, AAV-delivered CRISPR/Cas9 clock knockdown in mouse VTA dopaminergic neurons

Authors: *Y. JU¹, A. CRISWELL², S. SIANATI³, J. STRALKA⁸, O. C. GONZALEZ⁴, Y. MA⁵, J. ZHAO⁹, Y. KOZOROVITSKIY¹⁰, J. A. KAUER⁶, L. DE LECEA⁷, L. E. FENNO¹¹; ¹Psychiatry department, ²Univ. of Texas at Austin, Dell Med. Sch., Austin, TX; ³Psychiatry and behavioral Sci., Stanford Univ., Redwood City, CA; ⁴Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ⁵Psychiatry and Behavioral Sci., Stanford Univ., Los Altos, CA; ⁷Psychiatry, ⁶Stanford Univ., Stanford, CA; ⁸Stanford Univ. Sch. of Med., Stanford, CA; ⁹Northwestern Univ., Chicago, IL; ¹⁰Neurobio., Northwestern Univ., Evanston, IL; ¹¹Psychiatry & Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Bipolar disorder (BD) is a common, severe, and life-long psychiatric condition characterized by episodes of both mania and depression, often accompanied by circadian rhythm disruption. Genome-wide association studies (GWAS) have implicated several genetic risk factors, including the core circadian regulator *Clock*, but functional links between these genes and BD-relevant brain circuits are poorly understood. To address this, we developed a Cre-dependent AAV-delivered CRISPR/SaCas9 system to knock down *Clock* specifically in dopaminergic neurons of the ventral tegmental area (VTA), a region associated with sleep, reward, and mania-like behaviors. We designed and screened sgRNA candidates targeting *Clock* and validated editing efficiency both *in vitro* and *in vivo*, revealing consistent indel patterns across platforms. Our results demonstrated that a high-titer combination of *Clock1* and *Clock2* sgRNAs achieved the most robust knockdown at both mRNA and protein levels. Additionally, we observed titer-dependent effects on editing efficiency, with greater gene disruption at higher viral concentrations. Ongoing efforts are focused on building a next-generation single-vector platform delivering multiplexed sgRNAs to further enhance editing efficiency and scalability. This approach enables precise, cell-type-specific functional interrogation of BD-associated genes and offers a flexible toolkit for dissecting the genetic architecture of neuropsychiatric disorders.

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Poster

PSTR351: Mood Disorders: Neural Mechanisms

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Program #/Poster #: PSTR351.14/JJ2

Topic: H.05. Mood Disorders

Support: Breakthrough Discoveries for Thriving with Bipolar Disorder (BD²)

Title: Crispr-mediated deletion of bipolar disorder risk gene CLOCK in VTA neurons in mouse models

Authors: *J. A. KAUER^{1,2}, Y. JU³, J. ZHAO⁴, S. SIANATI¹, O. C. GONZALEZ², J. STRALKA², A. CRISWELL³, Y. MA², C. LUFF², C. EGEBJERG², L. DE LECEA², Y. KOZOROVITSKIY⁴, L. E. FENNO³;

²Psychiatry and Behavioral Sci., ¹Stanford Univ., Stanford, CA; ³Psychiatry & Neurosci., Univ. of Texas at Austin, Austin, TX; ⁴Neurobio., Northwestern Univ., Evanston, IL

Abstract: Bipolar disorder (BD) is characterized by alternating episodes of mania and depression, with disrupted sleep/wake cycles as a hallmark feature. While GWAS studies have identified numerous BD-associated genes, connecting these genetic variants to specific neural circuits and behaviors remains a significant challenge. Here we used a novel Cre-dependent AAV-SaCas9 system to knock down CLOCK, a core circadian regulator, specifically in dopamine (DA) neurons of the ventral tegmental area (VTA)—a region critically involved in mood regulation, sleep, and reward processing, and linked to mania-like behaviors in mouse models (e.g., Mukherjee et al., 2010; doi: 10.1016/j.biopsych.2010.04.031). Both CLOCK mRNA and protein were significantly decreased with our CRISPR approach. We then monitored resulting behaviors, sleep/wake cycles, neural activity, and corticosterone levels. Using GRAB-DA sensor photometry, we found that CLOCK KD in VTA dopamine neurons alters DA release in the NAc, with fewer but larger events. CLOCK KD mice exhibited reduced anxiety-like behavior, spending more time in the center of an open field. In testing learned helplessness, CLOCK KD mice increased escape failures, consistent with increased helplessness. Following a 12 hr-long sleep disruption, SD (Wu et al., 2024; doi: 10.1016/j.neuron.2023), CLOCK KD animals did not increase their locomotor activity, unlike control mice. We are also assessing social and aggressive behaviors using the resident-intruder paradigm in CLOCK KD mice before and after SD to assay experience-induced behavioral transitions. To assess the impact of CLOCK KD on sleep dynamics, we performed home cage continuous 48hr EEG/EMG recordings in control and CLOCK KD mice. We observed no differences in the time spent in each brain state (NREM, REM, Wake) or mean bout lengths of each state. EEG power spectral analysis revealed a reduction in delta (1 - 4Hz) power during NREM in CLOCK KD, specific to NREM bouts during the light period (ZT 0-12). Therefore, while sleep architecture remained intact in the CLOCK KD mice, sleep quality may be reduced. CLOCK KD increased the intrinsic excitability of DA neurons in brain slices, as evidenced by a depolarized resting membrane potential and more action potentials per unit of injected current. In control mice, SD similarly elevated DA neuron excitability, reaching levels comparable to those observed in CLOCK knockdown cells. This observation suggests that CLOCK KD mimics the effects of SD stress on DA cell excitability, potentially contributing to mania-like behavior. Taken together, CLOCK KD specifically in VTA dopamine neurons alters mouse behavior, sleep and neuron excitability.

Disclosures: J.A. Kauer: None. Y. Ju: None. J. Zhao: None. S. Sianati: None. O.C. Gonzalez: None. J. Stralka: None. A. Criswell: None. Y. Ma: None. C. Luff: None. C. Egebjerg: None. L. de Lecea: None. Y. Kozorovitskiy: None. L.E. Fenno: None.

Poster

PSTR351: Mood Disorders: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR351.15/JJ3

Topic: H.05. Mood Disorders

Support: Orion Research Foundation
Sigrid Jusélius Foundation
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Title: Temperature regulates TrkB and associated neurotrophic signaling pathways

Authors: *S. KOHTALA¹, G. GONZÁLEZ HERNÁNDEZ³, M. ROSENHOLM⁴, N. MATSUI⁵, W. THEILMANN², M. SARPARANTA², S. V. ROZOV⁶, T. P. RANTAMAKI¹;
¹Div. of Pharmacol. and Pharmacotherapy, Fac. of Pharm., Univ. of Helsinki, Helsinki, Finland;
²Univ. of Helsinki, Helsinki, ; ³1. Univ. of Helsinki, Finland 2. Univ. of Cádiz, Spain, Helsinki, ;
⁴Univ. of Copenhagen, Copenhagen, ; ⁵Tokushima Bunri Univ., Tokushima, ; ⁶Biomedicine, Helsinki.

Abstract: The mechanisms underlying rapid antidepressant-induced activation of TrkB neurotrophin receptors remain incompletely understood. Here, we show that TrkB phosphorylation triggered by various antidepressant and sedative/anesthetic drugs consistently coincides with hypothermia in adult male mice. This activation was independent of electroencephalographic slow-wave activity and unaffected in Bdnf Val66Met knock-in mice, which exhibit impaired activity-dependent BDNF release. Notably, active maintenance of core temperature blocked drug-induced TrkB activation, as well as phosphorylation of downstream targets such as p70S6 kinase (mTOR pathway) and GSK3 β . Similar temperature-linked increases in TrkB, GSK3 β , and p70S6K phosphorylation were observed during deep recovery sleep after sleep deprivation—a physiological state associated with a drop in body temperature. Preliminary data further indicate that TrkB phosphorylation fluctuates in alignment with the diurnal rhythm of core temperature, and that physiologically relevant changes in brain temperature are sufficient to regulate TrkB signaling. Together, these findings suggest that both drug-induced and physiological brain temperature changes are not merely correlational but may causally regulate TrkB and related signaling pathways involved in synaptic plasticity. This work underscores the importance of considering thermoregulatory states as a key experimental variable in preclinical research.

Disclosures: S. Kohtala: None. T.P. Rantamaki: None.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.01/JJ4

Topic: H.05. Mood Disorders

Support: JPB Foundation
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Title: A point process framework for predicting postictal generalized EEG suppression in electroconvulsive therapy

Authors: *G. H. KANG¹, M. S. BREAULT², M. AREGAWI¹, S. ORGUC¹, S. SEINER³, R. J. HORVATH⁴, M. HENRY⁵, E. N. BROWN⁶;

²The Picower Inst. for Learning and Memory, ¹MIT, Cambridge, MA; ³Psychiatry, McLean Hosp., Belmont, MA; ⁴Dept. of Anesthesia, Critical Care, and Pain Med., Massachusetts Gen. Hosp. / Harvard Med. Sch., Lexington, MA; ⁵Massachusetts Gen. Hosp., Boston, MA; ⁶Brain and Cognitive Sci., MIT/Mass Gen. Hospital/Harvard Med. Sch., Cambridge, MA

Abstract: Electroconvulsive therapy (ECT) is a medical treatment in which a brief electrical stimulus is applied to the scalp to induce a controlled seizure under general anesthesia. It has been used for decades to treat psychiatric conditions, one of which is treatment-resistant depression (Salik & Marwaha, 2022). Despite its clinical effectiveness, ECT elicits variable responses and causes adverse cognitive effects such as memory loss and confusion (Blanken et al., 2024). By better understanding and anticipating the brain's physiologic response to ECT, we could reduce this variability, personalize ECT, and improve both safety and efficacy. One promising lead lies in postictal generalized EEG suppression (PGES), a transient period of reduced EEG activity following the ECT-induced seizure. Studies have found that more robust PGES is associated with better clinical outcomes (Langroudi 2023, Azuma 2007). Yet, we lack a framework for anticipating when suppression occurs or what drives its variability across patients. In this work, we built a predictive model using EEG data from 15 ECT patients, defining PGES as an EEG amplitude drop below 10 μ V within a 1-second bin (Hickman 2020). Given the sparse, time-localized nature of PGES onsets, we modeled them using a point process framework with a discrete-time Poisson distribution. This approach allowed us to preserve temporal granularity, estimate covariate effects on the instantaneous rate of suppression onset, and convert predicted intensities into binary alerts using cross-validated thresholds. To construct predictors, we simulated effect-site concentrations of methohexital, succinylcholine, and propofol using individualized pharmacokinetic models based on patient age, sex, height, and weight. We also extracted dynamic EEG features, including interhemispheric correlation and delta-alpha power ratios, and incorporated lagged suppression history to capture local temporal context. Our population model achieved sensitivities over 80% and mean Poisson deviances under 0.4,

suggesting that suppression onset is not random but shaped by evolving physiology. Our next step is to screen more physiologic, pharmacologic, and EEG spectral covariates with rigorous cross-validation to identify the most informative predictors and refine the model. This framework sets the stage for adaptive treatment protocols that respond to the brain in real time, ultimately helping clinicians minimize treatment response variability, optimize timing of delivered anesthetics and seizure, and personalize ECT delivery in a data-driven way.

Disclosures: **G.H. Kang:** None. **M.S. Breault:** None. **M. Aregawi:** None. **S. Orguc:** None. **S. Seiner:** None. **R.J. Horvath:** None. **M. Henry:** None. **E.N. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PASCALL.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.02/JJ5

Topic: H.05. Mood Disorders

Support: National Science and Technology Council, Taiwan: NSTC 110-2926-I-A49-001-MY4
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National Science and Technology Council, Taiwan: NSTC 111-2634-F-A49 -014
National Science and Technology Council, Taiwan: NSTC 112-2634-F-A49 -003

Title: Utilizing EEG microstates as predictive biomarkers for antidepressant efficacy

Authors: ***Y.-W. HUANG**^{1,2}, **A.-C. YANG**^{2,5,6,3,4},

²Inst. of Brain Sci., ³Sch. of Med., ⁴Digital Med. and Smart Healthcare Res. Ctr., ¹Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; ⁵Dept. of Psychiatry, ⁶Dept. of Med. Res., Taipei Veterans Gen. Hosp., Taipei, Taiwan

Abstract: Background: Major depressive disorder (MDD) is a prevalent psychiatric disorder characterized by mood disturbances, cognitive impairment, and disrupted daily functioning, often linked to aberrant brain connectivity and neural dysregulation. Clinically, serotonin-

reuptake inhibitors (SSRIs) such as fluoxetine (commonly known as Prozac) is frequently used for treatment. However, some patients exhibit poor responses to fluoxetine, highlighting the limitations of current pharmacotherapy in terms of efficacy and individual variability. This underscores the necessity for predictive biomarkers. Electroencephalogram (EEG) microstate analysis captures transient, stable patterns of cortical activity that reflect underlying cognitive networks. This study aims to (1) identify baseline EEG microstate sequence features distinguishing fluoxetine responders from non-responders and (2) characterize microstate-based network alterations across different MDD severity levels. **Methods:** 121 moderate-to-severe MDD patients (age 20-69) were recruited in this study. All participants received 20 mg/day fluoxetine for six weeks. Patients were grouped according to treatment efficacy and severity, based on scores from the Hamilton Rating Scale for Depression (HAM-D-17). A total of 219 recordings were categorized into four severity levels: euthymia (n=28), mild depression (n=40), moderate depression (n=33), and severe depression (n=118). The resting-state EEG data decisively identified distinct microstates using a modified K-means clustering algorithm, demonstrating apparent differences in inter-group microstate metrics. For Aim 2, one-way ANOVA with Tukey's HSD post-hoc tests compared time coverage, mean duration, and occurrence rate across the four severity groups. **Results:** This study identified four microstates similar to the well-known canonical microstates (A-D). For Aim 2, the results showed significant differences in occurrence rates of microstate D among the groups. The mild depression group experienced a reduced occurrence compared to the euthymia group ($p_{\text{adj}}=0.032$). In contrast, the severe depression group showed a higher occurrence than the mild depression group ($p_{\text{adj}}=0.025$). No other microstate metric reached significance (all $p_{\text{adj}}>0.05$). **Conclusions:** The occurrence rate of microstate D systematically varies with MDD severity, positioning it as a promising EEG biomarker for illness staging. We are now completing Aim 1 to test whether baseline microstate features can predict individual fluoxetine response. Our goal is to integrate both aims into a predictive framework for personalized SSRI therapy.

Disclosures: Y. Huang: None. A. Yang: None.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.03/JJ6

Topic: H.05. Mood Disorders

Support: University of Pennsylvania Department of Psychiatry

Title: Escitalopram pharmacokinetic and pharmacodynamic interactions influencing treatment response in depression and anxiety disorders

Authors: *P. BURKAT;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Selective serotonin reuptake inhibitors (SSRIs) are first line medications for certain mental health conditions. Mechanisms of symptom reduction are complex and depend on serotonin reuptake transporter (SERT) occupancy and antagonism. The SSRI escitalopram exhibits highest SERT selectivity compared to others. Two determinants of treatment response include polymorphisms in cytochrome P450 2C19 (CYP2C19) and SERT amino acid moieties involved in escitalopram binding and conformational changes linked to reuptake inhibition. The findings of this study provide insight into PK/PD interactions influencing response to escitalopram in major depression and anxiety disorders. **Methods:** Data and initial parameter values were obtained from PubMed and DrugBank searches. PBPK models for escitalopram and the metabolite desmethylescitalopram were developed with PK-Sim software, and PD models were developed with the Monolix Suite 2024 platform. SERT occupancy was calculated for 10 and 20 mg escitalopram doses delivered every 24 hours over 56-168 days and correlated to clinical response in major depression, generalized anxiety, social anxiety, and obsessive-compulsive disorders. SERT occupancy was additionally calculated from steady state trough escitalopram plasma concentrations for CYP2C19 polymorphisms. Steady state escitalopram unbound interstitial brain concentrations were calculated from plasma concentrations, and used to estimate serotonin reuptake inhibition for a 10 mg dose. **Results:** Escitalopram plasma concentration for 50% maximum SERT occupancy is 7 nM. Steady state SERT occupancy is 76% for 10 mg escitalopram and 83% for 20 mg. Symptom reduction score shifts for major depression, generalized anxiety, social anxiety, and obsessive-compulsive disorders correspond to an increase in occupancy with this dose change. Trough SERT occupancy for pooled CYP2C19 phenotypes is 70%, 61% for the ultrarapid phenotype, and 82% for the null phenotype ($p < 0.05$). Trough serotonin reuptake is 70% for 10 mg escitalopram pooled CYP2C19 unbound interstitial brain concentrations, and 54% for 20 mg escitalopram. Serotonin reuptake for the SERT D98E mutation is 98% (10 mg escitalopram). Calculated serotonin reuptake is 60% (76% SERT occupancy) and 44% (83% occupancy). **Conclusions:** Symptom reduction in depression and anxiety disorders with an increase in escitalopram dose correlates with a shift in SERT occupancy. CYP2C19 polymorphisms significantly influence SERT occupancy, and SERT mutations may render treatment resistance if present in individuals with major depression and anxiety disorders.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

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Program #/Poster #: PSTR352.04/JJ7

Topic: H.05. Mood Disorders

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Opinions, interpretations, conclusions, and recommendations are those of the author(s) and are not necessarily endorsed by the Department of Defense.

Title: Depression improvement and changes to gut microbiota in veterans with Gulf War Illness after one month on the Low Glutamate Diet

Authors: *A. A. MAURY¹, K. F. HOLTON²;

¹Dept. of Neurosci., ²Depts. of Hlth. Studies and Neurosci., American Univ., Washington, DC

Abstract: Gulf War Veterans experience depression at nearly twice the rate of non-deployed veterans. Depression is a persistent symptom of Gulf War Illness (GWI), a chronic, multi-symptom condition affecting the CNS, immune, and gastrointestinal systems. Dysregulation of glutamatergic neurotransmission and dysbiosis in the gut have both been implicated in depression. Prior research demonstrated a significant reduction in depression symptoms after one month on the low glutamate diet, but microbiome changes were not analyzed. The objective of this study was to confirm that the low glutamate diet reduces depression symptoms in this population, and to examine whether or not species-level microbial changes are associated with changes in depression scores. Veterans with GWI (N = 51) were recruited from across the country. Participants completed assessments before and after 1-month on the low glutamate diet. This diet restricts consumption of excitotoxic food additives (e.g., those containing free glutamate or aspartate), and emphasizes nutrient-dense, antioxidant-rich whole foods. Depression symptoms were assessed using the Center for Epidemiologic Studies Depression scale (CES-D) and stool samples were analyzed using 16S rRNA long-read sequencing. Alpha diversity (Shannon, Simpson, Chao1) and beta diversity (Bray-Curtis) were calculated using the R package “vegan” in R Studio. Differential abundance was assessed using ALDEx2 ($\gamma = 0.5$). CES-D changes were compared using paired *t*-tests and Spearman correlations were run to compare taxa and depression change. CES-D scores significantly decreased from (mean (SD)) at baseline of (24 (9.5)) to post-diet (17 (10.4)), $p < 0.0001$. A significant shift in microbial community composition was observed from baseline to post-diet (PERMANOVA: $R^2 = 0.80$, $p = 0.001$; Bray-Curtis median = 0.74), while alpha diversity remained stable. ALDEx2 identified *Streptococcus thermophilus* as one of the most differentially abundant taxa post-diet (uncorrected $p = 0.007$; FDR = 0.31). *S. thermophilus* abundance was significantly correlated with CES-D improvement ($p = -0.24$, $p = 0.04$). Certain strains of this species are known to support gut-brain axis signaling through production of neuroactive compounds and short-chain fatty acids (SFCA). These findings suggest that a low-glutamate diet may improve depression and beneficially alter the microbiome in GWI, potentially through microbial pathways linked to gut-brain signaling.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

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Topic: H.05. Mood Disorders

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Title: Subjective reports during ultrasonic neuromodulation of subcallosal cingulate cortex predict subsequent clinical response in treatment-resistant depression

Authors: *B. COOPER¹, T. RIIS², D. FELDMAN², V. KOPPELMANS³, J. KUBANEK⁴, B. J. MICKEY⁵;

¹MSTP, Univ. of Utah, SALT LAKE CITY, UT; ²Biomed. Engin., ³Univ. of Utah, Salt Lake City, UT; ⁴Biomed. Engin., Univ. of Utah, 36 S Wasatch Dr, UT; ⁵Dept. of Psychiatry, Univ. of Utah Sch. of Med., Salt Lake City, UT

Abstract: **Background** Hyperactivity of the subcallosal cingulate cortex (SCC) is implicated in treatment-resistant depression (TRD). We recently developed and validated a novel low-intensity focused-ultrasound (LIFU) device that can directly modulate the SCC. Here we describe the immediate subjective reports of participants who received repeated, brief ultrasonic stimulation of the SCC and evaluate whether these immediate reports predict subsequent changes in mood and depression severity. **Methods** 17 individuals with TRD participated in two study visits one week apart. Active LIFU and sham LIFU, respectively, were delivered at the two visits in randomized order. Each visit included 15-18 trials, each lasting 30-180 seconds. In total, 574 sonications were delivered (289 active, 285 sham). All sonications were targeted to the SCC in a subject-specific, MRI-guided manner. Immediate subjective self-reports of sonication effects were scored as “positive”, “neutral” or “negative” in valence. Change in mood state was measured with the Sadness Subscale of the Expanded Positive and Negative Affect Schedule at the end of each study visit. Change in depression severity was measured 24 hours later with the 6-item Hamilton Depression Rating Scale (HDRS-6). Data were analyzed with generalized mixed models. **Results** The response distribution was 21% positive, 74% neutral, and 5% negative for active LIFU stimulation of SCC, compared to 2% positive, 97% neutral, and 1% negative for sham stimulation, demonstrating a significant increase in non-neutral response rate ($p<0.001$), skewed towards positive valence ($p<0.001$) with active stimulation. Ten participants demonstrated a significantly elevated sham-normalized positive response rate to active stimulation ($p<0.018$, binomial test), while seven showed no difference between conditions ($p>0.05$). Rate of positive response to active stimulation correlated marginally with improvement in Sadness score at the end of the stimulation visit ($r^2=0.21$, $p=0.073$) and significantly with improvement in HDRS-6 score at 24 hours ($r^2=0.41$, $p<0.006$). **Conclusions** These findings

demonstrate for the first time that direct ultrasonic neuromodulation of the SCC can elicit immediate subjective experiences and that the valence of these immediate subjective changes is more likely to be positive than negative for LIFU stimulation responders. The observed correlations between responses to brief sonication trials and subsequent clinical improvements suggest that transient changes in patient-reported experiences might be useful for guiding both invasive and non-invasive circuit-targeted interventions for neuropsychiatric disorders.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

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Program #/Poster #: PSTR352.06/JJ9

Topic: H.05. Mood Disorders

Support: NIMH MH106775
DARPA HR00112320032

Title: Transient oscillatory bursts as biomarkers for phase-locked rTMS treatment in major depression disorder

Authors: ***R. GONG**¹, **X. SUN**³, **J. DOOSE**¹, **S. HUFFMAN**¹, **C. PING**¹, **G. DOYLE**², **A. WILLIAMS**¹, **L. HONG**⁴, **R. GOLDMAN**⁶, **T. R. BROWN**¹, **M. S. GEORGE**⁷, **P. SAJDA**⁵, **L. MCTEAGUE**¹;

²Brain Stimulation, ¹Med. Univ. of South Carolina, Charleston, SC; ³Columbia Univ., New York, NY; ⁴Columbia Univ., new york, NY; ⁵Biomed. Engin., Columbia Univ., New York, NY;

⁶Univ. of Wisconsin–Madison, Madison, WI; ⁷Dept Psych, MUSC, Charleston, SC

Abstract: Personalized repetitive transcranial magnetic stimulation (rTMS) has gained increased attention as a promising treatment for patients with major depressive disorder (MDD). One such individualized approach is using electrophysiological features as temporal TMS targets at the left dorsolateral prefrontal cortex (DLPFC) to improve therapeutic outcomes. However, the relationship between transient oscillatory features and the efficacy of individualized rTMS strategies remains unclear, limiting insight into the underlying neuroplasticity mechanisms. We

conducted a randomized, double-blind clinical trial involving 23 MDD patients receiving 6 weeks (30 sessions) of rTMS treatment. Participants were randomly assigned to either a SYNC or UNSYNC group. In the SYNC group (N=12, 9F, 44.0 ± 12.5 yrs), we applied closed-loop phase-locked rTMS, synchronizing the stimulation pulses based on each patient's individual prefrontal alpha phase. The individual alpha phase was extracted from pre-treatment concurrent electroencephalography (EEG) - functional magnetic resonance imaging (fMRI) - single pulse TMS recording. In contrast, the UNSYNC group (N=11, 8F, 43.5 ± 13.5 yrs) received rTMS pulses at randomized phases. Five minutes resting-state EEG data were recorded before and after each rTMS session. Occurrence rates and durations of transient oscillatory bursts in theta (4-7 Hz), alpha (8-12 Hz), and beta (13-30 Hz) frequency bands were extracted from these EEG recordings. Our findings revealed that the pre-treatment theta burst occurrence rates at the left DLPFC correlated significantly with depression severity assessed prior to the rTMS treatment. Notably, pre-treatment theta burst occurrence rates in occipital region and beta burst occurrence rates in prefrontal region were able to predict treatment response specifically to phase-locked rTMS. While rTMS produced short-term effects by generally increasing oscillatory burst occurrence rates and durations across multiple frequency bands and scalp regions after each rTMS session, only the SYNC group presented weekly modulation in alpha burst occurrence rates. In specific, we observed that long-term increases in alpha burst occurrence rates over central scalp regions in respond to phase-locked rTMS were associated with greater improvements in clinical outcomes. In addition, preliminary data from our ongoing clinical trial with accelerated phase-locked rTMS protocol (30 sessions in 5 days) also showed comparable findings. Overall, our findings underscore transient oscillatory burst dynamics as valuable biomarkers for predicting and tracking clinical response to phase-locked rTMS in MDD.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

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Program #/Poster #: PSTR352.07/Web Only

Topic: H.05. Mood Disorders

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Osaka Metropolitan University (OMU)Strategic Research Promotion
Project (Young Researcher) OMU-SRPP2023_YR07

Title: State-dependent analysis of extracellular vesicles' mitochondrial DNA copy number in patients with bipolar disorder

Authors: *Y. KAGEYAMA, S. OKURA, Y. DEGUCHI, K. INOUE;
Osaka Metropolitan Univ. Grad. Sch. of Med. Dept. of Neuropsychiatry, Osaka, Japan

Abstract: Background: Mitochondrial DNA copy number in the blood of patients with bipolar disorder (BD) may reflect the disorder's pathophysiology, treatment response, cognitive function, cellular aging, and inflammatory status. Circulating cell-free mtDNA exists in two forms: freely circulating and membrane-enclosed, such as those within extracellular vesicles (EVs). EVs play a role in intercellular communication and can contain mitochondria-derived components, including mtDNA. However, how EV-associated mtDNA (EV mtDNA) relates to BD pathophysiology remains unclear. In this study, we measured and compared plasma total mtDNA and EV mtDNA in the depressive and remitted states of the same BD patients to investigate their potential role in distinguishing between disease states from the inflammation perspective. **Methods:** Plasma samples were collected from 21 BD patients during both depressive and remitted episodes. Total circulating EVs were isolated from 500 μ L of plasma using two rounds of ultracentrifugation. MtDNA levels in plasma and EVs were quantified by real-time quantitative PCR targeting two mtDNA regions (ND1 and ND4). Plasma IL-6 levels were measured using ELISA as an indicator of inflammation. **Results:** EV mtDNA accounted for $33.0 \pm 0.10\%$ of plasma total mtDNA. A strong correlation was found between plasma total and EV mtDNA ($p < 0.0001$, $r = 0.94$). There were no significant differences in EV mtDNA between depressive and remitted states ($p = 0.42$) or plasma IL-6 levels ($p = 0.45$). No significant correlations were observed between IL-6 and EV mtDNA ($p = 0.35$, $r = 0.022$) or IL-6 and total plasma mtDNA ($p = 0.32$, $r = -0.16$). **Conclusions:** Our findings demonstrated a strong positive correlation between plasma total and EV mtDNA in BD patients. However, EV mtDNA was not significantly altered between mood states or inflammation. The results underscore the necessity for more research to clarify the role of EV mtDNA in the pathophysiology of BD.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.08/JJ10

Topic: H.05. Mood Disorders

Title: Ropanicant (SUVN-911), an $\alpha 4\beta 2$ nAChR antagonist: Results from a Phase-2a Study Evaluating Safety and Efficacy in Participants with Moderate to Severe Major Depressive Disorder

Authors: *V. PALACHARLA, A. MOHAMMED, V. GOYAL, J. RAVULA, S. JETTA, R. SUBRAMANIAN, S. PANDEY, P. JAYARAJAN, A. K. SHINDE, V. BENADE, R. NIROGI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: Ropanicant (SUVN-911) is a potent and selective antagonist of $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChR). In animal studies, Ropanicant was orally bioavailable in rats, mice, and dogs, with favorable brain exposure in rats and dose-dependent occupancy of brain $\alpha 4\beta 2$ receptors. Ropanicant exhibited antidepressant-like effects in various preclinical models of depression. Safety pharmacology, genotoxicity, and general toxicity profiles support its therapeutic development. In a Phase-1 clinical study, Ropanicant was found to be safe and well tolerated. An open-label, parallel-group Phase-2a study (NCT06126497) was conducted at multiple centers across the United States to evaluate the safety and efficacy of Ropanicant in patients with moderate to severe major depressive disorder (MDD). Following a screening period of up to four weeks, participants received treatment for two weeks. A total of 41 patients were randomized in a 1:1:1 ratio to receive Ropanicant at 45 mg once daily (QD), 30 mg twice daily (BID), or 45 mg BID. Safety assessments included monitoring of adverse events, physical examinations, vital signs, electrocardiograms, clinical laboratory tests, and evaluation of suicidal ideation and behavior using the Columbia-Suicide Severity Rating Scale. Efficacy was assessed by changes from baseline in the Montgomery-Åsberg Depression Rating Scale (MADRS) and the Clinical Global Impression-Severity (CGI-S) scale. Pharmacokinetics was evaluated on Day 1 and Day 14 in patients receiving BID dosing. Ropanicant administered at 45 mg QD, 30 mg BID, or 45 mg BID was safe and well tolerated in patients with moderate to severe MDD. Changes in the MADRS total scores were analyzed using an analysis of variance model via the SAS Proc Mixed procedure. By Day 14, the MADRS total score showed significant reductions from baseline across all treatment groups: -12.7 for the 30 mg BID group, -10.5 for the 45 mg QD group, and -10.4 for the 45 mg BID group. The overall improvement in MADRS scores across treatment arms was highly statistically significant ($p < 0.0001$). On Day 14, the mean (standard deviation) change in CGI-S scores from baseline was -1.3 (1.06) for the 30 mg BID group, -1.0 (0.91) for the 45 mg BID group, and -0.8 (0.93) for the 45 mg QD group. The statistically significant improvements in MADRS scores suggest potential antidepressant benefits of Ropanicant. Based on these findings, a Phase 2b randomized, double-blind, placebo-controlled, parallel-group, multicenter trial (NCT06836063) has been initiated to further evaluate the efficacy and safety of Ropanicant in participants with MDD.

Disclosures: **V. Palacharla:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **A. Mohammed:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Goyal:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **J. Ravula:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **S. Jetta:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Subramanian:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **S. Pandey:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **A.K. Shinde:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences Ltd.

Poster

PSR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.09/JJ11

Topic: H.05. Mood Disorders

Title: The effects of LSD and its non-hallucinogenic analogue 2-Br-LSD on murine behavior, sleep patterns, and quantitative EEG

Authors: *K. MARSZALEK¹, M. DOMZALSKA¹, J. R. HUXTER², C. CAYIR², R. RAMAROHETRA², E. SOKOLOWSKA¹;

¹Transpharmation Ltd., Warsaw, Poland; ²Transpharmation Ltd., Sandwich, United Kingdom

Abstract: Pharmacotherapy for depression and anxiety faces challenges such as delayed onset of action, the necessity for long-term treatment, and treatment-resistant patient populations. Psychedelics offer an exciting, novel approach to tackling these issues. Nevertheless, intense hallucinogenic effects can limit therapeutic applications, highlighting the need for the development of non-hallucinogenic but therapeutically effective alternatives (Lewis et al., 2023). Here we compare the effects of LSD (Lysergic acid diethylamide) and its analogue 2-Br-LSD on sleep, neural activity and behaviour in male C57Bl/6 mice. LSD was dosed subcutaneously at 0.03, 0.1, and 0.3 mg/kg. 2-Br-LSD was administered intraperitoneally at 0.1, 0.3, and 1 mg/kg. Wireless electroencephalography (EEG) was used to monitor vigilance states and brain activity for 24 hours following dosing. Automatic sleep-wake scoring was performed, and the hourly distribution of wakefulness, NREM-sleep and REM-sleep was analysed. Hallucinogenic effects were measured in terms of head twitch responses (HTR). Locomotion and rearing were assessed using the LABORAS system. LSD at all tested doses induced HTR in mice in a dose-dependent manner, but did not alter locomotor activity. LSD selectively reduced REM sleep in a dose-dependent manner, while sleep bout-length and sleep latency were unaffected. In NREM sleep, low EEG frequencies (delta, theta, beta) were reduced by LSD administration. During waking, gamma was reduced by the highest dose (0.3 mg/kg). In contrast, 2-Br-LSD had no effect on the number of HTRs, but at 0.3 and 1.0 mg/kg, significantly reduced HTRs induced by DOI (2,5-Dimethoxy-4-iodoamphetamine). 2-Br-LSD significantly reduced locomotor activity. 2-Br-LSD did not affect the amount of REM sleep, but at the highest dose (1 mg/kg), we saw reduced NREM sleep latency and increased NREM bout-length. 2-Br-LSD significantly reduced beta power during NREM sleep, but did not consistently alter gamma power. The lack of HTRs after 2-Br-LSD administration demonstrates its non-hallucinogenic properties, while attenuation of DOI-induced HTRs indicated its 5-HT_{2A} receptor occupancy. While certain characteristics of LSD were recapitulated with 2-Br-LSD, such as the suppression of beta oscillations during NREM sleep, others like REM sleep suppression were not. The results indicate new ways in which physiological biomarkers can be used to track both hallucinogenic and non-hallucinogenic effects in this exciting new class of therapeutics.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

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Program #/Poster #: PSTR352.10/JJ12

Topic: H.05. Mood Disorders

Support: Research Council of Finland
Lundbeck Foundation
Sigrid Jusélius Foundation

Title: Multi-modal electrophysiology of default mode network dynamics in a mouse model of depression

Authors: ***R. BALIN**¹, E. CASTREN²;

¹HiLIFE Neurosci. Ctr., ²Neurosci. Ctr., Univ. of Helsinki, Helsinki, Finland

Abstract: Major Depressive Disorder (MDD) is a leading cause of disability, yet current antidepressants have significant limitations. The default mode network (DMN) shows aberrant activity in MDD patients, potentially underlying symptoms like rumination. Investigating DMN neurophysiology in animal models is crucial for advancing preclinical research. We have investigated functional connectivity and oscillatory dynamics of the DMN in a corticosterone-induced depression model in mice, utilizing a novel, high-density intracranial and cortical electrophysiology approach.

C57BL/6JRj mice received corticosterone via drinking water for 21 days (treatment group, n=3) or standard housing (control group, n=3). Neural activity from anterior and posterior DMN nodes was recorded pre- and post-treatment using two Neuropixels probes and two cortical micro-electrocorticography (μECoG) grids. Analyses included phase-locking value (PLV) in theta (4-8 Hz) and alpha (8-12 Hz) bands, and dynamic functional connectivity (DFC).

Preliminary data suggest that corticosterone treatment modulates DMN synchrony. Post-treatment, the treated group exhibited decreased PLV in posterior DMN regions and increased PLV in anterior DMN regions compared to baseline and controls. A dissociation in functional connectivity between anterior and posterior DMN was also observed in the treatment group, with DFC analysis indicating a decrease in posterior DMN connectivity and a subtle increase in anterior DMN connectivity. μECoG data further supported a dissociation in PLV between rostral (ACC) and caudal (Rsp) cortical DMN nodes post-treatment. Control animals did not show comparable alterations.

These initial observations suggest that chronic corticosterone exposure in mice may induce a dissociation within the DMN, characterized by potentially decreased posterior and increased anterior synchrony and connectivity. This innovative electrophysiological platform warrants further investigation with larger cohorts to validate these tentative findings and explore their implications for MDD pathophysiology and treatment.

Disclosures: **R. Balin:** None. **E. Castren:** 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.; Research contract with Kasvu Therapeutics, Inc.. E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Kasvu Therapeutics, co-founder, board member.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

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Program #/Poster #: PSTR352.11/JJ13

Topic: H.05. Mood Disorders

Support: KIST intramural Grant 2E33701
KIST intramural Grant 2E33711

Title: Mosapride exerts a rapid antidepressant effect by promoting the internalization of the TREK1-HTR4 complex

Authors: *S. LEE^{1,2}, J.-Y. PARK², E. HWANG¹;

¹Brain Sci. Inst., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; ²Dept. of Integrated Biomed. and Life Sciences, Col. of Hlth. Sci., Korea Univ., Seoul, Korea, Republic of

Abstract: Depression remains a significant global mental health challenge, with over 30% of patients exhibiting treatment-resistant depression (TRD). Selective inhibition of the two-pore domain potassium channel TREK1 has emerged as a promising antidepressant strategy; however, the regulatory mechanisms governing TREK1 function, particularly its interaction with G protein-coupled receptors (GPCRs), are not yet fully elucidated. In this study, we established a cell-based screening platform employing a bimolecular luminescence complementation (BiLC) assay to monitor the co-internalization of TREK1-GPCR complexes. Through this platform, we identified 5-hydroxytryptamine receptor 4 (HTR4) agonists as modulators of TREK1 membrane localization. The direct interaction between TREK1 and HTR4 was validated both in vitro and in vivo using bimolecular fluorescence complementation (BiFC), BiLC, and proximity ligation assays (PLA). Treatment with the HTR4 agonist mosapride induced co-internalization of TREK1 and HTR4. Electrophysiological patch-clamp analysis revealed that mosapride suppressed TREK1 channel activity in an HTR4-dependent but PKA/PKC-independent manner. Furthermore, oral administration of mosapride for five consecutive days ameliorated lipopolysaccharide (LPS)-induced depressive-like behaviors in mice and preserved hippocampal neurogenesis and CREB signaling. Collectively, these findings uncover a novel mechanism by which HTR4 regulates TREK1 internalization and suggest a potential therapeutic approach for TRD through GPCR-based modulation of channel trafficking.

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Poster

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Program #/Poster #: PSTR352.12/JJ14

Topic: H.05. Mood Disorders

Support: Stanley Family Foundation

Title: The potential of GSK3 inhibitors as a therapeutic approach for bipolar disorder

Authors: *L. YANG¹, M. WEIWER¹, M. H. SHENG², J. Q. PAN³;

²Stanley Ctr. for Psychiatric Res., ¹Broad Inst., Cambridge, MA; ³Stanley Ctr. for Psychiatric Res., Broad Inst., Acton, MA

Abstract: Bipolar disorder (BD) is a chronic psychiatric illness with limited therapeutic options due to the adverse effects of standard mood stabilizers such as lithium and valproate. At therapeutic-relevant concentrations, one of the main lithium targets is glycogen synthase kinase 3 (GSK3), which exists as two paralogs: GSK3 α and GSK3 β . Despite extensive efforts, existing GSK3 inhibitors remain limited by inadequate kinome and isoform selectivity, as well as suboptimal pharmacokinetic properties for CNS applications. We identified a series of GSK3 inhibitors with enhanced kinome selectivity and CNS penetrance. This includes BRD7355, a highly selective, potent, and brain-penetrant dual GSK3 α/β inhibitor, as well as paralog-selective inhibitors BRD8412 (α -selective) and BRD6921 (β -selective), enabling isoform-specific interrogation of GSK3's role in lithium-mimetic activity. These tool compounds were evaluated in rodent behavioral paradigms relevant to BD. BRD7355 and the reference dual inhibitor PF-04802367 (PF-367) were efficacious in hyperdopaminergic models, including amphetamine-induced hyperlocomotion (AIH) and the open field test in Grin2a KO mice—a genetic schizophrenia risk model. Both compounds also reduced immobility in the forced swim test (FST), indicating antidepressant-like activity consistent with lithium's mood-stabilizing effects. To assess isoform contribution, BRD6921 (GSK3 β -selective) was tested in the same paradigms. It was effective in AIH and FST, and showed a trend toward efficacy in Grin2a KO mice, at doses producing brain C_{max} near its GSK3 β IC₅₀ and below GSK3 α IC₅₀—suggesting that selective GSK3 β inhibition may be sufficient to recapitulate key therapeutic effects of lithium. Ongoing studies are evaluating BRD8412 (GSK3 α -selective) in parallel assays. Additional work aims to assess the efficacy of these inhibitors across other genetically driven models to further define the therapeutic landscape of isoform-specific GSK3 inhibition.

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Poster

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Program #/Poster #: PSTR352.13/JJ15

Topic: H.05. Mood Disorders

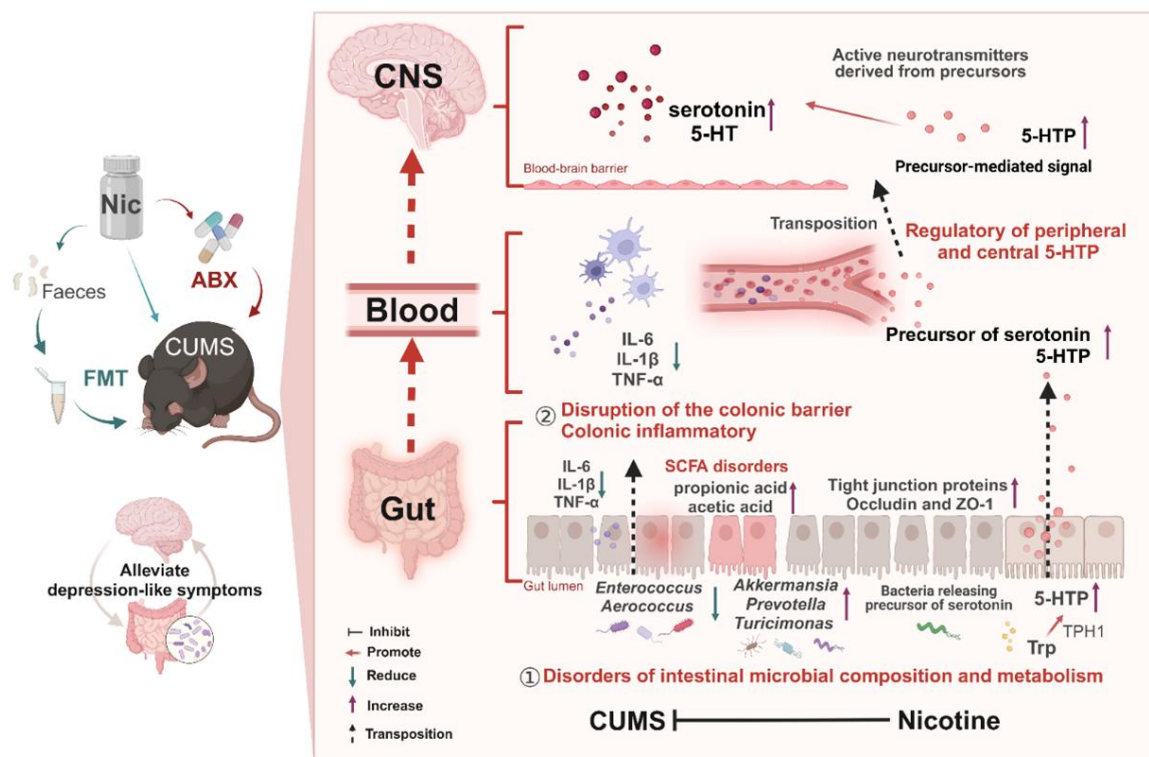
Support: 2023100CB0060
2023000CB0030

Title: Nicotine Alleviates Depression-like Behaviors Through Gut Microbiota-Dependent Serotonin Regulation

Authors: X. GAO^{1,2}, *J. ZHANG^{1,3}, W. MU^{1,3}, X. LI^{1,3}, Y. LI^{1,3}, Y.-N. MA^{1,3}, Q. LI^{1,2}, Z. NI¹, H. CHEN^{1,2,3}, H. HOU^{1,2,3},

¹Beijing Life Sci. Acad., Beijing, China; ²China Natl. Tobacco Quality Supervision and Test Ctr., Zhengzhou, China; ³Key Lab. of Tobacco Biol. Effects, Zhengzhou, China

Abstract: Depression is a severe mental disorder with limited effective treatments. While most antidepressants target central serotonin (5-HT) per the monoamine hypothesis, alternative mechanisms remain underexplored. In this study, we investigated whether nicotine exerts antidepressant-like effects via gut microbiota-mediated serotonin pathways. Male C57BL/6J mice underwent six weeks of chronic unpredictable mild stress (CUMS) to induce depressive-like behaviors. From week 3, CUMS mice received daily nicotine (0.2 mg/kg) with or without a broad-spectrum antibiotic cocktail to deplete gut flora; unstressed controls received saline or nicotine. Behavioral assays showed that nicotine reversed CUMS-induced anhedonia, despair, and hypoactivity, but antibiotics abolished these benefits, indicating a microbiota-dependent mechanism. Metagenomic sequencing revealed CUMS-associated dysbiosis: reduced α -diversity, loss of SCFA-producing genera (*Akkermansia*, *Turicimonas*, *Prevotella*), and increased *Enterococcus* and *Aerococcus*. Nicotine partially restored microbial diversity, enriched SCFA-biosynthesis pathways, and normalized cecal acetate and butyrate levels. Concurrently, CUMS disrupted colonic tight junctions (decreased ZO-1, occludin) and elevated proinflammatory cytokines (IL-1 β , IL-6, TNF- α); nicotine repaired barrier integrity and attenuated inflammation. Nicotine also normalized peripheral and hippocampal 5-HT and 5-HTP concentrations. Fecal microbiota transplantation from nicotine-treated donors into antibiotic-depleted CUMS recipients recapitulated microbial, barrier, SCFA, serotonin, and behavioral improvements. These findings suggest that nicotine alleviates stress-induced depression by reshaping gut microbial communities, promoting SCFA production, and enhancing serotonin availability in both peripheral and central compartments, highlighting its potential as a microbiota-targeted therapeutic agent.



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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

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Program #/Poster #: PSTR352.14/JJ16

Topic: H.05. Mood Disorders

Title: Discovery of aminopyrazole-based multitarget antidepressants: Virtual screening, molecular docking, and preliminary SAR and toxicity studies.

Authors: *I. Y. ATTAH¹, S. DANSO², B. AMO², I. MENSAH³, A. WORTSI³, J. MOFOR², S. OPOKU², C. BOITEY², R. P. BINEY^{3,4};

¹Dept. of Pharmaceut. Chemistry, Sch. of Pharm. and Pharmaceut. Sci., Univ. of Cape Coast, Cape Coast, Ghana; ²Sch. of Pharm. and Pharmaceut. Sciences, Univ. of Cape Coast, Ghana, Cape Coast, Ghana; ³Drug Discovery for Neuropsychiatric Disorders Lab, Univ. of Cape Coast, Ghana, Cape Coast, Ghana; ⁴Dept. of Pharmacotherapeutics and Pharm. Practice, Sch. of Pharm. and Pharmaceut. Sciences, Univ. of Cape Coast, Ghana, Cape Coast, Ghana

Abstract: Depression is a debilitating mental health disorder that significantly impairs the quality of life of individuals at all stages of life. It poses a global therapeutic challenge due to the delayed onset of action, limited efficacy, and side effects of current antidepressants. In the absence of a unifying theory of depression, emerging evidence supports a multitarget approach for developing novel antidepressants with improved therapeutic profiles. In this study, we used in silico docking-based screening to evaluate in-house compound libraries against key antidepressant targets: serotonin transporter (SERT, PDB ID: 5I73), norepinephrine transporter (NET, PDB ID: 8WTX), monoamine oxidase (MAO) enzyme (PDB ID: 2Z5X), and the 5-HT_{2A} receptor (PDB ID: 6A93). Using Maestro V13.5 of Schrödinger Suite 2023-1, compounds were prioritized based on binding scores relative to target native ligands. 75 hits of diverse scaffolds showed good binding across all four targets. The hits were further screened for toxicity at 100 µM using a brine shrimp (*Artemia salina*) lethality assay, with 11 compounds showing toxicity (LD₅₀ ≤ 25 µM). Amongst the non-toxic hits (at 100 µM) with favorable docking scores, we focused on a series of 12 aminopyrazole scaffolds due to their ease of synthesis. Also, preliminary structure-activity relationship (SAR) analysis performed for the pyrazoles revealed a strong binding affinity, particularly at MAOA, compared to the other targets. These pyrazoles will advance to *in vivo* antidepressant assays in mouse and zebrafish models while further optimization, including synthesis of novel derivatives, is underway to enhance potency and efficacy. Our work highlights aminopyrazoles as promising multitarget leads for depression therapy, combining computational insights with experimental validation to accelerate the discovery of novel, safer, and more effective antidepressants.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

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Topic: H.05. Mood Disorders

Support: Alexander von Humboldt, Equipment grants
CNPq
Capes

Title: Flies to the screening of potential antidepressants: fluoxetine as a proof of concept

Authors: F. BOZ ECKERT^{1,2}, F. TRICHES^{1,2}, G. ZURCHIMITTEN^{1,2}, M. SIDLOSKI¹, *C. LINO DE OLIVEIRA^{3,2};

¹UFSC, FLORIANÓPOLIS, Brazil; ²PhD in Pharmacol. UFSC, Florianópolis, Brazil; ³CFS-CCB-UFSC, Florianópolis, Brazil

Abstract: The selective serotonin (5-HT) reuptake inhibitors (SSRIs) are among the most effective and safe drugs helping to treat psychiatric disorders. However, mechanisms of SSRIs remain mostly illusive, leading sometimes to inefficacy, side effects, and slow development of new compounds. Innovative research pipelines may circumvent these limitations. In this project, *D. melanogaster* is the core resource leading to a new research platform to develop antidepressants. Here, a proof of concept simulated in *D. melanogaster* the screening of a known SSRI (fluoxetine, FLX) investigating its: 1) affinity to the *D. melanogaster* 5-HT transporter (dSERT), 2) consequences on 5-HT metabolism, 3) the alleged sexual side effects, 4) the putative anti-stress properties. Constant affinity (K_i), 5-HT metabolism (5-HT positive cells), sexual function (courtship latency), or preference index (PI) were estimated from outcomes of the molecular docking, 5-HT immunohistochemistry, mating test, and sucrose preference test, respectively. Treatments with FLX or vehicle were in the medium. Stressed flies undergone 3-day combination of cold, heat, food deprivation, and cycle inversion and unstressed ones were undisturbed. For chiral FLX (-a, -b) docked against monoamines transporters in each software (AutoDock Vina and DockThor 10 replicates/assay), the K_i were (nM): dSERT (-a: 0.48, -b: 0.34), hSERT (-a: 0.09, -b: 0.06), dDAT (-a: 0.28, -b: 0.27). Mating test (n = 2-3 pairs/group) revealed that, compared to doses 0 or 3 mM, FLX 30 and 10 mM delayed on average two times the latency for males courtship. Compared to vehicle (n = 6-22 brains/group), the number of 5-HT positive cells in the brain of flies treated with FLX 10 mM were, on average, two times larger in females and four times smaller in males. In unstressed flies (n = 30 males, n = 39 females), PIs were similar in both sexes across doses (mean, 95% CI = 0.16, 0.02 - 0.3, vehicle; 0.20, 0.05 - 0.33, FLX 2.5 mM; 0.25, 0.11 - 0.39, FLX 5 mM, -0.004 CI -0.15 - 0.14, FLX 10 mM). In stressed females, low doses of FLX enlarged PI (2.5 mM, n = 32, mean = 0.62, 95% CI 0.47 - 0.73; 5 mM, n = 27, mean = 0.56, 95% CI 0.37 - 0.7) as compared to vehicle (n = 29, mean = 0.12, 95% CI -0.10 - 0.34). In stressed-flies treated with FLX 10 mM, females had smaller PI (n = 26, mean = -0.012, 95% CI -0.25 - 0.23) than males (n = 22, mean = 0.37, 95% CI 0.13 - 0.57). These data suggest that, as in vertebrates, FLX may reduce sexual drive in flies. Moreover, anti-stress effects of FLX in flies were dose- and sex-dependent. In contrast to vertebrates, FLX may be an unselective 5-HT-dopamine reuptake inhibitor in flies. These studies require confirmation and independent replication assays.

Disclosures: F. Boz Eckert: None. F. Triches: None. G. Zurchimitten: None. M. Sidloski: None. C. Lino De Oliveira: None.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

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Program #/Poster #: PSTR352.16/JJ18

Topic: H.05. Mood Disorders

Title: Episodic organization of activity during amphetamine withdrawal: Effects of antidepressant

Authors: *I. M. WHITE¹, W. WHITE²;
²Psychology, ¹Morehead State Univ., Morehead, KY

Abstract: Rats increase activity for several hours after psychostimulant, followed by decreased activity 13-24 hours after treatment. The reduced activity is thought to resemble depressive symptoms of early psychostimulant withdrawal in humans. Previously, we reported that low-to-medium doses of escitalopram, a SSRI commonly used to treat depression in humans, blocked the amphetamine-induced acute withdrawal. Typically, in rats bouts or episodes of activity occur approximately every two hours, for about 20 minutes in duration. In this study, we used a fine-grained analysis to further assess the capacity of escitalopram to normalize bout organization during amphetamine-induced acute withdrawal. Female Wistar rats were given saline, amphetamine (2.0 mg/kg), escitalopram, or amphetamine followed by escitalopram (2.5 and 5.0 mg/kg). All injections were done near the light-onset of a 12/12-hour light/dark cycle. Activity was measured by distance traveled in open fields for 24 hours following treatments, and four bout parameters were analyzed: the number of bouts, the time between bouts, the duration of bouts, and the amplitude of bouts. Amphetamine reliably reduced activity 13-24 hours post-treatment by producing small changes in all bout parameters. Escitalopram given 15 minutes after amphetamine normalized bout organization during acute withdrawal. Our findings suggest that antidepressants may partially reverse acute psychostimulant withdrawal ("crash") by restoring the typical activity pattern and that psychostimulant withdrawal and depression may share similar neural mechanisms.

Disclosures: I.M. White: None. W. White: None.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

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Program #/Poster #: PSTR352.17/JJ19

Topic: H.05. Mood Disorders

Support: 90104238

Title: Ketamine-conjugated glucose dendrimer mitigates stress-induced depressive-like symptoms in a rat model of treatment-resistant depression

Authors: *M. P. AVALOS, J. ALLENDE LABASTIDA, P. VYAS, T. AHMED, L. VALLIYAPPAN, G. LEE, W. LIYANAGE, K. PARIKH, R. KANNAN, S. KANNAN;
Johns Hopkins Univ., Baltimore, MD

Abstract: Clinical and pre-clinical studies have reported a high prevalence of depression in patients diagnosed with post-traumatic stress disorder (PTSD). In addition to the complex pathophysiology of this dual disorder, the efficacy of current antidepressants is limited due to the delay in treatment response and low response rate in partial responder or treatment-resistant patients, a condition that remains an unmet need. S-ketamine has shown potential as a fast-acting

antidepressant for refractory depression; however, the clinical concerns linked to the ketamine treatment are the unwanted side effects (e.g., dissociation, abuse liability, and respiratory depression at high doses, among others). Recent studies from our lab revealed that drug-conjugated glucose dendrimers display selective targeting of injured neurons showing potential for restoring these neuronal disturbances. This study, aimed at improving the therapeutic intervention for treatment-resistant depression, attempts to evaluate the efficacy of a novel glucose dendrimer-ketamine nanoparticle in a rat model of treatment-resistant depression induced by stress. A comparative study was performed between Sprague Dawley (SD) rats and Flinders Sensitive Line (FSL) rats. Rats were exposed to a prolonged stress session (2 h of restraint stress followed by 15 min of forced swimming). To mimic flashbacks commonly observed in patients diagnosed with PTSD, rats were exposed to 2 h of restraint stress, as reminders, on days 7 and 14 of the experimental protocol. After the last reminder, animals underwent a systemic treatment regimen of twice-a-week IP injections of 10 mg/kg (on a drug basis) of KET, GD-KET, or saline until the end of the experimental timeline. Behavioral tests were performed to monitor depressive-like behaviors. GD-KET showed efficacy in (1) mitigating depressive-like symptoms in both SD and FSL rats, (2) ameliorating treatment-resistant depression in FSL rats, (3) targeting selectively brain regions associated with depression like medial Prefrontal Cortex and ventral Hippocampus and, (4) unlike KET, it did not show off-target effects like respiratory depression and sedation even at 300 mg/kg IP in healthy animals. Future in vivo studies will be performed to explore the abuse liability of GD-KET. Our results demonstrate potential to improve the treatment of refractory depression and associated comorbidities.

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Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.18/JJ20

Topic: H.05. Mood Disorders

Title: Pharmacological characterization of novel lead 5-HT_{2A} receptor agonists with non-hallucinogenic potential and translational antidepressant-like profiles

Authors: *C. A. BOWEN¹, T. C. ROBERTSON¹, E. ANDRZEJAK¹, T. A. KHAN¹, R. B. PERNI², H. B. JANSSENS¹, A. AUSTENOVA¹, J. D. S. HOLT¹, A. L. HALBERSTADT³, G. F. SHORT, III¹;

¹Atai Therapeutics, Inc., New York, NY; ²JMD Pharma Creativity, LLC, Marlborough, MA;

³UCSD, La Jolla, CA

Abstract: Effective, rapid and lasting treatments for treatment-resistant depression remain a significant unmet medical need. Accumulating research suggests that psychedelic 5-HT_{2A} receptor (5-HT_{2AR}) agonists, such as psilocybin's active metabolite psilocin, produce rapid and lasting antidepressant activity after a single dose. Preclinical neuroplasticity and behavioral studies also support antidepressant-like effects of non-hallucinogenic 5-HT_{2AR} agonists that may offer more convenient dosing to a broader patient population. Thus, research was focused on pharmacological characterization of two novel 5-HT_{2AR} agonists with CNS drug-like properties, a non-hallucinogenic profile and in vivo antidepressant-like activity. Artificial intelligence (AI)-driven de novo drug design followed by medicinal chemistry structure-activity relationship development yielded structurally distinct lead molecules, EGX-J & EGX-I, exhibiting potent human and rodent 5-HT_{2AR} activation. EGX-I showed promising 5-HT_{2AR} over 5-HT_{2B} receptor (5-HT_{2BR}) selectivity, which may reduce potential safety risks associated with prolonged 5-HT_{2BR} activation (e.g., cardiac valvulopathy). Both leads were brain penetrant, did not induce the mouse head-twitch response (HTR) and attenuated the HTR induced by the hallucinogen (±)-2,5-dimethoxy-4-iodoamphetamine (DOI), indicative of in vivo 5-HT_{2AR} interactions with little or no hallucinogenic potential. In a rat drug discrimination assay, neither EGX-J nor EGX-I fully substituted for psilocybin, suggesting their subjective effects are distinct from this known hallucinogen. Unlike EGX-I, EGX-J reduced immobility in a mouse forced swim test (FST) 24h after dosing, supporting durable antidepressant- and psilocybin-like activity in this screening assay. Translational antidepressant-like effects were demonstrated using electroencephalography (EEG)-based measures of rapid eye movement (REM) sleep in Wistar Kyoto (WKY) rats, which exhibit increased REM sleep consistent with symptoms observed in clinical depression. Both leads suppressed REM sleep, indicative of antidepressant-like responses similar to psilocybin in this model. 24h after dosing to rats, EGX-J and psilocybin produced distinct profiles of effects on ex vivo medial prefrontal cortex layer V pyramidal neuron activity, suggesting differential neural mechanisms may underlie their actions. Lead optimization is underway identifying promising development candidates with increased 5-HT_{2AR} selectivity and oral bioavailability, while maintaining a non-hallucinogenic profile and translational antidepressant-like efficacy.

Disclosures: C.A. Bowen: A. Employment/Salary (full or part-time): Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. T.C. Robertson: A. Employment/Salary (full or part-time): Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. E. Andrzejak: A. Employment/Salary (full or part-time): Former employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. T.A.

Khan: A. Employment/Salary (full or part-time): Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **R.B. Perni:** A. Employment/Salary (full or part-time): Consultant for atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **H.B. Janssens:** A. Employment/Salary (full or part-time): Former employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **A. Austenova:** A. Employment/Salary (full or part-time): Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **J.D.S. Holt:** A. Employment/Salary (full or part-time): Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **A.L. Halberstadt:** A. Employment/Salary (full or part-time): Consultant for atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company. **G.F. Short:** A. Employment/Salary (full or part-time): Employee of atai Life Sciences, Atai Therapeutics, Inc., is an atai platform company.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.19/JJ21

Topic: H.05. Mood Disorders

Support: Institute for the Brain and Cognitive Sciences (IBACS)
University of Connecticut Research Foundation

Title: Assessing the effort-based motivational effects of the atypical dopamine transport inhibitor Vanoxerine (GBR 12909) in rat and mouse models

Authors: ***J. N. MILLER**¹, M. F. DAVIS¹, G. A. EDELSTEIN², A. ECEVITOGU⁴, K. FLORA¹, A. O'BOYLE¹, M. CORREA⁵, J. D. SALAMONE³;

¹Dept. of Psychology, ²Dept. of Psychological Sci., ³Dept Psychological Sci., Univ. of Connecticut, Storrs, CT; ⁴Dept. of Psychological Sci., McLean Hospital/Harvard Med. Sch., Belmont, MA; ⁵Psicobiologia. Univ. Jaume I, Castello, Spain

Abstract: Motivational dysfunction is a common symptom subset found in psychopathologies such as depression and other disorders. It is characterized by fatigue, anergia, apathy, and reduced effort expenditure. These debilitating symptoms are highly treatment resistant, and serotonin selective transport inhibitors are relatively ineffective at treating motivational dysfunction. Evidence indicates that facilitating dopamine (DA) transmission by inhibiting the DA transporter (DAT) can enhance exertion of effort in humans and rodent models. GBR 12909 (Vanoxerine) is a DAT inhibitor with an atypical binding profile. Vanoxerine has low abuse liability, and is used in the UK for treating stimulant dependence. To assess Vanoxerine's ability to attenuate motivational dysfunction, rodent models of effort-based choice were used. The fixed ratio 5 (FR5)/chow choice task gives rats a high effort option (FR5 lever pressing reinforced by preferred high carbohydrate pellets) vs. the low-effort option of approaching and consuming the concurrently available, less preferred lab chow. At baseline, male and female rats show high lever pressing output and low intake of lab chow. The VMAT-2 inhibitor tetrabenazine (TBZ)

produces a low-effort bias in male and female rats, shifting choice from lever pressing to chow intake. TBZ is used for these studies because it produces depressive symptoms, including fatigue and apathy, in humans. Vanoxerine was able to significantly reverse the low-effort bias induced by TBZ in both male and female rats, with females tending to show a reversal at lower doses than males. A second experiment assessed the effort-related effects of vanoxerine using mouse touchscreen procedures. Mice were trained on a progressive ratio (PROG)/food pellet choice task in which the high-effort option was rearing up to press a touchscreen panel reinforced by strawberry milkshake. Vanoxerine significantly increased selection of the high-effort PROG panel pressing in male CD1 mice. Overall, results in both rat and mouse models show that Vanoxerine enhanced exertion of effort, consistent with the idea that atypical DAT inhibitors have therapeutic potential as an adjunct therapy for treating the motivational dysfunction symptoms seen in depression and other disorders.

Disclosures: J.N. Miller: None. M.F. Davis: None. G.A. Edelstein: None. A. Ecevitoglu: None. K. Flora: None. A. O'Boyle: None. M. Correa: None. J.D. Salamone: None.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.20/JJ22

Topic: H.05. Mood Disorders

Support: CT Institute for the Brain and Cognitive Sciences
University of Connecticut Research Foundation

Title: Evaluation of Antidepressants for Attenuation of Effort-Related Motivational Dysfunction in Male and Female Rats: the Importance of Dopamine

Authors: *G. EDELSTEIN¹, A. ECEVITOGLU³, J. MILLER¹, A. BESIREVIC¹, A. GOLDHAMER¹, S. PAPANIKOLAOU¹, T. SHRIRAM¹, A. NIP¹, A. BONAIUTO¹, N. CYR¹, S. NOWACKI¹, M. CORREA⁴, J. D. SALAMONE²;

²Dept Psychological Sci., ¹Univ. of Connecticut, Storrs, CT; ³Dept. of Psychological Sci., McLean Hospital/Harvard Med. Sch., Belmont, MA; ⁴Psicobiologia. Univ. Jaume I, Castello, Spain

Abstract: Major depressive disorder (MDD) is characterized by anhedonia, low mood, anxiety, and motivational impairments. Motivational dysfunction symptoms are extremely debilitating and are highly resistant to commonly prescribed antidepressants. Monoamine transport (MAT) inhibitors are the most common treatments for depression, but across drugs there are many different profiles of action on distinct MAT proteins. For example, serotonin transport (SERT) inhibitors are the most widely prescribed antidepressants, yet evidence indicates that these drugs (also known as SSRIs) are relatively ineffective at treating motivational dysfunctions. In contrast, drugs acting on dopamine (DA) and norepinephrine (NE) transporters (DAT and NET

respectively), such as bupropion, are reported to be more effective at treating motivational dysfunction. Sex differences in MDD prevalence and symptom presentation have been reported, but research assessing variations in treatment response is lacking. Rodent tests of effort-based choice are used as models for assessing the effort-related motivational effects of drugs, including antidepressants. The present studies assessed the ability of various drugs that inhibit MAT for their ability to reverse the low-effort bias induced by the VMAT-2 inhibitor tetrabenazine (TBZ) in male and female rats. TBZ is used for these studies as it produces depressive symptoms, including fatigue and apathy, in humans. In rats tested on the fixed ratio 5/chow feeding choice task, TBZ shifts choice, reducing lever pressing but increasing chow intake. Results show that compounds that inhibit DAT can significantly reverse the behavioral effects of TBZ in male and female rats. In contrast, compounds that selectively inhibit NET or SERT fail to do so. Moreover, female rats tend to show a different dose/response curve following administration of DAT inhibitors, with effects seen at lower doses than in male rats. Microdialysis methods are being used to assess potential differences in the neurochemical effects of these MAT inhibitors in male and female rats, to compare with the behavioral results. In summary, facilitation of DA transmission is critical for the attenuation of effort-based motivation dysfunctions, but sex differences must be taken into account.

Disclosures: G. Edelstein: None. A. Ecevitoglu: None. J. Miller: None. A. Besirevic: None. A. Goldhamer: None. S. Papanikolaou: None. T. Shriram: None. A. Nip: None. A. Bonaiuto: None. N. Cyr: None. S. Nowacki: None. M. Correa: None. J.D. Salamone: None.

Poster

PSTR352: Treatment and Drug Discovery in Mood Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR352.21/KK1

Topic: H.05. Mood Disorders

Support: IBACS MBNF

Title: Mouse touchscreen methods for assessing effort-based decision making based on physical effort: the role of individual differences in modulating drug effects

Authors: *J. D. SALAMONE¹, M. F. DAVIS¹, J. N. MILLER¹, G. A. EDELSTEIN², A. D. O'BOYLE¹, K. D. FLORA¹, F. L. ZAKAS¹, M. CORREA³;

¹Psychological Sci., ²Psychological Sciences, Behavioral Neurosci., Univ. of Connecticut, Storrs, CT; ³Psicobiologia. Univ. Jaume I, Castello, Spain

Abstract: Motivational dysfunctions such as fatigue, anergia, apathy, and avolition are common symptoms in psychopathology. As marked by commonly used rating scales, these symptoms are seen in people with depression, schizophrenia, Parkinson's disease, and other disorders.

Moreover, studies involving human tests of effort-based decision-making (EBDM) based on physical effort have shown that people with depression, schizophrenia, or Parkinsonism have a

bias towards selection of low-effort choices. The development of these human tests of EBDM followed years of research with rats, which characterized the neurochemistry, and pharmacology of effort-based choice. Mouse procedures also have been used to assess response allocation based on physical effort choice, including lever pressing and maze tasks with barriers and running wheels. More recently, mouse touchscreen tests have been adapted for use in assessing EBDM based on physical effort. Mice can be trained to panel press for a preferred reinforcer, in this case strawberry milkshake (Ensure Plus). For the present studies, CD1 mice were initially trained on a fixed ratio 1 (FR1) schedule in which the response was pressing a lit square target on the bottom of the touchscreen. Then, to impose a physical effort challenge, the target was elevated so that the mice had to rear up to press the panel. After several weeks of FR1 training, mice were then shifted to a FR2 schedule. As is typical in operant conditioning procedures, mice responded to the increase in the ratio requirement by elevating their response rates. Panel pressing rates stabilized after several more weeks of FR2 training, after which mice were given the option of panel pressing for the more preferred milkshake or approaching and consuming the less preferred freely available alternative (Bio-serv pellets). At this point, substantial individual differences in performance were revealed. While some mice continued to show high levels of panel pressing, others showed a substantial drop in panel pressing. Animals were divided into two groups by a median split, and the separation between high and low responders was consistent over several days of training. Following this FR2/pellet choice training, mice were tested for the effects of the DA D2 antagonist haloperidol (vehicle, 0.05, 0.1, and 0.15 mg/kg IP). Only the high-performance group showed the classic shift from high-effort to low-effort behavior commonly seen in rodent studies after administration of DA antagonists (i.e., decreased panel pressing/increased pellet intake). Ongoing studies are using these methods to assess additional mouse strains (e.g., C57BL6), and other pharmacological interventions.

Disclosures: J.D. Salamone: None. M.F. Davis: None. J.N. Miller: None. G.A. Edelstein: None. A.D. O'Boyle: None. K.D. Flora: None. F.L. Zakas: None. M. Correa: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.01/KK2

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant 5R00MH121355
Brain & Behavior Research Foundation Young Investigator Award
Bruce/Jones Graduate Fellowship in Addiction Biology

Title: Examining the role of maternal immune activation on alcohol intake in adulthood

Authors: *J. L. PALMER¹, J. J. DONEGAN²;

¹Psychiatry & Behavioral Sci., Univ. of Texas at Austin, Interdisciplinary Neurosci. Program, Austin, TX; ²Psychiatry, Dell Med. Sch. at UT Austin, Austin, TX

Abstract: Schizophrenia is a psychiatric disorder with debilitating symptoms, including hallucinations, delusions, and cognitive difficulties. Many people with schizophrenia engage in substance use, with approximately one in four people developing an alcohol use disorder (AUD) in their lifetime. The co-occurrence of schizophrenia and AUD makes the symptoms of both disorders harder to manage, while alcohol use also decreases medication adherence in those with schizophrenia, which further compounds treatment difficulties. Although the exact cause of schizophrenia is unknown, there are both hereditary and environmental risk factors that can increase one's likelihood of developing this disorder. In the human literature, there is a correlation between viral infection during pregnancy and the rate of those offspring developing a psychiatric disorder later in life. One way in which we model this in the lab is by using a maternal immune activation (MIA) paradigm, whereby pregnant mouse dams are injected with the viral mimetic Poly(I:C) to stimulate a transient immune response. MIA can create lasting anatomical and behavioral deficits in the pups, and our lab has found that in adulthood, male MIA offspring drank significantly more alcohol in a binge-like drinking paradigm compared with control males. Currently, I am investigating potential mechanisms for this increased drinking behavior by focusing on the innate immune receptor toll-like receptor 3 (TLR3) in the dorsal striatum, a region involved in impulsivity, habit formation, and reward processing. Previous studies have demonstrated that stimulation of TLR3 with Poly(I:C) prior to alcohol exposure increases alcohol intake specifically in male mice, whereas others have shown that knocking down TLR3 in the dorsal striatum reduces consumption. My hypothesis is that MIA mice have increased expression of TLR3 in the dorsal striatum, which is leading to increased alcohol intake in the males. The goal of this study is to interrogate the mechanisms that contribute to increased alcohol intake so that improved intervention strategies can be developed.

Disclosures: J.L. Palmer: None. J.J. Donegan: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.02/KK3

Topic: B.09. Glial Mechanisms

Title: Effects of Social Isolation and Sex on the Propensity to Binge Drink and Subsequent Neuroinflammation

Authors: *M. AZUMA¹, M. CAIN²;

¹Kansas State Univ., Manhattan, KS; ²Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Currently, in the United States, binge drinking (BD) accounts for nearly all forms of excessive drinking and can cause white matter degradation and inflammation. While the rates of BD have been decreasing among young adults, BD remains a risk factor for developing alcohol use disorder and females are now reporting higher rates of BD than males. In addition, social isolation is a risk factor for BD. We hypothesized that social isolation would increase ethanol

consumption and preference would be augmented in socially isolated female rats. At postnatal day 21, 64 male and female Long Evans rats were randomly assigned to the pair-housed standard condition (SC) or isolated condition (IC) for 30 days. This study used a repeated measures design using a modified Intermittent Access to Ethanol (IAE) paradigm to assess BD; rats had 16 hours of access to ethanol and water 3 times a week for 6 weeks after the rearing period. A water control group was included. In addition, a sucrose preference test was administered to 64 male and female Long Evans rats before and after the IAE period. Sucrose preference results showed that isolated female rats displayed the lowest sucrose preference regardless of ethanol exposure. IAE consumption results suggest that rats, regardless of sex or rearing condition, escalate ethanol drinking over the 6 weeks and female rats consumed more ethanol than male rats. IAE preference showed that all rats had an increase in ethanol preference over the 6 weeks and that isolated rats showed a greater preference for ethanol regardless of sex. To determine if ethanol exposure induces inflammation and white matter degradation, we are quantifying TNF-alpha, myelin, and microglia expression in the hippocampus and prelimbic cortex using immunofluorescence. The effects of social isolation and ethanol on corticosterone are being analyzed in fur samples taken before and after IAE exposure. These results suggest that social isolation increases preference to ethanol, however it does not increase ethanol consumption. When examining sex differences, we found that female rats will consume more ethanol than male rats despite not having an ethanol-preference. If our hypothesis is supported, it suggests that ethanol-induced increase in microglia and inflammation in the prelimbic cortex is associated with the increase in ethanol consumption in female rats and the increase in ethanol preference in isolated rats is related to an increase in inflammation in the hippocampus.

Disclosures: M. Azuma: None. M. Cain: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.03/KK4

Topic: H.08. Drugs of Abuse and Addiction

Support: NIAAA R01 AA031463

Title: Differential influences of gonads and sex chromosome complement on intermittent access ethanol drinking and aversion resistance using the Four Core Genotypes mouse model

Authors: *D. G. LEWIS, B. H. SWINFORD, J. P. KRENZ, M. J. WITTLICH, C. G. SCRIBNER, A. K. RADKE;
Miami Univ., Oxford, OH

Abstract: Men and women display distinct patterns of alcohol use: men maintain higher rates of general use and alcohol use disorder (AUD), while women exhibit vulnerabilities to related health issues and progress to AUD at faster rates. Within rodent models, females typically

consume more ethanol (EtOH) than males. In the Four Core Genotypes (FCG) mouse model, the Sry gene, which directs the development of testes, is translocated from the Y chromosome onto an autosome. This model allows for exploration of the individual contributions of gonads and sex chromosome complement on EtOH consumption and aversion-resistant drinking. Our lab has previously explored drinking in FCG mice using drinking in the dark (DiD), continuous access (CA), and operant paradigms. In this study, 56 FCG mice were obtained via in-house breeding pairs, resulting in the following configuration: XX/Sry- (n = 18), XX/Sry+ (n = 13), XY/Sry- (n = 14), XY/Sry+ (n = 11). Mice had access to one bottle of 20% EtOH and one bottle of reverse osmosis water in a two-bottle choice, 24-hour, intermittent access (IA) drinking paradigm. Measures of interest included g/kg EtOH consumption and EtOH preference. Following fifteen drinking sessions, EtOH was adulterated with increasing concentrations of quinine (0, 40, 100, 200 mg/L), using consumption and preference to quantify aversion-resistant drinking. Sry- mice with ovaries consumed higher amounts of EtOH compared to the Sry+ mice with testes. Interestingly, this result applied to water consumption as well, with Sry- mice consuming more water than Sry+ mice. There were no significant differences regarding EtOH preference. Sry+ mice exhibited greater aversion resistance compared to Sry- mice. Within Sry+ mice, there was a trend for greater quinine resistance in XX vs. XY mice. These results provide evidence that EtOH drinking behaviors within an IA model are largely determined by gonadal influences, while sex chromosome complement may play a role in aversion resistant drinking. Future work using gonadectomies and analysis of hormone and alcohol levels in blood will further clarify this understanding.

Disclosures: D.G. Lewis: None. B.H. Swinford: None. J.P. Krenz: None. M.J. Wittlich: None. C.G. Scribner: None. A.K. Radke: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.04/KK5

Topic: B.09. Glial Mechanisms

Support: NIH Grant 5U01AA016651-19
NIH Grant 5T32AA007471-38

Title: Evaluating drinking behavior and microglial adaptations in a fluorescent reporter mouse model of chronic ethanol exposure

Authors: *T. J. LIME¹, H. AZIZ², D. CARRIZALES SAUCEDA¹, C. BABCOCK³, R. A. MANGIERI⁴;

²Pharmacol. and Toxicology, ¹Univ. of Texas, Austin, Austin, TX; ³Col. of Pharm., ⁴Pharmacol., Univ. of Texas at Austin, Austin, TX

Abstract: Traditional preclinical work on brain adaptations in response to alcohol has focused mainly on neurons, but there is growing interest in the role of glia in the development of excessive alcohol consumption. The current study utilized both male and female fluorescent microglia reporter mice (*C57BL/6-Tmem119em2(EGFP)Gfng/J*; N_M= 36, N_F= 28) to evaluate blood ethanol concentrations, escalation of drinking behavior, and electrophysiological adaptations following administration of a 2-bottle choice (2BC) and chronic intermittent ethanol vapor (CIE) paradigm. The alcohol-naïve group was given two H₂O bottles during 2BC, and ethanol vapor was omitted from their chambers during CIE. Blood ethanol concentrations (BEC) were measured daily for all mice following each 16-hour CIE session. Voluntary ethanol consumption was calculated as grams of ethanol consumed per kilogram of body weight. Our CIE protocol produced an average BEC of 38.89 mM and a total range of 27.21-48.2 mM. A paired t-test of means revealed that these mice drank significantly more ethanol during the final 5-day 2BC bout than they did during the final 5 days of baseline 2BC ($t_{29} = 4.86$, $p < 0.001$). 24 hours following the final CIE session, a subset of mice were euthanized, and brain slices were prepared for electrophysiological studies of microglia in the Nucleus Accumbens (NAc). This region was targeted due to evidence showing robust neuronal adaptations in the NAc following 2BC+CIE which correlate with excessive ethanol consumption. Electrophysiological data was acquired from both ethanol-treated and naïve mice via *ex vivo* whole-cell patch-clamp using two separate recording stations (rigs). A Two-Way ANCOVA, with rig as a covariate, revealed a main effect of rig ($F_{1,67} = 42.28$, $p < 0.001$) and an interaction effect of ethanol treatment by sex ($F_{1,70} = 3.99$, $p = 0.05$) on the resting membrane potential (E_m) of NAc microglia. Within-sex One-Way ANCOVAs with rig as a covariate revealed a significant main effect of ethanol treatment on E_m of NAc microglia specific to female mice ($F_{1,30} = 5.22$, $p = 0.03$). Additionally, microglia were subjected to hyper- and depolarizing current steps and their voltage responses were recorded. A 3-Way ANOVA revealed a main effect of sex on elicited voltage responses to current injection ($F_{1,71} = 4.108$, $p = 0.0464$). These data indicate the *Tmem119-eGFP* mouse strain escalate their drinking following a 2BC+CIE paradigm, and that ethanol exposure elicits electrophysiological adaptations in NAc microglia in a sex specific manner. The sex-specificity of the observed adaptations may point to microglia as a mediator of widely reported sex differences in voluntary ethanol drinking in mice.

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Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.05/KK6

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH-R01-AA028782
NIH-P60-AA011605

Title: Chronic intermittent ethanol exposure in mice induces region-specific and time-dependent shifts in excitatory/inhibitory balance throughout the limbic pathway

Authors: *C. MODRAK¹, S. P. FACCIDOMO², C. W. HODGE³;

²Bowles Ctr. Alcohol Studies, ¹Univ. of North Carolina - Chapel Hill, Chapel Hill, NC;

³Psychiatry, Univ. North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Alcohol possesses highly reinforcing properties in which a subset of individuals are susceptible to escalated alcohol consumption, craving, and progression to alcohol use disorder (AUD). Several neural mechanisms underly the onset and progression of AUD, however an increased focus has been placed on the role of excitatory/inhibitory (E/I) balance via AMPAR and GABA activity. We have previously shown that GluA1-containing AMPAR activity and associated proteins including TARP γ -8, CaMKII, and PSD-95 throughout the amygdala (AMY), nucleus accumbens (NAc), and medial hippocampus (HPC) are implicated in the reinforcing properties of alcohol, as well as dependence and escalation of intake. Despite this, how downstream signaling pathways are altered in these regions following alcohol dependence remains to be fully elucidated. Here, we sought to assess such alterations in a chronic intermittent ethanol (CIE) model in mice. Thirty-eight female C57BL/6J mice underwent CIE vapor exposure 4 days a week for 3 weeks. Sessions were 16 hours in duration with mice receiving exposure either to ethanol vapor (n = 20) or air (n = 18). Mice were then sacrificed 24 or 72 hours following the last CIE session, and changes in gene expression in the AMY, NAc, and HPC were assessed using RT-PCR. At 24 hours, mRNA expression for GluA1 and TARP γ -8 in the AMY and NAc were upregulated, while PSD-95 and PKC γ were upregulated and GluA2 was downregulated in the AMY only. Consequently, at 72 hours, mRNA expression for GluA1, TARP γ -8, and PSD-95 remained upregulated, in addition to upregulation of GluA2, CaMKII, gephyrin, and the α -1 GABA_A subunit in the AMY, with no effects in the NAc aside from a downregulation of PKC- λ . Interestingly, in the HPC, GluA1, CaMKII, TARP γ -8, and the α -1 GABA_A subunit were all downregulated at 24 hours, while only GluA2 was downregulated at the 72-hour time point. These results reveal a dynamic, region-specific, and time-dependent shift in E/I balance during alcohol dependence. Particularly within the AMY and NAc, this suggests that there is a shift in E/I balance during the progression of alcohol dependence, initially relying on Ca²⁺-permeable AMPAR-mediated mechanisms, before shifting to more inhibitory, and Ca²⁺-impermeable AMPAR mechanisms over time. Future research will focus on investigating whether such mechanisms are critical for alcohol craving and relapse.

Disclosures: C. Modrak: None. S.P. Faccidomo: None. C.W. Hodge: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.06/KK7

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

Support: NIH grant AA020098

Title: Divergent responses in prefrontal cortical and hippocampal blood-brain barrier protein expression in animal models of nondependent drinking and alcohol dependence

Authors: A. VANDEKERKHOFF¹, S. JUNG², H. NONOGUCHI², D. KHARIDIA², *C. D. MANDYAM²;

¹VA San Diego Healthcare Syst., La Jolla, CA; ²VA San Diego Healthcare Syst., San Diego, CA

Abstract: Background: The prefrontal cortex (PFC) and hippocampus are important for the development of alcohol addiction. Neuronal activity in these regions are regulated by immune responses, and neuroimmune responses could be assisted by disruption of the blood-brain barrier (BBB). The detrimental effects of chronic alcohol consumption, abstinence and relapse to alcohol drinking on BBB markers in the PFC and hippocampus have been minimally explored. Moreover, how and if these effects are different or divergent in alcohol dependence and nondependent conditions is not known. Here we seek to answer these questions. Methods: Adult female and male rats were made ethanol dependent by chronic intermittent ethanol vapor (CIE) and ethanol drinking (ED) procedure or only experienced ED as nondependent drinking. CIE rats were euthanized during acute withdrawal (6-8h after CIE), protracted abstinence (2 weeks) or after relapse session and nondependent rats were euthanized at matched time points. Brain tissue homogenate of the PFC and hippocampus were analyzed for BBB disruption via Western blotting. This allowed us to associate the levels of drinking in male and female nondependent and dependent rats and expression of proteins important for tight junctions (Cld5) and adherens junctions (Cdh5). NF-κB, a marker of inflammation was also evaluated in each of these conditions. Results: CIE increased ED, with rats having higher ethanol consumption during CIE and relapse to ethanol drinking sessions compared with pre-CIE sessions. ED was not altered in nondependent rats. Under both conditions, females consumed more ethanol than males. Western blotting indicated enhanced expression of Cdh5 in CIE rats, an effect that was significant during acute withdrawal in males and females in the PFC and hippocampus. In the PFC, Cdh5 levels decreased below control levels during abstinence and were stimulated after the relapse session. The reduction in Cdh5 was associated with increases in activated NF-κB during abstinence. This effect was specific to the PFC and was not observed in the hippocampus. Cld5 was reduced in both brain regions in both sexes under CIE conditions and the reduction was more pronounced during acute withdrawal. Nondependent drinking did not alter Cdh5 or Cld5 in either brain regions in either sex. Conclusions: These results reveal significant effects of ethanol dependence in adherens junction protein Cdh5, and its association with neuroimmune factor NF-κB in the PFC, and emphasize their possible role in relapse to ethanol seeking in ethanol dependent rats.

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Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

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Program #/Poster #: PSTR353.07/KK8

Topic: H.08. Drugs of Abuse and Addiction

Support: AA028549
AA026999
AA006420

Title: Evidence of a functional interaction between orexin/corticotropin-releasing factor and orexin/dynorphin transmission in the infralimbic cortex following alcohol dependence: mediation of alcohol-seeking behavior

Authors: *F. J. FLORES RAMIREZ¹, R. MARTIN-FARDON²;

¹Psychology, California State Univ. San Marcos, San Marcos, CA; ²The Scripps Res. Inst., San Diego, CA

Abstract: Stress contributes to the chronic relapsing nature of alcohol use disorder (AUD), given its enduring ability to elicit craving and precipitate relapse, even after long periods of self-imposed abstinence. Although not thoroughly understood, anatomical, pharmacological, and behavioral data suggest that orexin (OX), corticotropin-releasing factor (CRF), and dynorphin (DYN) interact, particularly in the infralimbic cortex (IL). Functional interactions between these three systems in the IL may be critical for the etiology and pervasiveness of compulsive alcohol seeking in dependent subjects that may render them vulnerable to relapse. To test this hypothesis, male and female Wistar rats were trained to self-administer 10% alcohol for 3 weeks and then made dependent via chronic intermittent alcohol vapor exposure for 6 weeks. Following extinction training (12 sessions), the rats received an intra-IL microinfusion of the OX_{1/2} receptor antagonist suvorexant (15 µg/0.5 µl/side), the CRF₁ receptor antagonist CP154,526 (0.6 µg/0.5 µl/side), the κ-opioid receptor antagonist nor-binaltorphimine (norBNI; 4 µg/0.5 µl/side), or a combination of suvorexant+CP154,526 or suvorexant+norBNI. The rats were then tested for the footshock stress-induced reinstatement of alcohol-seeking behavior. In nondependent rats, only CP154,526 prevented the reinstatement of alcohol-seeking behavior, an effect that was abolished by suvorexant co-administration. In dependent rats, suvorexant, CP154,526, and norBNI attenuated alcohol-seeking behavior, and suvorexant was the most effect among all the treatments. Interestingly, the co-administration of CP154,526+suvorexant or norBNI+suvorexant attenuated suvorexant's effect. Increases in *Crhr1* and *Hcrtr1* mRNA expression in the IL were found in alcohol-dependent rats only. These results demonstrate a functional interaction between OX/CRF/DYN receptor signaling in the IL in subjects with a history of alcohol dependence.

Disclosures: F.J. Flores Ramirez: None. R. Martin-Fardon: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

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Program #/Poster #: PSTR353.08/KK9

Topic: H.08. Drugs of Abuse and Addiction

Support: NIAAA R01 AA031463

Title: Exploring the effects of *gprasp1* shRNA knockdown in alcohol drinking behaviors

Authors: *L. G. O'LALDE¹, R. ZEGARELLI², D. LEWIS¹, C. SCRIBNER¹, M. WITTLICH¹, A. K. RADKE²;

²Psychology, ¹Miami Univ., Oxford, OH

Abstract: Alcohol Use Disorder (AUD) is a prevalent public health issue in the United States. While AUD significantly affects both men and women, data from both clinical and pre-clinical populations highlight significant sex differences in the prevalence of the disorder. A novel factor found to affect alcohol drinking is the escape of a process called X-chromosome inactivation (XCI). Genes that escape XCI in females may be expressed at twice the rate of other genes, which could drive sex differences in behavior. One gene shown to escape XCI in the mouse brain is the G protein-coupled receptor associated sorting protein 1 (*Gprasp1*). This study examines this candidate gene through genetic knockdown in the medial prefrontal cortex (mPFC), an area known to coordinate a diverse range of higher-order behaviors like decision making and behavioral inhibition, and the nucleus accumbens (NAc), which is known for its role in motivation and goal-directed behavior. For the mPFC, 13 female C57BL/6J mice received a custom-made AAV expressing shRNA for the mouse *Gprasp1* gene or a virus expressing a scrambled version of the shRNA sequence. In the NAc experiment, 28 male and 27 female mice were subjected to the same experimental procedure. Mice in both experiments completed a drinking in the dark (DID) paradigm, an established model of binge drinking known to induce an escalation of ethanol consumption. After 4 weeks of drinking, quinine, a bitter tastant, was added to ethanol in escalating concentrations (0, 100, 250, 500, 1000 μ M) to assess aversion resistance. Current results indicate that the shRNA knockdown of *Gprasp1* in the mPFC did not alter ethanol consumption or preference in females in the DID paradigm. Quinine-resistant ethanol consumption and preference also did not differ between the shRNA or scramble virus treatment groups. shRNA-mediated knockdown of *Gprasp1* in the NAc suppressed preference for ethanol after the first week of drinking. This effect persisted during quinine sessions and ethanol consumption also decreased. Targeted knockdown of *Gprasp1* in the mPFC is ongoing and predicted to reduce alcohol drinking behaviors in male mice. Overall, this study provides insights into *Gprasp1* as a regulator of alcohol drinking behaviors and a potential mediator of sex differences.

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Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.09/KK10

Topic: H.08. Drugs of Abuse and Addiction

Title: Sex-specific differences in alcohol drinking and preference in mice deficient for the habenular PKA subunit RII α suggest changes in reward appraisal and negative valence

Authors: *E. LONDON^{1,2}, E. LESKO², A. VLACHOS², A. CACCAVANO³, G. A. VARGISH², C. J. MCBAIN²;

¹Eunice Kennedy Shriver Natl. Inst. of Child Hlth. an, Bethesda, MD; ²Section on Cell. and Synaptic Physiol., NIH/NICHD, Bethesda, MD; ³Section on Cell. and Synaptic Physiol., NIH/NICHD, Washington, DC

Abstract: Mice null for *Prkar2a* that codes for the PKA regulatory subunit II α (PKA RII α) have altered reward-related behaviors including reduced intake of palatable foods and increased voluntary running. These phenotypes are mediated by altered PKA signaling in medial habenula (mHb) where RII α is highly and specifically expressed (in brain). The septo-habenulo-interpeduncular pathway is critical in regulating negative emotions, mid-brain monoamines, value-based decision making, and as such, plays a key role in the cycle of addiction/withdrawal/relapse. We hypothesized that ethanol drinking behaviors would be impacted by the previously observed mHb defects in PKA localization and enzymatic activity in RII α ^{-/-} (KO) mice. We assayed ethanol preference, sensitivity and intake with two-bottle choice, drinking in the dark (DID) and intermittent access paradigms, and evaluated the impact of acute ethanol on c-Fos expression in mHb, lateral Hb (lHb) and IPN. Changes in key phosphorylation targets in both PKA-dependent and non-PKA-dependent signaling pathways after chronic alcohol exposure are being investigated. As sexual dimorphism is a documented phenomenon among PKA-dysregulation-associated phenotypes and alcohol and drug-related behaviors, we studied ethanol effects in both sexes. We found no differences in ethanol sensitivity between genotypes but identified divergent intake patterns in the KO mice when given 24h versus limited 4h access during the dark phase. During DID with 10% EtOH, KO females consumed significantly more ethanol and escalated their intake compared to same-sex WT littermates, while male KO mice did not significantly escalate intake as their WT counterparts did. During 24h EtOH access, female KOs drank less 10% EtOH than WT and male KOs drank more than male WT littermates suggesting genotype- and sex-dependent differences in both the timing of drinking bouts and negative affect experienced after ethanol drinking. Acute ethanol exposure induced c-Fos expression in lHb significantly more in KO than WT mice while there was minimal basal or ethanol-induced c-Fos expression in mHb. These data provide new insight into the role of habenular PKA signaling and the RII α subunit in ethanol intake patterns and preference.

Disclosures: E. London: None. E. Lesko: None. A. Vlachos: None. A. Caccavano: None. G.A. Vargish: None. C.J. McBain: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.10/KK11

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

Support: NIH Grant AA012404
NIH Grant AA020926
NIH Grant K00 AA029955

Title: Molecular mechanisms underlying TLR7-induced increases in voluntary alcohol consumption in C57BL/6J mice

Authors: *M. M. FRISKE;
Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Alcohol drinking leads to an activation in the innate immune system causing a neuroinflammatory response. We hypothesize that neuroinflammation induces a positive feedforward loop resulting in increased alcohol consumption. The toll-like receptor 7 (TLR7) is an endosomal receptor that induces the expression of proinflammatory cytokines via MyD88, IRF7 and NF-kB. Chronic activation of TLR7 by the endogenous synthetic agonist R848 leads to escalated ethanol consumption and preference. However, the molecular mechanism explaining how this neuroinflammatory process is leading to increased drinking and which brain regions may drive this phenotype is poorly understood. In this study, C57BL/6J mice were treated with i.p. injections of R848 in an every-other day schedule for 20 days. After 10 days of no treatment, the animals were sacrificed for single nucleus RNA-Seq in the prefrontal cortex (PFC) and the amygdala (AMG) (snRNA-Seq, n= 4-5/treatment per region) as well as for Xenium spatial transcriptomics (ST). The snRNA-Seq data revealed more differentially expressed genes (DEGs) in the AMG (n_{DEGs}= 312) compared to PFC (n_{DEGs}= 65). DEGs in both brain regions were mainly detected in inhibitory (AMG: 129, PFC: 20) and excitatory (AMG: 80, PFC: 11) neurons. Among glial cells, AMG astrocytes had the most DEGs while in the PFC glial cells seemed to be affected equally. In AMG astrocytes, we identified multiple DEGs being involved in blood-brain barrier (BBB)-regulatory mechanisms, such as Cldn5, Mecom, and Nrg1. This finding is supported by Xenium ST where AMG-containing slices validate the expression pattern of these BBB-associated DEGs in astrocytes of the AMG as well as adjacent brain regions. Furthermore, BBB-regulatory genes have been found to be altered across other cell types in both brain regions with a particular emphasis on the BBB-regulating Wnt signaling (e.g., Notch3, Top2a, Aldoc). Our data suggests that chronic TLR7 activation via R848 initiates neuroinflammatory processes that induce long-term BBB dysfunction in both a cell type-specific and nonspecific manner. These transcriptome alterations may underlie escalated alcohol consumption.

Disclosures: M.M. Friske: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.11/KK12

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH/NIAAA R01 AA030859-01 (Ron)
NIH/NIAAA R37 AA016848 (Ron)

Title: Bdnf in ventrolateral orbitofrontal cortex to dorsolateral striatum circuit keeps moderates alcohol consumption and gates habit

Authors: *S. GUNASEKARAN¹, J. MOFFAT³, Y. EHINGER², D. RON³;
¹UCSF, San Francisco, CA; ²UCSF, San Francisco, CA; ³Dept. of Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: BDNF is a member of the neurotrophic family of growth factors which is highly expressed in the CNS and has a crucial role in shaping the structure and function of neurons. We previously discovered that BDNF signaling in the dorsolateral striatum (DLS) is part of an endogenous signaling pathway that protects against the development of alcohol use disorder^{1,2}. We further showed that dysregulation of *BDNF* levels in the cortex or dysfunctional BDNF signaling in the DLS results in the escalation of alcohol drinking and in compulsive alcohol use^{1,2}. The major source of BDNF in the striatum is the cortex³. We identified a small ensemble of BDNF-positive neurons in the ventrolateral orbitofrontal cortex (vLOFC), a region involved in decision-making and reward learning, that extend axonal projections to the DLS⁴. We also found that *BDNF* expression is reduced in the vLOFC of male but not female mice after long-term cycles of binge alcohol drinking and withdrawal. We hypothesized that BDNF in vLOFC-to-DLS circuit may play a role in limiting alcohol drinking and that heavy alcohol use disrupts this protective pathway. To test this possibility, we utilized the Cre/DIO system to overexpress BDNF only in vLOFC-to-DLS projecting neurons. We found that BDNF in vLOFC-to-DLS neurons reduces alcohol intake and preference. BDNF-positive neurons from motor cortex (MC) also project to the DLS and a sparse population of BDNF-positive neurons in the vLOFC project to the dorsomedial striatum (DMS). We therefore determined whether BDNF in MC-to-DLS and vLOFC-to-DMS alters alcohol consumption. We found that BDNF overexpression in vLOFC or MC projecting neurons to DLS and DMS, respectively, did not alter alcohol consumption, emphasizing the specificity of BDNF in vLOFC-to-DLS projecting ensemble in controlling alcohol intake. In contrast, BDNF in vLOFC-to-DLS circuit did not alter sucrose drinking, suggesting selectivity for alcohol. Given the DLS role in habitual behaviors, we hypothesized that BDNF in vLOFC-to-DLS circuitry controls alcohol intake by gating habitual alcohol seeking. We found BDNF overexpression in vLOFC-to-DLS circuit and systemic administration of BDNF receptor TrkB agonist, LM22A-4, biases habitually trained mice towards goal-directed alcohol seeking in operant self-administration. Together, our data suggests that BDNF in a small ensemble of vLOFC-to-DLS neurons gates alcohol intake and attenuates habitual alcohol seeking. References 1. Ron, D., & Berger, A. *Psychopharmacology*, 2018, 235(6). 2. Egervari, G., et al. *Trends in neurosciences*, 2021, 44(12). 3. Altar, C. A., et al. *Nature*, 1997. 389(6653). 4. Ehinger, Y., et al. *eNeuro*, 2023. 10(5).

Disclosures: S. Gunasekaran: None. J. Moffat: None. Y. Ehinger: None. D. Ron: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.12/KK13

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant R01AA027660 (PN)

Title: Activation of TMEM16A channels alleviated alcohol withdrawal-induced anxiety-like behaviors in rats

Authors: G. R. CARDOSO¹, *P. N'GOUEMO²;

¹Physiol. and Biophysics, ²Howard Univ. Col. of Med., Washington, DC

Abstract: Alcohol withdrawal is a critical aspect of alcohol use disorder, occurring after significant reduction or cessation of heavy or prolonged alcohol consumption. Symptoms of alcohol withdrawal include seizures and anxiety-like behaviors. One key target of alcohol's action in the brain is calcium-activated chloride channels (CaCCs), which are activated by an increase in intracellular Ca^{2+} . Our preliminary data indicated that activating TMEM16A channels can reduce voluntary alcohol consumption and suppress alcohol withdrawal seizures (AWSs). However, the role of CaCCs on alcohol withdrawal-induced anxiety-like behaviors is unknown. To fill in this gap of knowledge, we investigated the effects of EACT and T16Ainh-A01, a potent TMEM16A channel activator and inhibitor, respectively, on anxiety-like behaviors in adult male and female rats submitted to withdrawal from chronic alcohol intoxication. Ten-week-old male and female Sprague-Dawley rats received three daily doses of ethanol (5 g/kg, 30% vol/vol from a 95% ethanol stock solution) or a vehicle control every 8 hours for 4 consecutive days. Anxiety-like behaviors were assessed using the open field, light-dark box, and elevated plus maze tests at three time points: 3 hours (before AWS susceptibility), 24 hours (when AWS susceptibility peaks), and 48 hours (when AWS susceptibility resolves). For pharmacological experiments, rats were evaluated at the 24-hour time point during ethanol withdrawal. The rats were randomly divided into eight groups, each consisting of male and female rats treated with either EACT (2.5, 5, or 10 mg/kg, p.o.) or T16Ainh-A01 (10 mg/kg, p.o.). The rat's behavior was recorded for 5 min using a video-monitoring system and analyzed using the ANYmaze software. Withdrawal from chronic ethanol exposure results in long-lasting anxiety-like behaviors observed from 3 to 48 hours after the last dose of ethanol. EACT reverses these anxiety-like behaviors in males and females 24 hours after ethanol withdrawal. Additionally, EACT continued to reverse anxiety-like behaviors at the 48-hour time point, with a more pronounced effect observed in females. In contrast, T16Ainh-A01 did not reverse ethanol withdrawal-induced anxiety-like behaviors. Our findings indicated that withdrawal from alcohol exposure can trigger anxiety-like behaviors and that activating TMEM16A channels can effectively reverse these behaviors.

Disclosures: G.R. Cardoso: None. P. N'Gouemo: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.13/KK14

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

Support: HK112-249

Title: The effects of alcohol on caspase-3 and iNOS in microglial cells

Authors: *J.-Y. WANG¹, S.-Y. CHEN²;

¹Dept Nursing (Basic Med. Sci), Hungkuang Univ., Taichung, Taiwan; ²Nursing, Hungkuang Univ., Shalu, Taiwan

Abstract: Alcohol (EtOH) is one of the most commonly abused substances. Excessive alcohol consumption induces neuroinflammatory response and impairment in learning and memory function. Central to this process are the activation of microglia, the resident immune cells of the central nervous system (CNS), and the subsequent induction of apoptotic pathways leading to neuronal damage. The key mediators of this neuroinflammatory cascade may be the caspase-3 and inducible nitric oxide synthase (iNOS), which are activated in response to EtOH-induced oxidative stress and inflammatory signaling. However, some data indicated the microglial activation was not positive correlation to neuroinflammation in EtOH-induced neurodegeneration. Up to now, the effects of EtOH on glial cells remain unclear. In this study, we wanted to estimate the cell injury and the neuroinflammation following alcohol exposure in (1) different brain areas (*in vivo*); (2) mixed glial cell culture (*in vitro*) of rats. Male SD rats fed with various concentration of alcohol for 1 week (Day 1 and 2: 1 %; Day 3 and 4: 5 %; Day 5, 6 and 7: 10 % alcohol). The diet of rats was restricted to decrease 20 % body weight. Then we started operating the behavioral experiment and estimated the memory functions by 8 arm maze. Rats were sacrificed after about 1 month and prepared brain section for immunocyto staining. The results of immunocyto staining of brain slices revealed that the number of neuron and astrocyte were decreased significantly (about 30 %); however, the activation of microglial cells was increased significantly (about 400 %) in alcohol treatment group of various brain area. The expression of caspase-3 and iNOS were significantly increased in prefrontal cortex, nucleus ambiguous (NAc) and hippocampus (CA1). *In vivo* data suggested that alcohol will impair the neuron and astrocyte, and alcohol will induce the activation of microglial cells to induce neuronal damage. *In vitro* experiment: rat cortical mixed glial cell cultures will be subjected to (1) Control; (2) Alcohol treated with 0.1, 0.5, 1 or 2 %; were treated for 1, 3 or 5 days. Cell density and morphology will be observed by phase-contrast microscopy. Cell injury will be assessed by MTT reduction. The production of NO will be measured to estimate the inflammatory responses. The *in vitro* data indicated that MTT reduction and NO production in treatment for 1, 3, or 5 days following alcohol exposure showed no significant difference compared to the control group.

Disclosures: J. Wang: None. S. Chen: None.

Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.14/KK15

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

Support: R01AG062716 (LKF)
R01AA025591 (KN)
T32 AA007471 (AAC, ERC)

Title: Heavy, binge-like alcohol leads to deficits in learning and memory and altered neuroimmune function in aged male rats

Authors: *A. A. CAMPANILE, E. R. CARLSON, L. SINGLETON, A. AGUILAR, K. NIXON, L. K. FONKEN;
Col. of Pharmacy, Div. of Pharmacol. & Toxicology, Univ. of Texas at Austin, Austin, TX

Abstract: Over 2 billion individuals will be aged 60 years or older by the year 2050. The current aging generation tends to drink more alcohol than previous generations, emphasizing the need to understand risks associated with alcohol use in aging populations. Aged individuals can experience considerable health consequences with alcohol use due to physiological changes that occur with aging, including increased risk for dementia. Thus, here we tested whether aged (25 mos) male Fisher 344 x Brown Norway rats exhibit short- or long-term deficits in learning and memory following binge-like alcohol exposure compared to adult controls (4 mos). Rats received either ethanol (25% w/v in Ensure Plus) or isocaloric (dextrose) control diet via oral gavage for two days (6 doses; modified Majchrowicz model), and then underwent testing in the Morris water maze (MWM; a hippocampal-dependent learning and memory test). Aged rats received 7.7 ± 1.2 g/kg/day which produced 397 ± 11 mg/dl blood ethanol concentrations (BEC) versus 8.9 ± 1.1 g/kg/day and 460 ± 18 mg/dl BEC in adults. BECs were significantly higher in adults compared to aged rats. Aged, ethanol-treated rats exhibited impairments at 35 days post-binge in the MWM compared to both adult ethanol and aged control rats, but not at an earlier time point (seven days post-binge). As changes in neuroimmune function are implicated in both aging and alcohol associated pathology, we hypothesized that altered neuroinflammation could impair neuronal activity in the hippocampus, leading to the learning deficits observed after alcohol exposure. To test this, we collected brains 90 min following MWM to assess c-Fos expression (neural activity) via immunohistochemistry and neuroimmune gene expression via qPCR. c-Fos⁺ cells were decreased in the dentate gyrus and CA1 hippocampal subfields in adult and aged ethanol-treated rats and aged controls compared to adult controls, demonstrating that aging and ethanol exposure reduced neural activity following MWM. Alcohol exposure in aged rats increased expression of the pro-inflammatory cytokine IL-1 β and microglia reactivity marker MHCII in the hippocampus. Several other inflammatory genes were elevated with aging irrespective of alcohol exposure, such as Nlrp3 and CD68. Overall, our findings suggest that the

combination of alcohol and aging can impact neuroimmune activity and impair learning and memory.

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Poster

PSTR353: Molecular Mechanisms of Alcohol's Effects on the Brain

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR353.15/KK16

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

Support: R01 AA025591
R01 AG062716
T32 AA007471

Title: Differential hippocampal neuroimmune reactivity in aged vs adult male rats following two-day binge-like alcohol exposure

Authors: *E. R. CARLSON¹, S. JOSEPH¹, N. A. SALEM², R. MAYFIELD³, L. K. FONKEN¹, K. NIXON¹;

¹Div. of Pharmacol. & Toxicology, ²Neurosci., ³WCAAR, The Univ. of Texas at Austin, Austin, TX

Abstract: The US population is aging, and this group drinks more alcohol than prior generations. Accordingly, there has been a >80% increase in those aged 65+ meeting diagnostic criteria for an alcohol use disorder (AUD). Heavy alcohol drinking in aged individuals worsens cognitive decline, may accelerate the aging process, and increases risk of dementia. Neuroimmune changes are implicated in both natural aging and alcohol-induced brain damage. Here, we investigated susceptibility of the aged brain to neuroimmune reactivity following excessive alcohol exposure in rats. Aged (~25 mos) and adult (~4 mos) male Fisher 344 x Brown Norway rats received alcohol [25% (w/v) ethanol, i.g.] or isocaloric control diet every 8 h for 2 days in a Majchrowicz model with doses titrated to intoxication behavior. Rats received similar amounts of ethanol (adult: 8.2 ± 2.4 g/kg/day, aged: 8.0 ± 1.6 g/kg/day) with similar blood ethanol concentrations (adult: 420 ± 10.0 mg/dl, aged: 416 ± 7.7 mg/dl). Rats were saline-perfused and brains harvested in a time course (T0, T2, T7, T28 days post-alcohol exposure) with one half flash frozen for gene expression and the other post-fixed in PFA for immunohistochemistry (IHC) in the hippocampus. Alcohol induced differential mRNA responses of pro-inflammatory IL-1 β and anti-inflammatory BDNF in adult vs aged rats (T0-7). Specifically, alcohol increased IL-1 β in adults while reducing higher basal BDNF in aging. Phagocytic CD68 mRNA expression was increased with age but reduced by alcohol exposure, suggesting blunted neuroimmune response. TagSeq analysis of T28 revealed strong aging effects independent of ethanol, with several hundred differentially expressed genes (DEGs),

$|\log_2FC| > 0.5$, $p < 0.05$) and upregulation of many immune-related pathways via GSEA. IHC analysis of CA3 revealed that alcohol induced aging-dependent reductions in MHCII+/Iba1+ microglia (overall higher in aging). In DG-ML, CD68+/Iba1+ microglia were increased in aging independent of alcohol. Moreover, alcohol affected IBA1+ cells age-dependently: aged rats had increased IBA1 at T7 vs ethanol-treated adults and aged controls. Alcohol also induced differential changes in vimentin density in DG-ML across age, with increased vimentin in aged rats at T2 vs ethanol-treated adults and aged controls, indicative of astrocyte reactivity. Thus, aged rats displayed greater basal hippocampal neuroimmune reactivity, yet the neuroimmune response following alcohol was blunted in aged rats. Understanding alcohol-induced changes in microglia function across the lifespan may lead to greater insight into how the neuroimmune system drives susceptibility to the damaging effects of alcohol.

Disclosures: E.R. Carlson: None. S. Joseph: None. N.A. Salem: None. R. Mayfield: None. L.K. Fonken: None. K. Nixon: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.01/KK17

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant DA046411

Title: Anhedonia After Intermittent Long-Access Nicotine Self-Administration in Rats

Authors: *A. W. BRUIJNZEEL, A. BEHNOOD-ROD, R. CHELLIAN;
Univ. of Florida, Gainesville, FL

Abstract: Tobacco use disorder is a chronic condition characterized by compulsive nicotine use and withdrawal symptoms after smoking cessation. Smoking is the leading preventable cause of morbidity and mortality worldwide. Smoking cessation leads to anhedonia, which is an inability to experience pleasure from previously enjoyed activities and is caused by dysregulation of the brain's reward and stress systems. It is also a key withdrawal symptom that contributes to relapse to smoking after a period of abstinence. To better understand the development of anhedonia, we investigated its onset and time course in rats that self-administered nicotine. Rats were implanted with intracranial self-stimulation (ICSS) electrodes to assess reward function and intravenous catheters for nicotine self-administration. Elevations in ICSS brain reward thresholds reflect decreased sensitivity to rewarding electrical stimuli, indicating anhedonia. The rats self-administered 0.06 mg/kg of nicotine intermittently, three days per week, for seven weeks. Brain reward thresholds were determined once a week 24 h after nicotine self-administration during weeks 1 to 3, and at 12, 24, and 48 h during weeks 4, 5, and 7. Elevations in brain reward thresholds were not observed during the first four weeks of nicotine self-administration. However, the brain reward thresholds were elevated in both weeks 5 and 7 at least 12 h after

nicotine self-administration, indicating that anhedonia emerges gradually and then persists. As withdrawal severity gradually increases, smoking cessation may become more challenging. Therefore, behavioral or pharmacological interventions soon after smoking initiation are critical to prevent the development of a tobacco use disorder.

Disclosures: A.W. Bruijnzeel: None. A. Behnood-Rod: None. R. Chellian: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.02/KK18

Topic: H.08. Drugs of Abuse and Addiction

Support: Support from Penn State Social Science Research Institute (SSRI)
NIH T32 DA017629

Title: Network connectivity supporting adaptive attentional control under nicotine withdrawal: a cognitive and affective demand manipulation

Authors: *X. YAN¹, A. S. WEIGARD², S. J. WILSON¹, Z. SHAPIRO³, H. GALLOWAY-LONG⁴, C. HUANG-POLLOCK¹;

¹Psychology, The Pennsylvania State Univ., University Park, PA; ²Psychiatry, Univ. of Michigan, Ann Arbor, MI; ³Psychology, Emory Univ., Atlanta, GA; ⁴Legacy Good Samaritan Med. Ctr., Portland, OR

Abstract: Introduction. Tobacco use is a leading preventable cause of disability and death, with nicotine withdrawal posing a key barrier to cessation. Withdrawal-related affective symptoms can worsen cognitive difficulties, yet the underlying neural mechanisms remain unclear. Dynamic interactions between the salience network (SN), default mode network (DMN), and frontoparietal network (FPN) support adaptive attentional control. Resting-state studies of withdrawal have linked increased SN-DMN coupling with the experience of craving, whereas task-based studies conducted outside the context of withdrawal have associated similar patterns with better performance under high cognitive demand. This contradiction highlights the need to clarify context-dependent network connectivity under nicotine withdrawal, particularly when cognitive and affective demands co-occur. **Methods.** We examined SN, DMN, and FPN dynamics in daily smokers (N = 18, mean age = 26.56, 55.6% women) during an N-back fMRI task following nicotine satiation and 12-hour abstinence. The task included 4 runs × 4 blocks with alternating 1- and 3-back levels to manipulate cognitive demand, and aversive odors in half the blocks to manipulate affective demand. Functional connectivity was compared across experimental and smoking conditions. **Results.** Under satiation, greater cognitive demand was associated with reduced within-DMN connectivity and increased SN-DMN coupling, with further SN-DMN increases under combined cognitive-affective demand — consistent with suppression of task-irrelevant processing. Cognitive load led to increased within-FPN

connectivity, but only under combined demand were concurrent increases in SN-FPN coupling observed, suggesting reactive control recruitment. Additionally, we observed greater DMN-FPN functional segregation under combined demand, potentially attributable to SN-driven regulation of both networks. During abstinence, however, the SN failed to modulate either FPN or DMN under the highest demand, indicating disrupted network coordination. **Conclusion.** SN coupling with DMN and FPN may reflect both salience signaling and reactive modulation. While prior studies link nicotine withdrawal with increased salience signaling, our data suggest that SN's modulatory capacity is also reduced — impairing toggling between DMN and FPN under high demand. These results underscore the importance of context-dependent connectivity analyses for understanding how affective and environmental demands shape attentional control and for informing precise risk prediction and intervention strategies.

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Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.03/KK19

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant U54-MD012393 [subproject 5378]
NIH T32DA043449

Title: Mesocortical and mesolimbic associations with anhedonia and impulsivity: A path to adolescent e-cigarette use

Authors: *B. SUTHERLAND¹, P. M. VIERA PEREZ², E. TRUCCO¹, L. D. HILL-BOWEN⁴, A. R. LAIRD⁵, M. T. SUTHERLAND³;

¹Psychology, ²Cognitive Neurosci., ³Florida Intl. Univ., Miami, FL; ⁴Dept. of Psychiatry and Behavioral Sciences, Vanderbilt Univ. Med. Ctr., Nashville, TN; ⁵Res. Imaging Ctr., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Introduction: Despite rate declines, clarifying factors for teen e-cigarette use at the neurobiological and psychological levels remains a priority. Brain regions along the mesocortical and mesolimbic dopaminergic paths are linked with mental operations impacting the initiation or continuation of substance use (SU) such as reward signaling, cognitive control, and decision-making. Psychologically, anhedonia (a reduction in pleasurable experiences) and impulsivity (acting with little forethought of consequences) are relevant for teen SU. We assessed the interrelations between activity within mesocortical/mesolimbic regions, anhedonia, impulsivity and e-cigarette use. **Methods:** We assessed 137 teens (48% female, 88% White, 85% Hispanic, M_{age}=14.9) from a larger study who completed a working memory (WM, n-back) and a reward processing task (Monetary Incentive Delay, MID) at wave(W)1. We focused on mesocortical

targets by assessing WM-related activity from the dorsolateral and dorsomedial prefrontal cortex (dlPFC, dmPFC) and mesolimbic targets by assessing MID-related activity from the left and right caudate. The Snaith-Hamilton Pleasure Scale quantified anhedonia at W1. Impulsivity (using the SU risk profile) and e-cigarette use days were assessed at W2 ~15 months later. Serial mediation models estimated the impact of brain activity on e-cigarette use via anhedonia and impulsivity covarying for sex, age, ethnicity, W1 impulsivity and lifetime e-cigarette use.

Results: When considering WM-related (mesocortical) activity, we identified an indirect effect for the dmPFC (but not dlPFC) model. Less dmPFC activity was linked with more anhedonia, which in turn predicted higher impulsivity, and higher impulsivity was linked with more e-cigarette use (indirect effect=-0.39, CI [-1.25, -0.01]). When considering MID-related (mesolimbic) activity, we did not detect indirect effects but rather observed a direct effect linking more left caudate activity during reward anticipation with more e-cigarette use (estimate=23.23, $p=0.03$). **Conclusions:** These outcomes highlight anhedonia and impulsivity as factors linking dmPFC activity and e-cigarette use. While PFC (mesocortical) regions were linked with anhedonia and impulsivity, mesolimbic regions did not show a mediated, but rather a direct effect to e-cigarette use. PFC regions are linked with cognitive processing influencing apathy/pleasure and impulsivity possibly contributing to risky-decision making. Notably, the caudate (mesolimbic) is linked with reward signaling and incentive salience (“wanting”) likely underlying increased approach behaviors towards drug-related stimuli.

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Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.04/KK20

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant
TRDRP Grant

Title: Behavior and Whole-Brain Functional Connectomic Changes in Mice Following Passive Electronic Cigarette Inhalation.

Authors: *S. BONNET-ZAHEDI^{1,3}, L. L. CARRETTE¹, L. CHINA¹, T. LA¹, G. KELLEY¹, J. MOSQUERA¹, E. A. SNEDDON¹, K. SHANKAR⁴, D. KOUZMINOVA¹, K. BAI¹, S. FOROUTANI¹, M. BRENNAN¹, S. L. PLASIL¹, G. DE GUGLIELMO¹, B. LIM², O. GEORGE¹;

¹Psychiatry, ²Biol. Sci., Univ. of California San Diego, La Jolla, CA; ³Inst. de Neurosciences de la Timone, Marseille, France; ⁴Sanford Burnham Prebys Med. Discovery Inst., San Diego, CA

Abstract: Although several circuits mediating nicotine addiction-like behavior are well-characterized, the effects of nicotine on brain-wide functional networks remain unresolved. This knowledge gap persists due to the lack of high-resolution, whole-brain mapping of neuronal activity during distinct stages of nicotine use, including acute intoxication, dependence, and withdrawal. To address this gap, the present study aimed to (1) characterize whole-brain neuronal activity patterns across these stages at single-cell resolution and (2) predict behavioral and physiological outcomes using advanced network analysis.

A mouse model of nicotine dependence was developed using passive vapor inhalation of nicotine (15 mg/mL in 1:1 propylene glycol/vegetable glycerin) delivered via electronic cigarette technology. Transgenic mice expressing tdTomato in cholinergic neurons were exposed to nicotine, vehicle, or air in one of three paradigms: single exposure (1×1 hour), chronic exposure (3×1 hour per day, 5 days per week for 3 weeks), or tested 24 hours after the final chronic exposure. Behavioral assays assessed locomotor activity and anxiety-like behavior (open field and elevated plus maze), thermal nociception (tail immersion), and body temperature.

A single nicotine exposure increased exploratory activity and locomotion, whereas these effects diminished following chronic exposure. In contrast, withdrawal reduced exploratory behaviors, suggesting a withdrawal-associated anxiety-like phenotype. Body temperature decreased following a single exposure, normalized with repeated exposure, and increased during withdrawal. Only chronic exposure elevated hyperalgesia compared to vehicle and naïve controls.

These results validate the model by demonstrating stage-specific, addiction-relevant behavioral alterations. Whole-brain Fos mapping with light-sheet microscopy, combined with ClearMap2 analysis and cholinergic neuron labeling, has yielded single-cell resolution datasets. Ongoing network analyses aim to correlate functional connectivity with behavioral phenotypes to identify novel biomarkers and therapeutic targets for nicotine use disorder.

Disclosures: S. Bonnet-Zahedi: None. L.L. Carrette: None. L. China: None. T. La: None. G. Kelley: None. J. Mosquera: None. E.A. Sneddon: None. K. Shankar: None. D. Kouzminova: None. K. Bai: None. S. Foroutani: None. M. Brennan: None. S.L. Plasil: None. G. de Guglielmo: None. B. Lim: None. O. George: None.

Poster

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Program #/Poster #: PSTR354.05/KK21

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA 1U01DA058278
NIH S10OD019960

Title: Spatial transcriptomics revealed region-specific metabolic shift and developmental disruptions in rat brain after prenatal e-cigarette exposure

Authors: *W. CHEN¹, Z. CHEN¹, C. WANG²;
²Ctr. for Genomics, ¹Loma Linda Univ., Loma Linda, CA

Abstract: Prenatal e-cigarette exposure has been linked to various neurodevelopmental disorders. However, the effect of prenatal e-cigarette exposure on the brain spatial transcriptome remains unknown. In this study, we investigated spatial and single-nucleus gene expression in postnatal day 7 (P7) rat brains and the long-term effects on the transcriptome and behavior of 3-month-old offspring following prenatal e-cigarette exposure. Using 10x Visium spatial transcriptomic technology, we found that the caudate putamen and hypothalamus were the most transcriptionally affected brain regions at P7 after prenatal e-cigarette exposure. Our analysis revealed spatially distinct transcriptomic changes and region-specific metabolic shifts, i.e., enhanced glycerolipid metabolism in the caudate putamen, and impaired cholesterol biosynthesis in the cortex. RNA velocity analysis indicated region-specific developmental alterations, including delayed maturation of the corpus callosum and accelerated hypothalamic development. Single-nucleus RNA-seq revealed that the transcriptomic alterations were sustained at 3 months, accompanied by behavioral changes such as hyperactivity and heightened impulsivity in males. Cross-mapping of e-cigarette-induced differentially expressed genes with the human PsychENCODE database showed an association with psychiatric disorders (i.e., ASD). Our study provides spatially resolved transcriptomic alterations of the developing brain following prenatal e-cigarette exposure, offering critical insights into the cellular and molecular mechanisms underlying the increased risk of neurodevelopmental disorders caused by maternal e-cigarette use.

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Topic: H.08. Drugs of Abuse and Addiction

Support: R01DA048094
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UG3DA048510
T32AA013526

Title: Neural patterns of emotion regulation predict smoking duration

Authors: *R. ARNOLD¹, B. FROELIGER²;
²Psychological Sci., ¹The Univ. of Missouri, Columbia, Columbia, MO

Abstract: Smoking cigarettes is a major public health concern, as it contributes to morbidity, mortality, and health resource utilization. Despite widespread dissemination of prevention and

cessation efforts, 1 in 5 deaths in the United States can be attributed to smoking. Nicotine dependence (ND) is associated with disrupted cognitive control and emotional processes; however, little is known about the underlying mechanisms of cognitive control over emotional responding i.e., emotion regulation (ER) in ND. The current, confirmatory study examined behavioral task-related ER differences between nicotine sated adults who smoke (ND; $n=153$, Age $M=42\pm11$) and a demographically matched nonsmoking control group (CG; $n=193$, Age $M=40\pm13$) via rmANOVA. Among a subset who performed ER while undergoing fMRI (ND $=129$, $M=43\pm12$; CG $=77$, $M=38\pm14$), neural task-related ER differences were examined via classification MVPA with linear support vector machine using leave one out cross validation, and kernel ridge regression MVPA with 10 k-folds. Biological sex was examined and was found to be ns. Behavioral ER task performance revealed significant group (ND, CG) x Strategy (Reappraise, View) interactions in the negative and positive conditions ($F's>13$, $p's<0.001$). Assessment of fMRI BOLD ER task performance significantly predicted smoking status (ND, CG) during positive ($p=0.004$, 0.65 accuracy, 0.68 precision, 0.84 recall, F1 0.75) and negative ($p=0.04$, 0.65 accuracy, 0.66 precision, 0.81 sensitivity, F1 0.73) task conditions. Regression analyses revealed, broadly, that years smoking was significantly associated with differential patterns of activation in frontoparietal circuitry during the negative ($r=0.25$, $p=0.009$) vs. positive ER ($r=0.25$, $p=0.01$) conditions. This is the first reported study using brain and behavioral measures of ER to characterize and predict ND. Overall, these novel findings suggest that the ND group demonstrates worse ER efficacy as compared to the CG, reflected in discriminable ND group/CG neural activation, and these patterns of ER task-related neural activation predict smoking duration. Further, findings demonstrating unique patterns of activation associated with each ER valence may serve to inform further study of emotional processing and regulatory mechanisms, as well as translational approaches to employing predictive models for testing new treatments to remediate emotion dysregulation in nicotine dependence.

Disclosures: R. Arnold: None. B. Froeliger: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH-NIDA 5T32DA043469
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Title: Temporal dynamics of in vivo interpeduncular nucleus nicotine reveal dose-dependent and dose-order dependent differences in nicotinic excitation of aversion circuitry

Authors: *R. R. MANSOURI-RAD¹, D. S. MCGEHEE², C. MCQUILLEN³, A. LEE²;
¹Committee on Neurobio., ²Anesthesia, Univ. of Chicago, Chicago, IL; ³Pathology and Lab.
Med. department, Weill Cornell Univ., New York, NY

Abstract: Tobacco smoking persists as a leading cause of preventable death worldwide due to its primary addictive component: nicotine. Human experiential responses to initial exposures to nicotine can predict likelihood of future use and dependence. In mice, injection of an acute high dose of nicotine (1.5 mg/kg) reliably causes behavioral aversion. *Ex vivo* techniques implicate increases in interpeduncular nucleus (IPN) activity as a mediator of high-dose nicotine aversive responses, but the temporal dynamics of this response has not been investigated *in vivo*. We recorded IPN neuronal activity *in vivo* in mice using fiber photometry: implanting a fiber optic probe above the IPN where we virally expressed intracellular Ca^{2+} indicator, GCaMP6s, pan-neuronally in nicotine-naïve male mice. Ten mice were split equally between those exposed to the aversive high nicotine dose (1.5 mg/kg) as their first nicotine experience, and those first exposed to a rewarding low dose (0.5 mg/kg). Both groups were given the alternate dose 24 hours later. Unexpectedly, both nicotine doses caused an increase in IPN Ca^{2+} fluorescence relative to saline. Slope of the rising phase and peak response amplitude were positively correlated with dose. Even more surprisingly these increases were followed by a sustained suppression of IPN Ca^{2+} , the duration of which was also positively correlated with nicotine dose. Though these dose-dependent activity measures persisted regardless of dose presentation order, we also found an order-dependent difference in the duration of the sustained intracellular Ca^{2+} increase. Looming dark shapes above the mice induced robust increased Ca^{2+} responses in the IPN, and these responses were suppressed by aversive nicotine doses. To assess nicotine self-administration, we have implemented a two-bottle choice apparatus with real time lick-o-meter to test dose effects on nicotine consumption in both male (n=12) and female (n=13) mice. Interestingly, female mice show higher levels of nicotine consumption relative to male mice. Assessing similarities or differences in the IPN activity measures between male and female mice is a focus of ongoing investigations. Overall, these studies are providing insight into the behavioral impact of IPN activity, which may provide a more complete understanding the determinants of nicotine's behavioral valence and the progression to addiction.

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Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

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Topic: H.08. Drugs of Abuse and Addiction

Support: Tobacco Related Disease Research Program project grant (22RT-0103A, S.L.)

Title: Genetic manipulation of the *chrna2* gene results in age- and sex-dependent alterations in hippocampal-dependent learning and memory, with impacts in adult nicotine self-administration

Authors: *A. WELLS¹, C. MOJICA², C. D. FOWLER³, S. LOTFIPOUR⁴;

¹Univ. of California Irvine, Irvine, CA; ²Pharmacol., UC Irvine, Irvine, CA; ³Neurobio. and Behavior, ⁴Emergency Medicine, Pharmacology, & Pathology, Univ. of California, Irvine, Irvine, CA

Abstract: The nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel that is activated by the neurotransmitter acetylcholine and exogenous ligands such as nicotine. The alpha-2 nAChR, encoded for by the *Chrna2* gene, is located primarily within neuronal cells and is implicated in hippocampus-dependent learning and memory and nicotine-facilitated synaptic plasticity. Alpha-2 containing nAChRs are expressed within oriens lacunosum moleculare (OLM) GABAergic interneurons in the CA1 region of the hippocampus and are critical for modulating synaptic plasticity by regulating inputs from the CA3 region and the entorhinal cortex (EC). Using a dorsal CA1 hippocampal-dependent task of preexposure-dependent fear conditioning paradigm, we assessed whether genetic manipulation of the *Chrna2* gene (*Chrna2* -/- or *Chrna2* +/+) resulted in alterations to hippocampal-dependent learning and memory in adolescent female and male mice and female adult mice. We also assessed whether hyperexcitability of the *Chrna2* gene in male adult mice would result in differences in nicotine but not food reinforcement as compared with *Chrna2* +/+ (wild-type) mice. We found that adolescent and adult female *Chrna2* -/- mice demonstrated no significant differences in learning and memory behavior, while adult male *Chrna2* -/- mice demonstrated deficits in learning and memory behavior. Given that our prior studies found that male adolescent *Chrna2* -/- mice had deficits in learning and memory behavior, we then examined female adolescent *Chrna2* -/- mice and found they extinguished independent of drug treatment. In contrast, female adult nicotine-exposed *Chrna2* -/- did not extinguish but increased in freezing behavior. Independent of nicotine pre-treatment, male *Chrna2* +/+ adolescents, but not *Chrna2* -/-, mice extinguished while these effects were absent in adults. Hypersensitivity of *Chrna2* in adult mice resulted in diminished acquisition of nicotine self-administration as compared with *Chrna2* -/- and *Chrna2* +/+ male mice and a flattening of the nicotine dose-response curve at all nicotine doses as compared with *Chrna2* +/+ mice, with no impact on food reinforcement. Our study suggests that alpha-2 -ontaining nAChRs play age- and sex-dependent roles in nicotine modulated behaviors.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: TRDRP

Title: Chrna6 variations and nicotine response in early adolescence

Authors: *Y. HAJY HEYDARY¹, F. M. LESLIE³, S. LOTFIPOUR²;

²Emergency Medicine, Pharmacology, & Pathology, ¹Univ. of California, Irvine, Irvine, CA;

³Dept Pharmaceut. Sci., UC Irvine, Irvine, CA

Abstract: Adolescence is a critical period of neurodevelopment, making the brain particularly vulnerable to the long-lasting effects of nicotine, the psychoactive component in tobacco. Tobacco product use remains a significant public health concern among U.S. middle and high school students. Nicotine exerts its effects through activation of neuronal nicotinic acetylcholine receptors (nAChRs), with $\alpha 6$ -containing nAChRs playing a crucial role in nicotine-induced behaviors such as locomotor activity, self-administration, and dopamine release. A single nucleotide polymorphism (SNP), rs2304297, in the 3' untranslated region (UTR) of the CHRNA6 gene (encoding for $\alpha 6$ subunit) has been linked to enhanced smoking behavior in adolescent humans. Our lab has developed humanized rat lines where the rat CHRNA6 3' UTR is replaced with the human sequence, allowing for in vivo investigation of this SNP. These humanized lines exhibit sex- and genotype-dependent effects on nicotine-induced locomotor activity and anxiety-like behavior. This project investigates the molecular mechanisms underlying these observed sex- and genotype-dependent differences in nicotine response. RNA sequencing on the Ventral tegmental area (VTA) reveals nicotine-induced alterations in gene expression. For instance, in nicotine-treated $\alpha 6$ CC female rats, upregulation of several nAChR subunits (including $\alpha 6$, $\alpha 5$, $\alpha 3$, and $\beta 3$) and genes involved in dopaminergic signaling (Slc6a3, Th, and Drd2) are observed in the VTA. Furthermore, our studies identified lysophosphatidic acid receptor 4 (LPAR4) as a gene of interest in nicotine-treated $\alpha 6$ CC male rats, showing a positive correlation with time spent in the center in the open field test. We also explore the novel regulatory role of miRNA-365 in modulating CHRNA6 expression. In vitro luciferase assays demonstrated that the G carrier group exhibited increased luciferase activity compared to the C carrier group, suggesting potential differences in miRNA regulation of each variant. These findings highlight how genetic variations, sex, and nicotine exposure interact to influence gene expression patterns in the VTA, providing critical insights into the development of nicotine sensitivity and addiction vulnerability.

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Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

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Topic: H.08. Drugs of Abuse and Addiction

Support: University of Chicago Quad Undergraduate Scholars Program
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University of Chicago The Grossman Institute of Neuroscience

Title: Using virtual reality to characterize the effects of nicotine on drug-place associations in hippocampal CA1 and mouse behavior

Authors: *S. M. KONOW;
Univ. of Chicago, Chicago, IL

Abstract: Substance use disorders affect millions, with relapse being a large challenge to recovery. Places associated with drug consumption can precipitate relapse, even after years of abstinence from drugs. The neural mechanisms behind these drug-place associations remain unclear. We developed a paradigm that mimics an extinction-renewal paradigm in head-fixed mice using virtual reality (VR) environments to learn more about those associations. Head-fixed mice were trained to explore a VR environment and received oral nicotine rewards through a lick spout, and we tracked licking behavior as an indicator of an association between nicotine and the environment. After training, we placed the mice in a novel VR without providing rewards to extinguish licking. Then, we returned the mice to the familiar VR without a reward and observed that the mice renewed licking. We also wanted to understand how drug-place associations were formed. We performed calcium imaging of hippocampal cells using a 2-photon microscope while mice navigated a familiar and novel environment with either a water or nicotine reward. We found that nicotine-rewarded mice formed drug-place associations that resulted in cellular activity that was distinct from the water-rewarded conditions and that nicotine-rewarded mice showed higher rates of licking during renewal, which is indicative of context-induced drug-seeking behavior. These results show that we can use VR with 2-photon microscopy to study drug-place associations in head-fixed mice, allowing us to study the effect of addictive drugs in experimentally well-controlled contexts. Using this method, we can gather behavioral and cellular data and longitudinally track large populations deep in the brain with high resolution, which will generate deeper insights into the neurobiology of addiction.

Disclosures: S.M. Konow: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

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Program #/Poster #: PSTR354.11/LL5

Topic: H.08. Drugs of Abuse and Addiction

Support: Key Project of Beijing Life Science Academy 2024100CB0090

Title: Chronic low-dose nicotine administration improves cognition in mouse

Authors: Y. LIU^{1,2}, X. LI^{1,2}, H. WANG^{1,2}, Y. TIAN^{1,2}, X. CUN¹, S. HAN^{1,2}, L. CUI^{1,2}, H. CHEN^{2,1}, *H. HOU^{2,1};

¹China Natl. Tobacco Quality Supervision & Test Ctr., Zhengzhou, China; ²Beijing Life Sci. Acad., Beijing, China

Abstract: Nicotine has been proven to significantly improve cognition in Alzheimer's disease (AD) mice, however, whether nicotine affects cognition in normal mice and the dose-effect relationship remain unclear. To explore the effects of nicotine on cognitive capacity, mice were divided into six groups, a saline control group and five nicotine administration groups with different doses (0.125, 0.25, 0.5, 1.0, 2.0 mg/kg). The mice received daily subcutaneous injections of nicotine. Acute administration was a single dose and chronic administration lasted for three weeks. Cognitive performance was assessed via novel object recognition (NOR) and Y-maze tests. Long-term memory was evaluated using Barnes maze test. The results showed that acute nicotine administration at all tested doses did not significantly elevate the recognition index (RI) in the NOR test but significantly reduced spontaneous alternation percentage in the Y-maze test, suggesting that acute nicotine exposure may impair spatial cognition of mice by inducing aversive responses. Chronic nicotine administration at the dose of 0.25 mg/kg markedly enhanced short-term memory, as indicated by increased RI in the NOR test, while had no significant effect on spatial cognition shown by the results of Y-maze test. Chronic nicotine administration at the dose of 0.25 mg/kg also promoted long-term memory, as evidenced by increased target hole exploration time of mice in the Barnes maze test. In conclusion, chronic nicotine administration rather than acute administration significantly improved cognitive behaviors in mice. Chronic low-dose (0.25 mg/kg) nicotine administration dramatically enhanced both short-term and long-term memory abilities in mice, while exerted no significant effect on spatial memory. This suggests that the cognitive improvement of nicotine in mice is influenced by the administration mode and dose.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: Underlying studies were supported by Philip Morris Products S.A. (PMP)

Title: Separating nicotine effects on attention and reinforcement in rats by dose and kinetic: go low and slow

Authors: *E. D. LEVIN¹, C. WELLS², M. STOUT², A. HAWLEY⁴, E. ANDONOV², A. H. REZVANI³;

¹Duke Univ. Med. Ctr., Durham, NC; ³Dept. of Psychiatry, ²Duke Univ., Durham, NC; ⁴SDuke Univ., Durham, NC

Abstract: Background: Nicotine is the principal psychoactive chemical in tobacco and is the main chemical driver of tobacco addiction. Nicotine is reinforcing and has a range of other effects in the nervous system. Numerous studies have found that nicotine increases accuracy in tests of cognition including attention. Is it possible to achieve cognitive improvement with nicotine while minimizing the adverse effect of drug reinforcement? In attempts to separate nicotine's cognitive improvement from reinforcing effects receptor subtype elective nicotinic drugs have been studied. However, combined nicotinic receptor subtype actions, such as achieved with nicotine, may be needed to improve cognition. Another possible way to separate pharmacological effects is by changing dose and kinetics of administration. We have evaluated the effects of low nicotine doses on self-administration and attention, reported here. Previously, we tested the effects of slow nicotine infusion with osmotic minipumps with a similar to zero-order kinetic delivery as transdermal nicotine patches in humans. **Methods:** In young adult female Sprague-Dawley rats, we assessed the effects of nicotine on reinforcement in a two-lever operant apparatus FR-1 at doses of 3, 10 and 30 µg/kg/infusion (IV) in one-hour sessions. In a separate set of rats, we assessed nicotine effects on attention in a standard operant visual signal detection task with low acute doses, of 10-40 µg/kg (SC) in one-hour sessions. **Results:** At a low dose of 3 µg/kg/infusion, rats self-administered nicotine at very low rates compared with higher doses of 10 and 30 µg/kg/infusion. The same total dose range of nicotine that is self-administered at 3 µg/kg/infusion was found in the companion study to significantly improve attentional accuracy. In previous studies, we have found in rats that chronic slow zero-order nicotine infusion with sc osmotic minipumps at 5 mg/kg/day significantly decreased nicotine self-administration. This same chronic infusion dose significantly increased attentional accuracy in the visual signal detection test of attention. **Conclusions:** These data suggest that nicotine effects on reinforcement and attention can be dissociated with low dose and slow kinetic nicotine delivery. Nicotine delivered at low doses or slow rates significantly improves attentional performance in Sprague-Dawley rats. The low dose range of nicotine that improves attention in rats is self-administered at only very low rates. Nicotine delivered with a zero-order kinetic via osmotic minipump similar to that achieved by the nicotine skin patch significantly improves attention and significantly reduces nicotine self-administration.

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Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

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Program #/Poster #: PSTR354.13/LL7

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH NIDA-IRP

Title: Sex- and dose-dependent behavioral effects of acute subcutaneous nicotine in mice

Authors: *X. MIN¹, Z. YE², S. IKEMOTO³;

¹Behavioral Neurosci. Res. Br., Natl. Inst. on Drug Abuse, Natl. Inst. of Hlth., Baltimore, MD;

²NIH/NIDA/IRP, Intramural Res. Program of NIDA, NIH, Baltimore, MD; ³Behav Neurosci Br, NIDA / NIH, Bel Air, MD

Abstract: Nicotine exerts complex effects on emotional and cognitive functions, which are influenced by both dose and sex; however, the detailed behavioral profiles following acute peripheral administration remain incompletely characterized. In the present study, we systematically evaluated the effects of acute subcutaneous nicotine administration on anxiety-like behavior and spatial memory in male and female mice using the elevated plus maze (EPM) and object location memory (OLM) procedures. In the EPM, low-dose nicotine (0.02 mg/kg, salt form) significantly increased the time spent in the open arms in both male and female mice compared to saline controls, indicating reduced anxiety-like behavior. In contrast, high-dose nicotine (0.5 mg/kg) significantly decreased open arm time, suggesting increased anxiety-like behavior at this dose. Interestingly, in the OLM task, nicotine produced differential effects between males and females depending on the dose. Low-dose nicotine (0.02 mg/kg) significantly increased relative time spent with the object at a new location in male mice, suggesting improved location memory, but exerted no significant effect in female mice. In contrast, high-dose nicotine (0.5 mg/kg) impaired discrimination between familiar and novel locations in males while enhancing such discrimination in females. These results demonstrate that acute nicotine produces dose- and sex-dependent effects on both anxiety-related behavior and spatial memory. The divergent behavioral outcomes provide a foundation for future investigations into the underlying neural mechanisms mediating nicotine's complex actions on emotional and cognitive processes.

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Poster

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Program #/Poster #: PSTR354.14/LL8

Topic: H.08. Drugs of Abuse and Addiction

Title: Effects of Acetylcholine Receptor Antagonism via Dihydro- β -erythroidine on Nicotine Conditioned Place Preference Following Adolescent Nicotine Exposure

Authors: A. BALDWIN¹, S. LEAGUE², A. DEAN³, *G. M. FERNANDEZ²;

¹Neurosci., ²Psychology, ³Biochem., Christopher Newport Univ., Newport News, VA

Abstract: Adolescence is a critical period for neural development, often characterized by risk-taking and reward-seeking behaviors, which result from uneven maturation rates across brain structures (Larsen & Luna, 2018). While areas such as the mesolimbic dopamine pathway, which is involved in reward processing, are fully mature at the start of early adolescence, other structures within the mesocortical pathway responsible for cognition continue to undergo key developmental changes (Spear, 2000). Nicotine exposure during adolescence, which has increased due to the popularity of electronic nicotine delivery (END) systems, has been linked to increased susceptibility to developing substance use disorders. These behavioral disruptions have been correlated with changes in cholinergic signaling, specifically $\alpha 4\beta 2$ nicotinic acetylcholine receptor (nAChR) activity, within the developing mesocortical pathway (Kundu et al., 2025). This project pharmacologically manipulates the cholinergic system, and examines its effect on nicotine-context association observed in conditioned place preference (CPP). Sprague Dawley rats were injected subcutaneously with twelve doses of 0.4 mg/kg nicotine (equivalent to one pack of cigarettes, or one END cartridge) or saline during adolescence (postnatal day 28- 49). Animals were tested as adults using a biased, nicotine CPP paradigm in which three exposures to 0.4 mg/kg nicotine were associated with a non-preferred context. Each conditioning session also included a co-administered, subcutaneous injection of 5 mg/kg dihydro- β -erythroidine (DH β E; an $\alpha 4\beta 2$ nAChR antagonist) or vehicle (dimethylsulfoxide). We replicated prior research which found DH β E attenuated CPP in adult cohorts. Our preliminary results indicate that animals pretreated in adolescence with nicotine and co-administered DH β E during CPP formed a significant drug-cue association, contrary to previous research in which DH β E attenuated CPP. These results suggest that adolescent nicotine exposure leads to unique, long-term disruptions in cholinergic system functioning.

Disclosures: A. Baldwin: None. S. League: None. A. Dean: None. G.M. Fernandez: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.15/LL9

Topic: H.08. Drugs of Abuse and Addiction

Support: T32IR4866
NIDA F30 DA062492

Title: Cannabidiol Transiently Reduces Nicotine Vapor Self-Administration in Mice

Authors: *A. S. MOGUL¹, K. N. LAUMANN¹, C. D. FOWLER²;
²Neurobio. and Behavior, ¹Univ. of California, Irvine, Irvine, CA

Abstract: Tobacco products containing nicotine remain among the leading causes of preventable deaths worldwide, despite public health efforts to reduce their use. Most individuals who use nicotine products struggle to quit successfully. First-line cessation aids, including nicotine

replacement therapy, varenicline, and bupropion, show limited long-term efficacy. *Cannabis Sativa* has long been used for its medicinal properties and is also widely available as a recreational drug. Of its main constituents, delta (9)-tetrahydrocannabinol (THC) is considered the main psychoactive component, whereas CBD fails to exhibit evidence of addiction liability in pre-clinical models. CBD has been shown to act on multiple targets which may mitigate its effects on drug reinforcement and withdrawal behaviors, and we have previously shown that acute oral administration of CBD decreases intravenous self-administration in mice. It is unknown, however, if this result is reproducible across other routes of nicotine administration with additional sensory cues, such as nicotine vapor self-administration. Here, we examined the effects of both acute and chronic oral CBD. During 21- day chronic administration, we observe a transient decrease in nicotine self-administration during the first week, but not the second or third weeks of testing. Together the data help to elucidate the behavioral effects of CBD administration on nicotine intake behaviors and the potential of CBD as a nicotine cessation therapeutic.

Disclosures: A.S. Mogul: None. K.N. Laumann: None. C.D. Fowler: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.16/LL10

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA Grant R03DA058238

Title: Psilocybin facilitates the effect of punishment on nicotine intake

Authors: *K. REYES¹, E. A. RAKOWSKI², N. AMISSAH¹, E. AKINYELE¹, M. J. MURAWSKI¹, P. J. MEYER¹;

¹Psychology, ²Pharmacol. & Toxicology, Univ. at Buffalo, Buffalo, NY

Abstract: Nicotine use disorder is a chronic, relapsing disorder that is a leading cause of preventable and harmful health repercussions globally. Despite availability of treatments, many individuals are unable to remain abstinent and are susceptible to relapse. Studies of psilocybin as a potential intervention suggest that this drug can promote abstinence from smoking. However, there are no published studies showing that psilocybin alters nicotine intake in animal models. In an initial study done in our lab, we found that psilocybin surprisingly increased motivation for nicotine measured by a progressive ratio test. Since there were no negative consequences associated with nicotine, rats had no incentive to reduce nicotine intake. Therefore, we tested the effect of psilocybin on nicotine intake when drug use is associated with negative consequences by pairing infusions with mild foot shocks. A total of 67 male and female Sprague-Dawley rats were trained to self-administer nicotine over 12 daily sessions, then were tested in a shock baseline session where 30% of infusions were paired with mild foot shocks (0.3 mA). Following

this, rats received an intraperitoneal injection of psilocybin (1 or 4 mg/kg) or saline, and were left undisturbed for 24 hours. Rats were then tested under the same punishment conditions for two sessions to assess treatment effects. A shock-free session was then conducted, followed by a 7-day forced abstinence period. On the 7th day, rats received a second psilocybin or saline injection. 24 hours following the injection, rats were tested in a cue-induced nicotine-seeking session, followed by a progressive ratio test to assess motivation. We found that psilocybin enhanced the reduction in intake and responding during the post-psilocybin shock session, compared to the pre-psilocybin session, but only in females treated with 1 mg/kg of psilocybin. We found no effect of psilocybin on cue-seeking. These findings suggest that psilocybin can facilitate a reduction in nicotine intake when paired with a negative consequence. These effects likely reflect psilocybin's ability to promote neural and behavioral plasticity in a way that could support lasting changes in drug-directed behavior under aversive conditions. Further studies will examine whether psilocybin facilitates responding for alternative reinforcers at the expense of drug reinforcers.

Disclosures: K. Reyes: None. E.A. Rakowski: None. N. Amissah: None. E. Akinyele: None. M.J. Murawski: None. P.J. Meyer: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.17/LL11

Topic: H.08. Drugs of Abuse and Addiction

Support: DePauw University

Title: Nicotinic Acetylcholine Receptors in Nicotine-Seeking and Avoidance Behavior of Larval Zebrafish

Authors: *H. SCHNEIDER, T. VO, G. EGGLESTON, K. DAVIS, A. WAJID, S. BAE, A. LE; DePauw Univ., Greencastle, IN

Abstract: The use of tobacco products remains one of the leading causes of preventable diseases worldwide and in the United States. Although the use of tobacco products in the United States has declined about 28 million adults in the United States still smoke. Both environmental and genetic factors contribute to nicotine use behavior. To identify targets for potential pharmacotherapeutics that could lower nicotine use behavior, we used a nicotine seeking and avoidance behavioral assay to test larval zebrafish with non-functional (knockout) alleles of nicotinic acetylcholine receptor subunit genes alpha 3 (chrna3) and alpha 5 (chrna5). The knockout of chrna3 and chrna5 genes resulted in differential shifts of nicotine seeking behavior of larval zebrafish. The chrna3 gene knockout resulted in significantly more time in the nicotine compartment compared to wildtype larval zebrafish at 10 micro molar nicotine. A similar trend was observed in chrna5 gene knockout mutants. In contrast, at high nicotine concentrations (100

micro molar) chrna5 gene knockout mutants spent significantly less time in the nicotine compartment while a similar trend was observed for chrna3 gene knockout mutants. The decrease in time spent in the nicotine compartment in knockout mutants was aligned with an increase in the time spent in the water compartment (avoidance) in the behavioral profile of each cohort. The gene knockouts impacted the percentages of nicotine seeking and avoiding larval zebrafish slightly. We saw a higher percentage of nicotine seekers at 10 micro molar nicotine in chrna3 mutants and an increased percentage of nicotine avoiders at 100 micromolar nicotine in chrna3 and chrna5 mutants. Overall, the results indicate that both chrna3 and chrna5 genes play a role in nicotine-seeking and avoidance behavior in larval zebrafish.

Disclosures: H. Schneider: None. T. Vo: None. G. Eggleston: None. K. Davis: None. A. Wajid: None. S. Bae: None. A. Le: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.18/LL12

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH NIDA R01 Grant DA051831
TRDRP T32IR4866

Title: Targeting 5-HT2A as a Novel Therapeutic Approach for Nicotine Addiction

Authors: *K. N. LAUMANN¹, C. D. FOWLER²;
²Neurobio. and Behavior, ¹Univ. of California, Irvine, Irvine, CA

Abstract: Recent studies have highlighted the potential of classical psychedelics to provide therapeutic benefits for psychiatric conditions. However, little is known about their potential role in treating substance use disorders. Classical psychedelics exert their effects primarily through actions on the 5-HT2A receptor, yet few studies have evaluated their capacity to modulate nicotine consumption. Importantly, the 5-HT2A receptor is expressed in key brain regions implicated in nicotine's actions, including the medial prefrontal cortex and the medial habenulo-interpeduncular pathway. In this study, we investigated the effects of DOI, a 5-HT2A and 5-HT2C agonist, on intravenous nicotine self-administration in male and female mice. To avoid the potential confounding effects of acute exposure, DOI was injected after a daily nicotine self-administration session, and we evaluated the subsequent effects 24 hours thereafter. We found that DOI exerted a dose-dependent reduction in nicotine intake. These results bridge a critical gap in the literature, offering evidence for the effects of psychedelics on nicotine reinforcement and supporting its potential utility as a therapeutic avenue for nicotine cessation. Further research is warranted to elucidate the precise molecular and circuit-level mechanisms underlying these effects.

Disclosures: K.N. Laumann: None. C.D. Fowler: F. Consulting Fees (e.g., advisory boards); scientific advisory board a therapeutic development company, GATC Health.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.19/LL13

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA DA039658 to CDF
TRDRP T32DT5202 to MB

Title: Taming and Triggering Nicotine Craving through Lynx2

Authors: *M. R. BAUTISTA¹, V. LALLAI², J. FOWLER¹, C. D. FOWLER³;
²Neurobio. and Behaviour, ³Neurobio. and Behavior, ¹Univ. of California, Irvine, Irvine, CA

Abstract: Significance: Nicotine addiction and nicotine-related diseases continue to be the leading cause of preventable death worldwide. Despite the availability of pharmaceutical and behavioral interventions, few individuals are successful in achieving long-term nicotine cessation. This thereby emphasizes the need to explore the neurobiological mechanisms underlying nicotine-related behaviors. Of interest, endogenous allosteric modulators acting on the nicotinic acetylcholine receptor (nAChR) have recently emerged as a novel target for drug development. Lynx2 is a negative allosteric modulator of the nAChR and has been shown to significantly decrease nAChR activity in the presence of an agonist, impacting downstream nAChR-mediated behaviors. Methods: In these studies, we assessed the global and region-specific impact of lynx2 in incubation of nicotine craving following nicotine self-administration and abstinence in mice. First, *lynx2* knockout mice and their wildtype littermates were assessed for alterations in nicotine seeking with the incubation of craving protocol following abstinence. Thereafter, wildtype mice were examined following viral mediated knockdown of *lynx2* or a scrambled vector into the medial prefrontal cortex (mPFC) prior to nicotine abstinence. Lastly, to examine time-specific effects of *lynx2* in the mPFC, *lynx2* was knocked down prior to nicotine self-administration. Results: Our data suggest that global knockout of *lynx2* prevents the incubation of nicotine craving, which is the increase in nicotine seeking following abstinence. In contrast, region-specific knockdown of *lynx2* in the mPFC increases the magnitude of nicotine seeking compared to scrambled vector control mice. Interestingly, *lynx2* knockdown in the mPFC prior to nicotine self-administration replicates the prevention of incubation of nicotine craving observed in knockout mice. Conclusion: Our data suggest that global knockout of *lynx2* prevents the incubation of nicotine craving, which is the increase in nicotine seeking following abstinence. However, region-specific and time-specific knockdown of *lynx2* illustrates the various roles that *lynx2* expressed in the mPFC play in both attenuating and exasperating cued incubation of nicotine craving. Together, these findings illustrate *lynx2*'s dynamic role in

nicotine relapse related behaviors and lay the foundation for our understanding of nAChR allosteric modulators in mediating drug seeking behaviors.

Disclosures: **M.R. Bautista:** None. **V. Lallai:** None. **J. Fowler:** None. **C.D. Fowler:** F. Consulting Fees (e.g., advisory boards); GATC Health.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.20/Web Only

Topic: H.08. Drugs of Abuse and Addiction

Title: Effect of nicotine exposure on stress threshold in rat models

Authors: ***R. C. BARNET**¹, C. DAUGHERTY², T. ZAVAR¹, E. IBRAHIM³;

¹Col. William & Mary, Williamsburg, VA; ²col William & Mary, Williamsburg, VA; ³Col. of William and Mary, Williamsburg, VA

Abstract: Rates of smoking across different demographics have greatly lowered but nicotine use remains a problem, now in the form of e-cigarettes and nicotine pouches (Sun et al., 2021). Nicotine can have paradoxical effects; smokers report they smoke to reduce stress, yet the psychoactive component of nicotine has stimulant properties increasing the release of stress hormones and other physiological markers of stress (Rohleder & Kirschbaum 2006). Moreover, nicotine has been previously found to induce both anxiogenic and anxiolytic profiles (increased anxiety and decreased anxiety, respectively), which can be dose dependent and dependent on the nature of the anxiety test (File et al. 1998). The Light Enhanced Startle (LES) paradigm (Walker & Davis, 1997) measures innate anxiety in rats elicited by bright light analogous to being in an open field during daylight hours when risk of predation might be enhanced. In LES, bursts of white noise which elicit startle reactions are presented in the presence vs. absence of a bright light (light bulb). Elevated startle in Light compared to Dark conditions is taken to reflect anxiety. Differing intensities of light (light bulb wattage) is akin to varying levels of environmental stressors. Two experiments were conducted in order to compare effects of acute (i.p.) nicotine exposure on anxiety measured in LES, under conditions in which the magnitude of the environmental stressor (light bulb wattage) was varied. As reported by Walker and Davis (1997), Experiment 1 revealed that increasing light intensity monotonically increased the magnitude of LES. Experiment 2 examined the effect of acute nicotine on LES under different levels of environmental stress (different light intensities). Lower doses of nicotine (0.15 mg/kg) induced an anxiolytic response in both males and females in the moderate (25-W) but not low intensity (15-W) stress condition. Higher doses (0.40 mg/kg) induced an anxiogenic effect in males but not females. The sex-dependent effects are discussed in terms of the posterodorsal region of the medial nucleus of the amygdala and the encapsulated region of the bed nucleus of the stria terminalis being 85% and 97% larger, respectively, in males (Hines et al. 1992). In addition, males exposed to the high 0.40 mg/kg nicotine dose tested under low environmental

stress (15-W bulb) demonstrated a level of anxiety (LES) equivalent to the saline treated male rats tested under conditions of moderate environmental stress (25-W bulb). This latter finding suggests, in the presence of nicotine, a “low level” environmental stressor can produce anxiety levels commensurate with a “highly” stressful environmental threat.

Disclosures: R.C. Barnet: None. C. Daugherty: None. T. Zavar: None. E. Ibrahim: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.21/LL14

Topic: H.08. Drugs of Abuse and Addiction

Title: Environmental enrichment attenuates enhanced nicotine-seeking behavior following exposure to chronic variable stress

Authors: *J. J. CORTRIGHT, K. STEELE, S. STEELE, D. ANDERSON, I. BLETT, E. JOHNSON;

Psychological Sci., Univ. of Wisconsin - River Falls, River Falls, WI

Abstract: Drug addiction is a major public health and serious economic concern in the United States costing taxpayers billions of dollars annually. Preclinical and clinical research indicates that individuals exposed to stress are more vulnerable to drug addiction (Koob, 2008; Ouimette et al., 2007). For example, acute stress exposure increases and reinstates nicotine-seeking behavior as measured by conditioned place preference (CPP) (Leão et al., 2009). Moreover, chronic unpredictable stress increases the response to nicotine-rewarding properties in the CPP test (Biala et al., 2018) and, more recently, we have shown the exposure to variable stress produces an enhanced, or sensitized, response to nicotine in an animal model of addiction (Cortright et al., in preparation). Contrary to the effects observed following exposure to variable stress, both running and environmental enrichment (EE) reduce CPP for cocaine (Mustroph et al., 2016). Based on the interactions observed between EE and chronic stress on learning and memory (Wright & Conrad, 2008), it is reasonable to postulate similar interactions on the rewarding properties of nicotine. To date, only one study (Biala et al., 2018) has assessed the effects of chronic stress on the rewarding effects of nicotine in the CPP model and none have measured the potential of EE in attenuating this effect. Therefore, this study will further investigate and determine whether EE can reverse, or abolish, the enhancement in the rewarding properties of nicotine following exposure to chronic variable stress. One group of animals will be exposed to 20 days of randomized stressors either during adolescence or early adulthood. Another group of animals will be exposed to four weeks of EE. An additional group will be exposed to 20 days of randomized stressors followed by four weeks of EE. Control animals will not receive any exposure treatment. Following exposure treatment animals will undergo place preference conditioning to nicotine (0.175 mg/kg, base, s.c.). Data indicate that animals exposed to chronic variable stress exhibit higher place preference for nicotine compared to controls, an

effect that is attenuated by exposure to EE. Further data analysis will consist of comparisons between sex and adolescence versus early adulthood stress exposure. Therefore, in addition to providing evidence of the contributions of stress and environmental conditions to successful cessation of drug-seeking, the results obtained can have important implications for the use of environmental stimulation together with pharmacological and behavioral therapies in the treatment of addiction.

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Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.22/LL15

Topic: H.08. Drugs of Abuse and Addiction

Title: Epigenetic effects of offspring of adult rats exposed to chronic variable stress on enhanced nicotine-seeking behavior

Authors: *K. G. STEELE, S. STEELE, J. J. CORTRIGHT;
Psychological Sci., Univ. of Wisconsin - River Falls, River Falls, WI

Abstract: Substance use remains highly prevalent, with products like vapes and e-cigarettes becoming one of the most widely accepted forms of substance use. Nicotine remains very relevant, as tobacco and other vaping products are continually being consumed worldwide, and exposure to tobacco products can alter behavior and neurobiology from generation to generation (Goldberg et al., 2021). Exposure to chronic stress has been found to impact human development and the development of disease, resulting in an increased risk of exhibiting substance-seeking behavior (Jenkins et al., 2021). The effects of parental exposure to stimuli on subsequent developmental changes have previously been studied using models of fear conditioning in rats whose parents were exposed to odorous or shocking stimuli. Such studies utilized conditioned fear responses to acetophenone, which activates an odorant receptor (Olfr151), in paternal rats prior to conception to analyze transgenerational and multigenerational effects on the following generations. Altered epigenetic marks, specifically DNA hypomethylation in sperm of F0 and F1 generations, were found to attribute to transgenerational inheritance of behavioral and neuroanatomical changes in offspring without prior exposure to the odor (Dias & Ressler, 2014). Few studies, however, have investigated the epigenetic effects on enhanced nicotine seeking behavior of offspring of adult rats exposed to chronic variable stress. Therefore, this study will investigate the effects of prenatal maternal chronic variable stress on conditioned place preference to nicotine in subsequent generations and the epigenetic processes involved. One group of female rats will be exposed to 20 days of randomized stressors, while control rats will not receive any exposure treatment. Following exposure treatment, animals will be bred, and offspring will undergo place preference conditioning to nicotine (0.175 mg/kg, base, s.c.). To

date, preliminary data indicate that animals bred from a mother exposed to chronic variable stress exhibit higher place preference for nicotine compared to animals bred from controls. In addition to providing evidence of how the experiences of a parent before conceiving offspring can influence the function of the nervous system and behavior of offspring, the results obtained can have important implications for the treatment of addiction.

Disclosures: K.G. Steele: None. S. Steele: None. J.J. Cortright: None.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.23/LL16

Topic: H.08. Drugs of Abuse and Addiction

Title: Exercise Intervention for the Gateway Effect of Nicotine on Subsequent Cocaine Self-Administration

Authors: *R. ROBERTS^{1,2}, C. V. PHAM⁴, K. BASTANIFAR³, S. LOTFIPOUR^{5,2};

¹Univ. of California Irvine, Irvine, MD; ²Exptl. Pathology, ³Pharmaceut. Sci., Univ. of California Irvine, Irvine, CA; ⁴Charlie Dunlop Sch. of Biol. Sci., ⁵Emergency Medicine, Pharmacology, & Pathology, Univ. of California, Irvine, Irvine, CA

Abstract: In 2024, nearly 20% of middle and high school students in the U.S. reported having tried a tobacco product. Nicotine, the active compound in these tobacco products, has long been established as the precursor for other drugs of abuse via the gateway effect. Adolescence is marked by an increased vulnerability to long-term changes on the brain including the effects of nicotine and other drugs of abuse. Our lab has demonstrated that an initiation dose of nicotine during adolescence enhances subsequent cocaine self-administration in rats, which does not occur when nicotine initiation occurs in adulthood. Furthermore, there are no effective age-appropriate strategies for nicotine use disorders nor are there FDA-approved therapies for cocaine use disorder. This necessitates exploring other interventions such as exercise, which has demonstrated promising results in blunting reward seeking-behavior for various drugs of abuse. However, there is limited data regarding the efficacy of exercise as an intervention during adolescence especially as it relates to multiple drug use. To determine whether exercise can mitigate this vulnerability to nicotine, Wild-Type Sprague Dawley rats will undergo the 10-day nicotine pretreatment paradigm paired with 2 hours of daily wheel running, then followed by cocaine intravenous self administration (IVSA). I will test the hypothesis that early life exercise will disrupt the gateway effect, leading to lower reinforced responses during cocaine IVSA. The preliminary results from the male rats indicate successful replication of the gateway effect in the nicotine pretreated-sedentary rats. Additionally, there is evidence for exercise as a disruptor of the gateway effect exhibited by lowered cocaine self-administration in the exercising rats compared to their sedentary counterparts This ongoing research will contribute to the development of non-drug, age-appropriate therapeutic interventions for adolescents and serve as

a foundation for investigating the underlying methods of these observations. Further studies will provide novel mechanistic insight to understanding nicotine's gateway effects to support more efficient prevention and intervention therapeutics.

Disclosures: **R. Roberts:** None. **C.V. Pham:** None. **S. Lotfipour:** A. Employment/Salary (full or part-time): University of California Irvine.

Poster

PSTR354: Nicotine: Behavioral and Cognitive Effects

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR354.24/LL17

Topic: H.08. Drugs of Abuse and Addiction

Title: From genes to behavior: nAChR subunit alleles modulate anxiety, memory, and locomotion in humanized rodents

Authors: ***B. M. TIMMERMAN**¹, **S. LOTFIPOUR**²;

¹Biol., ²Emergency Medicine, Pharmacology, & Pathology, Univ. of California, Irvine, Irvine, CA

Abstract: The subunit composition of nicotinic acetylcholine receptors (nAChRs) can impact pharmacology, neurochemistry and behavior. The *CHRNA6* gene, encoding the $\alpha 6$ nAChR subunit, has been shown to be critical for the modulation of dopamine release, locomotion, and reward related behaviors. In humans, a single nucleotide polymorphism (SNP) in the *CHRNA6* gene 3'-untranslated (UTR) region has been shown to be associated with increased nicotine/tobacco use during adolescence. This work is supported by our animal studies using a CRISPR-Cas9 genetically engineered humanized *CHRNA6* 3'UTR rat line. Previous findings illustrate that risk allele carriers (GG) have enhanced nicotine plus cue-primed reinstatement behavior in male adolescent rodents, which predict tissue level neurotransmitters in brain reward-related regions. Interestingly, baseline tissue neurotransmitter levels in the *CHRNA6* 3'UTR SNP line exhibit a developmental maturation, with adult neurotransmitter levels differing from those of adolescents based on genotype, brain region, and sex. The current studies aim to evaluate whether adult behaviors in the *CHRNA6* 3'UTR SNP line exhibit differences in anxiety-like behavior, spatial memory, fear conditioning, and locomotor activity. Our preliminary findings illustrate that risk allele GG subjects display impaired spatial learning and memory in the Morris Water Maze, heightened conditioned responses during cued & contextual fear conditioning and increased locomotor activity in the Elevated Plus Maze. These findings provide new insight into how genetic variations in an untranslated region of the *CHRNA6* gene can impact adult behaviors.

Disclosures: **B.M. Timmerman:** None. **S. Lotfipour:** None.

Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.01/LL18

Topic: H.05. Mood Disorders

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NIH grant U01NS128660
One Mind – COMPASS Rising Star Award (A.C.K.)
NIH fellowships F30MH129085

Title: Psilocybin's lasting action requires pyramidal cell types and 5-HT_{2A} receptors

Authors: *L. SHAO¹, C. LIAO¹, N. K. SAVALIA¹, Q. JIANG², A. C. KWAN³;
¹Cornell Univ., Ithaca, NY; ²Cornell Univ., ITHACA, NY; ³Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Psilocybin, a serotonergic psychedelic, has shown rapid and long-lasting therapeutic potential for psychiatric disorders. While its capacity to promote dendritic spine growth is well documented, how these structural changes translate to cell-type-specific circuit mechanisms remains unclear. Here, we dissected the cortical pyramidal circuits underlying psilocybin's effects using in vivo two-photon imaging, chemogenetic perturbation, and cell-type-specific electrophysiology in the mouse medial frontal cortex. We demonstrate that a single dose of psilocybin (1 mg/kg, i.p.) alleviates stress-induced behaviors. Remarkably, chemogenetic silencing of pyramidal tract (PT) neurons—subcortically projecting excitatory cells—abolishes the behavioral benefits of psilocybin, while inactivation of intratelencephalic (IT) neurons has no effect. Structurally, psilocybin increases dendritic spine density in both PT and IT neurons; however, only PT neurons show enhanced synaptic calcium transients and elevated firing rates following treatment. Targeted knockout of 5-HT_{2A} receptors abolishes psilocybin's effects on stress-related behaviour and structural plasticity. Collectively, this study highlights a cortical cell-type-specific and 5-HT_{2A} receptor mechanism that may underlie psilocybin's long-term therapeutic potential.

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.02/LL19

Topic: H.05. Mood Disorders

Support: Ekam Imaging

Title: Treating repetitive mild head injury with psilocybin: A preclinical MRI study

Authors: *E. K. BRENGEL^{1,2}, B. AXE², A. MAHESWARI², M. I. ABEER⁴, R. J. ORTIZ⁵, T. J. WOODWARD⁶, R. UTAMA², B. RODRIGUE⁷, N. J. CAVALLARO^{2,3}, C. SAWADA², S. BALAJI², P. P. KULKARNI^{1,2}, A. AGUILAR VALLES⁷, H. B. BRADSHAW⁶, M. A. GITCHO⁴, C. F. FERRIS^{1,2,3};

¹Dept. of Psychology, ²Ctr. for Translational Neuroimaging, ³Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA; ⁴Biol. Sci., Delaware State Univ., Dover, DE; ⁵Northern Illinois Univ., Dept. of Psychology, Aurora, IL; ⁶Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; ⁷Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: Psilocybin (PSI), a psychedelic 5HT-2A agonist, has garnered attention as an effector of neuroplasticity. While its therapeutic effects have been hypothesized to extend to traumatic brain injury (TBI), it has not yet been tested. In this study, short-term and lasting effects of PSI treatment on brain health following repetitive mild TBI (rmTBI) were examined using an array of preclinical assays from neuroradiology, molecular biology, and behavior. Adult female Wistar rats ($N=24$) were housed on a reverse light-dark cycle and subjected to awake closed-head momentum exchange mTBI or sham injury once daily for three days, followed by PSI or vehicle injection. On Day 3, plasma samples were collected for quantification of circulating small lipid biomarkers before rats received diffusion weighted imaging (DWI) on a 7T MRI scanner for microstructural examination. Cognitive and motor behaviors were assessed with Head Twitch, Open Field, Novel Object Recognition, Rotarod, and Beam Walk tests. Three weeks later, microstructural recovery was assessed with DWI, cerebrovascular reactivity was measured by fMRI with CO₂ challenge, and circuitry was measured with awake functional connectivity scanning (FC). Brains were collected for protein analysis of neurodegeneration, neuroinflammation, and neuroplasticity markers. Day 3 DWI indicates vasogenic edema is induced globally by rmTBI and partially reduced by PSI ($p<0.00005$). This pattern was reflected in the prefrontal cortex, sensorimotor cortex, and hippocampus ($p<0.05$), while PSI completely prevented vasogenic edema in the thalamus, basal ganglia, and olfactory system ($p<0.005$). By Day 22, all microstructural changes were resolved; however, lasting functional alterations were observed. fMRI indicates a hyperactive BOLD response to 5% CO₂ challenge is induced by prior rmTBI. This functional alteration is limited globally by PSI and prevented entirely in the prefrontal cortex and olfactory system ($p<0.0005$). FC indicates significant loss of midbrain DA system connectivity due to rmTBI is prevented and enhanced beyond control levels by PSI. Protein analysis indicates PSI may prevent phosphorylated tau buildup, promote BDNF signaling, and promote neuroprotective astrocytosis and microgliosis. Behavioral effects were minimal. These findings establish a body of evidence supporting the therapeutic potential of PSI for rmTBI. Future studies will examine the mechanistic role of BDNF, assess the efficacy of delayed PSI treatment, and deploy myelin water fraction MRI to longitudinally track changes in myelination in vivo. Preprint: <https://doi.org/10.1101/2025.02.03.636248>

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.03/LL20

Topic: H.05. Mood Disorders

Support: NIH R01 Grant DA056660
Brain PRIME UAB Fellowship

Title: Tabernanthalog: A Novel Psychedelic-inspired Compound for Chronic Pain and Opioid Use Disorder

Authors: *A. M. SCHWARZ^{1,2}, J. A. HEINSBROEK², J. PETERS³, M. VANHART², K. SARNER²;

¹Univ. of Alabama in Birmingham, Birmingham, AL; ²Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; ³Neurobio., U. of Alabama at Birmingham, Birmingham, AL

Abstract: Opioid use disorder remains a major health issue and is complicated by high rates of comorbid chronic pain. Ibogaine, a psychedelic-like compound found in the African shrub *Tabernaemontana iboga*, attenuates opioid use and relapse in human studies and reduces chronic pain in pre-clinical work. However, ibogaine's therapeutic utility is limited due to it producing prominent hallucinations and cardiotoxic side effects. Tabernanthalog (TBG) is a nonhallucinogenic, non-cardiotoxic, and non-rewarding analog of ibogaine. If TBG can provide analgesia alongside the anti-addictive properties without the adverse effects of ibogaine, it would have significant therapeutic potential. To test TBG's analgesic effects, we used an inflammatory model of chronic pain in mice and assessed TBG's efficacy in analgesia and pain-augmented opioid seeking. Male and female mice were injected with Complete Freund's Adjuvant (CFA; 50µL) in their left hind paws to elicit robust inflammation and pain. After one week, mice were given TBG (40mg/kg; i.p.) or vehicle (saline), and pain behavior was measured using von Frey. TBG did not reduce pain in the short term, but two days after treatment, TBG-treated mice showed decreased pain-like behaviors compared to controls. These data indicate that TBG produces delayed analgesic effects in a chronic inflammatory pain model. We previously demonstrated that TBG attenuates opioid seeking behavior and sought to determine if it could do so in a mouse model of pain-augmented opioid seeking. One week before CFA injections, mice

were first trained to self-administer sucrose. One week after CFA treatment, mice received TBG or vehicle and started daily heroin self-administration. Two days following the injection on increasing effort requirement across days (fixed ratio FR1, FR3, and progressive ratio). Preliminary data indicate that female but not male CFA mice showed long-term reduction in opioid seeking after a single injection of TBG. Our data show strong promise for the combined treatment of comorbid opioid use disorder and chronic pain with TBG. Ongoing studies are examining the underlying mechanisms responsible for these effects and the generalizability to other models of chronic pain.

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

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Program #/Poster #: PSTR355.04/LL21

Topic: H.05. Mood Disorders

Support: NIH R01 MH136708 to JRM
NIH Grant R01 MH133849 to JDM

Title: Sustained rescue of cognitive flexibility with psilocybin following chronic unpredictable stress in rats

Authors: *J. J. HENNESSEY^{1,2}, D. B. NOWAK¹, D. R. OLIVEIRA, Sr.¹, J. MCCORVY³, J. R. MANTSCH¹;

¹Pharmacol. and Toxicology, ²Med. Scientist Training Program, ³Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Rationale: Psychedelic-assisted therapy (PAT) shows immense potential for the treatment of a wide range of neuropsychiatric disorders. However, much is still unknown about the neural mechanisms of action of psychedelics. Cognitive flexibility has been shown to be impaired in many neuropsychiatric diseases, including depression, and PAT has been shown to promote cognitive flexibility in both the short- and long-term in these patients. We sought to model cognitive flexibility in animals to investigate the pharmacological and neural basis of long-term promotion of cognitive flexibility with psychedelics. Methods: Male Sprague-Dawley rats (8-10 weeks old upon arrival) were trained on a within-session attentional set-shifting task and then exposed to 21d of chronic unpredictable stress (CUS) while training continued. 12 hours after the last stressor animals received a single dose of psilocybin (1 mg/kg, i.p.) or saline. Performance on the original within-session attentional set-shifting task was assessed 20min and 24h post-injection; on days 5-9 animals were challenged with a novel extradimensional shift and a reversal of this new rule. Results: CUS exposure did not affect familiar extradimensional set-shifts but increased trials to criterion on the novel extradimensional shift. Psilocybin did not alter

familiar shifts at 20min or 24h in any group, yet normalized novel-shift performance in CUS rats relative to stressed controls on days 5-9. Effects on reversal learning were variable. All groups successfully acquired the novel tone rule, indicating this paradigm can be used in future set-shifting experiments. **Conclusions:** Chronic stress selectively disrupts the capacity to adopt new attentional sets while sparing well-rehearsed behavioral strategies and set-shifts between those familiar strategies. A single exposure to psilocybin produced a sustained rescue of this higher-order flexibility, extending prior acute findings to a disease-relevant model. This work advances understanding of the cognitive consequences of stress and provides a highly translational model for studying the effects of psychedelics on models of disease states in rodents.

Disclosures: **J.J. Hennessey:** None. **D.B. Nowak:** None. **D.R. Oliveira:** None. **J. McCorvy:** 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

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.05/LL22

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: A single administration of psilocybin is ineffective in reversing PTSD-like sequelae in a rat model of stress-enhanced fear learning

Authors: ***K. M. KING**, M. J. SAVINI, N. M. MOLNAR, E. J. SCHNEIDER, S. E. STRAUB, J. PARKER, L. P. PUPPEL, A. K. AL-OLIMAT, A. M. BACH, P. R. ZOLADZ;
Psychology & Educ., Ohio Northern Univ., Ada, OH

Abstract: Recent work has generated a great deal of enthusiasm about using psychedelics to treat major depression. Traditional antidepressants take several weeks to exert therapeutic effects, and at least one-third of patients fail to respond at all. In contrast, psychedelic substances, such as psilocybin, have demonstrated rapid, long-lasting antidepressant effects after only a single administration. There is also work suggesting that psychedelics may exert anxiolytic effects and be useful in treating more complex psychiatric conditions, such as post-traumatic stress disorder (PTSD). However, less is known about the impact of these agents on PTSD-related symptoms. Thus, we aimed to determine whether a single administration of psilocybin could reverse PTSD-like sequelae induced by stress-enhanced fear learning in rats. Adult male and female Sprague-Dawley rats were placed in Context A (circular, white conditioning box) and exposed to several uncontrollable footshocks (Stress) or no footshocks (No Stress). The next day, the rats were placed in Context B (square conditioning box with clear Plexiglas sides); all rats underwent contextual fear conditioning and were given a single mild electric footshock. Four weeks later, the rats were injected with 1 mg/kg psilocybin (PSIL) or vehicle. The next day, the rats began a battery of behavioral tests that assessed their freezing behavior in Context B, anxiety-like behavior on the elevated plus maze (EPM), locomotor

activity in the open field test (OFT), and neophobic behaviors in a novel object recognition assessment. Upon re-exposure to Context B, stressed rats exhibited significantly greater freezing than non-stressed rats, verifying the classic stress-enhanced fear learning phenomenon. Relative to controls, stressed rats also exhibited fewer overall arm entries on the EPM and traveled less distance in the OFT. All of these effects were independent of sex, and none were reversed by PSIL. Overall, these findings indicate that a single administration of PSIL is ineffective at reversing PTSD-related sequelae that result from stress-enhanced fear learning.

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.06/MM1

Topic: H.05. Mood Disorders

Title: Acute and durable miniscope-based neurobehavioral profiling of 5-HT_{2A} receptor agonists with hallucinogenic and non-hallucinogenic potential in mouse medial prefrontal cortex

Authors: C. A. BOWEN¹, E. ANDRZEJAK¹, D. CHENG², S. HUANG², N. REN², L. BELLIER², J. J. NASSI², *G. SHORT¹;

¹atai Therapeutics, Inc., New York, NY; ²Inscopix, Inc., Mountain View, CA

Abstract: Psychedelic 5-HT_{2A} receptor (5-HT_{2AR}) agonists such as psilocin have been reported to produce rapid and lasting antidepressant effects in humans. Preclinical neuroplasticity and behavioral studies also support antidepressant-like activity of non-hallucinogenic 5-HT_{2AR} agonists. The medial prefrontal cortex (mPFC) is associated with antidepressant effects and expresses 5-HT_{2AR}. We tested the hypothesis that 5-HT_{2AR} agonists exert acute and durable effects on mPFC neuronal activity that may mediate rapid and lasting antidepressant activity. We injected AAV1.CaMK2.GCaMP6f into the anterior cingulate cortex of 13 adult male mice and implanted them with a microendoscopic GRIN lens at the same site. After surgical recovery, 5-HT_{2AR} agonists with (psilocin, EGX-K) or without (2-Br-LSD, EGX-J) hallucinogenic potential were tested in freely behaving mice. Vehicle and 3 doses of each compound were assessed in sessions separated by at least 72h. In each session, we recorded miniscope-based one-photon calcium imaging of mPFC pyramidal neurons and synchronized movies of open field locomotion for 10 min prior to compound injection (baseline) and for 10 min after dosing at T_{max} (acute effect) and 24h (durable effect). Testing of individual compounds was separated by at least 1 week. Neurobehavioral profiles of effects relative to vehicle were computed and compared across compounds. Psilocin, 2-Br-LSD and EGX-J dose-dependently reduced locomotion acutely, while EGX-K increased it. Psilocin, 2-Br-LSD and EGX-K dose-dependently increased average neuronal spike rate acutely, while EGX-J reduced it. Psilocin and 2-Br-LSD dose-

independently reduced the correlation of neural activity between cells and disrupted the correlation structure acutely, while EGX-J and EGX-K had no consistent effects. No durable effects were detected following any test condition. For psilocin and 2-Br-LSD, we applied multi-session registration to paired acute and 24h recordings to confirm the absence of any durable effects at the single cell level. We also applied PCA and hierarchical clustering analyses to compare the rich neurobehavioral profiles across compounds. While psilocin and 2-Br-LSD showed similar effects, EGX-J and EGX-K exhibited distinct and strikingly opposing effects across metrics, suggesting that different mPFC mechanisms may underlie the action of these 5-HT_{2A}R agonists. The absence of any effects at 24h suggests that durable changes in mPFC pyramidal neuron activity are unlikely to mediate the lasting antidepressant effects of these 5-HT_{2A}R agonists. Future work may include longer-term evaluation of neural activity and the effects of repeated dosing.

Disclosures: **C.A. Bowen:** A. Employment/Salary (full or part-time): atai Life Sciences. **E. Andrzejak:** A. Employment/Salary (full or part-time): atai Life Sciences. **D. Cheng:** A. Employment/Salary (full or part-time): Inscopix. **S. Huang:** A. Employment/Salary (full or part-time): Inscopix. **N. Ren:** A. Employment/Salary (full or part-time): Inscopix. **L. Bellier:** A. Employment/Salary (full or part-time): Inscopix. **J.J. Nassi:** A. Employment/Salary (full or part-time): Inscopix. **G. Short:** A. Employment/Salary (full or part-time): atai Life Sciences.

Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.07/MM2

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Modulation of Psilocybin's Behavioral Effects by Carbamazepine: Evidence for Pharmacologic Interactions in Rats

Authors: ***M. SAKIB**¹, **E. ZOLALI**¹, **J. WILKERSON**⁴, **L. R. MCMAHON**², **S. OBENG**³;
¹Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX; ²Office of Res., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; ³Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX; ⁴Dept. of Pharmaceut. Sci., TTUHSC, Amarillo, TX

Abstract: Psychedelics, especially psilocybin, are being actively investigated for their potential in treating refractory psychiatric conditions such as major depressive disorder, posttraumatic stress disorder, and substance use disorder. With over 50 psilocybin-related clinical trials recruiting (clinicaltrials.gov) and millions of individuals in the U.S. affected by these disorders, understanding drug interactions that may affect psilocybin efficacy or safety is crucial. This study examined the interaction between psilocybin and carbamazepine, a CYP450 enzyme inducer commonly prescribed for neuropsychiatric disorders. Using a drug discrimination procedure, male (n=4) and female (n=4) Sprague Dawley rats were trained to differentiate the 5-HT_{2A} agonist DOI (0.32 mg/kg) from vehicle. Psilocybin, DOI, saline, M100907 (a selective 5-HT_{2A} antagonist) were administered intraperitoneally 30 minutes before DOI. DOI significantly increased the number of correct responses compared to vehicle, saline, and M100907. Psilocybin significantly reduced the number of correct responses compared to vehicle, DOI, and saline. DOI and psilocybin significantly reduced the number of incorrect responses compared to vehicle, saline, and M100907. DOI and psilocybin significantly increased the number of omissions compared to vehicle, saline, and M100907. DOI and psilocybin significantly increased the number of failures compared to vehicle, saline, and M100907. DOI and psilocybin significantly increased the number of correct responses compared to vehicle, saline, and M100907. DOI and psilocybin significantly increased the number of correct responses compared to vehicle, saline, and M100907.

HT2A antagonist), and carbamazepine were administered alone and in combination. Both DOI (ED₅₀ = 0.13 mg/kg) and psilocybin (ED₅₀ = 0.45 mg/kg) elicited dose-dependent drug-appropriate responding. Pretreatment with M100907 shifted the discriminative stimulus dose-response functions of both DOI and psilocybin to the right by 6.5 and 2.0-fold, respectively, suggesting 5-HT_{2A} receptor mediation. In contrast, co-administration with carbamazepine produced leftward shifts (10.2-fold for psilocybin; 5.8-fold for DOI), indicating enhanced discriminative stimulus effects. Notably, carbamazepine did not substitute for DOI. These results imply that carbamazepine may enhance psilocybin's behavioral effects through pharmacokinetic (e.g., enzyme interaction) or pharmacodynamic mechanisms. Such interactions could potentially elevate psilocybin's bioavailability or receptor-mediated effects, raising important safety considerations in clinical settings. Ongoing work is focused on delineating the relative contributions of these mechanisms.

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

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Program #/Poster #: PSTR355.08/MM3

Topic: H.05. Mood Disorders

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Title: Psilocybin triggers an activity-dependent rewiring of large-scale cortical networks

Authors: *Q. JIANG^{1,2}, A. C. KWAN², L. SHAO², S. YAO⁴, N. K. SAVALIA³, P. A. DAVOUDIAN⁵, G. TIAN⁷, H. LAI⁹, K. BEIER⁸, H. ZENG⁶;

¹BME, Cornell Univ., ITHACA, NY; ²Biomed. Engin., Cornell Univ., Ithaca, NY; ³Biomed. Engin., Cornell Univ., ITHACA, NY; ⁴Mol. Genet., Allen Inst. for Brain Sci., Seattle, WA;

⁵Allen Inst. for Brain Sci., New Haven, CT; ⁶Allen Inst. for Brain Sci., Seattle, WA; ⁸Physiol. & Biophysics, ⁷Univ. of California Irvine, Irvine, CA; ⁹The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Psilocybin has shown promise for treating mental disorders by inducing structural and functional plasticity in the brain. Using monosynaptic rabies tracing, we mapped the brain-wide distribution of inputs to pyramidal neurons in the dorsal medial frontal cortex of mice. We found

that psilocybin reshapes connectivity in a network-specific manner, enhancing inputs from perceptual and medial regions to subcortical targets while reducing recurrent cortico-cortical loops. The rewiring pattern differs between pyramidal tract (PT) and intratelencephalic (IT) neurons, with opposing changes in their presynaptic input sources. Furthermore, drug-evoked spiking activity plays a crucial role in determining whether specific inputs are strengthened. These findings provide insight into how psilocybin reorganizes large-scale cortical networks and suggest neural activity modulation as a potential approach to shaping psychedelic-induced plasticity.

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.09/MM4

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Discovery of novel 5-HT_{2A} receptor partial agonist without hallucinogenic effects

Authors: ***L. ZHAO**, H. JIA, D. CHEN, C. ZHANG, D. XU, Y. QU, J. H. SHEN;
NeuShen Therapeut., Shanghai, China

Abstract: The potential of psychedelic substances—such as psilocybin and MDMA—to treat mental disorders has recently attracted considerable attention. These compounds can produce long-lasting reductions in depression and anxiety with just one or two doses. However, their therapeutic use is limited by intense hallucinogenic effects, which necessitate close clinical supervision. The principal psychedelics are either tryptamines or phenylethylamine derivatives, and they are believed to exert their psychological effects by activating the serotonergic 5-HT_{2A} receptor via both Gq and β -arrestin2 signaling pathways. Crucially, 5-HT_{2A}-Gq efficacy appears necessary for psychedelic activity: studies suggest that only compounds whose Gq-mediated calcium-flux efficacy exceeds 70% of the 5-HT E_{max} produce hallucinogenic effects. NS-079 is a novel 5-HT_{2A} partial agonist designed to deliver therapeutic benefits without inducing hallucinogenic side effects. It demonstrates a 5-HT_{2A} EC₅₀ of 30 nM and a Gq E_{max} of less than 50%, with negligible activity at 5-HT_{2B} receptors (EC₅₀ > 10 μ M). In cultured rat cortical neurons, NS-079 robustly promotes dendritogenesis and spinogenesis. In vivo, NS-079 elicits rapid and robust antidepressant effects in both the chronic unpredictable mild stress (CUMS) and learned helplessness (LH) models. Notably, NS-079 does not induce a head twitch response (HTR) in mice, confirming its non-hallucinogenic profile as a 5-HT_{2A} agonist, and it shows no abuse potential in conditioned place preference (CPP) assays.

Overall, NS-079 offers a favorable pharmacological profile compared to psilocybin and shows significant therapeutic potential for mood disorders and other indications. It is currently undergoing IND-enabling studies, with further details to be presented.

Disclosures: **L. Zhao:** A. Employment/Salary (full or part-time): NeuShen Therapeutics. **H. jia:** A. Employment/Salary (full or part-time): NeuShen Therapeutics. **D. Chen:** A. Employment/Salary (full or part-time): NeuShen Therapeutics. **C. Zhang:** A. Employment/Salary (full or part-time): NeuShen Therapeutics. **D. Xu:** A. Employment/Salary (full or part-time): NeuShen Therapeutics. **Y. Qu:** A. Employment/Salary (full or part-time): NeuShen Therapeutics. **J.H. Shen:** A. Employment/Salary (full or part-time): NeuShen Therapeutics.

Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.10/MM5

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Support: NIH Grant ZIA DA000523
Alexander Shulgin Research Institute (ASRI)

Title: Effects of deuteration or fluorination of MDMA-like compounds on monoamine transporter activities

Authors: ***M. H. BAUMANN**¹, D. WALTHER¹, P. F. DALEY², N. V. COZZI³;
¹IRP, NIDA, NIH, Baltimore, MD; ²Alexander Shulgin Res. Inst., Lafayette, CA; ³Univ. of Wisconsin, Madison, WI

Abstract: 3,4-Methylenedioxymethamphetamine (MDMA) is an empathogen compound that has shown promise in treating post-traumatic stress disorder (PTSD). Here, we explored the *in vitro* pharmacology of deuterated or fluorinated analogs of MDMA and its cathinone congener, methylone. Compounds were tested for their abilities to inhibit monoamine neurotransmitter uptake and stimulate release at transporters for dopamine (DAT), norepinephrine (NET), and serotonin (SERT) in rat brain synaptosomes. The compounds were also evaluated for metabolism by human liver microsomes. MDMA and its *N*-ethylated analog (MDEA) were fully efficacious uptake inhibitors at DAT, NET, and SERT, with preference for SERT. Methylone and its *N*-ethylated analogue (ethylone) were fully efficacious uptake inhibitors, with similar potencies across transporters. For MDMA and methylone analogs, deuteration was well tolerated, whereas fluorination reduced uptake inhibition potency or eliminated activity altogether. MDMA and MDMA-d3 were fully efficacious substrate-type releasers at all transporters. MDEA was a fully efficacious releaser at SERT, a partial releaser at NET, and devoid of releasing activity at DAT. When the ethylamino of MDEA incorporated a single fluorine substitution (MDEA-F), transporter-mediated releasing activity was abolished. Methylone and methylone-d3 were fully

efficacious releasers at all transporters, while ethylone and ethylone-d5 were fully efficacious releasers at SERT, partial releasers at NET, and devoid of releasing activity at DAT. When the ethylamino of ethylone incorporated two fluorine atoms (ethylone-F2), releasing activity was lost. MDMA, MDMA-d3, and the cathinones were metabolically stable in liver microsomes, but MDEA-F was rapidly metabolized. In summary, deuterated analogs of MDMA and methylone retain uptake inhibiting and releasing properties at monoamine transporters, while fluorinated versions exhibit greatly diminished or complete loss of activity.

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Poster

PSTR355: Translational Studies of Hallucinogens and Related Compounds

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR355.11/MM6

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Office of the Vice President Research Brown University
P20 GM130414

Title: Development of active placebo for MDMA-assisted therapy randomized controlled trials

Authors: L. NUNEZ SEVERINO¹, *C. L. HAASS-KOFFLER²;

¹Brown Univ., Jamaica, NY, ; ²Brown Univ., PROVIDENCE, RI

Abstract: The empathogen 3,4-methylenedioxymethamphetamine (MDMA) is a psychoactive substance that has been studied in recent years in conjunction with assisted therapy (AT) to treat psychiatric disorders. Within many studies utilizing MDMA-AT, blinding procedures have served as a challenge in compromising outcomes. In addition to subjective response, transient increases in blood pressure in the MDMA condition were indicative of patient assignment, potentially compromising blinding. The goal of this study was to optimize low doses of MDMA to be utilized as an active placebo in future MDMA randomized controlled trials (RCT). We first conducted a scoping review of published RCTs that used different doses of MDMA in conjunction with therapy on patients with post-traumatic stress disorder (PTSD) and healthy volunteers. Then, we extracted data on blood pressure, blinding outcomes, and adverse events to monitor the effect of multiple doses of MDMA. Six studies were included that collected data on changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP). Three studies consisted of individuals with PTSD (n=66) and three of healthy volunteers (n=81) with prior MDMA use. All studies showed a dose-response increase on hemodynamics parameters after MDMA administration. In individuals with PTSD, doses between 30-40 mg were sufficient to increase SBP above 10 mmHg. In healthy volunteers, doses between 38-75 mg were needed to increase SBP by 10 mmHg. Similarly, in individuals with PTSD, doses between 15-38 mg were sufficient to increase DBP above 5 mmHg. In healthy volunteers, doses between 37.5-120 mg were needed to increase DBP above 5 mmHg. Blinding efficacy was reported in three studies

utilizing individuals with PTSD and one study utilizing healthy volunteers with previous MDMA use. Correct condition guess rate was 52% for participants and 77% for investigators. Within the studies utilizing participants with PTSD diagnoses, there was no significant increase in adverse events following administration of low doses of MDMA. These findings indicate that low-dose MDMA (40 mg) can serve as an active placebo for future RCT examining MDMA-AT in patients with PTSD by transiently increasing blood pressure and improving the integrity of the blinding procedures without impacting the occurrence of adverse events.

Disclosures: C.L. Haass-Koffler: None.

Poster

PSTR356: Mood and Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR356.01/MM7

Topic: H.05. Mood Disorders

Support: Hope for Depression Research Foundation
Pritzker Neuropsychiatric Disorders Research Consortium

Title: The neuroendocrine profile during the trier social stress test in college freshmen offers insights into the emergence of anxiety and depression symptoms

Authors: *C. A. TURNER¹, H. KHALIL³, V. MURPHY-WEINBERG², F. LI², A. ONICA², K. ARAKAWA², L. WEINBERG², C. STACK⁴, J. F. LOPEZ⁵, S. J. WATSON, Jr.⁶, H. AKIL²;
¹Univ. of Michigan, Willis, MI; ²Univ. of Michigan, Ann Arbor, MI; ³The Univ. of Michigan, Ann Arbor, MI; ⁴Univ. of Michigan, Ann Arbor, MI; ⁵Univ. Michigan, Ann Arbor, MI; ⁶MNI, Michigan Neurosci. Inst., Ann Arbor, MI

Abstract: This study explores the relationship between current and future anxiety and depression in relation to the Trier Social Stress Test (TSST) in a large cohort of college freshmen at the University of Michigan (N=338; Females=184, Males=154). We previously showed that a combination of psychiatric instruments (Affect Score) at the start of the freshmen year could predict who would develop depression symptoms during the year. We replicated this relationship in four additional years. We collected a number of self-report questionnaires at the start of the freshmen year, including the GAD-7 for anxiety symptoms and the PHQ-9 for depression symptoms. We performed the TSST during the school year and collected follow-up mood questionnaires quarterly. The subjects also wore Fitbits to collect heart rate during the TSST. Individuals with depression symptoms during the TSST showed a blunted cortisol (CORT) stress response, but this was not predictive of depression. However, individuals who developed anxiety symptoms at some point during the freshmen year had a higher Pre-TSST CORT response during the TSST even after accounting for the baseline Affect Score, suggesting an anticipatory hyperresponsiveness. Heart rate was important in anxiety but was not predictive. We also found interesting differences in the response to the TSST with a smaller group of females on birth

control who showed higher CORT levels throughout the TSST. Thus, current depression alters the stress response, elements of the TSST stress response are important for predicting future anxiety, and the Affect Score is an independent modifier of the anxiety risk.

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Poster

PSTR356: Mood and Cognition

Location: SDCC Halls B-H

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Program #/Poster #: PSTR356.02/MM8

Topic: E.05. Auditory and Vestibular Systems

Support: DFG He 7857/1-1

Title: Investigating Hemodynamic Proxies of Neural Excitation-Inhibition Balance Across Human Cortex

Authors: *R. CATENA^{1,2}, M. ALAVASH³, B. HERRMANN⁴, J. OBLESER²;
²Dept. of Psychology, ³Ctr. of Brain, Behavior, and Metabolism (CBBM), ¹Univ. of Lübeck, Lübeck, Germany; ⁴Rotman Res. Inst., Univ. of Toronto, Toronto, ON, Canada

Abstract: Theoretical models and experimental evidence argue that the balance between excitatory and inhibitory activity is crucial for optimal functioning of the brain. Many studies have attempted to measure “excitation-inhibition ratio” (E-I) non-invasively using EEG or fMRI. However, expression of E-I across the human cortex has remained understudied. Addressing the cortical heterogeneity of the E-I is important in studying whether and where the balance in neural dynamics is altered in healthy aging, under pharmacological interventions, or during tasks. Here, we address this question using resting state data from the Human Connectome Project and two previously established measures of E-I. Specifically, we ask (1) how estimates of E-I map across the entire cortex and (2) how these maps relate to receptor-density maps of neurotransmitters glutamate (NMDA-receptor) and GABA. Neuroimaging data were parcellated according to the Desikan-Killiany atlas with 68 cortical parcels. The first measure we used was a metric from complex systems dynamics, called the Hurst exponent (H). A recent study predicted a negative correlation between H and excitatory activity in fMRI BOLD signals (Trakoshis et al. 2020). More recently Fotiadis et al. (2023) showed that during resting state primary sensory-motor areas have higher H than other areas, which is associated with more inhibition. The second measure is derived from the parametric feedback-inhibition control model (pFIC) as described in Zhang et al. (2024). We mapped the cortical distribution of E-I derived from pFIC at rest and observed higher E-I in primary sensory-motor areas, in a specular way to the pattern shown by Fotiadis et al. (2023). We additionally calculated the H of the same fMRI time series and found a conflicting positive correlation with the E-I from the pFIC model. Further, we consulted PET-derived

NMDA and GABA neurotransmitter maps (Hansen et al. 2022, Dukart et al. 2018) and correlated the E-I from the pFIC model with the NMDA and GABA receptor densities. Across cortical parcels, we observed only a moderate negative correlation between the cortical E-I and NMDA density, which is non-trivial considering the excitatory role of glutamate and its important role in driving GABAergic interneurons. These seeming inconsistencies deserve further attention but provide partial support for the results of Fotiadis et al. (2023). In sum, several E-I measurement methods (data- or model-driven) from non-invasive hemodynamic recordings are now available. However, currently, they provide partly inconsistent cortical E-I distributions, which calls for cross-validation across different E-I measures.

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Poster

PSTR356: Mood and Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR356.03/MM9

Topic: H.05. Mood Disorders

Title: Strengthening emotional regulation through nature: Effects of a psychoeducational intervention in a green setting among graduate students

Authors: *S. MORALES¹, T. CIBRIAN-LLANDERL², Y. CAMPOS-USCANGA³, M. BARRADAS-MOCTEZUMA⁴;

¹Inst. de Investigaciones Cerebrales, ²Inst. de Neuroetologia, ³Inst. de Salud Publica, Univ. Veracruzana, Xalapa, Mexico; ⁴Univ. Veracruzana, Instituto de Investigaciones Cerebrales, Xalapa.

Abstract: Graduate education involves high academic, financial, and personal demands that increase the risk of emotional dysregulation and psychological distress. This quasi-experimental study examined the preliminary effects of a psychoeducational intervention focused on emotional regulation, implemented in a green environment, in comparison to a classroom-based intervention and a no-intervention control group. Thirty-four graduate students from a public university in Mexico were initially assigned to three groups: green setting (n = 18), classroom (n = 8), and control (n = 8). After attrition, post-intervention analyses included 17 participants. The intervention consisted of four in-person sessions based on cognitive-behavioral strategies, including mindfulness, cognitive restructuring, and self-compassion practices. Psychometric instruments were administered pre- and post-intervention to assess academic stress (SISCO), emotional dysregulation (DERS-15), and affective symptoms (DASS-21). Wilcoxon tests were used for within-group comparisons and Kruskal-Wallis tests for between-group differences. Preliminary results indicate that the green group showed significant improvements in depression (p = .034), anxiety (p = .036), difficulty in goal-setting (p = .034), and emotional non-acceptance (p = .048). No significant changes were observed in the classroom group. The control group showed increases in stress symptoms (p = .016) and emotional non-acceptance (p = .048). At the

end of the study, the green group reported lower scores in depression, anxiety, and emotional dysregulation compared to the control group. These preliminary findings suggest that integrating psychoeducational strategies into natural settings may enhance their effectiveness in improving emotional well-being among graduate students. Nature-based interventions represent a viable and low-cost option to promote mental health in demanding academic contexts.

Disclosures: S. Morales: None. T. Cibrian-Llenderal: None. Y. Campos-Uscanga: None. M. Barradas-Moctezuma: None.

Poster

PSTR356: Mood and Cognition

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR356.04/MM10

Topic: H.05. Mood Disorders

Support: Beca CONACYT 931445
SIREI (TCLL 43200202477) SNI (TCLL-210785)

Title: The impact of the COVID-19 pandemic on perceived stress, anxiety, depression, and empathy performance: a comparative study

Authors: *E. ACOSTA-MARI¹, Y. CAMPOS-USCANGA³, H. ACOSTA⁴, R. TRIANA-DEL RIO⁵, R. L. CASTILLO LÓPEZ⁶, T. CIBRIAN-LLANDERL²;

²Inst. de Neuroetología, ¹Univ. Veracruzana, Xalapa, Mexico; ³Inst. de Salud Pública Univ. Veracruzana, Xalapa, Mexico; ⁴Inst. de Investigaciones de Inteligencia Artificial Univ. Veracruzana, Xalapa, Veracruz, Mexico; ⁵Ctr. for Neural Sci., New York Univ., New York, NY; ⁶Dirección, Clínica Psicológica Bienestart, Xalapa, Veracruz, Mexico

Abstract: Background: Three years after the initial SARS-CoV-2 outbreak, COVID-19 remains a major global health burden. WHO defines ‘post COVID-19 condition’ as symptoms lasting ≥ 2 months, appearing ≥ 3 months post-infection, without alternative explanation. In primary care data, Long COVID was defined as ≥ 1 pre-defined symptom between 90-365 days after infection, absent in the 180 days prior, to minimize misclassification.

Objective: To assess stress, anxiety, depression, and empathy scores across three groups in Mexico to examine the pandemic's impact.

Method: Cross-sectional, descriptive, observational design. Participants: 126 pre-pandemic (Dec 2019), 158 post-pandemic with COVID-19, and 105 post-pandemic without COVID-19 (Feb-Nov 2023). Informed consent was obtained. Instruments: Perceived Stress Scale, Beck Anxiety and Depression Inventories, Reading the Mind in the Eyes, and Davis Interpersonal Reactivity Index (emotional and cognitive empathy).

Results: Significant differences were found across groups in stress ($\chi^2=54.40$, $DF=2$, $p<0.05$), anxiety ($\chi^2=2.19$, $DF=2$, $p<0.05$), and depression ($\chi^2=6.10$, $DF=2$, $p<0.05$). Lower empathy scores were observed on RME ($\chi^2=-4.38$), IRI-PT (-14.40), IRI-FS (-8.28), IRI-EC (-11.16),

and IRI-PD (-4.55), all $p < 0.05$.

Conclusion: The pandemic and SARS-CoV-2 exposure negatively affected mental health and social cognition.

Acknowledgments: SIREI (TCLL 43200202477), SNI (TCLL-210785), CVU 931445. Acknowledgement to SIREI (TCLL 43200202477) SNI (TCLL-210785) and CVU 931445. Conclusions: The pandemic and exposure to the SARS-CoV-2 virus are associated with a greater negative impact on mental health and social cognition. Acknowledgement to SIREI (TCLL 43200202477) SNI (TCLL-210785) and CVU 931445

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Poster

PSTR356: Mood and Cognition

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Program #/Poster #: PSTR356.05/MM11

Topic: H.05. Mood Disorders

Support: SEHCIHTY (No. CVU 1087378)
SIREI (TCLL 43200202477)
SNI (TCLL-210785)

Title: Intranasal oxytocin modulates emotional processing during social cognition tasks across genders

Authors: *L. L. CRUZ ZAVALA¹, R. TRIANA-DEL RIO⁴, J. FERNANDEZ-RUIZ⁵, G. A. CORIA-AVILA², T. CIBRIAN-LLANDERL³;

¹Neuroethology, Univ. Veracruzana, Xalapa, Mexico; ²Inst. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Ver, Mexico; ³Inst. de Neuroetologia, Univ. Veracruzana, Xalapa, Mexico; ⁴Ctr. for Neural Sci., New York Univ., New York, NY; ⁵Univ. Nacional Autonoma Mexico, Mexico D.F., Mexico

Abstract: Intranasal administration of oxytocin (*IN OXT*) may modify fundamental social behaviors in humans but has faced obstacles due to lack of study replication, reliable methodological development, and transparent experimental practices. This study investigated the effects of intranasal oxytocin (IN-OXT) on social cognition in 60 neurotypical adults (50% female) using a double-blind, placebo-controlled design. The assessment tools used were the Davis Interpersonal Reactivity Index (IRI) comprising four subscales: two measuring affective empathy (perspective-taking and fantasy) and two assessing cognitive empathy (empathic concern and personal distress). The Reading the Mind in the Eyes Test (RMET) was used, with performance analyzed through total correct answers, accuracy on easy (*FAC*) and difficult (*DIF*) items, and recognition of positive (*POS*), negative (*NEG*), and neutral (*NEU*) emotional items. Participants received either 24 IU IN-OXT (n=30) or placebo (n=30), with cognitive testing

conducted 40 minutes post-administration. Intranasal oxytocin administration demonstrated statistically significant enhancements across multiple measures of social cognition. On the *IRI*, the *OXT* group showed superior performance in both cognitive empathy (Perspective Taking: *OXT* $M=27.2\pm3.42$ vs *PLB* $M=25.4\pm3.64$, $t(58)=1.90$, $p<.05$, $d=0.51$) and affective empathy (Empathic Concern: *OXT* $M=26.0\pm3.84$ vs *PLB* $M=23.3\pm3.46$, $t(58)=2.86$, $p<.05$, $d=0.28$), while displaying reduced personal distress (*OXT* $M=14.6\pm3.06$ vs *PLB* $M=16.6\pm3.88$, $t(58)=-2.18$, $p=.017$, $d=0.57$). These effects extended to emotion recognition as measured by the *RMET*, where the oxytocin group achieved higher total scores (*OXT* $M=26.5\pm2.52$ vs *PLB* $M=24.9\pm2.80$, $t(58)=2.33$, $p<.05$, $d=0.590$), with particularly strong advantages in recognizing difficult (*DIF*: *OXT* $M=9.1\pm1.61$ vs *PLB* $M=7.7\pm1.29$, $t(58)=3.8$, $p<.05$, $d=0.299$) and negative emotional expressions (*NEG*: *OXT* $M=12\pm1.62$ vs *PLB* $M=10.3\pm2$, $t(58)=3.62$, $p<.05$, $d=0.760$), indicating enhanced sensitivity to subtle social cues. This study concludes that intranasal oxytocin administration can modify performance in various empathy and social cognition tests, particularly in areas related to cognitive empathy and recognition of complex emotions.

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Poster

PSTR356: Mood and Cognition

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Program #/Poster #: PSTR356.06/MM12

Topic: H.05. Mood Disorders

Support: SECIHTI (CVU: 1227361)
SIREI (TCLL 43200202477)
SNI (TCLL-210785)

Title: Effect of a Psychoeducational Intervention on Stress and Treatment Adherence in Patients with Breast Cancer

Authors: *A. RODRÍGUEZ MONTERO¹, T. CIBRIAN-LLANDERL²;

²Inst. de Neuroetologia, ¹Univ. Veracruzana, Xalapa, Mexico

Abstract: Breast cancer is one of the most prevalent types of cancer worldwide, and those affected face various physical, emotional, economic, and social challenges that hinder treatment adherence. This study aimed to evaluate the effect of a psychoeducational intervention on stress and treatment adherence in women with breast cancer. A longitudinal, quasi-experimental, and correlational study was conducted with 62 patients treated at the "Dr. Miguel Dorantes Mesa" State Cancer Center in Xalapa, Veracruz, Mexico. Stress was assessed using the Perceived Stress Scale (PSS-10) and the Measure of Current Status (MOCS), while treatment adherence was evaluated with the Breast Cancer Treatment Adherence Scale (EAT-CaMa). Statistical analysis included descriptive statistics, Spearman's correlation, Wilcoxon signed-rank test, and effect size

estimation. The results showed improvements in capacity and well-being perception ($W=8.06$ $P<0.05$, g de Hedges= 0.30). Additionally, an increase in physician-patient communication ($W=9.0$ $P<0.05$, g de Hedges= 0.35) and the use of active coping strategies, particularly the fighting spirit was observed ($W=2.75$, $P<0.05$, g de Hedges= 0.06). Notably, while global perceived stress levels did not show significant changes, hospital-related stress decreased. In conclusion, the psychoeducational intervention promoted treatment adherence behaviors by improving physician-patient communication, coping strategies, and peer emotional support. Although no significant changes were observed in overall perceived stress, hospital-related stress was reduced, strengthening therapeutic adherence.

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Poster

PSTR356: Mood and Cognition

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Topic: H.05. Mood Disorders

Support: SUBVENCION 833490

Title: Empathy as an indicator of depression and psychosocial risk in adolescence

Authors: *L. CASTAÑEDA LANDA¹, S. ZAMORA LUGO², J. FERNANDEZ-RUIZ⁴, T. CIBRIAN-LLANDERLAL³;

²Inst. de Neuroetología, ³Inst. de Neuroetología, ¹Univ. Veracruzana, Xalapa, Mexico; ⁴Univ. Nacional Autonoma Mexico, Mexico D.F., Mexico

Abstract: Structural changes in the adolescent brain driven by simultaneous hormonal and physical cascades produce a temporal mismatch between limbic maturation and prefrontal control. This reorganization window may alter social cognition, understood as the ability to detect and respond to others' mental states. The present study examined the combined impact of mental-health factors (anxiety, depression and psychosocial risk) on social-cognition performance in adolescents. Two hundred students (16.4 ± 0.8 years) completed the Beck Anxiety Inventory (BAI), Beck Depression Inventory-II (BDI-II) and the Problem-Oriented Screening Instrument for Teenagers (POSIT). Social cognition was assessed with the Empathy Quotient-10 (EQ-10), the Reading the Mind in the Eyes Test (RMET) and a 20-item Facial Emotion Recognition Test (FERT). The study adhered to the Declaration of Helsinki and was approved by the Universidad Veracruzana ethics committee. Because data were non-normal (Shapiro-Wilk, $p < .05$), Kruskal-Wallis tests with Dunn-Bonferroni post hoc and Spearman correlations were applied ($\alpha = .05$). Empathy differed significantly across depression levels ($H = 8.84$, $p = .032$) and psychosocial-risk strata ($H = 16.94$, $p < .001$). The lowest EQ-10 quartile showed the highest median BDI-II ($Md = 20.6$) and POSIT ($Md = 28.0$) scores. By contrast, RMET and FERT accuracy did not vary with depression, anxiety or sex. Females scored higher

than males on anxiety ($W = 3\,079.5$, $p < .001$), depression ($W = 3\,179.5$, $p < .001$) and psychosocial risk ($W = 3\,921$, $p = .008$), yet no sex differences emerged in any social-cognition test. Empathy-quartile distributions differed by depression category ($\chi^2 = 14.9$, $p = .021$) but not by anxiety ($\chi^2 = 6.1$, $p = .41$). Among social-cognition components, empathy showed the strongest and most consistent association with depression and psychosocial risk. Although females reported higher symptomatology, their social-cognition performance matched that of males. These findings identify empathy as a sensitive behavioral marker of affective vulnerability in adolescence and support its prioritization as an indicator for preventive programs targeting youths with elevated psychosocial risk and depressive symptoms.

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Poster

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Topic: H.05. Mood Disorders

Support: 5R01MH120168
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BX004475

Title: From blood to brain: structural network modularity correlates with platelet biomarker in depression

Authors: *A. GUNAY¹, A. LEOW², M. M. RASENICK³, O. A. AJILORE²;

¹Univ. of Illinois at Chicago, Chicago, IL; ²Psychiatry, Univ. of Illinois Chicago, Chicago, IL;

³Dept Physiol, Biophysics, U. Illinois Col. of Med., Chicago, IL

Abstract: The G protein Gs α is predominantly localized within lipid rafts in platelets of individuals with Major Depressive Disorder (MDD). Effective antidepressant treatment promotes its translocation out of rafts, enhancing coupling to adenylyl cyclase (AC). Platelet Gs α -AC coupling, indexed by prostaglandin E1 (PGE1)-stimulated cAMP, inversely correlates with depression severity (QIDS-C16) in MDD. Recent trials suggest psychedelics exert antidepressant effects by altering brain dynamics (e.g., modularity, global connectivity, entropy). Preliminary findings indicate that psychedelics also mobilize Gs α from lipid rafts, but on distinct timescales. These findings prompted an exploratory analysis of the link between peripheral Gs α signaling and large-scale brain network organization. Here, we investigated whether structural brain network modularity measured from DTI correlates with peripheral platelet biomarker signaling

particularly Gs α compartmentalization in MDD patients. Participants (ages 25-50) meeting DSM-5 criteria for MDD were recruited at the University of Illinois Chicago. Each completed two sessions, two weeks apart, with DTI scans and blood draws collected at both visits. Louvain modularity was computed from fiber count matrices using the Brain Connectivity Toolbox (n=22), and betweenness centrality was assessed across all scans (n=44). Platelet cAMP levels were measured following PGE1 and PGE2 stimulation as a marker of Gs α -AC coupling. FDR correction was applied to regional correlation analyses. Significant inverse correlations were found between DTI-derived modularity and cAMP responses to PGE1 ($r = -0.43$, $p = 0.03$) and PGE2 ($r = -0.53$, $p = 0.007$). These associations strengthened after controlling for symptom severity, with PGE1 ($r = -0.51$, $p = 0.01$) and PGE2 ($r = -0.61$, $p = 0.002$), suggesting an independent neurobiological relationship between biomarker and modularity. Betweenness centrality analysis revealed three non-hub regions (left inferior frontal gyrus, left supramarginal gyrus, and cerebellar vermis) significantly associated with cAMP responses ($q < 0.05$). Notably, the brain regions correlating with the peripheral biomarker were not central hub regions, suggesting that altered connectivity in non-hub areas may play a key role in this link. In future analyses, we aim to investigate these non-hub regions further, exploring their specific contributions to depression. These results provide novel link bridging macroscale brain metrics and microscale molecular signaling abnormalities in MDD, with potential implications for a blood biomarker for depression.

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Poster

PSTR356: Mood and Cognition

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR356.09/MM15

Topic: H.05. Mood Disorders

Support: NIH R61 MH128492

Title: Reduction of Negative Attentional Bias in Depression with Real-Time Neurofeedback

Authors: ***N. LUBIANIKER**¹, **B. WOODS**³, **F. NITCHIE, IV**³, **A. S. BATZDORF**⁵, **A. C. MENNEN**⁶, **Q. LIN**⁸, **K. NORMAN**⁷, **N. B. TURK-BROWNE**², **Y. I. SHELIN**⁴;
²Psychology, ¹Yale Univ., New Haven, CT; ⁴Psychiatry, ³Univ. of Pennsylvania, Philadelphia, PA; ⁵Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA; ⁶Princeton Neurosci. Inst., ⁷Princeton Univ., Princeton, NJ; ⁸Ctr. for Neurosci. Imaging Res., Ctr. for Neurosci. Imaging Research, Inst. for Basic Science, Korea, Suwon, Korea, Republic of

Abstract: Individuals with Major Depressive Disorder (MDD) show an attentional bias toward negatively valenced stimuli and thoughts. In this double-blind, randomized controlled study, we applied a validated closed-loop neurofeedback procedure designed to reduce this bias. Internal attentional states were decoded in real time from functional magnetic resonance imaging (fMRI) data. Participants were shown overlaid negative face and neutral scene images with variable opacity values, and were instructed to attend to the neutral scene while ignoring the negative face. When their decoded attentional state indicated a failure to attend to the scenes, the negative faces became more opaque, thus externalizing the brain's momentary attentional state. Forty-eight participants with MDD were randomly assigned to either the real neurofeedback group (N = 24) or to a sham control group who received feedback yoked to a participant in the training group (N = 24). Each participant completed three fMRI neurofeedback sessions. The main pre-registered outcome measure quantified the extent to which participants get "stuck" in the most negative attentional state. This occurs when they attend to the negative face and see it grow increasingly opaque but then are unable to draw their attention back to the neutral scene. We hypothesized that, relative to the sham control group, the real neurofeedback group would learn to escape the most negative attentional state from before to after training. We used bootstrap resampling to estimate a sampling distribution for the key interaction between group (real vs. sham) and session (pre vs. post). Consistent with our hypothesis, real neurofeedback training reduced the probability of getting stuck in a negative state. These results replicate and extend prior work, demonstrating the efficacy and dosing needed to remediate negative attentional bias in MDD with neurofeedback from real-time fMRI.

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Poster

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Topic: H.05. Mood Disorders

Support: NIH Grant R21 MH126197-01A1

Title: Reference point pathology: a mechanism and marker for major depressive disorder

Authors: *A. VITTALA, L. WU, D. YAN, D. LIEBERS, E. TELL, X. SONG, K. LOUIE, C. M. RAI0, D. IOSIFESCU, P. W. GLIMCHER;
NYU Langone, New York, NY

Abstract: Prospect theory, the mathematical theory of decision-making established by Kahneman and Tversky, posits that people decide if a stimulus is rewarding by comparing it to a fluctuating and unconscious benchmark called the reference point. Reinforcers above the reference point are experienced as positive, and those below as negative. Decades of behavioral experiments have shown that a wide range of reward-related behaviors are reference-dependent and that people can appropriately set their reference point based on their expectations about the current environment.

What would happen if the reference-setting mechanisms went awry? If a person were unable to appropriately adapt their reference point and ended up with a pathologically elevated reference point, then they would experience many objectively positive stimuli as negative. Psychiatrists would term this phenomenon anhedonia: the inability to enjoy previously rewarding experiences. Importantly, anhedonia is one of the defining features of major depressive disorder (MDD), and the presence of this common symptom suggests that a maladaptive response to rewards is a key feature of MDD.

We hypothesized a defining feature of MDD may be a pathologically elevated reference point, leading to lower subjective valuation of all stimuli and therefore a loss of interest in previously rewarding activities. To test this hypothesis, we used a gamified foraging task to assess the reference point in controls (n=70) and participants diagnosed with MDD (n=50). We found that participants with MDD (vs. controls) showed a systematic 50% elevation of the reference point (t-test, $p < 0.001$). In addition, individual reference points strongly correlated with depression severity as measured by the gold-standard Montgomery-Asberg Depression Rating Scale ($r = 0.71$, $p < 0.001$).

We used a second task to test the hypothesis that the reference point adapts to the reward environment pathologically in patients with MDD, perhaps driving the pathological elevation observed at steady-state. Using this task, we found that when participants were switched to a new reward environment, participants with MDD show slow, or undetectable, resetting of their reference point compared to controls.

Together, these results suggest that anhedonia in depression may reflect a pathology in reference point setting, and they provide the foundation for more objective, neuroeconomics-based measurement of depression.

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Poster

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Program #/Poster #: PSTR356.11/MM17

Topic: I.04. Executive Functions

Title: Effect of Neurofeedback Training on Depression Scores and Prefrontal Cortical Activity in Depression: A Randomized Control Study

Authors: *K. TIWARI¹, M. S. PHATAK, Sr.², S. JAISWAL³, A. PATIL⁴;

¹All India Inst. of Med. Sci., Nagpur, India; ²Indira Gandhi Govt, Med. College, Nagpur, Nagpur, India; ³All India Institute of Med. Sci., Nagpur, India; ⁴Physiol., All India Inst. of Med. Sci. Nagpur, Nagpur, India

Abstract: Depression is a prevalent mood disorder often accompanied by cognitive impairment and disrupted prefrontal cortical activity. While pharmacological treatments are standard, they are not universally effective and may cause adverse effects. As a non-pharmacological alternative, this study evaluated functional Near-Infrared Spectroscopy (fNIRS)-based neurofeedback—a non-invasive, portable, and patient-friendly intervention enabling real-time self-regulation of brain activity. Despite its advantages over EEG and fMRI, the clinical utility of fNIRS in depression remains underexplored. A single-center, open-label randomized controlled trial was conducted at AIIMS Nagpur. Thirty-eight patients with major depressive disorder (ICD-10; HDRS ≥ 8) were randomized into neurofeedback (N) or sham (S) groups. Both groups received four weekly 20-minute sessions targeting the dorsolateral prefrontal cortex (DLPFC). The N group received real-time HbO feedback; the S group received pre-recorded signals. Depression severity was measured on Days 1, 29, and 57 using the Hamilton Depression Rating Scale (HDRS). Prefrontal cortical activity was assessed using fNIRS at rest and during cognitive tasks. Data were preprocessed using NIRXLab and MATLAB, and analyzed with SPSS and GraphPad Prism. The neurofeedback group showed a 31.2% HDRS reduction post-intervention (vs. 5.8% in sham) and 29.1% at follow-up (vs. 9.1% in sham). A significant Group $\times$ Time interaction ($F = 124.5$, $p < 0.001$) confirmed HDRS improvement. Verbal fluency improved significantly in the neurofeedback group ($p < 0.001$), with a greater number of words generated vs. sham ($p < 0.001$). N-back accuracy ($p = 0.017$) and reaction time ($p < 0.001$) improved significantly in the neurofeedback group; between-group differences were also significant ($p < 0.01$ and $p = 0.004$, respectively). Emotional Stroop incongruent trial accuracy ($p < 0.001$) and reaction time ($p < 0.001$) improved post-training, though between-group differences were non-significant. Neurofeedback significantly increased HbO ($p = 0.044$), HbR ($p = 0.001$), and HbT ($p = 0.010$) during the N-back task. At rest, only HbT increased ($p = 0.0446$). Between-group differences post-intervention were significant for resting-state HbT ($p = 0.0195$). No significant changes were observed in VFT or Emotional Stroop responses. **Conclusion:** fNIRS-based neurofeedback significantly reduces depressive symptoms and improves prefrontal hemodynamics and cognitive performance. Further studies should explore optimal protocols and underlying neural mechanisms.

Disclosures: K. Tiwari: None. M.S. Phatak: None. S. Jaiswal: None. A. Patil: None.

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.01/MM18

Topic: B.07. Network Interactions

Title: Intracranial multi-electrode recording of burst suppression patterns in humans during general anesthesia induction

Authors: ***J. NATARAJ**¹, M. S. BREAU², A. SEYYED MOUSAVI³, J. OLAYA⁴, M. LIKER⁴, E. N. BROWN⁶, T. D. SANGER⁵;

¹Electrical Engin. and Computer Sci., Univ. of California, Irvine, Irvine, CA; ²The Picower Inst. for Learning and Memory, MIT, Brookline, MA; ³Univ. of California Irvine, Irvine, CA;

⁴Neurosurg., ⁵Res., Children's Hosp. of Orange County, Orange, CA; ⁶Brain and Cognitive Sci., MIT/Mass Gen. Hospital/Harvard Med. Sch., Cambridge, MA

Abstract: General Anesthesia (GA) is a reversible drug-mediated condition comprised of unconsciousness, amnesia, antinociception, and immobility with maintenance of physiological stability during a surgical procedure. Electroencephalography (EEG) studies have shown that brain dynamics vary based on anesthetic dose and class, patient age, and patient health. Higher doses result in burst suppression, a deeper state of unconsciousness characterized by alternating periods of high amplitude oscillations and isoelectricity. In this study, we observed subcortical burst suppression dynamics using GABAergic anesthetics in three pediatric subjects who underwent placement of intracranial electrodes for staged deep brain stimulation. We recorded EEG and local field potentials (LFPs) from the brainstem and thalamus: pedunculopontine nucleus (PPN), ventral intermediate nucleus (VIM), and ventralis oralis anterior and posterior nuclei (Voa/Vop). To study burst suppression dynamics in these normally inaccessible brain regions, we performed multitaper spectral analysis of data recorded during burst suppression mediated by propofol alone, and the combination of propofol and sevoflurane. EEG and observed subcortical regions show synchronous burst suppression patterns in two patients, implying possible spatial homogeneity of burst suppression. Interestingly, alpha band activity (8-12 Hz), typically associated with lighter levels of unconsciousness, persisted across all areas during bursts and suppressions. However, total spectral power and alpha power were lower during suppression. Early versus late burst suppression activity showed variability in alpha power and time spent in suppression, suggesting that burst suppression is a dynamic state guided by changes in underlying neurophysiology. This is consistent with computational models suggesting that burst suppression is a metabolic phenomenon, driven by a decrease in cerebral metabolic rate. A third patient with severe brain injury displayed asynchronous bursting and disappearance of thalamic alpha band activity. Patients with brain injury may be more susceptible to the burst suppressive state because their metabolic state may be less well-regulated. Our results show the global nature of burst suppression and shed light on the heterogeneity of dynamics within burst and suppressive periods over time. Better characterization of burst suppression could facilitate better anesthetic control, reducing postoperative cognitive impairment. To our knowledge, this work represents the first reported human recordings of brainstem and thalamic regions during burst suppression.

Disclosures: **J. Nataraj:** None. **M.S. Breault:** None. **A. Seyyed Mousavi:** None. **J. Olaya:** None. **M. Liker:** None. **E.N. Brown:** None. **T.D. Sanger:** None.

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.02/NN1

Topic: B.07. Network Interactions

Support: National Institute of General Medical Sciences Grant R01GM141792

Title: Sequential deactivation across the hippocampus-thalamus-mPFC pathway during loss of consciousness

Authors: *X. CHEN¹, S. R. CRAMER², X. HAN³, N. ZHANG⁴;

¹The Pennsylvania State Univ., state college, PA; ²Biomed. Engin., ³The Pennsylvania State Univ., State College, PA; ⁴The Pennsylvania State Univ., University Park, PA

Abstract: How consciousness is lost in states such as sleep or anesthesia remains a mystery. To gain insight into this phenomenon, we conducted concurrent recordings of electrophysiology signals in the anterior cingulate cortex and whole-brain fMRI in rats exposed to graded propofol, undergoing the transition from consciousness to unconsciousness. Our results reveal that upon the loss of consciousness (LOC), there is a sharp increase in low-frequency power of the electrophysiological signal. Additionally, simultaneously measured fMRI signals exhibit a cascade of deactivation across a pathway including the hippocampus, thalamus, and medial prefrontal cortex (mPFC) surrounding the moment of LOC, followed by a broader increase in brain activity across the cortex during sustained unconsciousness. Furthermore, sliding window analysis demonstrates a temporary increase in synchrony of fMRI signals across the hippocampus-thalamus-mPFC pathway preceding LOC. These data suggest that LOC might be triggered by sequential activities in the hippocampus, thalamus and mPFC, while wide-spread activity increases in other cortical regions commonly observed during anesthesia-induced unconsciousness might be a consequence, rather than a cause of LOC. Taken together, our study identifies a cascade of neural events unfolding as the brain transitions into unconsciousness, offering critical insight into the systems-level neural mechanisms underpinning LOC.

Disclosures: X. chen: None. S.R. Cramer: None. X. Han: None. N. Zhang: None.

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

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Program #/Poster #: PSTR357.03/NN2

Topic: B.07. Network Interactions

Support: UCSF Clinical and Translational Research Institute 137241F
Hellman Society Fellowship 7504921
UCSF Catalyst Award 7032439
Pediatric Epilepsy Research Foundation A144223

Title: Coin: a novel eeg metric for detecting blood pressure-dependent focal ischemia in intubated patients

Authors: *M. CAFFARELLI;
Neurol. & Pediatrics, Univ. of California, San Francisco, San Francisco, CA

Abstract: **OBJECTIVE:** Extracorporeal membrane oxygenation (ECMO) carries a high risk of ischemic stroke. Right carotid artery (RCA) cannulation is associated with stroke in the right hemisphere, which can be detected using Electroencephalography (EEG). Our group developed the EEG Correlate Of Injury to the Nervous System (COIN) index, a symmetry metric can visualize and quantify stroke. A value of '0' implies a symmetric study and increasingly negative values are associated with stroke. This case assessed whether COIN can be used to recognize patients at risk for focal stroke. **METHODS:** Case of an RCA cannulation ECMO run with EEG evidence of ischemia and no infarct on head imaging. EEG was analyzed on MATLAB using the COIN index and correlated against mean arterial blood pressure (MAP) to assess hemodynamic dependence of the COIN signal. Fluctuations in COIN and MAP were compared by subtracting 3-hour from 1-hour moving averages of each trend. Correlation was assessed with Pearson's coefficient. **RESULTS:** Two year old male on ECMO who received EEG. COIN showed right hemispheric ischemia, and weakly correlated with MAP fluctuations ($r^2 = 0.18$). The patient survived with no cortical infarct and minimal neurological deficit. **DISCUSSION:** This case demonstrates the ability to measure blood pressure dependence of neurophysiologic function using COIN. COIN's sensitivity for regional power changes may be useful for studies quantifying the relative response to stimuli or ischemia using conventional scalp EEG.

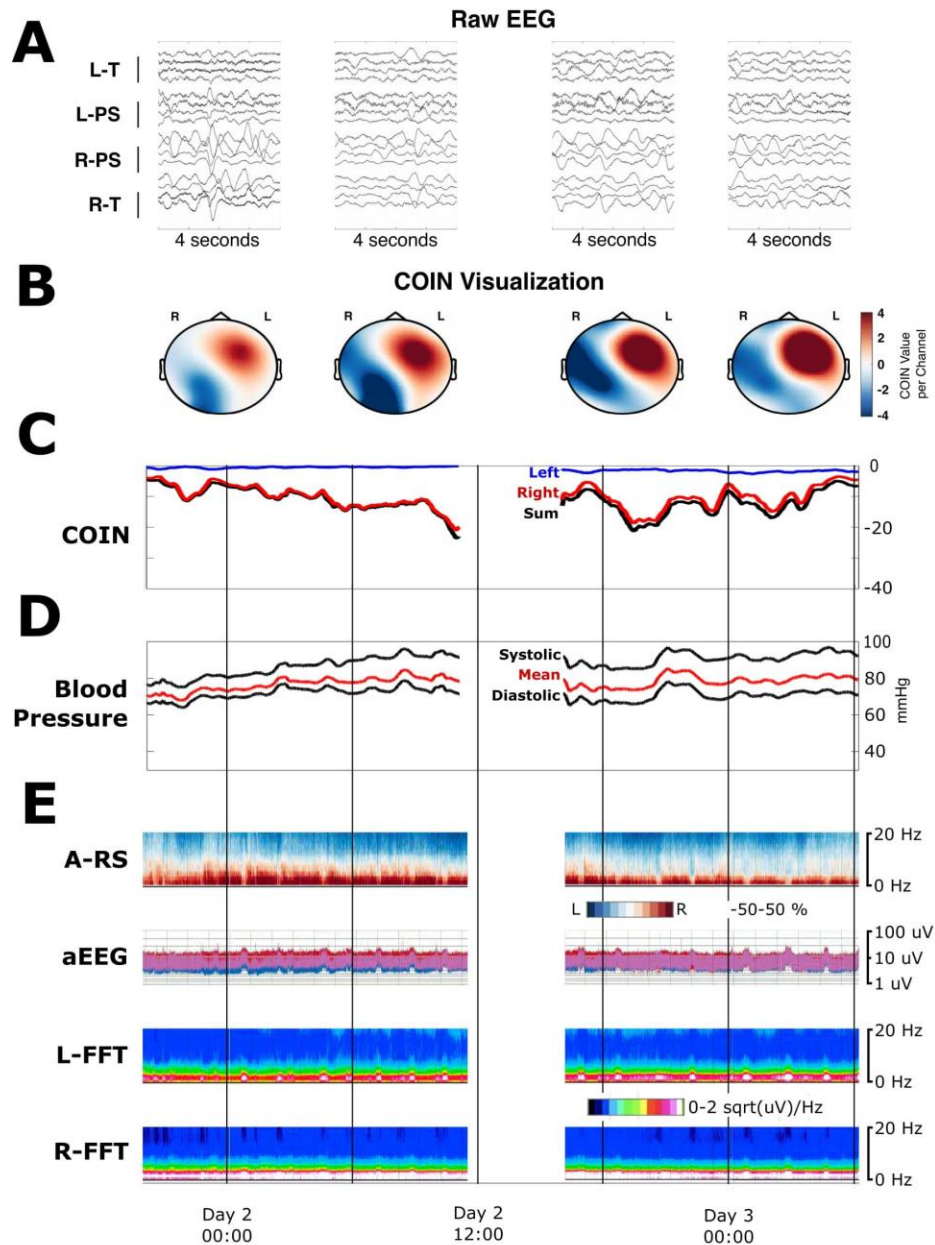


Figure 1: A) Raw EEG tracing from four representative 4-second epochs in the period before and after head imaging. B) COIN visualization from representative EEG epochs shows persistent right hemispheric attenuation across multiple bipolar channels. C) Continuous COIN trend shows dynamic trend in right and left hemispheric COIN values over 36 hours of EEG recording, with summary COIN values maintained above -20 for the majority of the recording. Recording time is relative to ECMO start, with pause in recording during imaging. D) Continuous blood pressure shows mean arterial pressure maintained above 65 mmHg before, and above 75 mmHg after, head imaging. E) Other quantitative trends derived from the EEG tracing show relative attenuation of alpha and beta power and augmentation of delta power in the right hemisphere. EEG, Electroencephalography; L, Left; R, Right; T, Temporal; PS, Parasagittal; COIN, Correlate Of Injury to the Nervous system; A-RS, Asymmetry - Relative Spectrogram; aEEG, amplitude-integrated EEG; FFT, Fast Fourier transform.

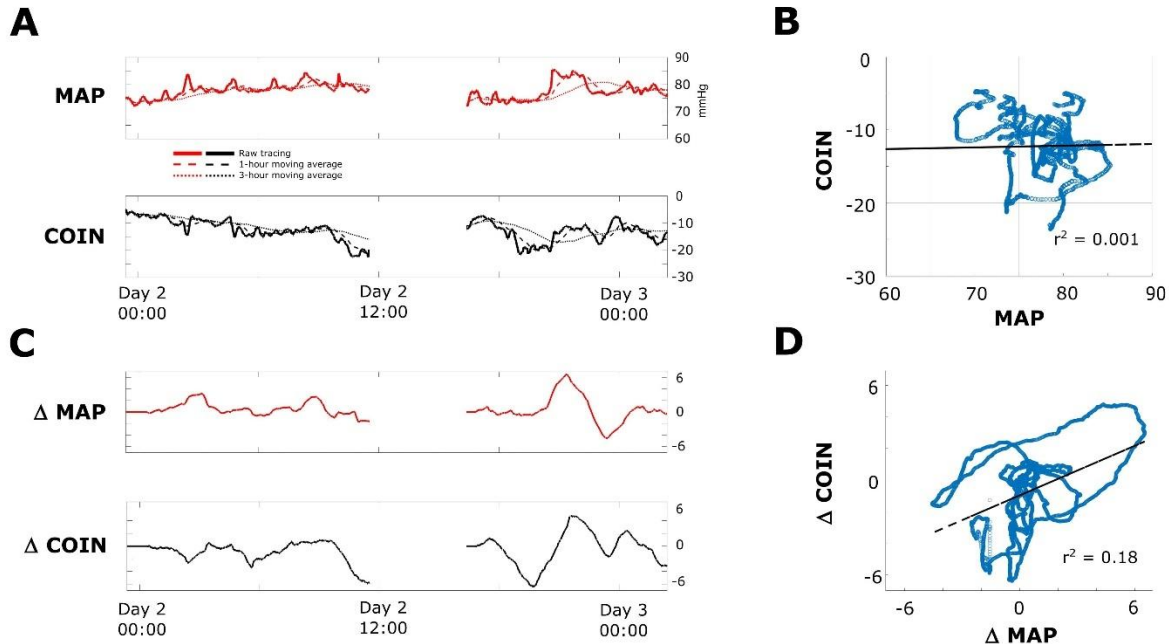


Figure 2: A) Continuous mean arterial pressure (MAP) and COIN values during the EEG recording timed relative to ECMO start. Raw MAP and COIN are overlaid with 1-hour and 3-hour moving average tracings. B) Scatterplot of raw MAP against COIN values shows no correlation ($r^2 = 0.001$). C) Δ MAP and Δ COIN tracings derived by subtracting 3-hour from 1-hour moving average tracings. D) Scatterplot of Δ MAP against Δ COIN shows a weak correlation ($r^2 = 0.18$).

Disclosures: M. Caffarelli: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor in Patent using EEG for stroke detection with no associated licensing agreement.

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.04/NN3

Topic: B.07. Network Interactions

Support: NSF Grant 2047529
U.S. Army Research Office Grant W911NF-23-1-0014
NIH Grant R21MH127567

Title: Sawtooth waves provide a time window for interregional communication during rapid eye movement sleep in humans

Authors: C. N. FLEMING¹, M. R. WITCHER², *S. VIJAYAN¹;

¹Virginia Tech., Blacksburg, VA; ²Virginia Tech. Col. of Med., Roanoke, VA

Abstract: Synchronization of oscillatory dynamics between different brain regions is a key mechanism by which the brain performs critical functions during sleep. Rapid eye movement (REM) sleep is marked by an oscillation called sawtooth waves (2 to 6 Hz), which stand out as distinct events against the persistent irregular, low voltage activity typical of this stage of sleep. Although sawtooth waves are one of the prominent features of REM sleep, relatively little is known about them and their functional role. Our working hypothesis is that sawtooth waves play an important role in mediating the processing of emotional memories. We set out to further characterize this waveform in a population of subjects with intractable epilepsy undergoing invasive recordings to localize the focus of their seizures; the implanted electrodes allow us to record directly from and analyze deep brain limbic structures involved in emotional processing. We examined and scored overnight sleep recordings from ten patients (6 females and 4 males) between the ages of 19 and 55 years and identified 41 REM episodes used for further analysis. We find that intracranial sawtooth waves are distributed across a wide range of both cortical and subcortical locations and report for the first time localized detections in the amygdala and hippocampus. Furthermore, we find that the period of time when sawtooth waves emerge on the scalp is a window where oscillatory dynamics between brain regions become synchronized. The synchronization of oscillatory activity between brain regions is thought to be an indication of communication between those regions. Therefore, this synchronization during the presence of sawtooth waves along with their presence in limbic structures (e.g., the amygdala), suggest that sawtooth waves may mediate the processing of emotional memories—one of the putative functions of REM sleep.

Disclosures: C.N. Fleming: None. M.R. Witcher: None. S. Vijayan: None.

Poster

PSTR357: The Neural Circuits of Consciousness.

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Program #/Poster #: PSTR357.05/NN4

Topic: B.07. Network Interactions

Support: R01 AG056015-01

Title: Characterizing the relationship between slow wave amplitude and phase amplitude coupling during sevoflurane-induced anesthesia

Authors: F. TIAN¹, M. HE², R. GUTIERREZ³, O. AKEJU¹, *P. L. PURDON²;

¹Massachusetts Gen. Hosp., Boston, MA; ²Stanford Univ. Sch. of Med., Palo Alto, CA; ³Univ. of Chile, Santiago, Chile

Abstract: Slow wave amplitude saturation and phase amplitude coupling have been proposed as potential markers of anesthesia-induced unconsciousness. Both of these markers reflect the same underlying mechanistic principle, namely that slow oscillations measured in the EEG reflect alternating "On" and "Off" states in neuronal populations that interrupt neuronal firing and thus

cortical function. In this study, we seek to characterize the relationships among slow wave amplitude and phase amplitude coupling parameters, ϕ_{mod} (preferred phase of coupling) and k_{mod} (strength of coupling), during sevoflurane-induced general anesthesia. We analyzed 64-channel EEG data from a previously recorded dataset in 12 subjects before, during and after sevoflurane-induced general anesthesia. The recording consisted of a 10 minute baseline period, followed by increasing stepwise end-tidal sevoflurane concentrations targeting subanesthetic (1.1%), general anesthetic (2.1%), and deep-general anesthetic (2.8%) levels, each of which was maintained for 15 minutes, followed by a 10 minute emergence period. Phase amplitude coupling between slow and alpha was identified predominantly in frontal regions of the brain. Strong correlations ($r=0.6\sim0.8$, $p<0.05$) were found between slow wave amplitude and ϕ_{mod} for frontal channels. Moderate to low level of correlations ($r=0.2\sim0.6$, $p<0.05$) were found between slow wave amplitude and k_{mod} across the scalp. We also characterized how sevoflurane altered slow wave amplitude, ϕ_{mod} , and k_{mod} using linear mixed-effects models. All three dependent variables are significantly influenced by the fixed effect of sevoflurane, which explained similar proportions of variance across the EEG measures. In addition, ϕ_{mod} showed a smaller intraclass correlation coefficient compared to slow wave amplitude and k_{mod} , suggesting a reduced variability across subjects. Our results suggest that slow wave amplitude and phase amplitude coupling are closely linked, and both could be used to monitor anesthesia-induced unconsciousness. It is known that there are substantial between-subject variations in the magnitude of anesthesia-induced slow wave activity, which make it challenging to incorporate it into clinical practice, requiring individualized assessment of the appropriate slow wave amplitude target for titration. The phase amplitude modulation parameters, ϕ_{mod} and k_{mod} , also track the depth of anesthesia, with ϕ_{mod} in particular showing reduced between-subject variation that might make it suitable for monitoring in a broad range of patient populations without the need to determine individualized titration targets.

Disclosures: **F. Tian:** None. **M. He:** None. **R. Gutierrez:** None. **O. Akeju:** None. **P.L. Purdon:** Other; PLP is a co-founder of PASCALL Systems, Inc., a start-up company developing closed-loop physiological control systems for anesthesiology.

Poster

PSTR357: The Neural Circuits of Consciousness.

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.06/NN5

Topic: B.07. Network Interactions

Support: P01GM118269
TR01GM104948

Title: Comparison of thalamocortical activity across sleep, sedation, and anesthesia

Authors: I. DALLA BETTA¹, M. A. WILSON³, E. N. BROWN⁴, *F. J. FLORES^{2,5};

¹Picower Inst. for Learning and Memory, ²MIT, Cambridge, MA; ³Picower Inst. for Learning

and Memory, Massachusetts Inst. of Technol., Cambridge, MA; ⁴MIT/Mass Gen. Hospital/Harvard Med. Sch., Cambridge, MA; ⁵Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Slow wave sleep (SWS), sedation, and anesthesia-mediated unconsciousness are all characterized by sustained low frequency, high amplitude oscillations observed in cortical activity. In all three states the presence of this cortical activity is accompanied by behavioral quiescence and loss of consciousness. During SWS these high amplitude cortical oscillations are associated with high amplitude thalamic oscillations. Many previous studies have highlighted the role of the thalamocortical system in the unconsciousness produced by these states, but none has compared its role across the three states. We recorded prefrontal cortical and higher order thalamic local field potential (LFP) during SWS, sedation induced by dexmedetomidine and midazolam, and anesthesia induced by propofol and isoflurane in freely moving rats. As expected, cortical and thalamic LFP power during sleep is higher than during wake at most frequencies below 20 Hz. Each drug produces unique spectral signatures in the cortex and the thalamus, all of which are different from SWS. In the cortex, propofol significantly increases power across most frequencies, while dexmedetomidine increases power in the delta band but reduces power in the gamma band. In the thalamus, all drugs reduce power in at least some frequency ranges when compared to sleep. While some of the conspicuous behavioral and neurophysiological traits of SWS, sedation, and anesthesia are similar—unconsciousness and increase in cortical LFP amplitude—the different actions of the drugs in the central nervous system result in important differences between, and even within, these states.

Disclosures: **I. Dalla Betta:** None. **M.A. Wilson:** None. **E.N. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PASCALL, Masimo. **F.J. Flores:** None.

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.07/NN6

Topic: B.07. Network Interactions

Support: NIH Grant R01

Title: A modulatory role of the nucleus reuniens in hippocampal-prefrontal communication

Authors: *S. SHAO¹, J. J. FERRERO¹, T. KAWANO², D. KHODAGHOLY¹, J. GELINAS¹;
¹UC Irvine, Irvine, CA; ²Toyohashi Univ. of Technol., Toyohashi, Aichi, Japan

Abstract: Memory consolidation requires functional synchrony between the hippocampus (HC) and the medial prefrontal cortex (mPFC). The nucleus reuniens (RE) has recently been reported as a structural and functional hub between HC and mPFC. The role of the RE in hippocampal-prefrontal communication during memory consolidation remains unclear. Here, we used *in vivo*

electrophysiology to monitor the hippocampal-thalamic-prefrontal network in rats during non-rapid eye movement (NREM) sleep. We identified ripple-like activity in the RE in the 100-180 Hz frequency range with an average duration of 45 ms, and was associated with robust recruitment of thalamic neural spiking. RE ripple activity was phase-locked to both hippocampal sharp wave-ripples (SWR) and mPFC ripples. Hippocampal ripples are known to couple with cortical ripples for facilitating memory consolidation. Although ripples were independently detected in all three structures, we found that coupling between HC and mPFC ripples necessarily involved co-occurring RE ripples. The coupling strength among HC, RE and mPFC ripples enhanced with the memory demand. These results suggest a modulatory role for the RE in oscillatory synchronization between the HC and the mPFC, with potential implications for network mechanisms of memory consolidation.

Disclosures: S. Shao: None. J.J. Ferrero: None. T. Kawano: None. D. Khodagholi: None. J. Gelinas: None.

Poster

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Topic: B.07. Network Interactions

Support: James S. McDonnell Foundation
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NINDS Grant R01NS128961
NINDS Grant DP2HD101400
NINDS Grant R21NS109627
NINDS Grant RF1NS115268
NINDS Grant U01NS086625

Title: Network-based autopsy of the human brain

Authors: *M. OLCHANYI¹, C. MAC DONALD², A. NOLAN², C. MAFFEI⁴, H. FREEMAN⁴, J. C. AUGUSTINACK⁵, L. ZOLLEI⁴, A. VAN DER KOUWE⁴, R. L. HAYNES⁹, J. MORA⁴, C. ANALORO⁴, L. DEDEN-BINDER⁴, J. NOLAN⁴, J. WILLIAMS RAMIREZ⁴, B. HEALY⁴, M. FROSCH⁴, B. R. ROSEN⁶, J. P. KELLY¹⁰, G. T. MANLEY¹¹, D. PERL¹², C. DESTRIEUX¹³, E. N. BROWN¹⁴, R. D. FOLKERTH¹⁵, H. C. KINNEY¹⁶, B. FISCHL⁷, K. DAMS-O'CONNOR¹⁷, C. D. KEENE³, B. EDLOW⁸;

¹MIT, Cambridge, MA; ³Pathology, ²Univ. of Washington, Seattle, WA; ⁵Radiology,

⁴Massachusetts Gen. Hosp., Boston, MA; ⁶Radiology, Massachusetts Gen. Hosp., Charlestown, MA; ⁷Massachusetts Gen. Hosp., CAMBRIDGE, MA; ⁸Neurol., Massachusetts Gen. Hosp., Boston, MA; ⁹Pathology, Children's Hosp Boston, Boston, MA; ¹⁰Neurol., Univ. of Colorado Sch. of Med., Aurora, CO; ¹¹Univ. of California, San Francisco, San Francisco, CA; ¹²Uniformed Services Univ., Bethesda, MD; ¹³Univ. de Tours, Tours, France; ¹⁴Brain and Cognitive Sci.,

MIT/Mass Gen. Hospital/Harvard Med. Sch., Cambridge, MA; ¹⁵Rehabil. and Human Performance, Brain Injury Res. Center, Icahn Sch. of Med. at Mount Sinai, New York, NY, USA, New York, NY; ¹⁶Dept Pathology, Children's Hosp, Boston, MA; ¹⁷Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY

Abstract: Disorders of consciousness (DOC) following traumatic brain injury (TBI) are often described as a "disconnection syndrome", as they are associated with white matter lesioning patterns in brain networks that are known to promote consciousness. Currently, most lesion localization studies aimed at matching microstructural injury to functional outcomes in DOC are restricted to two-dimensional histological sections, which fail to capture the multifocal, three-dimensional nature of axonal damage. To address this gap, we propose a multi-scale approach involving structural connectivity mapping with high-resolution diffusion MRI (dMRI), integrated with histology. As a proof of principle, we initially focus on the connectivity of brainstem Ascending Arousal Network (AAN) nuclei, a set of ten neurotransmitter-specific brainstem regions which are key to arousal modulation in consciousness. Using dMRI, we generate structural connectivity strengths between all AAN nuclei, the neuroanatomic boundaries of which are automatically segmented and refined with corresponding histological sections (Figure 1). This connectivity model is based on probabilistic tractography, including both mean connectivity strengths between the set of all AAN nuclei pairs as well as their inter-subject variability in 16 control brain specimens. We then compare this normative AAN connectivity matrix to corresponding connections in 15 acute and 26 chronic TBI brain specimens to characterize distinct losses of structural connections within AAN subgroups in DOC. We plan to publicly release all *ex vivo* neuroimaging data as well as paired digitized histopathological sections as part of the Network-Based Autopsy project.

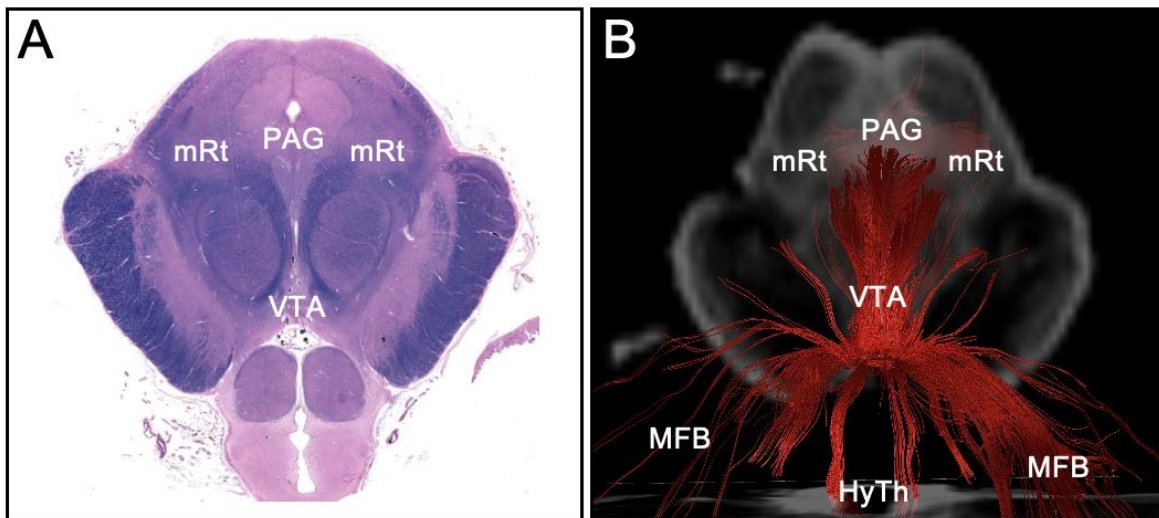


Figure 1: (A) An axial hematoxylin/eosin section in the midbrain of a normative *ex vivo* brain specimen displaying the neuroanatomic boundaries of rostral AAN nuclei, including the Ventral Tegmental Area (VTA), Periaqueductal Gray (PAG), and the Mesencephalic Reticular Formation (mRt). These boundaries are used to directly guide and refine the segmentation of AAN nuclei in dMRI space (B), from which AAN tractography is performed to assess structural connectivity patterns.

Disclosures: M. Olchanyi: None. C. Mac Donald: None. A. Nolan: None. C. Maffei: None. H. Freeman: None. J.C. Augustinack: None. L. Zollei: None. A. Van Der Kouwe: None. R.L. Haynes: None. J. Mora: None. C. Analoro: None. L. Deden-Binder: None. J. Nolan: None. J. Williams Ramirez: None. B. Healy: None. M. Frosch: None. B.R. Rosen: None. J.P. Kelly: None. G.T. Manley: None. D. Perl: None. C. Destrieux: None. E.N. Brown: None. R.D. Folkerth: None. H.C. Kinney: None. B. Fischl: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CorticoMetrics LLC. K. Dams-O'Connor: None. C.D. Keene: None. B. Edlow: None.

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.09/NN8

Topic: B.07. Network Interactions

Support: NIH Grant 3R42DA053075-03S1

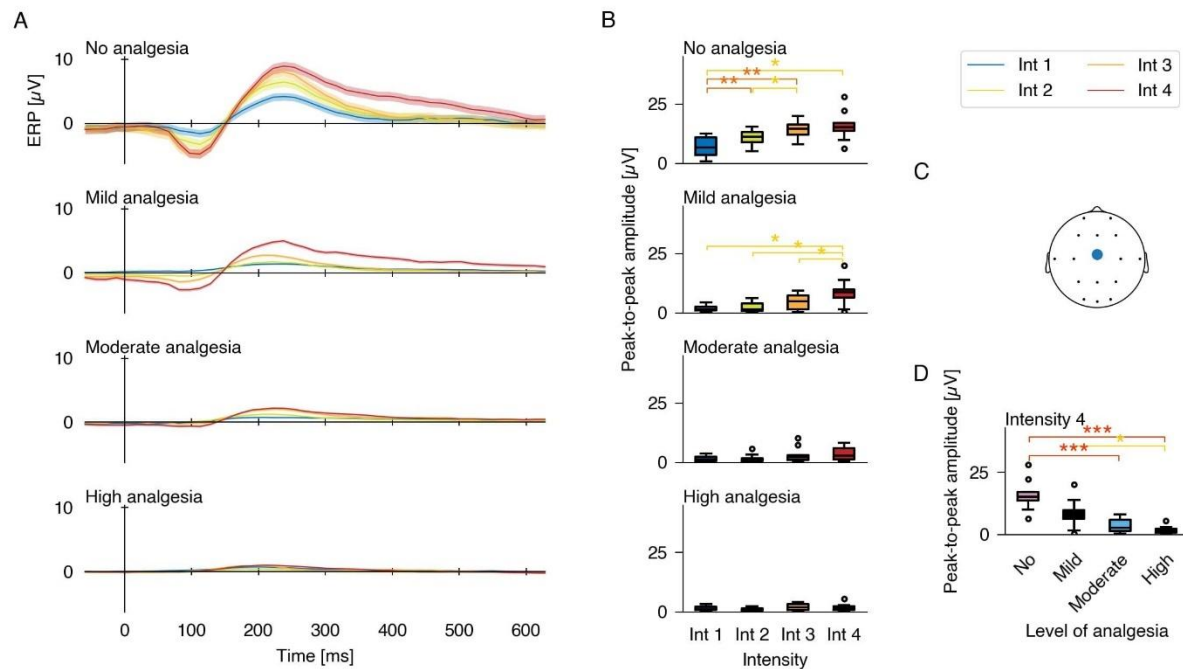
Title: Event Related Potential Biomarkers for Pain and Analgesia

Authors: *P. DAS¹, R. GUTIERREZ², J. I. EGANA³, P. L. PURDON⁴;

¹Natl. Brain Res. Centre, Gurgaon, India, Manesar, Guragon, India; ²Univ. de Chile, Chile, Santiago, Chile; ³Anestesiología y Medicina Perioperatoria, Hosp. Clinico Univ. De Chile, Santiago, Chile; ⁴Stanford Univ. Sch. of Med., Palo Alto, CA

Abstract: Objective biomarkers for pain have been long sought after. Although pain is ultimately a subjective assessment, tools to objectively measure it are clearly useful for guiding interventions specific to an individual patient's pain presentation, for tracking their efficacy, and for understanding different contributors to pain including emotional and psychosocial factors. Yet, finding such objective markers remains challenging. Neurophysiological methods such as the electroencephalogram (EEG) and its derivative event-related potentials (ERPs) have long been proposed as a plausible pain biomarker. In this context, the limited spatial resolution of EEG/ERPs does not hinder the measurement of the essential brain dynamics that signal varying degrees of pain. However, conventional approaches to ERPs have low signal to noise requiring significant amounts of data to produce an acceptable effect estimate that may be infeasible in clinical or even experimental research applications. Here we introduce a novel methodology for the analysis of ERPs, capable of extracting microvolt-level somatosensory stimuli from the complex background of larger amplitude brain oscillations. This approach utilizes a novel multi-level state-space model, hereafter referred to as state-space ERPs (SS-ERPs). We investigated pain-evoked neural activity in human participants and further examined the influence of analgesia, induced by the ultra-short acting opioid remifentanyl, on these electrophysiological signals. Our findings demonstrate that SS-ERPs exhibit robust correlations with both perceived pain intensity and analgesic effects. Notably, we have shown that SS-ERPs represent a potent tool for the detection of pain and the assessment of analgesic efficacy, offering superior

discriminative ability, requiring fewer stimulus repetitions compared to conventional ERP techniques, and demonstrating applicability at the individual subject level. These results hold significant potential implications for diverse areas within clinical pain management and research.



Disclosures: **P. Das:** None. **R. Gutierrez:** None. **J.I. Egana:** None. **P.L. Purdon:** Other; PLP is a co-founder of PASCALL Systems, Inc., a start-up company developing closed-loop physiological control systems for anesthesiology., PASCALL Systems, Inc..

Poster

PSTR357: The Neural Circuits of Consciousness.

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR357.10/NN9

Topic: B.07. Network Interactions

Support: R01GM141792
R01NS085200

Title: Multimodal decoding of consciousness recovery using fMRI, pupil dynamics, and electrophysiology

Authors: *X. HAN¹, X. CHEN², N. ZHANG³;

¹The Pennsylvania State Univ., State College, PA; ²The Pennsylvania State Univ., state college, PA; ³The Pennsylvania State Univ., University Park, PA

Abstract: Regaining consciousness (ROC) following anaesthesia is a complex neurophysiological process involving widespread and coordinated changes across the brain. In this study, we employed simultaneous electrophysiology, pupillometry, and resting-state fMRI (rsfMRI) in rats to comprehensively characterise the spatiotemporal dynamics underlying ROC. Using a pupil-based criterion to define the moment of ROC, we identified a sharp dilation of pupil size that reliably marked this transition point. Electrophysiological recordings in the cortex and hippocampus revealed a significant decrease in delta (1-4 Hz) and theta (4-8 Hz) band power around ROC, indicating a shift toward desynchronized cortical activity. Concurrently, rsfMRI showed reduced intra-cortical functional connectivity (FC), suggesting a breakdown of local cortical integration. In contrast, the thalamus exhibited a transient increase in internal FC around ROC, potentially reflecting its role in reinitiating cortical communication. Thalamo-cortical FC increased robustly, highlighting the re-establishment of long-range interactions critical for conscious awareness. Additionally, we observed elevated FC between the basal ganglia and both the hypothalamus and insular cortex, implicating these subcortical regions in the consciousness-related aspects during awakening. Collectively, these findings offer a comprehensive view of the brain state transition underlying consciousness recovery, revealing the coordinated neurophysiological and network-level dynamics across distributed brain regions.

Disclosures: X. Han: None. X. chen: None. N. Zhang: None.

Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR358.01/NN10

Topic: I.05. Working Memory

Title: Goal-setting improves visual working memory performance and modulates neural signatures of effort and storage

Authors: *O. KOZLOVA, K. C. ADAM;
Psychological Sci., Rice Univ., Houston, TX

Abstract: Visual Working Memory (VWM) is the ability to hold information actively in mind, and it has a strictly limited capacity of only 3-4 items. Because VWM capacity is a key “bottleneck” for complex cognition, there is theoretical and practical interest in maximizing VWM performance. In previous work, we showed that using performance feedback and “goal setting” can produce improvements to VWM performance. Specifically, we awarded small monetary bonuses when participants achieved a performance goal (cued on each trial, e.g., “Remember 3!”) and we found that an “optimal goal” (i.e., the goal condition tailored to match an individual’s VWM capacity) improved VWM performance but increased metacognitive errors. Here, we conducted an EEG experiment (n=20) to test which underlying cognitive processes support VWM performance improvements, and metacognitive deficits, with optimal goals. Participants performed a VWM task, and we manipulated whether they received an

optimal goal (i.e., “Remember 3”!), versus a goal that was too difficult (“Remember 5!”) or too easy (“Remember 1!”). We predicted that processes altered by goal-setting should appear “U-shaped” in favor of the “optimal goal” (Goal 3>Goal 5=Goal 1), whereas processes related to simple effort will lead to monotonic increases in neural signals (Goal 5>Goal 3>Goal 1). First, we found monotonic increases in tonic pupil dilation with goal conditions, suggesting that participants exerted more effort as the performance goal increased (Goal 5=Goal 3>Goal 1; $p<.01$). However, we found a “U-shaped” pattern for posterior alpha power suppression ($p<.05$), suggesting that participants maintained more items in VWM for optimal performance goals. Finally, we performed exploratory analyses of factors that may lead to trade-offs between performance improvements and poorer metacognitive accuracy with optimal goals. Together, these findings demonstrate that goal-setting modulates neural signatures of successful VWM storage (i.e., posterior alpha power suppression), and that a simple effort account cannot explain performance enhancements related to performance feedback and goal setting (i.e., pupil dilation markers of effort were greatest for the supra-capacity Goal 5 condition, but performance was highest for the optimal Goal 3 condition).

Disclosures: O. Kozlova: None. K.C. Adam: None.

Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR358.02/NN11

Topic: I.05. Working Memory

Support: NSF 2050833

Title: Neocortical traveling waves track the removal of information from visual working memory

Authors: *E. F. ESTER;
Univ. of Nevada, Reno, Reno, NV

Abstract: Working memory (WM) enables the storage and manipulation of information no longer present in the environment. To avoid exceeding WM capacity limits, information held in WM should be purged when it is no longer relevant. Top-down control over the contents of WM have been linked with neural oscillations, yet the specific mechanisms responsible for removing task-irrelevant information from WM remain opaque. Here, we hypothesized that the removal of task-irrelevant WM content is mediated by top-down spatially coherent oscillatory signals - traveling waves - that link human frontal cortex with occipitoparietal areas implicated in WM storage. We tested this hypothesis by re-analyzing data from three published EEG experiments that used a neural measure (the contralateral delay activity, or CDA) to track the removal of task-irrelevant information from WM (Tsubomi et al., 2024). We identified a feedback (frontal-to-occipital), mid-frequency (~8-12 Hz) traveling wave that emerged over electrode sites

contralateral to the visual hemifield containing remembered information approximately 650 ms after this information became task-irrelevant. The temporal characteristics of this signal closely tracked reductions in CDA amplitudes thought to index the removal of task-irrelevant WM content. These results implicated neocortical traveling waves in top-down control over the contents of WM.

Disclosures: E.F. Ester: None.

Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR358.03/NN12

Topic: I.05. Working Memory

Support: NIH ROIMH087214
ONR N00014-12-1-0972

Title: Mechanisms of Output Gating for Visual Working Memory

Authors: *C. ZHAO¹, T. ADEKOYA², S. HORWITZ¹, E. AWH¹, E. K. VOGEL¹;

¹Univ. of Chicago, Chicago, IL; ²UC Irvine, Irvine, CA

Abstract: Working memory tasks often require comparing remembered visual arrays to test displays, yet little is known about how people edit the contents of working memory at test. Across two experiments, we used contralateral delay activity (CDA) as a neural index of working memory load to examine how memory representations are selectively accessed during retrieval. In Experiment 1, when a single test item was probed, CDA amplitudes increased with larger set sizes, indicating that untested items were still actively maintained. This suggests minimal editing based on spatial location. In Experiment 2, however, when items belonged to distinct mnemonic categories, CDA amplitudes at test were reduced, consistent with selective editing based on category relevance. Supporting this, analysis of the P3 old-new effect revealed that decision speed and strength were influenced by the number of items maintained at test. Together, these results demonstrate that while people do not edit their working memory load based on spatial cues, they do engage in selective editing based on categorical relevance, enabling more efficient retrieval of task-relevant information.

Disclosures: C. Zhao: None. T. Adekoya: None. S. Horwitz: None. E. Awh: None. E.K. Vogel: None.

Poster

PSTR358: Working Memory and Flexible Cognition

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR358.04/OO1

Topic: I.05. Working Memory

Support: NEI R01-EY025872

Title: Cross-modal recruitment of sensorimotor cortices during a unimodal visual working memory task

Authors: ***L. J. ETTENSOHN**, S. PARK, J. T. SERENCES;
Psychology, UCSD, La Jolla, CA

Abstract: Working memory (WM) involves the temporary storage and manipulation of information in different sensory domains (i.e. visual, haptic, auditory). Research focused on unimodal haptic and visual WM demonstrates that detailed mnemonic representations are sustained in sensory encoding areas over short delays. Here, we use fMRI to measure object-selective response patterns in both visual cortex and sensorimotor cortices to determine whether haptic representations are recruited during purely visual WM tasks, especially when observers are familiarized with the haptic properties of the visual objects. Object-selective responses during a delayed match to sample task were collected before and after 3 days of behavioral training. During behavioral training, objects were divided into three sets: one set for haptic training without visual feedback, one set for visual training without haptic feedback, and one 'control' set of objects that was not trained in between scans. To control for modality and amount of experience, we used novel objects from the asymmetric greeble family (Sheinberg & Tarr, 2009). Using this approach, we compared the impact of modality and amount of experience on object-selective response patterns. Behaviorally, both haptic and visual training increased accuracy on the visual WM task, and univariate mean BOLD responses were also modulated by training, particularly in the visual condition. Finally, the identity of the visual stimuli could be decoded based on activation patterns in both early visual and somatosensory/motor cortex, with improvements in decoding accuracy associated with training. Collectively, these results suggest that there is a cross-modal recruitment of somatosensory and motor cortices in support of a purely visual WM task.

Disclosures: **L.J. Ettensohn:** None. **S. Park:** None. **J.T. Serences:** None.

Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

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Program #/Poster #: PSTR358.05/OO2

Topic: I.05. Working Memory

Support: SUNY System Administration under SUNY Research Seed Grant Award 231056

Title: Working Memory Representations of Visuospatial Sequence Structure in Early Visual and Posterior Parietal Areas

Authors: *L. JIANG¹, J. TEPAN², A. KHIPOVSKA², H.-C. LEUNG²;

¹Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ²Dept. of Psychology, State Univ. of New York, Stony Brook, Stony Brook, NY

Abstract: How does the brain represent visuospatial sequences for guiding future actions? While some suggest that sequences are stored item-wise, others propose that they are represented as compressed structures to enhance processing efficiency. To investigate the neural basis of such structured representations, we collected 12 runs of high-resolution multi-band fMRI data (TR = 1 s, voxel size = 2.3x2.3x2.3 mm³) from nine healthy young adults performing a serial-order, memory-guided saccade task. During this task, four visual images were presented sequentially in different spatial locations and ordinal positions; each stimulus was displayed for 500 ms with a 500-ms inter-stimulus interval. Participants were instructed to retain the stimuli locations over a delay period of 8 s or 10 s and then shift their gaze to the remembered location of the cued target within a 1-s response window. We extracted the GLM-modeled beta maps from each individual, and utilized representational similarity analysis (RSA) to examine the multivariate brain activity patterns varying by the spatiotemporal sequence structures across the task events. Our results showed distinguishable spatial patterns of brain activations in correspondence to the spatial (shape, eccentricity) and temporal-order of the stimulus sequence structure, particularly in early visual areas (V1, V2) during stimulus display (encoding) and in retinotopic regions of the posterior parietal cortex during the retention interval. These findings suggest that sequential visuospatial inputs are represented in working memory according to their spatiotemporal structure, and that different cortical areas, early visual area vs. posterior parietal cortex, play differential roles in representing the structured sequences across time.

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Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR358.06/OO3

Topic: I.05. Working Memory

Support: NIH award 1R01EY036843-01

Title: Adaptive offloading: Visual working memory content is flexibly represented in hand and eye movements

Authors: *S. YANG¹, Y. DONG², A. KIYONAGA³;

¹Cognitive Sci. Dept., Univ. of California San Diego, La Jolla, CA; ²Cognitive Sci., UCSD, San Diego, CA; ³Cognitive Sci., UCSD, La Jolla, CA

Abstract: Working memory (WM) information content is widely distributed across the brain. This distributed encoding supports representations at varying levels of complexity and abstraction that can be flexibly recruited to best serve upcoming behavior. For instance, information may be represented relatively more strongly in the visual cortex when the WM task requires finer visual details. If cortical representations are dynamically recruited based on task demands, then associated peripheral sensors and effectors may also reflect this flexibility. Indeed, recent studies have shown that oculomotor and physiological signals from the peripheral nervous system - such as saccade biases and pupil modulations - can track simple WM features during delay periods. But the functional role of these signals, and their sensitivity to cognitive and motor demands remain unclear. Here, we test whether WM stimulus features can be read out from patterns of eye and hand movements during the WM delay, and whether such signatures are modulated by response modality. We asked participants (n=36) to remember the orientations of grating patches and recall their angles after a brief delay. Critically, we manipulated the motor task demands by varying the response format across experimental blocks: using a digital stylus, participants were asked to either draw a line or adjust a wheel to report the remembered orientation. While the WM stimulus and memory-relevant feature were the same in both cases (i.e., angle), these ‘draw’ and ‘wheel’ conditions were expected to engage different manual action plans and necessitate different levels of memory precision. Via eye- and stylus-tracking, we found that remembered orientations were decodable from both gaze and micro-hand movements during the blank WM delay. However, decoding strengths varied by response format: gaze tracked memorized features relatively better in the ‘wheel’ condition (vs. ‘draw’), while hand movements were more informative in the ‘draw’ condition (vs. ‘wheel’). Individuals who showed greater ‘wheel’ benefits in gaze-based decoding also showed greater ‘draw’ benefits in hand-based decoding, suggesting a strategic processing shift to the more relevant system. As in, drawing may entail a more elaborate manual plan, thereby recruiting the hand more during WM maintenance. These findings suggest that recruitment of the peripheral nervous system during WM maintenance may be adaptively tailored to subtle differences in response demand. In sum, visually encoded WM contents may be flexibly distributed to the most task-relevant motor effectors, balancing WM representations across peripheral signals according to behavioral demands.

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Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

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Program #/Poster #: PSTR358.07/OO4

Topic: I.05. Working Memory

Support: NIH Grant F32MH124268

Title: Cerebellar TMS disrupts spatial working memory representations in visual and frontoparietal cortex

Authors: *J. A. BRISSENDEN¹, M. VESIA², T. G. LEE¹;

¹Psychology, ²Kinesiology, Univ. of Michigan, Ann Arbor, MI

Abstract: Despite extensive research focused on identifying the specific brain structures and mechanisms involved in working memory (WM), much of the prior literature has relied on correlational methods to explore the neural substrates of WM. We sought to investigate the causal role of brain areas spanning the cerebral cortex and cerebellum in WM using a combination of functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation. Across multiple sessions, we applied continuous theta-burst stimulation to perturb activity in cerebellar lobule VIIb, intraparietal sulcus (IPS), frontal eye fields, and a somatosensory control site immediately prior to the performance of a continuous report spatial working memory task in an fMRI scanner. A baseline fMRI session collected structural MRI, resting-state, and population receptive field (pRF) mapping scans. We investigated how stimulation impacted delay-period spatial tuning properties across a network of spatial WM areas. We found that disruption of cerebellum causally impacted spatial decoding in higher-order visual cortex, IPS, and frontal cortex. Further analysis primarily attributed this disruption of spatial decoding to a causal influence of the cerebellum on the response gain of cerebral cortical areas. We also demonstrated that cerebellar disruption abolished the sustained relationship between spatial decoding in visual areas and behavior during the maintenance period.

Disclosures: J.A. Brissenden: None. M. Vesia: None. T.G. Lee: None.

Poster

PSTR358: Working Memory and Flexible Cognition

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Program #/Poster #: PSTR358.08/OO5

Topic: I.05. Working Memory

Support: NIH award 1R01EY036843-01

Title: Flexible gaze reinstatement during working memory for natural scenes

Authors: *Y. DONG, A. KIYONAGA, S. YANG;
UCSD, San Diego, CA

Abstract: Prominent working memory (WM) models assert that feature-specific WM content is stored in sensorimotor cortical activations, and newer evidence suggests that even peripheral oculomotor signals carry WM information. For instance, during a WM delay, small gaze biases veer toward locations in memorized visual space. Yet the specificity and functional relevance of

such oculomotor WM signatures is unclear. In long term memory, gaze patterns at retrieval have been found to recapitulate the spatiotemporal pattern from encoding, and the degree of such gaze reinstatement may functionally relate to performance. Here, we ask whether oculomotor patterns during WM exhibit a similar degree of spatiotemporal specificity and relevance to function. We tracked participants' eye gaze during encoding and WM maintenance of naturalistic images, and we manipulated whether they would be tested on the visual or semantic features of the image. In two experiments, we found that gaze patterns during a blank WM delay resembled the spatiotemporal sequence of eye movements observed during visual encoding of the same image. Gaze patterns during WM may therefore track the identity of complex, natural images that are held in mind. Moreover, we found a stronger degree of such WM gaze reinstatement when the task prioritized precise visual information as compared to semantic categories. Peripheral oculomotor signatures of WM content are therefore malleable to how the content will be used. Lastly, we examined the time course of this WM gaze reinstatement, using representational similarity analysis. Gaze patterns during WM, especially during encoding, followed the representational structure of the stimulus space - meaning that more visually similar images evinced more similar gaze patterns. During the WM delay, this signal was modest, but it ramped up and distinguished between visual and semantic task conditions as the WM probe approached. This suggests that oculomotor WM signals may prospectively anticipate behavioral task demands. Collectively, these results highlight a potential role for the earliest levels of visual processing in WM content representation, and suggest that ocular WM signals are functionally flexible to upcoming demands.

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Poster

PSTR358: Working Memory and Flexible Cognition

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR358.09/OO6

Topic: I.05. Working Memory

Support: FWO

Title: How the brain tracks serial order in working memory; spatial and abstract coding mechanisms

Authors: *G. CRISTOFORETTI^{1,2}, R. BOTTINI¹, Y. XU³, S. VIGANÒ³, W. FIAS²;
¹Cimec, University of Trento, Trento, Italy; ²Dept. of Exptl. Psychology, Ghent Univ., Ghent, Belgium; ³Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: The ability to track sequence order in working memory (WM) is fundamental to human cognition, supporting language, action planning, and recall. Yet its neural coding remains debated. Classical theories propose abstract positional markers that bind items to symbolic sequence slots. In contrast, recent evidence points to a spatial foundation, with ordinal positions anchored to internal spatial axes (e.g., left-to-right continua) and accessed via covert shifts of

attention. Crucially, these accounts may not be mutually exclusive. Here, we used fMRI and searchlight multivoxel pattern analysis (MVPA) to dissociate spatial and abstract representations of serial order in WM. Participants memorized four-digit sequences presented either from left to right or right to left, then completed a dot-probe detection task designed to assess position retrieval. Although spatial layout was incidental to the task, behavioral responses revealed direction-dependent biases, indicating that serial order was spontaneously encoded along a spatial reference frame. MVPA showed that item position could be decoded in the left intraparietal sulcus (IPS) for left-to-right sequences and in the right IPS for right-to-left sequences, supporting the existence of direction-specific codes reflecting egocentric spatial anchoring. In contrast, cross-direction decoding succeeded in the left supramarginal gyrus (SMG), consistent with a direction-invariant, abstract ordinal code. Parametric modulation analyses further revealed that activity in medial parietal cortex and anterior hippocampus scaled linearly with item position, reflecting a distance-based relational signal, likely arising from internal sequence scanning. These findings support a flexible, multi-format architecture for serial order in WM, in which the brain dynamically integrates spatial attention, symbolic codes, and relational structure, bridging mechanisms of working memory, sequence processing, and cognitive mapping.

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Poster

PSTR358: Working Memory and Flexible Cognition

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Topic: I.05. Working Memory

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Title: Tracking the neural signatures of selecting sensory and motor contents in working memory

Authors: *I. ECHEVERRIA-ALTUNA^{1,3,5}, S. BOETTCHER⁴, F. VAN EDE⁶, K. E. WATKINS⁴, A. NOBRE^{2,3};

²Dept. of Psychology, ¹Yale Univ., New Haven, CT; ⁴Exptl. Psychology, ³Univ. of Oxford,

Oxford, United Kingdom; ⁵BCBL, Donostia, Spain; ⁶Vrije Univ. Amsterdam, Amsterdam, Netherlands

Abstract: Visual and motor contents within working memory can be flexibly and dynamically prioritized to guide adaptive behavior. Such prioritization is accompanied by modulations in frequency-specific electroencephalography (EEG) activity. Lateralized posterior alpha-band (8-13 Hz) modulation tracks changes in internal attention to item locations, whereas lateralized central mu/beta-band (8-30 Hz) modulation tracks changes in response-plan prioritization. The limited spatial resolution of EEG, however, precludes resolving the cortico-subcortical networks underlying alpha and mu/beta modulations during internal attention. To identify and compare the networks controlling internal visual and motor selection associated with these EEG markers, we completed a combined simultaneous functional magnetic resonance imaging (fMRI)-EEG study. Participants held two items (tilted bars) in working memory. In turn, items appeared on the left or right side and required a left- or right-hand response, with location and hand manipulated orthogonally. In half the trials, an informative retro-cue prompted participants to prioritize one encoded visual item and its associated action plan and, with the passage of time, to shift their focus to the other spatial location and response plan. In the other half, retro-cues were uninformative, so neither visual nor motor prioritization was possible. The EEG analyses replicated patterns of contralateral alpha modulation for spatial selection and contralateral mu/beta modulation for motor selection. Analysis of fMRI data revealed the engagement of frontal and parietal areas associated with internal attention control. Additionally, we found lateralized modulation of visuo-parietal areas during internal location selection and of primary motor cortex during action prioritization. Ongoing analyses will probe for subcortical-cortical systems tracking shifts in internal spatial and motor attention. The results offer interesting clues about the sources underlying the modulations of frequency-specific activity that accompany flexible sensory and motor prioritization in working memory.

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Poster

PSTR358: Working Memory and Flexible Cognition

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Topic: I.05. Working Memory

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MH122023

Title: Phase burst coupling reveals functional working memory networks

Authors: ***B. KUNDU**¹, T. DAVIS⁵, S. S. NAIR², P. HACKETT³, E. H. SMITH⁶, J. D. ROLSTON⁷, V. OMELYUSIK⁴;

²Electrical & Computer Engin., ³Neurosurg., ⁴Dept. of Electrical Engin. and Computer Sci.,

¹Univ. of Missouri, Columbia, MO; ⁶Neurosurg., ⁵Univ. of Utah, Salt Lake City, UT;

⁷Neurosurg., Brigham and Women's Hosp., Newton, MA

Abstract: Transient bursting in the high gamma (70-140 Hz) and beta (20-35 Hz) bands in the prefrontal cortex has been associated with working memory (WM). However, WM is likely related to activity from a network of frontotemporal areas. Here we analyzed local field potential coupling between beta phase and high gamma bursts in 26 frontotemporal regions of 13 epilepsy patients with intracranial depth electrodes while they performed a load-3 visual delayed recall task. We hypothesized that gamma bursts align to certain phases of the ongoing beta oscillation. We quantified this coupling by binning bursts by the phase of the beta wave on which they occur. Phase burst coupling (PBC) compares the entropy of the experimental phase-burst distribution over 18 phase bins with the entropy of the uniform distribution and reflects the degree to which bursts cluster at particular phases of the beta wave. We computed average PBC between channel pairs within each region and between regions during the encoding and maintenance periods of the task in correct trials and identified regions whose coupling was significantly different from the fixation baseline. Treating these PBC values as weight edges in a directed graph, we constructed encoding and maintenance PBC networks. We found that these networks were significantly different ($p = 0.003$, paired t-test) implying the maintenance period engages a different network than encoding period. A subgroup of subjects (6/13) were not required to maintain fixation during encoding. Analysis of group differences showed the subgroup had a smaller number of significant connections during both periods, and less evidence of top-down connections. However, there was no significant difference across the bottom-up connections common to both groups ($p > 0.1$ for both encoding and maintenance, paired t-test). These results indicate that maintaining items in extrafoveal locations requires top-down control versus foveating to memoranda (more significant PBC pairs). Extending the analysis, we found that the beta-gamma PBC network was significantly different from the theta-gamma (4-8 Hz) network during maintenance ($p < 0.001$, paired t-test) but not encoding ($p = 0.06$, paired t-test), which might indicate functional differences between the beta and theta rhythms.

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Poster

PSTR358: Working Memory and Flexible Cognition

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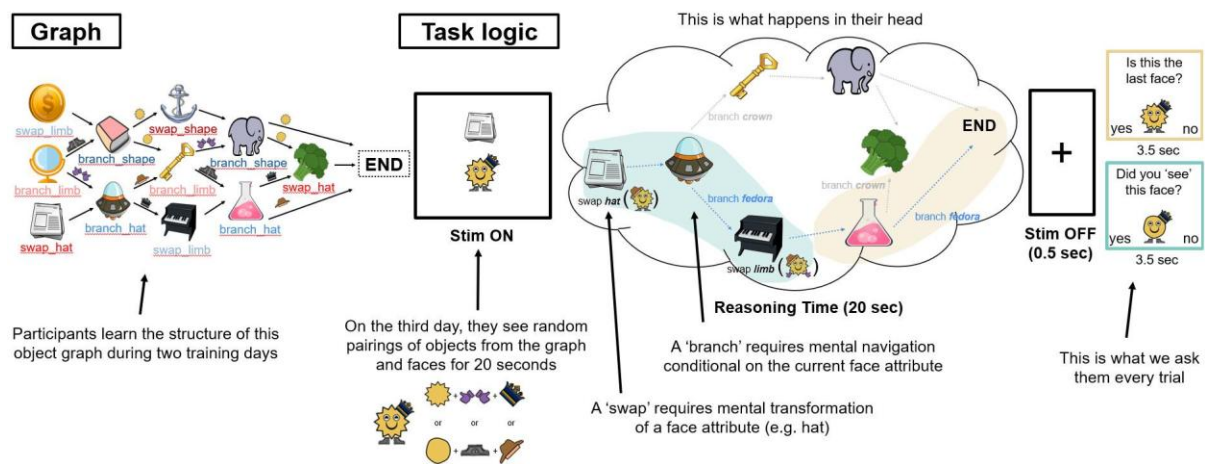
Title: Executing programs in replay

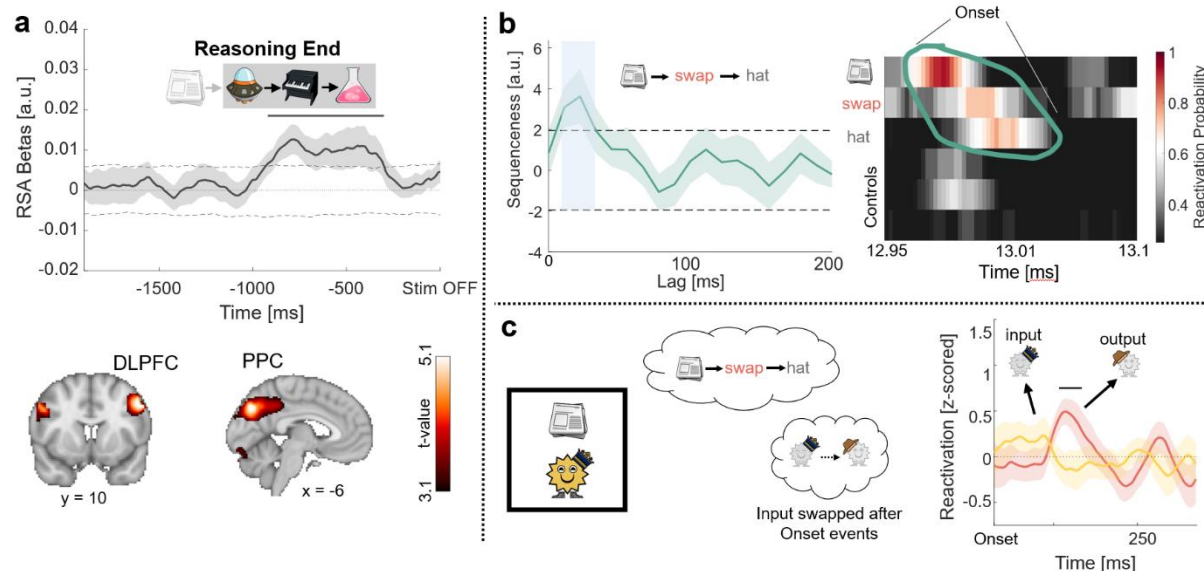
Authors: *S. VESELIC¹, L. LUETTGAU², S. W. KENNERLEY¹, T. E. BEHRENS³, Z. KURTH-NELSON², T. MULLER¹;

¹Univ. of Oxford, Oxford, United Kingdom; ²Univ. Col. London, London, United Kingdom;

³FMRIB Centre, Univ. Oxford, Oxford, United Kingdom

Abstract: A series of surprising experimental discoveries about replay in the brain have led to the proposal that replay is a general-purpose reasoning mechanism. Here we test two predictions of this theory: that replay events include representations of operations used for reasoning, and that the outcome of reasoning appears in the brain after replay events. We developed a magnetoencephalography (MEG) task where participants executed simple programs that operated on a cartoon face with three attributes (Figure 1, Task Logic). Each trial in the scanner contained a novel program not seen in training. While participants (N = 29) mentally executed these programs during Reasoning Time, we found neural representations of executed programs (Figure 2a) that localised to dorsolateral prefrontal and posterior parietal cortex. During reasoning, when neural representations of objects on the graph (e.g. Newspaper) spontaneously reactivated, abstract representations of their operations followed milliseconds later (Swap and Hat, Figure 2b). Aligning neural representations of face attributes (e.g. Fedora or Crown) to these events revealed they were correctly transformed shortly after (Figure 2c). Our results support the hypothesis that replay is a general-purpose reasoning mechanism.





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Poster

PSTR358: Working Memory and Flexible Cognition

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Program #/Poster #: PSTR358.13/Web Only

Topic: I.05. Working Memory

Title: Genetic Contribution of Phase Amplitude Coupling Entrained by Working Memory

Authors: *A. NAZARI¹, M. ESGHAEI², S. TREUE²;

¹German Primate Ctr., Gottingen, Germany; ²Cognitive Neurosci. Lab., German Primate Ctr., Gottingen, Germany

Abstract: A ubiquitous property of neural oscillatory activity throughout the brain is the coupling between low and high-frequency component, a property that is critical for higher order cognitive functions, such as working memory (WM). So far, the heritability of this coupling has remained unclear. We analyzed magnetoencephalography (MEG) data from the human connectome project dataset (HCP 1200 Release¹, collected from 82 subjects—consisting of monozygotic (MZ) and dizygotic (DZ) twins, and unrelated (UR) pairs—who performed a WM task. Local field potentials (LFPs) during the blank period, i.e., when the subjects maintained a previously presented image in memory, were filtered into multiple low and high frequency bands. Phase-amplitude coupling (PAC) was computed for low-high frequency pairs, using a novel method; we determined the time point where the Gamma power peak happens for each high-frequency band and extracted the lower frequency signal ± 100 ms around this peak. This

component -named Gamma peak-triggered low frequency- was averaged across trials for each recording site. The peak-to-peak magnitude difference of the Gamma peak-triggered low frequency was used to measure across-subject PAC similarity. We observed that PAC similarity was significantly higher among MZ twins compared to UR pairs across several frequency pairs. To investigate if the stronger PAC similarity is due to genetic similarity (and not age, gender, ...), we further analyzed same-sex DZ twins. The mean correlation of the DZ twins was compared to the MZ twins. Results revealed that DZ twins had lower PAC similarity compared to MZ twins, in a wide range of Gamma bands. To investigate if the PAC similarity among MZ twins is behaviorally relevant, we examined if working memory performance, measured by accuracy similarity between pairs, is similar among them. Our analysis showed a significant positive correlation between PAC similarity and accuracy similarity, suggesting that PAC similarity is behaviorally relevant. In summary, MZ twins show a stronger PAC similarity compared to both DZ twins and UR pairs. This suggests that PAC has genetic underlying mechanisms, especially in the context of its role in WM. Our results provide compelling evidence for the heritability of PAC as a foundational mechanism in high-level cognitive processing.¹. Van Essen DC, Ugurbil K, Auerbach E, et al. The Human Connectome Project: A data acquisition perspective. *Neuroimage*. Published online 2012. doi:10.1016/j.neuroimage.2012.02.018

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Poster

PSTR358: Working Memory and Flexible Cognition

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Topic: I.05. Working Memory

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Title: Attention orientation during multi item maintenance in human working memory

Authors: *B. BERGER^{1,3}, T. MINARIK⁴, M. RUVALCABA⁵, P. BRUNNER⁶, M. R. PAFF⁷, J. T. WILLIE⁸, J. J. LIN⁹, O. JENSEN¹⁰, R. T. KNIGHT²;

²Psychology and Neurosci., ¹UC Berkeley, Berkeley, CA; ³Psychology, Victoria Univ., Wellington, New Zealand; ⁴Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; ⁵Univ. of California, Berkeley, Santa Clara, CA; ⁶Dept. of Neurosurg., Washington Univ., St. Louis, MO; ⁷Univ. of California, Irvine, Irvine, CA; ⁸Neurolog. Surgery, Washington Univ.

Sch. of Med., Saint Louis, MO; ⁹Dept. of Neurol., Univ. of California, Davis, Sacramento, CA;
¹⁰Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom

Abstract: Working memory (WM), i.e. keeping information in mind for short periods of time and using this information in a goal directed manner, is essential for everyday life, yet it is still controversial how it is implemented in the human brain or how we could enhance impaired WM in neuropsychiatric disorders. Here we present findings from human non-invasive (MEG) and invasive intracranial electrophysiology (sEEG) highlighting a cortical and subcortical network for flexible multi-item WM maintenance. We performed a working memory retro cue paradigm with healthy young adults using MEG as well as in patients with refractory epilepsy using intracranial sEEG. Participants were presented with a lateralized face and Gabor grating followed by a short retention and a retro cue indicating which item to keep maintaining and which one to forget. A probe stimulus was presented after the post cue retention period and participants responded with a simple match/non-match button press. In the MEG we combined the paradigm with a novel approach by invisibly tagging visual stimuli presented on screen (RFT) that can be read out using electrophysiology and reliably distinguished the face tag from the Gabor tag. Alpha activity in MEG supported lateralized item maintenance whereas beta frequency uniquely distinguished between the maintenance of Gabor grating orientation and face identity in the MEG.

Beta activity in MEG differentiated working memory contents while slower oscillatory activity (theta and alpha frequency bands) supported long-range network formation. High frequency activity in sEEG (HFA; 70-150 Hz) supported both item perception and memory retention. Furthermore, HFA was increased before a stimulus onset providing an index of attention preparation. MEG also showed that the strength of the induced fast rhythmic signals (RFT) were modulated by and thus tracked covert attention during WM maintenance. Finally, the HFA signal in sEEG differentiated areas involved in item maintenance (parietal) or in attention allocation (dorsolateral and frontal eye field) with insular cortex showing HFA for both conditions as well as item perception.

Our findings are in accord with invasive animal models highlighting the relevance of beta oscillations for item maintenance in working memory and offer new insights into the temporal and spatial organization of WM in the human brain. By leveraging the strengths of MEG and sEEG we delineate complementary signatures of attention and memory.

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Poster

PSTR358: Working Memory and Flexible Cognition

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Topic: I.05. Working Memory

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Title: Brain-wide neural mechanisms of cross-task information generalization

Authors: *Y. WANG¹, C. T. LI^{2,3}, X. ZHANG³;

¹Inst. of Neuroscience, CAS, Shanghai, China; ²Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai City, China; ³Lingang Lab., Shanghai, China

Abstract: Cognitive flexibility is the cornerstone of adaptive behavior, enabling humans and other animals to rapidly learn the associations between external stimuli and to appropriately generalize the learned knowledge to solve different problems for continual task demands. Many studies have shown that both frontal cortex (PFC, OFC) and hippocampus play important roles in representations of task states. However, little is known about the neural mechanisms of cross-task information generalization through the whole brain. To address this question, we trained mice learning to associate sequential odors with rewards in tasks involving 2 WM (working memory) tasks and 1 NonWM (non-working memory) task. High-throughput electrophysiological recordings showed that neurons exhibit both task-specific and cross-task generalization encoding abilities. At the single-cell level, neurons showed diverse encoding patterns across tasks. At the population level, orbitofrontal cortex neurons demonstrated differentiated encoding during the learning stage but evolved cross-task generalization only between WM tasks during the expert stage. Whole-brain analysis revealed that while individual regions like the anterior olfactory nucleus showed distinct generalization patterns, overall neural populations exhibited more task-specific representations during learning and more consistent generalization during expertise. Using geometry analysis, we found that neural activity trajectories in a three-dimensional decoding space showed increased convergence between WM tasks and divergence between NonWM task and WM task during the expert stage. Above all, our research has expanded the global understanding of cognitive flexibility, making an important contribution to further in-depth understanding of this advanced function in the field.

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Poster

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Topic: I.05. Working Memory

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Title: Composing Representations and Transformation to Enable Flexible and Generalizable Cognition

Authors: *A. ARDALAN¹, J. D. COHEN², T. BUSCHMAN²;
¹Princeton Neurosci. Inst., Princeton, NJ; ²Princeton Univ., Princeton, NJ

Abstract: Cognition is highly flexible; we process a variety of stimuli and can generate a range of responses according to different tasks. We can also generalize to new stimuli and new tasks. Yet, the neural mechanisms that underly such flexibility remain unknown. Inspired by recent experimental observations, we propose a novel recurrent neural network (RNN) architecture that leverages the geometry of representations and transformation to allow for flexible sequencing of tasks. Our approach harnesses compositionality in three key ways: (1) composing representations to integrate different modalities and geometries, (2) composing dynamics to transition between regimes such as perception, memory maintenance, and subspace alignment, and (3) composing transformations to interpolate between tasks and support generalization. Our approach sheds light on how the brain may jointly achieve flexibility and generalization, while offering inductive biases relevant to building artificial general intelligence systems.

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Poster

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Topic: I.05. Working Memory

Support: Startup grant from U. Pittsburgh

Title: Intracortical spiking and local field potential activity in human primary somatosensory cortex during vibrotactile working memory

Authors: A. ABDALTAWAB¹, C. M. GREENSPON⁵, A. PERSELLO², R. A. GAUNT³, *D. MENDOZA-HALLIDAY⁴;

¹Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; ²Univ. of Pittsburgh, Pittsburgh, PA, ; ³Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; ⁴Univ. of Pittsburgh, PITTSBURGH, PA; ⁵Dept. of Organismal Biol. & Anat., Univ. of Chicago, Chicago, IL

Abstract: Humans and other animals can temporarily maintain mental representations of recent stimuli - a function known as working memory (WM). Numerous studies in non-human primates (NHPs) have shown that WM representations of sensory features are robustly encoded in the neuronal spiking activity in sensory association cortex and higher-level parietal and prefrontal cortical areas but are absent or very weak in early sensory areas. In contrast, human fMRI studies have reported that WM representations of visual features can be decoded from blood-oxygenation level-dependent (BOLD) patterns in primary visual cortex. These studies have proposed that neurons in primary sensory cortical areas are causally involved in the maintenance of visual WM representations, in contradiction with NHP electrophysiological studies. This discrepancy could be due to an inconsistency between signals (BOLD signals vs. single-neuron spiking) or a difference in WM mechanisms between humans and NHPs. To resolve this discrepancy, here we performed intracortical recordings of multi-unit activity and local field potentials (LFPs) in one human subject with two 32-channel multi-electrode arrays implanted over primary somatosensory cortex for clinical purposes, while they performed a vibrotactile WM task. The subject was presented with a 1.2-s vibrotactile sample stimulus with one of five amplitudes, followed by a 5-s delay period during which they had to remember the sample amplitude. A subsequent test stimulus was presented for 1.2 s, and the subject reported verbally which amplitude was higher - sample or test. A distractor vibrotactile stimulus was presented for 1 s mid-delay. We found that for nearly three quarters of all multi-units, firing rates during the sample were significantly different from baseline, and many were selective for the sample amplitude, as expected. In contrast, during the delay period, firing rates were not significantly different from baseline, and sample selectivity was virtually absent. For most channels, LFP power during the sample was different from baseline and was sample-selective in all frequency bands. During the delay, power in many channels was significantly different from baseline in several frequency bands, but this activity was not sample-selective. Interestingly, sample selectivity reappeared during the test period. Our results suggest that spiking activity and local field potentials in human primary somatosensory cortex do not contribute to the maintenance of vibrotactile representations in WM, but that these representations can return to this area to modulate subsequent stimulus responses when a sensory-memory comparison is required.

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Poster

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Topic: I.05. Working Memory

Support: NIH Intramural Research Program ZIAMH002783

Title: Hippocampus involvement in a dual-retrocue working memory task

Authors: *C. WALSH¹, J. GONZALEZ-CASTILLO², D. A. HANDWERKER², T. MORGAN³, P. BANDETTINI²;

²Section on Functional Imaging Methods, ³fMRI Core Facility, ¹Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Maintenance of information in working memory is fundamental for higher-order cognition. Research in both non-human primates and humans includes evidence that the maintenance of task-relevant information comes from persistent neural activity in the lateral prefrontal cortex. However, recent work has suggested that information can be maintained in the face of interruptions to this persistent activity, a phenomenon known as activity silent working memory (ASWM). Here, task-relevant information is held through persistent, recurrent activation in parietal and prefrontal regions. Information that is not currently task-relevant but may later become so is held in long-term memory state through sub-threshold patterns of reinforced synaptic weights, that are then brought into an active supra-threshold state by medial temporal lobe structures such as the hippocampus when they become task-relevant. To test the hypothesis that medial temporal lobe structures support working memory, the present study (7T fMRI scanning, 1.3mm³ voxels, TR=1s, TE=20ms) utilizes a dual retrocue paradigm. In this task, participants are initially shown a face and a scene to maintain across a short delay period, after which they are cued that they will be tested on one of the two images after an additional delay. After the first test, a second cue indicates which of the two images will be tested in the second half of the trial; critically, this second probe image is sometimes the same image category as the first probe (“stay” trials) and sometimes reflects the other image category (“switch” trials). Preliminary univariate analyses show increased activation in right posterior hippocampus during the second maintenance period of switch trials relative to stay trials, which could imply retrieval of the initially uncued image from an activated long-term memory state. Additionally, we observe greater univariate activation during the same second maintenance period in left anterior hippocampus for stay relative to switch trials, potentially indicating a refreshing of the information held in working memory. We plan multi-voxel approaches to provide more precise indications of specific memory features, thus giving more precision to these findings. We also plan to assess hippocampal-cortical functional connectivity as well as hippocampal activation and decoding performance in a post-scan memory test.

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Poster

PSTR358: Working Memory and Flexible Cognition

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Topic: I.05. Working Memory

Support: 5u01MH120447

Title: Preliminary analysis on behavioral and resting state connectivity data following theta burst stimulation

Authors: *I. SUN¹, D. F. GREGORY², M. PATEL³, N. L. BALDERSTON⁴;
²Psychiatry, ¹Univ. of Pennsylvania, Philadelphia, PA; ³Univ. of Pennsylvania Dept. of Psychiatry, Philadelphia, PA; ⁴Psychiatry, Univ. of Pennsylvania Department of Psychiatry, Philadelphia, PA

Abstract: Recent neuromodulation studies have shown compelling evidence that theta burst stimulation (TBS) of the dorsolateral prefrontal cortex (dlPFC) is involved with the cognitive process of emotion regulation, an underlying mechanism crucial to the treatment of mental illnesses. However, it is not yet explained how specific mechanistic changes in the brain contribute to this finding. This study aims to determine both resting state connectivity and task performance following two patterns of stimulation: intermittent (iTBS) and continuous (cTBS). We hypothesized that iTBS over cTBS would increase the functional connectivity between the left dlPFC and downstream regions and improve task performance. Our study aims to enroll 200 total participants to complete 3 sessions of either iTBS or cTBS, with functional scans performed about 24 hours after each TMS session. Participants are instructed to complete a variation of the Sternberg letter memory task while in the scan, for both TMS targeting and functional connectivity analyses. Throughout the task, participants are asked to either sort or maintain the letters seen each trial. Participants are required to attend six visits in total (including consent) throughout the study. This poster aims to present preliminary analysis of task performance in response to each visit and TMS frequency in a subsample of the participants (n=100). For both sort and maintain trials, we analyzed the effects of TMS frequency (iTBS and cTBS) and visit on accuracy and reaction time using a two-way ANOVA. For sort trials, simple main effects were found for accuracy with both TMS frequency ($p < 0.001$) and visit ($p < 0.01$). Additionally, a significant interaction was found between TMS frequency and visit for reaction time ($F(1,3) = 3.61$, $p = 0.013$). Simple main effects were found with visit ($p < 0.001$), but not with TMS frequency ($p = 0.815$). Moreover, for both maintain and sort trials, iTBS showed an overall increase in task performance with higher accuracy rate and lower reaction time compared to cTBS. The results support our hypotheses with expected interaction found between TMS frequency, visit, and task performance. With TBS being increasingly applied in the treatment of mental illness, it will be important to understand how TBS modulates network connectivity. As we understand more about the underlying mechanisms of the relationship between specific brain regions and emotion regulation, TBS can be widely applied to various mental illnesses in clinical treatment.

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Poster

PSTR358: Working Memory and Flexible Cognition

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Topic: I.05. Working Memory

Support: NARSAD young investigator award to Hamed Ekhtiari

Title: Real-time optimization of tACS using closed-loop tes-fMRI to modulate frontoparietal connectivity

Authors: *G. SOLEIMANI¹, H. EKHTIARI²;

¹Univ. of Minnesota, Minneapolis, MN; ²Dept. of Psychiatry, Univ. of Minnesota, Wayzata, MN

Abstract: Closed-loop transcranial electrical stimulation combined with functional MRI (tES-fMRI) provides a powerful method for personalizing and optimizing non-invasive brain stimulation in real time. We developed a closed-loop system that systematically adjusts transcranial alternating current stimulation (tACS) parameters—specifically frequency and phase—based on frontoparietal task-based functional connectivity (FC). To evaluate this approach, we used a working memory (WM) task as a test case due to its well-established association with frontoparietal connectivity, which can be modulated using theta-band tACS. Twenty healthy adults were randomized to receive 20 minutes of dual-site tACS via two 1×4 HD electrode montages over F4 and P4. During the training phase, stimulation parameters were iteratively updated using a simplex optimization algorithm. The **Boost group (n=10)** received stimulation intended to enhance frontoparietal FC (maximized FC), while the **Reduce group** received stimulation designed to decrease it (minimized FC). In the test phase, each group received its respective optimized or non-optimized parameters during the WM task, and performance was assessed via accuracy and reaction time. Results showed no significant differences during the training phase; however, in the test phase, the Boost group (0.96 ± 0.03) demonstrated significantly higher accuracy than the Reduce group (0.86 ± 0.05 ; $P < 0.01$), with no difference in reaction time. These findings demonstrate the feasibility and efficacy of a closed-loop tES-fMRI system for real-time optimization of tACS parameters. Modulating stimulation to enhance frontoparietal connectivity can lead to measurable improvements in working memory performance, supporting the potential of connectivity-informed stimulation protocols for cognitive enhancement.

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Poster

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FA9550-23-1-0024

Title: Reinforced inhibitory signaling strengthens neural persistence and working memory in *Drosophila*

Authors: *M. L. HEINTSCHEL^{1,2}, M. YAO^{4,3}, J. XU⁵, T. O. SHARPEE^{6,3}, D. GROVER¹;
¹Kavli Inst. for Brain and Mind, ²Bioengineering, ³Physics, UCSD, La Jolla, CA; ⁴Salk Inst. for Biol. Studies, San Diego, CA; ⁵Caltech, Pasadena, CA; ⁶CNL-T, Salk Inst., San Diego, CA

Abstract: Working memory (WM), the ability to maintain and manipulate a limited amount of information “in mind” for a brief period, is a crucial component of cognition. While persistent activity is considered essential for WM, the neural and cellular mechanisms that generate and sustain this persistence over extended timescales remain poorly understood. Emerging evidence suggests that inhibitory signaling plays a key role in this process across model systems, including in invertebrates such as *Drosophila*. Here, we aimed to determine the cellular inhibitory microcircuit and signaling dynamics that govern the neural mechanisms underlying a visual WM task through its concomitant physiological signature of trace conditioning; wherein the conditioned and unconditioned stimuli are separated by a temporal gap. Referencing the fly connectome, we found GABAergic (i.e., inhibitory) R2/R4m ring neurons within the Ellipsoid Body substructure of the Central Complex, previously reported to exhibit prolonged, oscillatory calcium transients during trace conditioning, heavily reciprocated synapses with concentric GABAergic R3/R4d ring neurons. The R3/R4d neural population was found to behave counter to R2/R4m neurons, with functionally necessary suppression of calcium activity during conditioning. To identify the cellular signaling mechanisms underlying these neural ON/OFF states, we selectively knocked down GABA signaling from R2/R4m (GAD1, vGAT) and to R3/R4d (Rdl), resulting in a complete loss of signal persistence and consequently no learning. In addition to GABA, we selectively knocked down glutamate (Glu; vGluT) and nitric oxide (NO; dNOS) which are known to interplay with GABA signaling. We discovered that a knockdown of Glu transport from R2/R4m neurons and NO production in R3/R4d neurons led to impaired performance and altered calcium dynamics, though these manipulations did not fully abolish activity as observed with GABAergic knockdown. Finally, using biosensors that provide a readout of glutamate and GABA levels in this circuit, we characterized the interplay between these neurotransmitters during the ‘ON’ state of task-relevant neurons. We will demonstrate evidence that reveals coordinated Glu and GABA dynamics that evolve over the course of conditioning. Together, these results uncover a functional inhibitory microcircuit where Glu, NO, and GABA coordinate in a task-dependent manner to regulate observed oscillatory neural persistence underlying WM.

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Poster

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Title: Modeling inhibitory signaling dynamics underlying working memory in *Drosophila* trace conditioning

Authors: *M. YAO^{1,2}, M. HEINTSCHEL^{3,4}, D. GROVER⁴, T. O. SHARPEE^{1,2};
¹Salk Inst. for Biol. Studies, San Diego, CA; ²Dept. of Physics, ³Dept. of Bioengineering, ⁴Kavli Inst. for Brain and Mind, UCSD, La Jolla, CA

Abstract: Trace conditioning, where a conditioned stimulus (CS) precedes a temporally separated unconditioned stimulus (US), requires transient working memory (WM) to bridge the delay. In *Drosophila*, EB ring neurons R2/R4m show persistent but distraction-sensitive activity during the trace interval (TI), supporting a WM-like role. Recent experiments reveal that R3/R4d neurons also modulate performance, suggesting a more complex inhibitory network supporting WM. To understand how these inhibitory subpopulations contribute, we built a biophysical computational model of the EB circuit, informed by molecular and circuit-level experiments. Specifically, optogenetic activation and targeted neurotransmitter perturbations revealed a *glutamate-NO-GABA* feedback loop between R2/R4m and R3/R4d as critical for trace conditioning, motivating our modeling framework. Our model extends a *Wilson-Cowan* architecture with synaptic and molecular signaling dynamics. It integrates four components: (1) a WM circuit with bi-stability; (2) dopamine-modulated Hebbian plasticity; (3) a nonlinear R3/R4d molecular pathway; and (4) temporal difference reinforcement learning (TDRL). Each mechanism maps to specific neural populations, with R2/R4m acting as a plastic inhibitory implementer and R3/R4d as a modulatory unit amplifying global inhibition through the glutamate-NO-GABA cascade. Simulations reproduced key experimental findings: optogenetic activation of R2/R4m facilitates CS retention, while activation of R3/R4d suppresses it. Hebbian learning asymmetrically shapes synaptic weights to form a stable attractor supporting CS-driven persistent activity. NO from R3/R4d acts on R2/R4m to increase GABAergic output strength, enhancing the unbalanced connectivity. Disrupting this crosstalk reduces synaptic asymmetry and impairs learning, consistent with experimental results. These dynamics suggest a division of labor: R2/R4m performs selective stimulus retention and learning, while R3/R4d stabilizes circuit by enhancing the selectivity. Simulations highlight distinct effects of GABA gain, NO synthesis rate, input currents and several other biophysical parameters. Such testable predictions offer a roadmap for future experiments involving timescale-sensitive or molecule-specific perturbations. Together, our model links molecular signaling, circuit dynamics, and behavior. By modeling inhibitory interactions, it shows how transient WM states emerge and stabilize within a minimal circuit. This work illustrates how neuromodulation and circuit heterogeneity together balance flexibility and robustness in temporally structured learning.

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Poster

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Topic: I.05. Working Memory

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Title: Dynamic changes in effective connectivity during decision making

Authors: *M. L. IOFFE¹, C. D. BRODY², D. W. TANK¹;

¹Princeton Univ., Princeton, NJ; ²Princeton Neurosci. Inst., HHMI / Princeton Univ., Princeton, NJ

Abstract: How does cortical connectivity support decision making? Here we probed the functional connectivity of excitatory pyramidal neurons in layer 2/3 retrosplenial cortex, with an all-optical simultaneous imaging and stimulation system while mice navigated a sensory discrimination maze in virtual reality. We measured effective connectivity both during and following maze navigation, which provided separate estimates of task-independent and task-dependent connectivity. When the distance between target and responder neurons is in the ~200um range, the interaction between the pair of excitatory neurons is expected to be mediated by interneurons. Curiously, these interactions were not always suppressive during the task. Task-dependent connectivity was modulated by the ongoing epoch, with large contributions from how specific subpopulations of excitatory neurons drove the rest of the population. For example, during the crucial decision-making epoch in the task, these interactions were net excitatory or inhibitory, dependent on which choice the sensory evidence indicated. Interestingly, this effect did not depend on which choice the target neurons themselves preferred. Our results suggest a functional role for interneuron circuits in mediating the divergence of neural trajectories during the decision-making epoch.

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Poster

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Topic: I.05. Working Memory

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Title: A shared cortical representation between similar tasks scales with working memory

Authors: *E. DIAMANTI¹, L. PINTO², M. SCHOTTDORF³, S. Y. THIBERGE¹, C. D. BRODY⁴, D. W. TANK¹;

¹Princeton Univ., Princeton, NJ; ²Dept. of Neurosci., Northwestern Univ., Chicago, IL; ³Univ. of Delaware, Newark, DE; ⁴Princeton Neurosci. Inst., HHMI / Princeton Univ., Princeton, NJ

Abstract: How does the structure of neural representations underlying cognitive processes differ from those underlying other task-relevant variables? We asked whether the cortex contains structured working-memory representations and how they differ from task representations not requiring working memory. We trained 9 transgenic mice to perform a working-memory task (towers) in virtual reality that combined navigation with sensory evidence accumulation. The towers-task alternated in blocks with a simpler (visually guided) task featuring the same virtual environment without requiring working memory. While mice performed the two tasks with the same motor behavior, we simultaneously imaged excitatory neurons in one visual area (AM), and two association areas, retrosplenial (RSC), and premotor (M2), using a random-access two-photon microscope. At first glance, single-neuron trial-averaged activity was indistinguishable between the two tasks. However, pairwise correlations dropped during working memory, especially in association areas. We linked the drop in pairwise correlations to both a linear and a nonlinear task subspace configuration, whereby the working-memory task subspace contained the simpler task subspace. Furthermore, nonlinear dimensionality reduction analyses provided further insight into the organization of the neuronal population onto the low-dimensional, task-induced structure ('manifold'). Namely, the firing fields of individual neurons were organized in an orderly manner on the manifold. Yet, the organization of firing fields differed between tasks, specifically for neurons forming sequences along the evidence dimension. These neurons formed firing fields on the manifold that became more disjoint during the working-memory compared to the simpler task. Even when treating the two tasks independently, we found that the underlying structure was the same, yet the working-memory task structure expanded in the two association areas, but not in AM. Crucially, the amount of overlap between manifold firing fields, and hence between task subspaces, reflected the degree by which behavior relied on working memory across mice. We propose the expansion of an otherwise common task structure, whereby, during working memory, individual firing fields become more disjoint. We thus provide a conceptual framework for how cognitive processes, such as working memory can shape the dynamics of distributed neural networks in cortex.

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Poster

PSTR359: Basic and Translational Circuit Mechanisms of Social Behavior

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Topic: I.06. Social Cognition

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Title: Social cognition deficits in the J20 mouse model of Alzheimer's disease

Authors: ***R. MORAIS-RIBEIRO**^{1,2}, D. PIMPINELLA², T. BOCK^{2,3}, M. MENDANHA¹, T. G. OLIVEIRA¹, S. A. SIEGELBAUM²;

¹Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Medicine, Univ. of Minho, Braga, Portugal; ²Dept of Neurosci., Columbia Univ., New York, NY; ³RWTH Aachen Univ., Aachen, Germany

Abstract: The hippocampal CA2 subregion plays major roles in regulating social behavior. It is required for the encoding, consolidation, and recall of social novelty recognition memory, which is essential to discriminate a novel from a familiar conspecific. CA2 dysfunction has been implicated in several neuropsychiatric disorders associated with abnormal social behavior, including epilepsy, schizophrenia, and different forms of dementia. Recent studies of the Tg2576 mouse genetic model of Alzheimer's disease (AD) reported a decrease in inhibitory synaptic transmission and synaptic plasticity in CA2 that contributes to social memory deficits. Here we explore a second genetic model of AD, the J20 mouse line, to determine whether social memory deficits and altered CA2 function are a common feature of AD mouse models. We found that male and female J20 mice show increased mobility in an open field test, in line with previously reported hyperactivity phenotype observed in these mice. Moreover, male and female J20 mice show relatively normal levels of sociability, the preference to explore a novel mouse inside a wire cup cage compared to an empty cup. We then subjected the mice to a CA2-dependent social novelty recognition task, in which a subject mouse explores an arena containing a novel conspecific and a previously encountered conspecific confined to separate wire cup cages. Social memory is manifested by the preference of the subject to explore the novel mouse. Male J20 mice show a significant impairment in this task. Strikingly, age-matched female J20 mice show a normal social memory behavior. To understand the mechanisms underlying the differences encountered within the J20 group, we collected the brains of the mice subjected to behavioral tests to measure the expression of cellular markers that could be associated with their performance. Interestingly, our preliminary results suggest that greater deltaFosB immunoreactivity, a readout of hippocampal chronic activity, may be associated with greater social memory impairment, manifested by the preference of the subject to explore the previously encountered conspecific in the social memory task. In electrophysiological experiments in ex vivo hippocampal slices, we have so far failed to observe a significant difference in either intrinsic electrophysiological properties of CA2 pyramidal neurons or in the amplitude of excitatory or inhibitory synaptic potentials evoked by electrical stimulation of the hippocampal or cortical inputs to CA2 in J20 compared to wild-type mice. Future experiments will explore whether the deficit in social memory is associated with altered in vivo activity of CA2 neurons in this AD mouse model.

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Poster

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Title: Neural circuits for valence updating in social memory

Authors: *N. SUTO^{1,2}, M.-Y. WANG², M. CHUNG², K. TAO², Z. HUANG², S. MORIKAWA², H. TAKEUCHI², S. KIRA³, T. OKUYAMA²;

¹The university of tokyo, Bunkyo-ku, Japan; ²The Univ. of Tokyo, Tokyo, Japan; ³Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Social animals, including humans, recognize and respond appropriately to both harmful and affiliative conspecifics within their group. As social relationships change dynamically, this adaptive behavior requires continuous updating of the social valence to specific familiar individuals. To investigate how such valence updating occurs in the context of negative interactions, the neural mechanisms underlying social avoidance behavior have been rigorously investigated using the chronic social defeat stress model. In the model, the defeated test-mice socially avoid not only the aggressor mouse but also other non-aggressor mice, whose behavioral phenotype we referred to as “general social avoidance”. However, the mechanisms by which defeated animals specifically avoid the individual who defeated them, rather than avoiding all individuals—a behavior we referred to as “specific social avoidance”—remain largely undefined. To address this, we established a novel behavioral paradigm to investigate neural mechanisms underlying specific social avoidance. Using the paradigm, we showed that the neural circuit from the ventral CA1 (vCA1) in the hippocampus, a storage region of social memory engram, to the basolateral amygdala (BLA), a critical site for emotional representation, is necessary for the association of social memory with negative emotional valence to elicit the specific social avoidance. Furthermore, optogenetic manipulation of social memory engram exhibited that the vCA1-BLA projection of social memory engram neurons is necessary and sufficient to associate social memory with negative valence. Finally, we found by Ca²⁺ imaging that a subset of BLA neurons exhibits distinct neural activities during social interaction with the aggressor compared to the non-aggressor, specifically in contexts where mice demonstrated specific social avoidance behaviors. Our findings suggest that, following social defeat, social

memory engram neurons in the vCA1 strengthened their connections with BLA neurons carrying negative valence.

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Title: Representation of sex-specific social memory in ventral CA1 neurons

Authors: *K. TAO, A. WATARAI, T. OKUYAMA;
Inst. for Quantitative Biosci. (IQB), The Univ. of Tokyo, Bunkyo, Tokyo, Japan

Abstract: Recognizing familiar individuals is crucial for adaptive social interactions among animals. However, the multidimensional nature of social memory encompassing sexual information remains unelucidated. We found that neurons in the ventral CA1 region (vCA1) of the mouse hippocampus encoded the identities and social properties, specifically sex and strain, of familiar conspecifics by using both rate and theta-based temporal coding. Optogenetic reactivation of social memories of females, but not males, induced place preference. Ablation of the upstream hippocampal dorsal CA2 region or the medial amygdala disrupted the representation of sex and the sexual dichotomy of social memory valence. Thus, vCA1 neurons employ dual coding schemes to represent the identities and social properties of familiar conspecifics as a cohesive memory.

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Topic: G.03. Stress and the Brain

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Title: Sharp Wave Ripples In Prairie Vole CA2 Over Social Bonding

Authors: *A. GHIMIRE¹, L. S. BUENO-JUNIOR², N. E. MILMAN³, C. J. TINSLEY⁴, M. M. LIM⁵, B. O. WATSON²;

¹Univ. of Michigan, Ann Arbor, Ann Arbor, MI; ²Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI; ³Behavioral Systems Neurosci., OHSU, Portland, OR; ⁴Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR; ⁵Veterans Affairs Portland Hlth. Care Syst., Portland, OR

Abstract: The hippocampus is crucial for integrating memories of “what”, “where”, and “when”. But what is the role of the hippocampus in memories of “who”? Though the role of the hippocampus in episodic memory and spatial navigation has been well investigated, its role in social memory—the ability of an individual to remember a conspecific—is less clear, especially for the formation of intense, long-lasting social bonds. Decades of single-animal studies of episodic and spatial memory have established a two-stage model of hippocampal memory formation based on an alternation between “online” encoding and “offline” consolidation. Online states occur during wake and involve attention, locomotion, sensorimotor integration, and theta oscillations (5-10 Hz) in hippocampal local field potentials (LFP). Offline states, on the other hand, occur during quiet wake, non-REM sleep, and involve quiet/appetitive behaviors such as immobility and eating, and are characterized by an increased occurrence of sharp wave ripples (SWRs) in hippocampal LFP. These SWRs are believed to play a crucial role in memory consolidation by “replaying” spiking patterns, thus continuing the plasticity processes that were initiated during preceding online states. Here, for the first time, we use multi-day behavior recordings synchronized with multi-day electrophysiologic recordings in the monogamous prairie vole to investigate hippocampal online and offline states over the formation of strong social bonds (pair bonding). We target the CA2 region of the hippocampus, as recent studies show it to be crucial for social memory. We observe SWRs during huddling in pair bonded prairie voles, a novel finding. Additionally, our preliminary data show differences in hippocampal LFP between periods of social engagement (awake, near and oriented towards each other) and periods of social disengagement (awake, oriented away from each other).

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Topic: I.06. Social Cognition

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Title: Hippocampal perineuronal nets disrupt astrocytes in a mouse model of social memory dysfunction

Authors: *P. KRASZEWSKI, W. TATERA, S. HASHMI, E. C. COPE;
Dept. of Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: The extracellular matrix surrounds all neuronal and glial structures, forming an interface for cell-to-cell communication. The specialized extracellular structures known as perineuronal nets (PNNs) enwrap certain neurons and play critical roles in synaptic plasticity and cognitive function. However, their impacts on glial plasticity remain unexplored. Here, we focused on the CA2 of the hippocampus, an area essential for social memory and rich in PNNs. We examined the CA2 of BTBR mice, an inbred wildtype model of social memory dysfunction, and found a reduction in GFAP and GLT1 immunoreactivity in astrocytes that coincided with increased *Wisteria floribunda agglutinin*+ PNNs, a pan-PNN marker. Reducing CA2 PNNs in BTBR mice improved social memory and rescued GFAP and GLT1 immunoreactivity, suggesting a link between excessive PNNs and astrocytic function. To test if social memory function is altered when astrocytes are impaired, we virally inhibited intracellular astrocytic calcium in mice using calcium extruder (CalEx) AAV injections into CA2. CalEx mice showed impairments in social memory, suggesting that astrocytic calcium is crucial for proper CA2 function. Since PNNs are composed of multiple components, we then explored the possibility that specific PNN elements restrict glial plasticity. One such component is chondroitin sulfate proteoglycans, which have many attached glycosaminoglycan chains. These chains carry sulfation at the 4 (C4S) and 6 (C6S) positions, each exerting differing effects on PNN plasticity. We observed a marked increase in C6S intensity and a decrease in C4S intensity in the CA2 of BTBR mice. BTBR mice with reduced PNNs maintained high C6S levels, suggesting that C6S+ PNN abundance may not directly account for the observed shifts in GFAP and GLT1 astrocytic marker expression. To test whether these effects are due to low C4S+ PNNs, we virally overexpressed carbohydrate sulfotransferase 11 (chst11), an enzyme involved in C4S synthesis, in CA2 neurons. Chst11 overexpression increased C4S and reduced WFA+ PNNs in both controls and BTBR mice. Ongoing studies are exploring whether increasing C4S+ PNNs rescues astrocytic markers and social memory dysfunction in BTBR mice.

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Poster

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Title: Microglial depletion promotes perineuronal net accumulation in the hippocampal CA2 region and impairs social memory

Authors: *B. SANCHEZ, S. LEE, C. M. REMY, E. A. CAMPBELL, E. C. COPE;
Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: Social memory is crucial for establishing social relationships and adapting to social contexts, with its dysfunction being a devastating feature of various disorders. While often overlooked, the hippocampal CA2 subregion and its connecting regions form an intricate circuit important for social memory. The CA2 has a striking abundance of perineuronal nets (PNNs), specialized extracellular matrix structures that ensheath particular cells. Disrupting CA2 PNNs impairs social memory and reducing excessive CA2 PNNs improves social memory in a mouse model of social dysfunction. PNNs are composed of various components that form a complex structure, which undergoes remodeling in response to environmental cues and disease-driven changes. Yet, the specific mechanisms regulating CA2 PNN abundance remain largely unexplored. Evidence shows microglia, the brain's resident macrophages, remodel PNNs, although this has not been explored in social memory circuits. Here, we fed mice PLX3397 chow to pharmacologically inhibit colony-stimulating factor 1 receptor to effectively deplete microglia. In the absence of microglia, we observed social memory deficits where mice were unable to distinguish between novel and familiar conspecifics, while object recognition remained unaffected. Notably, we observed increased intensity of *Wisteria floribunda agglutinin* (WFA), which labels the glycosaminoglycan chains of PNNs, in the CA2 after microglial depletion. These PNN effects were not observed across other hippocampal regions, suggesting the CA2 may be more vulnerable to microglial remodeling. Because PNNs uniquely surround excitatory pyramidal cells in the CA2 in addition to inhibitory PV cells, we checked whether PNN accumulation was cell-type specific. PLX-treated mice had a higher intensity of WFA+ PNNs around both cell types, although this was more pronounced around excitatory cells. Then, we examined whether microglial repopulation reverses CA2 PNN accumulation. After stopping PLX, we found that a restoration of microglial numbers coincided with reduced intensity of WFA+ PNNs in the CA2 to control levels. I examined endogenous PNN components using markers for various chondroitin sulfate proteoglycans (CSPGs), tenascin-R, and hyaluronic acid binding protein. While we did not find changes across all PNN components, we observed increased intensity of aggrecan, a major CSPG enriched in PNNs. This data suggests that microglia remodel CA2 PNNs, at least in part, by regulating CSPG accumulation. Ongoing studies are exploring whether microglia influence PNN formation and loss to better understand their role in regulating CA2 PNN abundance.

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Title: Basolateral Amygdala Contributions to Social Decisions in 16p11.2 Duplication Mouse Model

Authors: *E. OCASIO ARCE¹, A. ACHZIGER², E. ILLESCAS-HUERTA², C. DE PAULA CUNHA ALMEIDA², A. SHAH², J. VIGIL², S. DEHAUT², S. SAXENA³, N. PADILLA-COREANO²;

²Neurosci., ¹Univ. of Florida, Gainesville, FL; ³Dept. of Biomed. Engin. & Wu Tsai Inst., Yale Univ., New Haven, CT

Abstract: Social decision-making is fundamental for the functioning of social species and shaping all social interactions. Neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (SCZ) often impair social cognition, leading to significant disruptions in social behavior that affect millions daily. The 16p11.2 duplication(16p11.2dup/+), a copy number variant, increases the risk for several neuropsychiatric conditions such as ASD and SCZ. We used the 16p11.2dup/+ mouse model to study circuit-level dynamics during social decision-making. Recent work shows that the basolateral amygdala (BLA) plays a critical role in social decision-making (Scheggia et al., 2022). The 16p11.2 duplication disrupts the functional connectivity within the amygdala (Bristow et al., 2020), potentially impairing the BLA's ability to integrate motivational and social cue information. To test the hypothesis that the 16p11.2dup/+ mutation impacts social function by altering the BLA, our study has two main goals: first, to characterize social decision-making and sociability in 16p11.2dup/+ mice; and second, to determine whether these social impairments can be reversed through targeted activation of the BLA. We used a social decision-making paradigm to measure preferences between rewarding a partner (prosocial choice) or not (selfish choice). Our results showed that 16p11.2dup/+ mice do not develop a preference for the pro-social choice compared to Wild-Type (WT) mice. 16p11.2dup/+ mice had significantly fewer prosocial choices than WT mice (WT N=18, 16p11.2dup/+ N=16). Our 3-chamber assay results indicate that 16p11.2dup/+ mice exhibit deficits when interacting with novel and long-term familiar conspecifics (16p11.2dup/+ N=13) and WT female mice (WT N=13). Notably, when allowed to move freely, social deficits in time interacting were not observed in 16p11.2dup/+ mice (16p11.2dup/+ N=14). However, training a semi-supervised model representing the social behavior of pairs of animals indicates that differences exist in social interactions between the two genotypes. Our preliminary data suggest that chronic activation of the BLA via chemogenetics resulted in increased prosocial choices in our prosocial decision-making task in male 16p11.2dup/+ mice (N=6), but not in female mice (N=3). On the other hand, our data suggest that BLA activation in WT mice increases selfish choices (N=12). Ongoing analyses use machine learning to investigate what

behavioral changes contribute to a BLA-induced change in prosocial behavior. Altogether, our results may lead to mapping neural activity signatures underlying social dysfunction in psychiatric disorders.

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Poster

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Topic: I.06. Social Cognition

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Title: Physiological states and mPFC circuit dynamics track social valence, familiarity, and rank in mice

Authors: *S. SMITH¹, M. CUM¹, A. JAIN¹, A. LOPEZ GARCIA¹, T. HEEPS¹, N. PADILLA-COREANO²;

¹Neurosci., Univ. of Florida, Gainesville, FL; ²Neurosci., Univ. of Florida, GAINESVILLE, FL

Abstract: Long-lasting social relationships require the brain to acquire and recall social memories that integrate social valence, familiarity, and rank. These features not only shape behavior but also modulate internal physiological states. The medial prefrontal cortex (mPFC) is well-positioned to represent long-term social relationships, given its projections to subcortical structures that support social memory, valence, and dominance, including the nucleus accumbens (NAc), mediodorsal thalamus (MD), basolateral amygdala (BLA), and ventral hippocampus (vHPC) (Xing, et al., 2021; Zhou et al., 2017). Here, we used ECG and respiration recordings to investigate how social context influences autonomic responses in mice. Next, we employed in vivo electrophysiology and optogenetics to understand how prefrontal-subcortical circuits influence social recognition across social contexts. First, we investigated how autonomic signals reflect the valence of social interactions with conspecifics of varying familiarity and threat level. Subject mice were exposed to affiliative (juvenile), familiar (dominant or subordinate cagemates), or aversive (aggressive CD1) conspecifics, while recording heart rate and respiration via wireless loggers and intranasal pressure sensors. Our preliminary data show that these autonomic responses change during interactions with bully conspecifics. Ongoing analyses are using machine learning to identify differences related to valence and rank. Next, we recorded local field potentials in the BLA, MD, NAc, and vHPC, and single-unit activity in the mPFC during social interactions with a newly co-housed conspecific for 7 days, revealing how dynamics evolve with increasing familiarity. Finally, we used optogenetic inhibition to test the

necessity of prelimbic cortex (PL)→NAc core projections for social recognition of a highly familiar conspecific. We found that inhibition during recall did not impair social recognition of a cagemate, suggesting that this pathway is not required for social recognition of long-term relationships. Together, these studies highlight complementary physiological and circuit mechanisms through which the mPFC integrates social memory and internal state, providing insight into the neural basis of social cognition.

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Title: Semi-supervised model to identify neural circuit features contributing to social competition behavior

Authors: *Y. LU¹, R. IWATA³, E. ILLESCAS-HUERTA⁴, C. MARAIS⁵, M. CUM⁵, T. HEEPS⁶, S. SAXENA², N. PADILLA-COREANO⁵;

¹Biomed. ENgineering, ²Biomed. Engin., Yale Univ., New Haven, CT; ³RIKEN Ctr. For Developmental Biol., Kobe, Japan; ⁴Univ. of Florida, United States, FL; ⁵Neurosci., ⁶Univ. of Florida, Gainesville, FL

Abstract: Social hierarchies are fundamental organizational principles across social species that stem from collective experiences and shape group dynamics. The neural circuits that control social competition are not well understood. Recent work shows that the medial prefrontal cortex (mPFC) encodes competitive outcomes (win vs lose). To address if mPFC encodes the competitiveness (high vs. low competitive states) we recorded single units during social competition tasks where CD1 male mice competed for rewards (n = 12 pairs). Individual neurons specifically encoded winning, with those active during high competition (both strived to win) highly non-overlapping those active in low competition (only one sought the reward), suggesting that mPFC employs distinct encoding with possibly distinct circuits denoting competitive states. To explore potential circuits, we recorded local field potentials from mPFC, basolateral amygdala (BLA), lateral hypothalamus (LH), mediodorsal thalamus (MD), and ventral hippocampus (vHPC) then calculated power, coherence (CH), and Granger causality (GC) as neural features. Next, we trained a semi-supervised model using discriminative factor analysis to

identify network states predictive of outcome and competitiveness. Our results discover regional features that predict social interaction (Fig. 1) with more accurate results for outcome over competition level. Theta band features in mPFC-BLA, BLA-LH, and mPFC-LH were present in all states, but especially when predicting outcome. Competition level engaged vHPC-BLA and beta frequencies throughout the network. This identifies the features that dictate social competition behavior, distinguishing between outcome results and competitive drive.

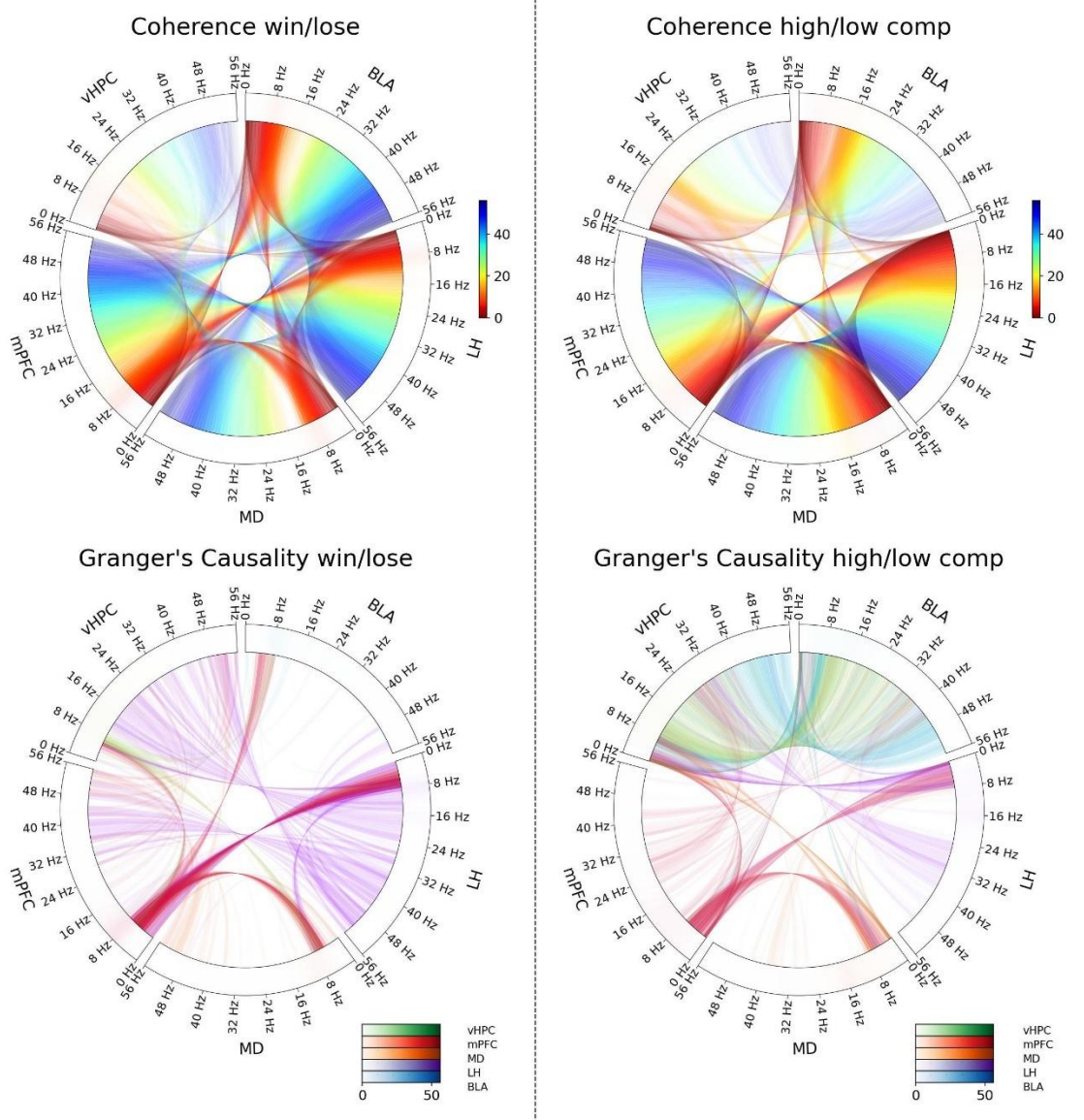


Fig. 1: A visual of the top 35% connections. The left network represents the supervised component trained to predict outcome and the right for competitiveness. The top plots represent CH between similar frequencies (indicated by color) while the bottom shows GC (color represents leading region). The lines' opacity indicates relative connection strength.

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Title: Pag inputs to vta modulate play-related behavior

Authors: *W. TANG¹, M. BRECHT²;
²BCCN Berlin, ¹Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Social play behavior is a light-hearted form of social interaction especially prominent during juvenile and adolescent stages of life in many mammalian species, including rats and humans. Play is believed to contribute to social, emotional, and cognitive health. The periaqueductal gray (PAG), and particular the lateral PAG columns have been identified as essential for play behavior in rats. However, the underlying neural circuitry and neurobiological mechanisms of play fighting in laboratory rats remain poorly understood. In this study, using whole-brain c-Fos mapping, fiber photometry, and optogenetic manipulation, we identified the PAG-VTA (ventral tegmental area) pathway as a key regulator of social play behavior. Specifically, the lateral and ventrolateral regions of the PAG (l/vlPAG) project to the VTA. During social play, a subset of l/vlPAG neurons projecting to the VTA, as well as both dopamine and non-dopamine neurons within the VTA, are activated. Optogenetic inhibition of PAG-VTA projections significantly reduced play behavior. In contrast, we observed little play-related activation raphe nuclei. We wonder if the PAG-VTA interaction contributes to the fun associated with play.

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Title: Social play representation in the periaqueductal gray of rats

Authors: *M. MUGNAINI¹, M. A. CONCHA², N. GLOVELI², M. BRECHT²;
¹Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany; ²BCCN Berlin, Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Despite being ubiquitous across species, play remains as one of the most poorly understood behaviours. It is characterized by voluntary, pleasurable actions that mimic functional behaviors, often occurring in exaggerated or non-functional contexts. In its social form, rats typically display sequences of stereotyped behaviors, like attempts to reach and contact each other's nape, that are accompanied by periods of intense locomotion. Recent studies have identified the periaqueductal gray (PAG) as a key structure involved in the expression of social play and associated affective responses. However, how individual play behaviors evolve over rapid timescales and how they are represented in the brain remains largely unknown. To address this, we conducted high-resolution video and ultrasonic vocalization recordings of rats engaged in social play, while simultaneously recording neural activity from the PAG and surrounding structures using high-density Neuropixels probes. We segmented the play dynamics using Hidden Markov models and found that quiet periods alternate with brief episodes of intense playful activity (~2.8 s), characterized by states of high locomotion and elevated ultrasonic vocalization - so-called "play bouts". We found that the PAG and adjacent structures exhibited moderate theta-range entrainment, and that the power of these oscillations correlated with ongoing play behavior. Furthermore, we describe how distinct columns of the PAG and nearby regions (i.e. superior colliculus and dorsal raphe) differentially encode play behavior. Our results indicate an orderly mapping of specific play behaviors onto columns of the PAG. Put together, these results confirm the critical role of the lateral PAG in social play.

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Poster

PSTR359: Basic and Translational Circuit Mechanisms of Social Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR359.12/PP13

Topic: I.06. Social Cognition

Support: NeuroCure Cluster of Excellence (Exc 2049-390688087)
European Research Council (BrainPlay ERC Synergy Grant)

Title: The effects of play deprivation on social play and the periaqueductal gray in rats

Authors: *N. GLOVELI, H. YILDIZ, M. BRECHT;

Bernstein Ctr. For Computat. Neurosci. Berlin, Humboldt Univ. Berlin, Berlin, Germany

Abstract: Humans, rats, and many other animals invest a substantial amount of time and energy in play, especially during the juvenile period. Despite the abundance of play behavior and its known contribution to social and cognitive development, various factors can lead to insufficient amounts of play, disrupting essential developmental processes. However, our understanding of the consequences restricting access to play on the behavioral and neural development of juvenile rats is limited. In the current study, we aim to investigate the behavioral and neural changes that occur when animals are not given sufficient opportunity to play. To significantly limit play while maintaining access to social interactions, we introduced a three-week play deprivation period during which juvenile female rats are housed with female adults, who are known to have a greatly reduced play drive. Following the deprivation period, we tested the impact of play deprivation in juvenile rats on their ability to play with age-matched partners and anxiety behavior using the elevated plus maze. Additionally, we examined the neural activity of the periaqueductal gray, a midbrain region involved in various instinctive, as well as tickling and play-related behaviors in rats. During behavioral testing, we recorded PAG activity in the freely moving rats using Neuropixels probes to investigate whether and how the neural activity of the PAG differs between play-deprived and non-play-deprived rats during social play. By monitoring home-cage behavior and quantifying the time spent playing in both play-deprived and non-play-deprived rats, we confirmed a pronounced reduction in play behavior when juvenile rats are housed with an adult. Furthermore, preliminary results show that play-deprived rats rarely engage in play with their play partners during the first days of behavioral testing, while non-play-deprived rats are quicker to engage in play. This distinction between groups tends to diminish after several days of testing, as play deprived rats increasingly engage in social play with their partners. Currently, we investigate the changes of periaqueductal gray activity associated with play deprivation. Our results indicate that play deprivation can affect the capacity for social behaviors, such as social play, and that repeated access to a play partner may counteract the effect of play deprivation.

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Poster

PSTR359: Basic and Translational Circuit Mechanisms of Social Behavior

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Program #/Poster #: PSTR359.13/PP14

Topic: I.06. Social Cognition

Support: NWO XL SoMeMe

Title: Input and output characteristics of shock observation engram neurons in the anterior cingulate cortex

Authors: ***Á. BABICZKY**¹, R. RAJENDRAN¹, S. PAPAS^{1,2}, X. FENG^{1,3}, V. GAZZOLA^{1,4}, C. KEYSERS^{1,4};

¹Netherlands Inst. for Neurosci., Amsterdam, Netherlands; ²Masters Brain and Cognitive Sci.,

³Swammerdam Inst. for Life Sci., ⁴Dept. of Psychology, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The evolutionary success of our species is highly dependent on our ability to learn from the experiences of others through observation. For instance, we can observe others burning themselves on a stove and consequently learn about the dangers of fire without the cost of burning ourselves. Although this phenomenon, social learning, attracted the attention of many behavioral- and neuroscientists in the previous decades, the underlying neuronal circuitry still remain poorly understood at the cellular level. Here we aimed to map the afferent and efferent connections at the whole-brain level of a subset of anterior cingulate cortex (ACC) neurons that are specifically activated during shock observation in mice. By combining cutting-edge engram labelling tools and monosynaptically-restricted transsynaptic tracing with deletion-mutant rabies virus, we were able to selective label and map different ACC neuron populations. Collected brains were processed with serial two-photon microscopy to achieve unbiased mapping of afferent- and efferent connections at the whole-brain level. We compared the connectivity map of shock observation engram neurons with that of neurons belonging to engrams activated by other behavioral conditions, such as conditioned fear recall and self-pain. Collectively, our preliminary data provide unique insights into how the experiences of others are processed in the ACC to enable animals to learn about the safety or the dangers of their own environment. Given tight homologies between pain observation activation in humans and rodents, our results might inform an etiological understanding of human social dysfunctions occurring in several neuropsychiatric disorders.

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Poster

PSTR359: Basic and Translational Circuit Mechanisms of Social Behavior

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Topic: I.06. Social Cognition

Support: National Institute of Health (5K08NS105938 to N.A.F.)
SFARI Bridge to Independence award (to N.A.F.)
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Title: Multiplexed encoding of social and spatial information in the prefrontal cortex

Authors: *N. L. JOHNSTON¹, S. FLATT², A. CHIKUNI², D. KWAK², N. A. FROST²;
¹Neurol., ²Dept. of Neurol., Univ. of Utah, Salt Lake City, UT

Abstract: Social interactions are highly complex and tightly regulated repertoires of behaviors critical for the survival of an individual. To facilitate flexible decision making during social interaction, the medial prefrontal cortex (mPFC) represents spatial, social, and contextual information in parallel. We have previously shown that the mPFC encodes contextual and social information in overlapping ensembles, but it is not well understood how the mPFC co-encodes social and spatial information at the cellular or network level. To address this, we utilized *in vivo* calcium imaging in the mPFC of mice exploring an open field with or without a conspecific. We then looked for individual neurons which encoded the relative (egocentric) or absolute (allocentric) position of environmental features or a conspecific. Using this approach, we identified four broad spatial cell types: place cells (PCs) and social place cells (SPCs) which encode the allocentric position of the self or other, and egocentric boundary Cells (EBCs) and egocentric social cells (ESCs) which encode the relative position of either objects or the conspecific. Together, these neurons made up ~46% of mPFC neurons, and did not emerge in shuffled data. We next calculated the egocentric vs allocentric preference and the social vs. non-social preference of our recorded cells, which revealed strong egocentric and social biases within the prefrontal microcircuit in social contexts. These results indicate a cellular substrate by which the mPFC co-encodes social and spatial information across reference frames.

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Topic: I.06. Social Cognition

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MOST 2022ZD0204900

Title: Neuronal representations of multi-social individuals in the prefrontal cortex

Authors: *T. LI¹, X. HE¹, X. ZHANG²;
¹Beijing Normal Univ., Beijing, China; ²State Key Lab. of Cognitive Neurosci. & Learning, Beijing, China

Abstract: Social interaction is a key aspect of mammal's behaviors. It is largely unknown how the brain encodes or memorizes respective identities of interacted multiple individuals with varied social experience. In the present study, we conducted *in vivo* electrophysiological recording of neuron population spiking activities in the mouse medial prefrontal cortex (mPFC)

when it was socially interacting with four other ones. We reveal that mPFC population neuronal activities are able to encode each social individual by stably allocating respective neural subspaces. Furthermore, with a new conditioned social learning paradigm, in which social behaviors of the test mice are associated with a milk reward, aversive air puff, and blank stimuli with four individuals, respectively, for 8 days, we find that such social associative learning significantly enlarges the angle of neuronal activity subspaces respectively representing the two individuals associated with the reward and aversive stimuli. The latter change is accompanied with a shift of social preference to conditioned individuals in the test mice. Interestingly, neural subspaces corresponding conditioning stimuli and social individuals are persistently orthogonal during the social learning. Taken together, our findings from recording and behavioral experiments strongly suggest that the mouse can recognize and memorize at least three different individuals, and social identities of interacted individuals are stably encoded by structured neuronal population activities in the mPFC.

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Poster

PSTR359: Basic and Translational Circuit Mechanisms of Social Behavior

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Program #/Poster #: PSTR359.16/PP17

Topic: H.06. Anxiety Disorders

Support: MH130862

Title: The novel role of CHD7 in regulating social behavior in postmitotic ACC neurons

Authors: *E. DEW¹, S. SAGGU², K. JIAO³, Q. WANG⁴;

¹Augusta Univ. Neurosci. Grad. Program, Augusta, GA; ²NEUROSCIENCES, Augusta Univ., Augusta, GA; ³Med. Col. of Georgia at Augusta Univ., Augusta, GA; ⁴Neurosci. and Regenerative Med., Med. Col. of Georgia, AUGUSTA, GA

Abstract: Social behaviors are the adaptive interactions between individuals that can promote or impact survival. The roles of neuronal circuits and their molecular regulators in controlling critical behaviors remain limited. Although epigenetic regulation in complex behaviors has been appreciated, the role of chromatin remodeling in regulation of social behaviors has been understudied. The anterior cingulate cortex (ACC) integrates autonomic input, memory, and emotion to regulate complex behaviors including social interaction. We identified enriched expression of CHD7, which is a SNF2-like ATP-dependent chromatin remodeling factor primarily necessary for development, in postmitotic excitatory neurons of the ACC in adult mice. The goal of our study is to uncover the role of *Chd7* in postmitotic ACC neurons. We employed *Chd7*^{loxp/loxp} mice and introduced Cre expression in excitatory neurons of the ACC or an ACC downstream target, bed nucleus of the stria terminalis (BNST), through an adeno-associated viral system. ACC deficiency of *Chd7* in male mice led to a lack of proper environmental threat

avoidance behavior in elevated zero maze and light/dark tests, along with decreased activity in the ACC. Furthermore, the male *Chd7* conditional knockout mice showed a lack of proper social threat avoidance behavior in a free social interaction test, as well as decreased activity in the ACC following the test. This is demonstrated by increased interaction initiation and increased participation in prosocial behaviors. However, there was no change in sociability in a three-chamber social interaction test. These data suggest a specific role of CHD7 in regulating social threat avoidance but not sociability per se. In addition, *Chd7* deletion in ACC-BNST-projecting neurons led to decreased social threat avoidance behavior in male mice and decreased neuronal activation following the free social behavior test in the ACC and BNST. Our data provide the first evidence demonstrating a critical role of CHD7 in regulating ACC circuit activity and threat avoidance behaviors, advancing our understanding of epigenetic regulation of complex behaviors.

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Poster

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Program #/Poster #: PSTR359.17/PP18

Topic: I.06. Social Cognition

Support: RS-2024-00438988

Title: Medial amygdala's role in abnormal social behavior in Shank2 haploinsufficiency

Authors: *H. YOON, E. HWANG, E. LEE;
Yonsei Univ. of medicine, Seoul, Korea, Republic of

Abstract: Social familiarity information is essential for maintaining stable and dynamic social networks, influencing behaviors such as keeping proximity, aggression, and mating. The medial amygdala (MeA) plays a key role in recognizing social cues, such as pheromones. It is involved in distinguishing familiar and unfamiliar conspecifics and modulating social behaviors accordingly. In this study, We identified a specific population of GABAergic, somatostatin-expressing neurons in the posteroventral subdivision of the MeA (MeApv), which are tagging social familiarity information. Based on these findings, We hypothesized that MeApv somatostatin-expressing neurons disinhibit MeApd GABAergic neurons, thereby modulating aggression. To test this hypothesis, we employed *Shank2* mutant mice, a well-established ASD model. *Shank2* haploinsufficient (*Shank2* HT) mice exhibited impaired social recognition and heightened aggression. Additionally, while the population of somatostatin-expressing neurons in the MeA was increased, MeApv somatostatin-expressing neuronal activity was absent, suggesting a potential disruption in the functional connection between social recognition and aggression, providing insights into the neural mechanisms underlying altered social behaviors in ASD.

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Poster

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Topic: I.06. Social Cognition

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Title: Restoring interbrain prefrontal theta synchronization reverses social deficits

Authors: E. HWANG¹, A. LEE², S. SHIN¹, H. YOON³, *E. LEE¹;

¹Yonsei Univ., Seoul, Korea, Republic of; ²Yonsei University, Col. of Med., Seodaemun-gu, ;

³Yonsei Univ. of medicine, Seoul.

Abstract: Social interaction requires the integration of information about oneself, others, and the shared environment. One proposed mechanism for this coordination is interbrain synchrony - correlated neural activity between interacting individuals -observed in both humans and animals. However, whether this synchrony plays a functional role in orchestrating social behavior remains unclear. Here, we show that theta-band synchronization in the medial prefrontal cortex (mPFC) is essential for naturalistic social interactions in mice. Using a genetic model of social dysfunction, we demonstrate that disruptions in mPFC synchrony correlate with impaired social behaviors. We identify a population of social behavior-encoding neurons that are directly linked to interbrain synchrony. Optogenetically enhancing theta synchronization restores both interbrain synchrony and social behaviors, while desynchronization impairs social interactions in wild-type mice. Our findings establish prefrontal theta synchrony as a fundamental mechanism for social behavior with therapeutic implications for disorders characterized by social deficits. More broadly, this work provides a framework for understanding how neural circuits across brains dynamically interact to shape behavior

Disclosures: E. Lee: None.

Poster

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Topic: I.06. Social Cognition

Support: CDMRP W81XWH-22-1-0333

Title: Brain-wide subnetworks encoding different cognitive functions in normal and autistic-like mice

Authors: *R. TERNER-ROTHKOFF, S. SHERER, A. GILAD;
Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Our brains mediate individual thoughts, experiences, and emotions, dynamically encoding complex information via billions of neurons across many brain areas. A certain cognitive function is represented by a brain-wide subnetwork, which partially overlaps with those for other cognitive functions. These subnetworks are thought to change over time and differ across individuals, especially during natural behavior. However, the dynamics of these subnetworks at the individual level are largely unknown. This becomes prominent when studying autism, a neurological disorder that displays large heterogeneity in symptoms, cognitive profiles, and underlying neural mechanisms across individuals. The mouse model has become a popular tool to study behavior, and several mouse models for autism have been developed. In this study, we implement a state-of-the-art multifiber approach that enables simultaneous neuronal measurement from dozens of cortical and subcortical cognitive-related areas such as amygdala, hippocampus, prefrontal cortex and more. This was done in both wild-type (WT) and autism-like (ASD; SHANK3) mouse models, while mice perform a range of freely moving behavioral tasks, including anxiety-related tasks (elevated plus maze and open field), a social interaction task (three-chamber test), and a memory task (novel object recognition). We present data from 16 WT and 22 ASD mice each performing multiple tasks. Our goal is to study brain-wide networks during different behavioral tests and compare them within and between WT and ASD mice. Behaviorally, ASD mice spent less time in open arms, the open field center, and near novel objects or social partners. In the elevated plus maze, WT mice showed increased activation in prefrontal cortex, amygdala, and hippocampus when in the open arms, while ASD mice showed increased activity in the caudate putamen, dentate gyrus, and infralimbic cortex. In the sociability test, ASD mice exhibited stronger activation in the nucleus accumbens shell near the stranger. In the social novelty task, this increase was specific to the novel stranger. In contrast, WT mice showed stronger activation in the dysgranular insular cortex (DI) and caudate putamen (Cpu), consistent with greater exploration of the novel social stimulus. Across all tasks, distinct brain-wide subnetworks were revealed, differing by task, genotype, and individual. We also observed substantial variability within ASD mice, highlighting the spectrum nature of the disorder. These results underscore the power of large-scale calcium imaging in freely moving animals and provide a framework for linking circuit-level dysfunctions to behavioral phenotypes in ASD.

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Poster

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Title: Dimorphism of autistic-like phenotypes and aberrant brain organization in *chd8*^{+/R2219*} mice

Authors: *Q. ZHANG¹, L. HUIXIAN², J. LUO³;

¹Dept. of Brain Sci. and Brain Medicine, Dept. of Psychology and Behavioral Science, Zhe, Hangzhou, China; ²Dept. of Brain Sci. and Brain Medicine, Dept. of Psychology and Behavioral Science, Zhe, Hangzhou, China; ³Zhejiang Univ. Sch. of Med., Hangzhou, China

Abstract: Autism spectrum disorder (ASD) affects approximately 0.6% of the global population with four times more in males than in females. However, the underlying mechanisms of sexual dimorphism remain poorly understood. *CHD8* is a strong ASD-risk gene that encodes a chromatin remodeler broadly expressed in various brain regions and cell types. Here, we characterized sexually dimorphic changes in mice carrying a heterozygous mutation in *Chd8* (*Chd8*^{+/R2219*}) by using the CRISPR-Cas9 approach according to the information on *CHD8* mutation from two autistic patients. *Chd8*^{+/R2219*} mice exhibited macrocephaly and autistic-like behaviors including social deficits, increased repetitive grooming, and heightened anxiety. Compared to wildtype, magnetic resonance imaging (MRI) analyses confirmed increased whole brain volume in *Chd8*^{+/R2219*} mice, without significant sex difference observed. Voxel-based morphometry (VBM) analysis revealed enlarged gray matter volume in bilateral hippocampi, with larger hippocampal volume correlated with more severe social deficits in male *Chd8*^{+/R2219*} mice. Functional connectivity analysis showed that the hippocampal-seeded connectivity with key regions involved in social processing, such as the amygdala, prelimbic, and orbitofrontal cortex collectively predicted the severity of social deficits in these mice. Taken together, this study indicates that *Chd8*^{+/R2219*} mice exhibit sexual dimorphism in autistic-like abnormalities and aberrant hippocampal volume and functional connectivity.

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Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

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Program #/Poster #: PSTR360.01/QQ2

Topic: I.08. Learning and Memory

Support: MRC grant
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Title: Neural mechanisms of causal inference

Authors: *S. NALLURU¹, L. GLITZ¹, S. ABBASI-RAD², A. HESS¹, W. CLARKE¹, J. CAMPBELL¹, J. O'REILLY¹, H. BARRON¹;

¹Univ. of Oxford, Oxford, United Kingdom; ²Harvard, Boston, MA

Abstract: Throughout our lifetime, we learn new information that can later be selectively recalled. A key mechanism enabling selective memory recall is the dynamic regulation of cortical excitability, which is thought to 'gate' memory expression and prevent memory interference. However, little is known about how cortical excitability affects the fidelity of memory consolidation, including the reactivation or 'replay' of memory that occurs during periods of rest or sleep in hippocampal-neocortical circuits. Here we test the hypothesis that changes in cortical excitability alter the coherence of memory reactivation. We predict that such changes in the coherence of memory reactivation may account for disordered thought, including the formation of spurious associations and inference, and/or impaired credit assignment. To test these predictions, we recruited healthy participants who all started with behavioural training on a novel computerized memory task which incorporates both causal and correlational associations. Then, we used anodal transcranial direct current stimulation (atDCS) to decrease inhibition in the neocortex during rest when offline replay-like reactivation is known to occur. Using 7T functional magnetic resonance imaging (fMRI) and spectroscopy (fMRS), we assessed neural evidence for spurious associations and causal misattributions, as a function of changes in cortical excitability. We found that atDCS reduces neocortical inhibition, thereby altering cortical excitability. This manifests as 'spontaneous' memory expression and disturbed sequences during rest, which is reflected in later behavioural performance. These results suggest that altered cortical excitability may affect offline memory reactivation, in turn contributing to disordered thought and the formation of false memories. Our findings therefore have important implications for our understanding of psychosis, where positive symptoms that include delusions and hallucinations may be attributed to causal misattributions and false memories that arise via disturbances in memory reactivation.

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Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

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Program #/Poster #: PSTR360.02/QQ3

Topic: I.08. Learning and Memory

Support: P50MH132642
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R01MH137624

Title: Memory replay as a neural mechanism for creativity

Authors: *P. CHENG¹, H. KAN¹, S. KASTNER²;

¹Psychology, ²Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Creativity is the driving force behind societal advancement and a defining feature of cognition that distinguishes humans from other species. Previous research suggests that creativity results from recombining memory contents (see Benedek et al., 2023 for a review). However, how creative thoughts emerge from dynamics of neural activity is unknown. We propose that creative thoughts arise from replay of memories in flexible and purposeful ways. Specifically, replay, driven by the hippocampus, may use abstract, high-level knowledge to guide reactivation of memories, with the sequential structure giving rise to new ideas. To test this, we developed a novel task where trained musicians compose new musical pieces using melody chunks they learned, in a way that respects chord progression in Western music theory. These melody chunks are newly composed, which preserve the underlying chords, the abstract knowledge that scaffolds musical composition. We paired our task with triaxial Optically Pumped Magnetometers (OPMs) to record whole-brain activity. OPMs provide superior spatial and temporal resolutions compared to conventional SQUID MEG, and are more tolerant of natural movements, making them ideal for studying real-world creativity. Applying regularized logistic regression to all channels, we decoded representations of melody chunks with an approximately 90% accuracy. Permutation tests show that after adding stimulus-absent data to the training, classifiers can consistently detect target melodies at the time point of their peak accuracy. These results demonstrate the classifiers' spatial and temporal specificity, validating their use to identify time-resolved replay of specific memories. When applied to the stimulus-absent period, these classifiers successfully detected reactivation of melody memories in a time-resolved manner while musicians were composing. This evidence suggests that memory replay supports musical creativity. Next, we plan to probe the sequential and hierarchical structure of replay to gain a deeper understanding of how replay supports creativity. Specifically, we will test whether these replayed sequences match more closely to the final composition, rather than the order of presentations; and whether replay reflects high-level knowledge (e.g., chords' constraint on melody development) and uses it to guide composition. This study seeks to shed light on the roles and functions of replay, which is not just to recall the past, but to imagine and create the future.

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Poster

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Program #/Poster #: PSTR360.03/QQ4

Topic: I.08. Learning and Memory

Support: U01 NS117839

Title: Phase-targeted stimulation affects functional connectivity in the human hippocampus

Authors: *Y. SALIMPOUR¹, T. ARGINTEANU², W. S. ANDERSON³;

¹Neurosurg., Johns Hopkins Univ., Baltimore, MD; ²Neurosurg., Johns Hopkins Sch. of Med., Baltimore, MD; ³Neurosurg., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Theta frequency range oscillations in the human hippocampus play a crucial role in memory formation. These oscillations help organize neural activity, enabling the encoding and retrieval of information. The phase of theta oscillations is particularly important because it facilitates neural communication. Research suggests that theta phase coding contributes to temporal integration, allowing the brain to sequence events in time, essential for memory processing. Additionally, the phase of theta oscillations is linked to interregional functional connectivity, ensuring efficient information transfer between various memory-related structures. A recent human study indicates that the hippocampal theta oscillation orchestrates the transition in functional connectivity, with opposite phase angles organizing the flow of information. Despite advances in understanding hippocampal theta oscillations, the precise mechanisms underlying theta phase synchronization and its impact on memory remain unclear. To address this gap, we applied phase-targeted stimulation in epileptic patients undergoing intracranial monitoring. Using depth electrodes, we delivered bipolar biphasic direct electrical stimulation to the hippocampus at specific theta phase angles, recording evoked responses via electrocorticography. As a measure of functional connectivity, we assessed whether hippocampal evoked responses varied based on the stimulation phase direction. Our findings reveal that in the human hippocampus, stimulation synchronized with the peak of the theta phase enhances both early and late components of the evoked response. In contrast, stimulation at the trough tends to suppress them. Furthermore, event-related dynamic phase-amplitude coupling analysis between the phase of the theta and the amplitude of gamma shows that theta-gamma interactions fluctuate depending on theta phase, demonstrating the critical role of theta oscillations in shaping hippocampal activity. Taken together, these results suggest that the phase of theta oscillation governs the transition of the functional connectivity in the human hippocampus, and phase-targeted stimulation is an effective technique to modulate functional connectivity and may influence memory-related neural processes. By targeting specific theta phases, phase-targeted stimulation may offer novel application strategies for treating memory-related neurological disorders.

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Poster

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Topic: I.08. Learning and Memory

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Title: Acetylcholine modulates theta phase synchronization in prefrontal-hippocampal dynamics during human memory formation

Authors: *S. CHENG¹, B. C. LEGA², Z. FU³;

¹Univ. of Texas Southwestern Med. Center, Dept. of Neurolog. Surgery, Dallas, TX;

²Neurosurg., ³Neurolog. Surgery, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Extensive evidence indicates that cholinergic signaling critically supports memory formation, and blocking cholinergic receptors with drugs (e.g., scopolamine) reliably impairs memory. Our prior work showed that scopolamine-induced memory deficits are linked to disrupted theta oscillations (2-10 Hz), including reductions in amplitude, changes in phase alignment, and altered connectivity within the hippocampus. Episodic memory relies on coordinated hippocampal-prefrontal cortex (PFC) interactions, yet how cholinergic modulation specifically alters these neural interactions remains unclear. We investigated the effects of cholinergic blockade on episodic memory by recording intracranial EEG from 11 patients with epilepsy (82 hippocampal, 260 PFC electrodes) performing a free-recall task under scopolamine and placebo injections. Data analyses were restricted to successfully recalled trials. Scopolamine significantly impaired memory performance ($t_{(10)}=5.782$, $p<0.001$). In the PFC, this memory deficits were accompanied by decreased theta power in a late time window of 0.8 to 1.5 s (permutation test, scopolamine vs. placebo, $p=0.002$; remembered trials only, $p=0.002$) and disrupted theta phase reset (remembered trials only, $p=0.014$). Furthermore, scopolamine reduced local theta (2-4 Hz) phase synchronization (scopolamine vs. placebo, $p=0.002$; remembered trials only, $p=0.002$) and theta-gamma phase-amplitude coupling (PAC) (scopolamine vs. placebo, $p=0.024$; remembered trials only, $p=0.002$) within the PFC. Notably, prefrontal-hippocampal synchronization in low theta frequencies was paradoxically enhanced during cholinergic blockade (scopolamine vs. placebo, $p=0.002$; remembered trials only, $p=0.002$), and hippocampal PAC was also significantly increased, especially during successful recall (scopolamine vs. placebo, $p=0.002$; remembered trials only, $p=0.002$). Our results suggest that cholinergic blockade disrupts local cortical synchronization essential for memory formation but triggers compensatory increases in prefrontal-hippocampal interactions and hippocampal PAC, potentially representing adaptive mechanisms aimed at preserving memory function.

Disclosures: S. Cheng: None. B.C. Lega: None. Z. Fu: None.

Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

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Program #/Poster #: PSTR360.05/QQ6

Topic: I.08. Learning and Memory

Support: NIH Grant U01NS117839
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Title: Prefrontal and medial temporal neurons encode ordinal information of event sequences in humans

Authors: *E. PAVARINO¹, J. ZHENG², I. SKELIN³, M. YEBRA⁴, M. L. DARWIN⁷, C. REED⁵, S. K. KALIA⁸, T. A. VALIANTE³, D. KRAMER⁹, J. A. THOMPSON¹⁰, A. MAMELAK⁵, G. KREIMAN¹¹, U. RUTISHAUSER⁶;

¹Harvard Univ., Boston, MA; ²Neurolog. Surgery and Biomed. Engin., Univ. of California Davis, Davis, CA; ³Univ. Hlth. Network, Toronto, ON, Canada; ⁴Neurosurg., ⁶Dept. of Neurosurgery, AHSP #6432, ⁵Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁷Neurosurg., CU Anschutz Med. Campus, Aurora, CO; ⁸Toronto Western Res. Inst., Toronto, ON, Canada; ⁹Univ. of Colorado Sch. of Med., Aurora, CO; ¹⁰Neurosurg., Univ. of Colorado, AMC, Aurora, CO; ¹¹Harvard Med. Sch., Boston, MA

Abstract: Humans remember episodic events as discrete units, yet how these memories are integrated into a chronological continuum remains unknown. While prefrontal and medial temporal regions are implicated in temporal recall, the specific neural mechanisms underlying this process in humans are unclear. We investigated this question by recording single-neuron activity and local field potentials in 23 drug-resistant epilepsy patients as they watched custom-filmed stimuli consisting of 25 video clips, each with four sequenced everyday events of variable length. Participants' memory was tested with scene recognition and temporal order discrimination tasks. We recorded 965 single units across patients. We identified "order-selective cells" (OSCs) in the hippocampus (12% of hippocampal population), amygdala (13%), and orbitofrontal cortex (10%) that responded selectively to specific event orders, regardless of content or absolute timing. At a population level, we discovered that event order was linearly decodable from neural activity alone, with OSCs being the major contributing features. Removing OSCs from the pseudopopulation dropped decoding accuracy to chance levels, highlighting their crucial role for temporal information encoding. Trial-averaged PCA trajectories revealed significant shifts in neural state-space between event orders. At the mesoscale, theta modulated its power along event sequences, with OSCs exhibiting theta phase precession at their preferred order. Critically, the strength of this precession during encoding predicted subsequent temporal memory performance. These findings reveal a neural mechanism for encoding event order in human episodic memory, showing how event structure shapes neural dynamics to transform discrete experiences into a coherent temporal narrative.

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Poster

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Topic: I.08. Learning and Memory

Support: BRAIN initiative U01NS117839

Title: Ground-truth memory signal in human hippocampal neurons facilitates behavioral and neuronal pattern separation

Authors: *N. KURILENKO¹, C. M. REED², W. S. ANDERSON⁴, T. A. VALIANTE⁵, A. N. MAMELAK³, U. RUTISHAUSER³;

²Neurol., ³Neurosurg., ¹Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁴Neurosurg., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ⁵Univ. Hlth. Network, Toronto, ON, Canada

Abstract: How does the human brain rapidly distinguish between novel stimuli and previously encountered, similar memories? It's long been theorized that the hippocampus supports this function via pattern separation, yet its applicability and neuronal correlates in humans remain hotly debated. We recorded from 3155 single neurons from four brain areas across 78 intractable epilepsy patients performing a recognition memory task. Patients performed with 68.45% accuracy. As expected, memory selective (MS) cells responded differently to familiar and novel stimuli and carried a memory strength signal (351 cells, 11%). We hypothesized that MS cells are reflective of pattern separation, e.g., their responses to similar stimuli would be dissimilar and decorrelated. First, based on behavior, we validated that the probability of incorrectly recognizing new images as "old" (false alarms) is due to new images being similar to previously studied. Both human similarity judgements and deep neural network (DNN)-based similarity metrics significantly correlated with the false alarm rate (ρ , humans: 0.3, DNN: 0.5, $p < 0.05$), suggesting that new images more similar to those previously studied are more likely to be mistakenly recognized as "seen before". Next, we examined MS cell activity during false alarms. Single-neuron decoding showed a subset of them—here termed pattern separation cells—reliably distinguished true old from false old stimuli (permutation $p < 0.001$), even when behavior was incorrect. Decoding of correctly identified new trials exceeded that of false alarms ($p < 0.001$, sign test), underscoring behavioral relevance. At the population level, we used demixed principal component analysis (dPCA) to separate ground truth from behavioral choice across MTL and MFC. Only the hippocampus allowed significant single-trial decoding of stimulus ground truth (permutation $p = 0.003$), including false alarms, where lures are misidentified behaviorally but still decoded as "new." Critically, analysis of decoder weights showed the decoder relied on pattern separation cells to decode ground truth. Finally, single-trial projections onto the ground-truth dPC axis in the hippocampus showed that true positive and true negative trials were more separable (by d-prime) than true positives and false alarms. In contrast, preSMA projections for true positives and false alarms overlapped, showing that only the hippocampus preserves stimulus fidelity despite behavioral errors. Together, these findings reveal a single-neuron substrate for pattern separation and demonstrate hippocampal-specific mechanisms that support pattern separation for highly similar visual inputs.

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Poster

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Topic: I.08. Learning and Memory

Support: NIH Grant R00NS126233

Title: Neurons Reflect Memory-Based Prediction in Humans

Authors: *J. MO¹, N. KURILENKO², I. SKELIN⁴, T. DO⁵, A. MAMELAK², T. A. VALIANTE⁴, D. BRANDMAN⁶, J. SHARMA⁶, U. RUTISHAUSER³, J. ZHENG⁷;

¹Univ. of California, Davis, Davis, CA; ³Dept. of Neurosurgery, AHSP #6432, ²Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁴Univ. Hlth. Network, Toronto, ON, Canada; ⁵UC Davis, Davis, CA; ⁶Neurolog. Surgery, UC Davis Hlth., Sacramento, CA; ⁷Neurolog. Surgery and Biomed. Engin., Univ. of California Davis, Davis, CA

Abstract: Objective: Memory allows us to use past experience to anticipate what comes next. The brain learns temporal regularities (e.g., lightning -> thunder) that support inference by analogy (e.g., fireworks -> sound). Although this ability is central to adaptive behavior, the underlying neural mechanisms remain poorly understood. We recorded single neuron activity with high spatiotemporal resolution, allowing us to probe the neural basis of predictive inference. We hypothesized that boundary cells, which signal unexpected transitions in an ongoing narrative, change their responses to reflect predictive inference.

Methods: We designed a memory-based prediction task (**Fig. 1a**) using naturalistic video clips. Each 4-event clip was presented three times (runs). Participants were asked to predict the next event at the end of event 2 and answered a recognition question at the end of event 4. In Run 3, clips were either identical to the original (repetition), structurally similar but visually distinct (generalization), or had a reversed event sequence (violation). We recorded 647 neurons from 12 patients with refractory epilepsy who had depth electrodes implanted for seizure monitoring.

Results: Behaviorally, participants had high recognition accuracy indicating engagement (**Fig. 1b, top**). Order prediction accuracy improved across Run 1 and 2 (**Fig. 1b, bottom**), reflecting memory-based prediction. In Run 3, order accuracy remained high for generalizations indicating successful predictive inference but dropped for violations (as hypothesized). At the neural level, we found 134 boundary cells (21% of total neurons) that responded to at least one event onset (boundary) in Run 1 with increased firing rates. By Run 3, 24% of these boundary cells (5% of total neurons) decreased their boundary responses in repetitions and generalizations but had persistent responses in violations (**Fig. 1c**).

Conclusions: Our findings suggest that neurons track event structure by signaling cognitive boundaries, with their boundary responses modulated by predictive inference.

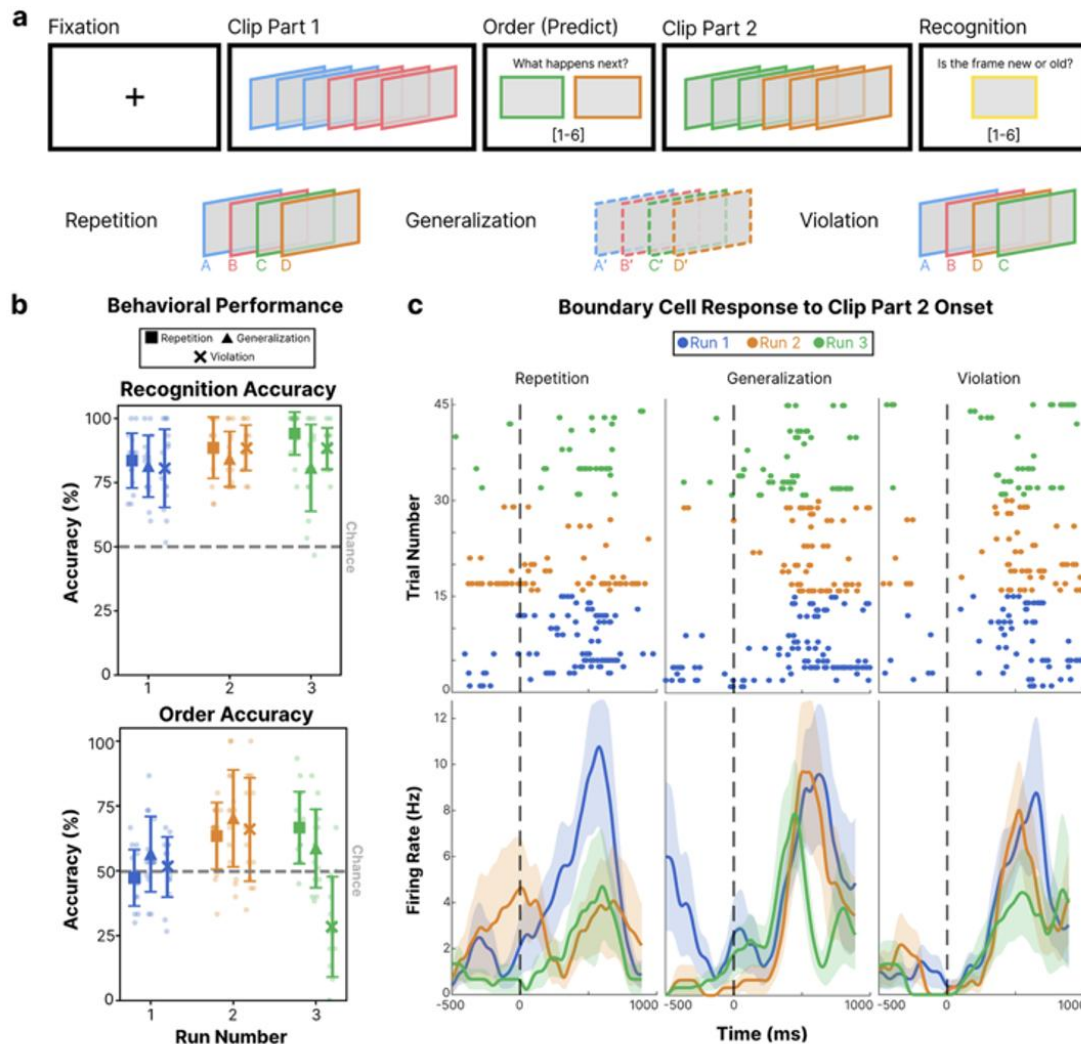


Figure 1. (a) Task schematic with three trial types. (b) Recognition accuracy (top) and order accuracy (bottom) across runs and trial types. (c) Example boundary cell (0 ms line marks Clip Part 2 event onset).

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Poster

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Topic: I.08. Learning and Memory

Support: NFRF - Brains in the Wild project

Title: Human Time Cells Support Working Memory Maintenance

Authors: *X. XIAO^{1,2,3}, U. RUTISHAUSER^{4,5}, T. A. VALIANTE^{2,1}, J. TAXIDIS^{6,1};
¹Univ. of Toronto, Toronto, ON, Canada; ²Univ. Hlth. Network, Toronto, ON, Canada; ³The Hosp. for Sick Children, Toronto, ON, Canada; ⁴Dept. of Neurosurgery, AHSP #6432, Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁵Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; ⁶Neurosciences and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Working memory, the active retention of information over short periods, is a fundamental cognitive function, yet its underlying neural mechanisms in humans remain poorly understood. Recent rodent studies have revealed sequences of “time cells” i.e. neurons spiking after a cue that encode both its identity and time since its presentation. These sequences form a potential mechanism for maintaining working memory in mice but whether they exist in the human brain is unclear.

To investigate this, we analyzed recordings from 902 neurons obtained from the medial frontal and medial temporal lobes of 21 neurosurgical patients performing the Sternberg working memory task (Kyzar et al., 2024). We observed neurons that consistently fired at their preferred time points during memory maintenance, forming temporally structured sequences. These sequences allowed for more accurate decoding of elapsed time compared to non-sequential neuronal ensembles, and their reproducibility was related to both memory load and task performance. Moreover, we identified neurons that responded during specific time windows after individual images, suggesting a potential mechanism for maintaining cue information during working memory.

Together, these findings demonstrate that temporally tuned firing in medial frontal and temporal regions supports human working memory, highlighting the integrative role of temporal coding in cognition.

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Poster

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Topic: I.08. Learning and Memory

Support: Center for Neural Science and Medicine at Cedars-Sinai Postdoctoral fellowship
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Title: Persistent activity in visual axis-tuned neurons during working memory in human ventral temporal cortex and medial temporal lobe

Authors: *J. XIAO, J. DAUME, A. MAMELAK, U. RUTISHAUSER;
Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Working memory is a core component of human cognition. It relies on the brain's ability to maintain information over delay periods, yet the neural mechanisms that support sustained representations of complex visual stimuli in the absence of visual input remain unclear. Here, we examined whether persistent activity during working memory is organized via axis-based coding in a visual feature space. Using intracranial recordings from 42 neurosurgical patients (49 sessions, 1871 neurons), we analyzed single-unit activity while participants performed a Sternberg working memory task. On each trial, participants viewed one or three images, maintained them over a 2.5-2.8 s delay, and indicated whether a probe image matched any of the previously presented items from that trial. To capture the visual features of each stimulus, images were processed with AlexNet to obtain embeddings from the fc6 layer. We then applied principal components analysis (PCA) and used the top 50 components, yielding a 50-dimensional feature space in which each image was represented as a point. Neurons were classified as axis-tuned if their firing rates correlated with the projection value of stimuli along a single specific axes (the preferred axis), and not with any of the orthogonal axes, in this feature space. Significant visual axis tuning was observed across the ventral temporal cortex (VTC) and medial temporal lobe (MTL), with 64% of visually selective cells in the VTC and 37% in the MTL. To test whether axis-tuned neurons exhibited persistent activity, we performed a median split of each neuron's projection values for the images maintained in WM and examined whether firing rates during the delay period correlated with the projection values. We found that axis-tuned neurons exhibited elevated delay-period activity when the stimulus in the working memory had a higher projection value, indicating sustained representation during memory maintenance. Surprisingly, this persistent activity was not limited to the MTL but was also observed in the VTC, a region not previously associated with sustained delay-period firing in humans. Notably, many of these neurons did not show selective persistent activity during the maintenance window under a conventional categorical framework, underscoring the increased sensitivity of our continuous feature-based framework for selecting neurons with persistent activity. Together, these findings reveal that axis-tuned persistent activity supports memory maintenance across both classical memory areas and high-level visual cortex, demonstrating the utility of axis-based models for uncovering neural dynamics during the working memory process.

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Poster

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Topic: I.08. Learning and Memory

Title: Saccade-related theta-reset is modulated by attention in humans

Authors: *K. TYNER¹, J. ZHENG², U. RUTISHAUSER³, D. KRAMER⁴, J. A. THOMPSON⁵;
¹Neurosurg., Univ. of Colorado Anschutz Med. Ctr., Aurora, CO; ²Neurolog. Surgery and Biomed. Engin., Univ. of California Davis, Davis, CA; ³Dept. of Neurosurgery, AHSP #6432, Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁴Univ. of Colorado Sch. of Med., Aurora, CO; ⁵Neurosurg., Univ. of Colorado, AMC, Aurora, CO

Abstract: Previous literature has established the importance of theta band activity in navigation and memory formation. Saccade-related theta-reset is seen during memory formation in non-human primates as well as in a limited number of patients with intracranial electroencephalography (iEEG) in both the hippocampus and lateral neocortex. One challenge has been disambiguating whether phase alignment (reset) resulting from saccadic eye movements is due to a motor response or if it may be a proxy of attentional processing. In this study, we evaluated whether theta-reset during an attention-dependent task was modulated by task demands reflecting a cognitive, top-down process. We enrolled 8 patients who underwent iEEG depth monitoring for seizure localization at the University of Colorado (ages 18-60, no cognitive deficits). Patients performed an episodic memory task in which they watched short video clips and asked whether the video takes place inside or outside. Each patient performed 90 trials. Eye tracking (1000 Hz) and local field potential (LFP) data (500 Hz) were simultaneously recorded. Channels with excessive line noise were removed, and remaining channels were bipolar referenced and bandpass filtered between 4-8 Hz. Saccade onset times were determined using custom algorithms, and the instantaneous phase of the LFP recording was extracted with Hilbert transform. Phases corresponding to the first 500 ms of the inter-trial interval (ITI) and 250 ms before to 250 ms after image onset and question presentation, as well as all saccades that occurred during the trial for channels located in the amygdala, anterior basal temporal, dorsal anterior cingulate, anterior hippocampus, posterior hippocampus, basal temporal, orbital frontal, and posterior basal temporal cortex were extracted. Phase consistency was examined by calculating inter-trial phase coherence (ITPC). The maximum ITPC value after each event onset was calculated, and significance was assessed using Kruskal-Wallis, and we corrected for multiple comparisons using Bonferroni correction. For each wire analyzed, the max ITPC of ITI, image onset, and question presentation was significantly higher than following saccades ($p < 0.05$), except for wires in the right basal temporal, right mid parietal, and right posterior basal temporal areas, where the max ITPC of ITI was not significantly different from those following saccades. Together, our results indicate that there is greater ITPC when an event occurs compared to saccades.

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Topic: I.08. Learning and Memory

Support: BRAIN initiative U01NS117839

Title: Semantic category neurons are more prevalent and respond earlier in anterior compared to posterior human hippocampus

Authors: *S. CHENG¹, N. KURILENKO², C. P. MOSHER¹, C. M. REED³, W. S. ANDERSON⁴, T. A. VALIANTE⁵, A. N. MAMELAK¹, U. RUTISHAUSER¹;

¹Neurosurg., ²Neurosurgery/Biomed. Sci., ³Neurol., Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁴Neurosurg., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ⁵Univ. Hlth. Network, Toronto, ON, Canada

Abstract: The human hippocampus extends up to 5 cm along its longitudinal axis. Despite theories attributing distinct functions to its anterior (aHPC) and posterior (pHPC) parts, there is little known difference in their functional properties at the electrophysiological level in humans. Developing a better understanding of the cognitive processes supported by various parts of the hippocampus along its long axis is critical, because surgical resection in epilepsy patients typically targets only the anterior part. To gain insights into the functional organization along the human hippocampal long axis, we analyzed a large dataset of single-neuron activity acquired from epilepsy patients implanted with hippocampal depth electrodes bilaterally. Patients performed a recognition memory task with visual stimuli. We localized the electrodes using an automated segmentation pipeline (FreeSurfer). We analyzed 1146 neurons: 939 in aHPC and 207 in pHPC. Of these, 207 neurons responded differentially to visual categories (visually selective (VS), 18%), which are a type of cell that represents semantic features of visual stimuli. 184 VS cells are localized to aHPC, 23 to pHPC. The proportion of VS cells was greater in aHPC than pHPC (21.9% vs 11.8%, chi-square $p = 0.002$). Next, we asked if the response properties of VS cells differ between aHPC and pHPC. We quantified response latencies by computing time-resolved ANOVA effect size. We found that the onset of the differential response of VS cells was on average 153 ms earlier in aHPC than pHPC (378 ms vs. 531 ms). aHPC VS cells latencies did not differ significantly from those in amygdala (AMY, KS test $p = 0.4$), while pHPC had significantly slower responses than both aHPC and AMY (KS test $p = 0.01$ and $p = 0.02$, respectively). To make sure that the differential latencies are not driven by laterality, we compared response onset of VS cells between left and right hemispheres in both aHPC and pHPC. We did not detect significant difference in either ($p = 0.8$ aHPC, $p = 0.99$ pHPC), suggesting that aHPC and pHPC have different temporal dynamics independent of their hemispheric location. Notably, firing rates of VS cells and ANOVA effect size were not different between HPC subregions (rank-sum test, $p = 0.8$, $p = 0.3$), indicating that the latency differences cannot be explained by neural statistics or tuning qualities. Together, these findings demonstrate for the first time, to our knowledge, differential response latency to visual stimuli along the human hippocampus longitudinal axis. We believe that this finding might indicate the greater involvement of pHPC into higher-level cognitive processes such as attributing visual input to its semantic meaning.

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Poster

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Topic: I.08. Learning and Memory

Support: R00NS126233
U01NS117839

Title: Neural signatures of auditory event segmentation in humans

Authors: *A. FLORES¹, V. WADIA², A. MAMELAK³, D. BRANDMAN⁵, J. J. LIN⁶, J. ZHENG⁷, U. RUTISHAUSER⁴;

¹Neurosci., Univ. Of California, Davis, Davis, CA; ²Cedars-Sinai Med. Ctr., Arcadia, CA; ⁴Dept. of Neurosurgery, AHSP #6432, ³Cedars-Sinai Med. Ctr., Los Angeles, CA; ⁵Neurolog. Surgery, UC Davis Hlth., Sacramento, CA; ⁶Dept. of Neurol., Univ. of California, Davis, Sacramento, CA; ⁷Neurolog. Surgery and Biomed. Engin., Univ. of California Davis, Davis, CA

Abstract: The human medial temporal lobe (MTL) plays a crucial role in segmenting continuous sensory experience into discrete events, a fundamental process for episodic memory formation. Event boundaries are contextual transitions that help structure continuous experience into mnemonic episodes, allowing for efficient encoding and retrieval of information. Prior work from our group (Zheng et al., 2022) has reported MTL neurons that signal event boundaries in the visual domain. Whether similar mechanisms exist in the auditory domain remains unknown. Understanding how the brain processes naturalistic spoken narratives is essential for uncovering the neural basis of memory formation in real-world settings, where auditory information plays a dominant role in communication and learning. In this study, we recorded 757 single neurons from 12 participants with drug-resistant epilepsy implanted with depth electrodes during seizure monitoring. Participants listened to seven brief stories (~30-60 seconds), each repeated six times, and freely recalled their content. Guided by Event Segmentation Theory (Zacks et al., 2007), we defined event boundaries as moments of high unpredictability derived from large language models (GPT-2 and sBERT) and validated with annotations from healthy subjects. Using story scripts as inputs, GPT-2 captures word-level prediction error quantified as Bayesian surprise, while sBERT reflects broader narrative transitions quantified as low cosine similarity between adjacent sentences. The distinct contributions of GPT-2 and sBERT suggest a hierarchical organization of event segmentation, where local semantic prediction errors and global narrative shifts may reflect parallel processes. We found that 65 of 757 neurons (8.59%) and 56 of 757 (7.40%) neurons respond to word-level event boundaries and sentence-level event boundaries, respectively. These neurons are largely located in the MTL and in the prefrontal cortex. In sum, our findings provide single-neuron evidence for event boundary detection during auditory narrative comprehension. By linking neural responses to computational measures of speech prediction, we advance the understanding of how the human brain segments and encodes experiences into memory.

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Poster

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NSF/UKRI (2342847)

Title: Human single-unit and mass neural signatures of auditory sequence coding in the mnemonic system

Authors: *R. M. CALMUS^{1,2}, Z. KOCSIS^{3,1}, J. I. BERGER¹, H. KAWASAKI¹, T. D. GRIFFITHS², M. A. HOWARD, III¹, C. I. PETKOV¹;

¹Dept. of Neurosurg., Univ. of Iowa, Iowa City, IA; ²Biosci. Inst., Newcastle Univ., Newcastle upon Tyne, United Kingdom; ³Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: There is substantial interest in identifying neuronal signals that carry traces of the sensory past and predictions about the future. However, outside of animal models, we lack insights into how site-specific neuronal activity within the human mnemonic system carries information reflecting the relational coding of learned sensory sequences over temporal delays. We conducted an auditory statistical learning task with a cohort of 11 neurosurgery patients during intracranial monitoring of refractory epilepsy. Patients listened to perceptual sequences of 3 nonsense words containing a dependency between two sounds in each sequence. Words were drawn from sets (A, B and X), with regularities between pairs of relevant (A and B) sounds often separated in time by uninformative (X) sounds. Each A-B sound pairing thus formed either an adjacent or non-adjacent dependency. We first analyzed site-specific single-unit activity and local field potentials (LFPs) from auditory cortex, hippocampus, and frontal cortex using traditional methods, demonstrating engagement of fronto-temporal sites including the hippocampus in the processing of the auditory sequences. In addition to univariate analyses, novel multivariate decoding analyses applied to both single-unit and LFP responses revealed evidence of distinct sequence item representations: hippocampal high-frequency oscillations and single-unit activity suggested time-compressed replay occurs transiently before and after key sounds in the sequence, while frontal cortex results provided evidence of a stimulus-driven relational code that incorporates ordinally coded position and item identity. Building on these findings, we characterize a variety of single-unit responses that, in concert with our decoding analyses, provide evidence for a distributed relational code underlying prospective and retrospective sequence item representation in the human hippocampus and frontal cortex. Our

results reveal critical roles for the mnemonic system in transforming sensory events into mental structures, providing insights into the single-neuron and mass-neural contributions to the maintenance and replay of sequential content in the human brain.

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Poster

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Topic: I.08. Learning and Memory

Title: Relationship Between Memory and Consciousness in Focal Seizures

Authors: *N. E. CAMPORA¹, A. NASIMBERA², M. M. VILLANUEVA³, S. KOCHEN⁴;
¹Hosp. El Cruce, Buenos Aires, Argentina; ²Neurosci., ³CABA, Hosp. de Alta Complejidad El Cruce Nestor Kirchner, CABA, Argentina; ⁴CONICET, capital federal, Argentina

Abstract: Introduction: Consciousness includes external interaction, subjective experience, and memory. The ILAE associates awareness with consciousness, assuming patients aware during seizures can recall and report the event. This study aims to characterize memory in seizures and its relation to loss of consciousness (LOC). **Methods:** Patients with refractory focal epilepsy undergoing video-EEG (VEEG) were included. Consciousness was assessed using the CSS scale (Arthuis et al.), and memory (aura and ictal events) via SPS (Campora et al.). **Results:** Of 230 seizures (79 patients), 119 were adequately evaluated. Complete amnesia occurred in 48.7% (58), partial memory in 36.1% (43), and full memory in 15.1% (18). Aura recall was adequate in 52.1% (62). Consciousness was preserved in 16.8% (20), profoundly altered in 59.7% (71), and moderately altered in 23.5% (28). **Aura as a Marker of Memory Preservation:** Aura recall was present in 100% of seizures with partial or full ictal memory and mostly absent in cases with profound (80.8%) or moderate (66.6%) LOC. Memory impairment was associated with greater LOC ($p < 0.001$) and sleep ($p < 0.0001$), but not with seizure duration, epileptogenic zone, or lateralization. **Relationship Between LOC and Memory:** Amnesia occurred in 72.4% of seizures with profound LOC and 53.6% with moderate LOC. Seizures without LOC had 65% adequate memory, though 35% showed memory deficits. Partial memory appeared in 40.8% of profound and 32.1% of moderate LOC cases. **Conclusions:** Aura recall is a key marker of memory preservation in focal seizures. Profound and moderate LOC significantly impair memory, especially during sleep. Even without LOC, memory can be affected. These findings underscore the need for further research into the neurophysiological mechanisms linking consciousness and memory in epilepsy.

Disclosures: N.E. Campora: None. A. Nasimbera: None. M.M. Villanueva: None. S. Kochen: None.

Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR360.15/QQ16

Topic: I.08. Learning and Memory

Title: Connectivities of the human hippocampal memory system with semantic cortical regions, and their functions in semantic learning

Authors: *E. T. ROLLS¹, C. YANG², C.-C. HUANG³;

¹Oxford Ctr. For Computat. Neurosci., Coventry, United Kingdom; ²Ctr. for Life Sci., Peking Univ., Beijing, China; ³Psychology, East China Normal Univ., Shanghai, China

Abstract: The hippocampal episodic memory system is implicated in the learning of new semantic representations (Rolls et al, 2025). To analyse the pathways involved, diffusion tractography, functional connectivity, and effective connectivity was measured in 171 Human Connectome Project Participants and analysed in the MCP-Multimodal Parcellation. The medial parahippocampal cortex region TF (which connects via the perirhinal and entorhinal cortex with the hippocampus) has connectivity with the inferior temporal visual cortex involved in object and face representations, TE2p, TE1p and FFC; with the orbitofrontal cortex (47m), which provides a route for reward value to be incorporated into memories; with the anterior temporal lobe semantic network including STSda, STSva, TE2a, and temporal pole TGd; with temporo-parieto-occipital junction regions TPOJ2 and TPOJ3 that are implicated in language; and with inferior frontal cortex regions IFSp and IFJa, which are highly connected with Broca's area regions 44 and 45. The lateral parahippocampal cortex regions PHA1-3 (which connect via the entorhinal cortex with the hippocampus and which represent spatial scenes with spatial view cells) have connectivity with the ventromedial cortical visual pathway regions VMV1-3 and ProStriate Cortex (ProS); with TPOJ3, which is a semantic region in the temporo-parietal region; with inferior parietal PGs (adjacent to and connected with PGi), which is connected with anterior temporal lobe semantic regions, and which may encode information about actions which is an important part of semantic representations; with some area 7 parietal and related regions involved in visuo-motor actions in space; and with reward-related ventromedial prefrontal cortex region 10r. It is proposed that some of these connectivities provide for the 'What' object or face, 'Where' spatial scene, and reward representations to reach the hippocampus to be combined into episodic memories. It is also proposed that connectivity from the hippocampal system to semantic cortical regions provides a route for the already chunked components used for each episodic memory to provide useful building blocks to form new semantic memories using slow semantic learning when related episodes are recalled close together in time (Rolls et al, 2025).

Rolls, ET, Zhang, C, Feng, J. (2025) Slow semantic learning in the cerebral cortex, and its relation to the hippocampal episodic memory system. *Cerebral Cortex* 35: bhaf107.

Rolls, ET, Treves, A. 2024. A theory of hippocampal function: new developments. *Prog Neurobiol.* 238: 102636.

Rolls ET. 2023. Hippocampal spatial view cells for memory and navigation. *Hippocampus* 33: 533-572.

Disclosures: E.T. Rolls: None. C. Yang: None. C. Huang: None.

Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

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Topic: I.08. Learning and Memory

Support: NIH Grant HD094623
NIH Grant HD059205
NIH Grant MH084164
NIH Grant MH121069
Stanford Maternal & Child Health Research Institute Postdoctoral Support Award

Title: Glutamatergic signaling shapes brain organization for mathematical and reading abilities in children: a cross-cohort neurotransmitter mapping study

Authors: *Y. ZHANG, H. CHANG, D. EL-SAID, V. MENON;
Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

Abstract: Mathematical and reading proficiencies are cornerstones of cognitive development and predictors of academic and professional success. Despite their importance, the molecular mechanisms underlying individual differences in these abilities remain largely unexplored. This study investigates the relationship between neurotransmitter systems and structural brain organization supporting math and reading abilities in children. We analyzed data from two independent cohorts: the CMI-HBN cohort (Child Mind Institute Healthy Brain Network; N=760) and a Stanford cohort (N=231), comprising children ages 7-14. Our analytical approach consisted of two key steps. We first derived whole-brain structural phenotypes associated with math and reading abilities using gray matter measurements and canonical correlation analysis. We then mapped these phenotypes onto an atlas of 19 neurotransmitter receptors and transporters using regression models. To ensure robust findings, we implemented a discovery-validation framework, with the larger CMI-HBN cohort serving as the discovery sample and the Stanford cohort providing independent validation. Three key findings emerged. First, the spatial distribution of N-methyl-D-aspartate (NMDA) receptors consistently and robustly mapped onto structural brain organization supporting both math and reading abilities across cohorts, highlighting the critical role of glutamatergic signaling in cognitive development. Second, NMDA receptor distribution patterns showed domain-specific characteristics - widespread across multiple functional networks for math ability, but more focally concentrated within visual networks for reading ability - suggesting both shared and distinct molecular mechanisms

underlying these cognitive domains. Third, these associations were specifically pronounced for NMDA receptors, while other neurotransmitter systems showed weaker, inconsistent, or non-replicable relationships, underscoring the privileged role of NMDA receptor-mediated excitatory signaling in learning and cognitive development. This work bridges a critical gap between molecular mechanisms and macro-level structural brain organization supporting academic skills. Our findings advance understanding of neurobiological foundations of learning and identify potential molecular targets for interventions in children with learning disabilities.

Disclosures: Y. Zhang: None. H. Chang: None. D. El-Said: None. V. Menon: None.

Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR360.17/QQ18

Topic: I.08. Learning and Memory

Title: Exploring the impact of semantic structure on generalization: a behavioral and fMRI investigation

Authors: *S. ZIN, S. SOLOMON;
Binghamton Univ., Binghamton, NY

Abstract: Concepts (e.g., bird, insect) can be understood as clusters of features (e.g., wings, feathers, antennae). These features co-occur in meaningful ways, and the pattern of co-occurrence determines the internal structure of the concept itself. While prior research suggests that this structure influences category learning, we do not yet know whether it also shapes generalization. To investigate this, we designed two novel insect categories, each defined by 11 discrete features (e.g., arms, wings, legs) and one of two structures: Modular categories contained tight clusters of highly co-occurring features and Lattice categories contained features that were more evenly distributed. This design allows us to test whether the internal structure of a category supports or hinders generalization. In a behavioral study, participants learned one Modular and one Lattice category in a Missing Feature task. In a subsequent generalization task, participants viewed novel exemplars composed of varying proportions of features from the Modular and Lattice categories and decided to which category the exemplar most likely belonged. The direct influence of internal category structure on generalization is assessed by examining asymmetries in categorization decisions, and analyzing behavior across the two tasks can reveal trade-offs between ease-of-learning and generalizability. Our results reveal an inverse relationship between learning performance and generalization, highlighting a potential trade-off. In a corresponding fMRI study, we use the same tasks described above to reveal the neural mechanisms underlying this learning and generalization behavior. By examining the multivariate similarities across exemplar and category representations, we will locate regions that express the output of the generalization process above and beyond the identity of category features. Taken

together, these experiments reveal the extent to which semantic generalization is impacted by a category's internal structure and how these processes are implemented in the brain.

Disclosures: S. Zin: None. S. Solomon: None.

Poster

PSTR360: Neural Mechanisms of Learning and Memory in Humans

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Program #/Poster #: PSTR360.18/QQ19

Topic: I.04. Executive Functions

Support: JSPS KAKENHI Grant Number23K24748

Title: Effects of task repetition on cognitive flexibility performance and brain activity in healthy young and older adults

Authors: *R. ASAHARA¹, D. HOSHI², M. FUKUIE², J. SUGAWARA^{2,3}, T. TARUMI^{2,3,4};
¹Natl. Inst. of Advanced Industrial Sci. & Technol. (AIST), Takamatsu, Kagawa, Japan; ²Natl. Inst. of Advanced Industrial Sci. & Technol. (AIST), Tsukuba, Japan; ³Univ. of Tsukuba, Tsukuba, Japan; ⁴Texas Hlth. Presbyterian Hosp. Dallas, Dallas, TX

Abstract: Repeated exposure to cognitive tasks has been shown to improve its performance, while decreasing the task-related brain activity. However, whether these task repetition effects differ between young and older adults remains unclear. The present study aimed to determine the repetition effects of inhibitory task on its performance and associated brain activity in healthy young and older adults. This is a secondary analysis of randomized crossover study that investigated the acute effect of aerobic exercise on cognition and brain activity. Seventeen young (8 women and 9 men, 25 ± 2 yr) and 19 older (10 women and 9 men, 62 ± 5 yr) adults participated in this study. Each participant underwent two experimental conditions (exercise and control) on separate days in random order. On each day, functional magnetic resonance imaging (fMRI) data were collected during task switching at three time points. In total, six fMRI measurements were performed per participant over two days. The main outcomes were task switching performance (error rates and reaction times) and task-related neural activity. Linear mixed model (LMM) was used to examine the main and interaction effects of task repetition and age. Repeated measures correlation analysis was used to examine the relationship between repetition-related changes in performance and neural activity. LMM revealed significant effects of task repetition and repetition $\times$ age on reaction time, but no effect of age was observed. Older adults improved reaction time with task repetition ($p < 0.05$), whereas no significant changes were observed in young adults. In contrast, no significant effect was found for error rate. fMRI data revealed significant effects of age and task repetition, with no effect of age $\times$ repetition. Older adults had greater task-related activation in frontal, parietal, and temporal, and posterior cortices compared with young adults, while no regions showed greater activation in young adults. Task repetition decreased brain activity in both age groups ($p < 0.05$), particularly in the

bilateral cerebellum, left middle occipital gyrus, left precentral gyrus, and bilateral middle frontal gyrus. No regions showed increased activation with task repetition. Repeated measures correlation analysis revealed a positive relationship between task repetition-induced decreases in reaction times and brain activity. Taken together, our results showing reduced brain activity coupled with improved cognitive performance suggest the enhancement of neurocognitive efficiency particularly in older adults.

Disclosures: **R. Asahara:** None. **D. Hoshi:** None. **M. Fukui:** None. **J. Sugawara:** None. **T. Tarumi:** None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR361.01/QQ20

Topic: I.08. Learning and Memory

Support: NSF CAREER 2441575
NIH DP1DA060838
NIH R00HD098371
UAB BRAIN PRIME program

Title: Developmental Regulation of Learning and Memory by Chromatin Remodeling in *C. elegans*

Authors: ***H. LEE**, H. SUN;

Dept. of Cell, Developmental and Integrative Biol., Howard Hughes Med. Institute, The Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Neuronal plasticity requires coordinated changes in gene expression, chromatin accessibility, and synaptic remodeling. While neuronal numbers remain largely stable post-development, circuit properties and transcriptional states evolve across life stages, potentially shaping learning capacity. However, how chromatin remodeling contributes to this developmental regulation of learning remains poorly understood.

Using the genetically tractable model *Caenorhabditis elegans*, we investigated how chromatin remodeling factors influence aversive olfactory learning across developmental stages, with a focus on the L4-to-adult transition—a critical period of transcriptional reorganization in chemosensory neurons such as AWC. Pharmacological inhibition of transcription, but not translation, during conditioning impaired learning, indicating that transcriptional activation is essential for memory acquisition. A functional screen of 73 chromatin remodeler mutants revealed several factors with stage-specific roles in learning. Notably, the CHD3 ortholog *let-418* was required for adult-stage learning but dispensable at L4, suggesting developmental modulation of chromatin accessibility or usage. Ongoing work aims to define how learning-induced molecular programs are implemented in specific circuit using neuron-specific

transcriptomic and chromatin profiling.

Together, our findings demonstrate that chromatin remodeling regulates experience-dependent transcription in a stage-specific manner, and support a model in which epigenetic mechanisms dynamically shape neuronal plasticity across development in post-mitotic neurons.

Disclosures: H. Lee: None. H. Sun: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR361.02/RR1

Topic: I.08. Learning and Memory

Support: NIH grant R01 NS019895
NIH grant R01 NS102490

Title: Balance of transcription activation and repression determines the efficacy of two-day training protocols to induce long-term synaptic plasticity

Authors: *R.-Y. LIU¹, Y. ZHANG³, P. D. SMOLEN⁴, J. H. BYRNE²;

²Dept. of Neurobio. and Anat., ¹McGovern Med. Sch. at UTHealth Houston, Houston, TX;

³Neurobio. & Anat., ⁴Dept. of Neurobio. and Anat., McGovern Med. Sch. of UTHSC At Houston, Houston, TX

Abstract: Improved learning is achieved when training trials are separated in time. However, the mechanisms underlying the effectiveness of distinct inter-training intervals, particularly those that involve repetitive training given over multiple days, are poorly understood. To address this issue, we exploited the technical advantages of long-term synaptic facilitation (LTF) of the *Aplysia* sensorimotor synapse, at which two blocks of spaced repetitive 5-HT treatment or four blocks of training, with blocks separated by 24 h, produce persistent (up to 7 d) LTF and long-term memory. we discovered the timing of the second block was critical to determine the efficacy of 2-block training. A second block consisting of a single 5-HT pulse 24 h after the first block of 5 pulses of 5-HT led to persistent LTF. However, this pulse was without effect if delivered at 18 h. This difference appears due in part to transcription factors activated by the first block. In particular, the transcription repressor CREB2 was active at 18 h but not 24 h, inhibiting induction of persistent LTF. Consistently, after blocking activation of CREB2 by inhibition of its upstream kinase p38 MAPK, persistent (2 d) LTF could be induced by the second block of a single pulse of 5-HT applied at 18 h. At 24 h, the activation CREB2 returned to baseline, releasing an inhibitory constraint for LTF. The release facilitated prolonged LTF, which also relies on activation of the transcription activator CREB1. The results suggest that the dynamic profiles of transcription factors serve as a cellular timer or clock that specifies different temporal domains of synaptic plasticity as well as setting the eligibility for a second learning trial to further consolidate a memory. These data and the dynamics of their upstream kinases were used

to build a computational model that delineated mechanisms of memory improvement by repetition. These results indicate that the timing of concurrent CREB1 and CREB2 activation determines the efficacy of a two-block training protocol, and that these dynamics are useful to predict training protocols to prolong LTF.

Disclosures: R. Liu: None. Y. Zhang: None. P.D. Smolen: None. J.H. Byrne: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.03/RR2

Topic: I.08. Learning and Memory

Support: NIH grant R01 NS019895

Title: Role of p90 ribosomal S6 kinase (RSK) in 5-HT-induced long-term enhancement of excitability (LTEE) in *Aplysia* sensory neurons

Authors: *R. CALVO^{1,2}, R.-Y. LIU³, J. H. BYRNE⁴;

¹McGovern Med. Sch. at UTHealth Houston, Houston, TX, United States, Houston, TX; ³Dept. of Neurobio. and Anat., ⁴Dept. of Neurobio. and Anat., ²McGovern Med. Sch. at UTHealth Houston, Houston, TX

Abstract: Long-term synaptic facilitation (LTF) and long-term enhancement of excitability (LTEE) are two cellular correlates of long-term memory (LTM). LTEE can be produced in isolated *Aplysia* sensory neurons (SNs) by repeated application of serotonin (5-HT), a neurotransmitter necessary for long-term sensitization. A computationally-designed Enhanced protocol with irregular inter-stimulus intervals (ISIs) between 5-HT applications produces greater LTF and LTM than the Standard protocol with uniform ISIs. RSK activation is important for LTF and LTEE. Interestingly, previous research indicates that RSK returns to baseline at 24 h following the Standard protocol, but remains elevated after the Enhanced protocol. Elevated RSK activity may contribute to prolonged LTF and LTEE for SNs treated with the Enhanced protocol. As a first step to test this hypothesis, a specific inhibitor of RSK, BI-D1870 (BID), was applied to SNs cultures at 17 h for 2 h to determine whether RSK inhibition would reduce activation of CREB1, a downstream target of RSK and important mediator of LTF and LTEE. CREB1 activation was measured 5 h after BID treatment, and 24 h after 5-HT. Four groups of SNs were used: 1) Vehicle (Veh), 2) Enhanced, 3) BID, and 4) Enhanced + BID. Blind procedures were used for experiments and data analysis. Immunofluorescence assays indicated that BID significantly attenuated 5-HT-induced pCREB1 levels in the SN nucleus at 24 h (one-way RM ANOVA, $F_{3,19} = 9.046$, $p < 0.001$). We next examined the effect of blocking the late activation of RSK on late LTEE (24, 48 and 72 h post-stimulus) induced by the Enhanced protocol. Two-way RM ANOVA indicated a significant difference in treatment among the groups ($F_{3,40} = 21.861$, $p < 0.001$), and the effect of treatment was not dependent upon the time

of measurement for 24, 48 and 72 h (treatment x time, $F_{6,40} = 0.372$, $p = 0.892$). Student-Newman-Keuls *post hoc* test indicated a difference between Enhanced and Veh ($p < 0.001$), Enhanced and Enhanced + BID ($p < 0.001$), and no difference between Enhance + BID and BID alone ($p = 1.00$) or between Enhance + BID and Veh ($p = 1.00$). These results suggest that the Enhanced protocol induces persistent LTEE up to 72 h, and that the maintenance of LTEE is dependent on the second wave of activation of RSK. Supported by NIH grant R01 NS019895

Disclosures: R. Calvo: None. R. Liu: None. J.H. Byrne: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.04/RR3

Topic: I.08. Learning and Memory

Support: NIH grant NS102490

Title: Dynamics of protein kinases in different brain regions after fear conditioning in rats

Authors: *Y. ZHANG¹, N. ELINSON-WATSON², P. D. SMOLEN¹, F. H. DO MONTE¹, J. H. BYRNE¹;

¹McGovern Med. Sch. of UTHSC At Houston, Houston, TX; ²Univ. of Texas at Austin, Austin, TX

Abstract: Learning and memory require the activation of multiple protein kinases and transcription factors with distinct temporal dynamics. Training protocols with intervals that maximize the interaction between specific kinases enhance both nonassociative learning in invertebrates (Zhang et al. 2011, 2013) and associative learning in rats (Zhang et al. 2023). To design better training protocols for associative learning, here we investigated the time courses of activation of kinases essential for fear acquisition in rats. We used western blotting to quantify the activation patterns of protein kinase A (PKA) and the mitogen-activated protein kinase (MAPK) isoform ERK at 0, 15, 30 and 45 min after a single trial of fear acquisition (one 30-s tone co-terminated with one electrical footshock). We measured dynamics of activation in four brain regions implicated in associative learning: the amygdala, the dorsal hippocampus, the midline thalamus and the medial prefrontal cortex. Compared to samples from untrained control rats (30 s tone, no footshock) examined at the same time points, kinase activities showed distinct temporal patterns in different brain regions. In the amygdala, one trial of fear conditioning induced an immediate and transient increase in PKA expression ($28.6 \pm 11.4\%$, $n = 9$; Wilcoxon Signed Rank Test with Bonferroni corrections, $p = 0.048$), which returned to control levels at 15 min ($3.5 \pm 11.9\%$, $n = 8$; $p = 1$). In contrast, ERK expression increased only at 30 min post-trial ($30.3 \pm 9.3\%$, $n = 9$; $p = 0.016$), returning to control levels at 45 min ($8.8 \pm 11.8\%$, $n = 8$; $p = 1$). Interestingly, one trial of fear conditioning was not sufficient to alter PKA and ERK activation in the other analyzed brain regions, when compared to untrained controls (all p values > 0.05). We

are using the dynamic patterns of kinase expression in the amygdala to develop biologically realistic models of fear conditioning in rats, which will help predict optimized protocols to enhance associative learning in mammals.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.05/RR4

Topic: I.08. Learning and Memory

Support: R15MH101703

Title: Alterations of brain extracellular vesicles in Angelman syndrome contribute to memory impairment

Authors: E. PENNA^{1,2}, *W. SU¹, M. BRAGA¹, H. SHIGEMITSU^{3,1}, K. TA^{4,1}, B. MARIN^{5,1}, M. BAUDRY¹, X. BI¹;

¹Western Univ. of Hlth. Sci., Pomona, CA; ²Univ. of Naples Federico II, Naples, Italy; ³Scripps Col., Claremont, CA; ⁴Univ. of California Berkeley, Berkeley, CA; ⁵Claremont McKenna Col., Claremont, CA

Abstract: Angelman syndrome (AS) is a rare neurodevelopmental disorder with severe developmental delay, lack of language skills, severe cognitive impairment, motor dysfunction, and often with comorbid epilepsy and autism spectrum disorder. Genetic/genomic studies have attributed the cause of AS to deficiency of UBE3A. Several mouse models of AS reproduce the debilitating features of the human disease, including impaired synaptic plasticity, reduced dendritic spine density, and lysosomal alterations. Emerging research has highlighted the role of extracellular vesicles (EVs) in brain development and function, with mounting evidence linking EV dysregulation to various neurological disorders. In this study, we performed proteomic analysis of brain EVs from wild-type (WT) and AS mice. Comparison of EV proteins secreted from cortical synaptosomes of WT mice with those from AS mice revealed 139 differentially expressed proteins (DEPs); 120 proteins were upregulated and 19 were downregulated in AS EVs compared to WT EVs. GO Enrichment analysis of DEPs indicated that upregulated proteins are mostly involved in protein synthesis processes, while the downregulated proteins are involved in synaptic activity, protein ubiquitination (including UBE3A) and signaling. Functionally, administration of WT-derived EVs restored hippocampal neuronal morphology and improved learning and memory in AS mice, providing compelling evidence for their therapeutic potential. Together, these findings suggest that EVs secreted from synapses of AS mice lack the protein components supporting normal synaptic structure and activity and that EVs could represent a promising strategy for treating this currently incurable disorder.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.06/RR5

Topic: I.08. Learning and Memory

Support: NIH Grant R15MH101703

Title: Activation of TRPML1 enhances hippocampal LTD and reduces remote memory recall and memory precision during the critical postnatal period

Authors: *M. ZHOU¹, J. SUN^{1,2}, I.-B. UDOH¹, W. SU¹, Y. LIU¹, A. SALEH¹, T. QUACH¹, W. KAIBAN¹, M. BRAGA¹, M. BAUDRY¹, X. BI¹;

¹Western Univ. of Hlth. Sci., Pomona, CA; ²UCLA, Los Angeles, CA

Abstract: Memory processing in early life is fundamentally distinct from that in adulthood due to ongoing brain maturation, particularly in hippocampal circuitry. Infants have limited capacity for memory storage, recall, and for the integration of spatial, temporal, and contextual components, because the hippocampal circuitry necessary for these functions is not yet fully developed. This phenomenon is generally referred to as infantile amnesia, as it is associated with limited memories for episodes taking place in early development. Recent evidence implicates factors such as neurogenesis and memory circuitry immaturity in infantile amnesia. Immature memory circuitry is also responsible for infantile mnemonic imprecision. However, research on infantile memory processes and their underlying molecular/cellular mechanisms has remained limited. The lysosomal transient receptor potential mucopolipin 1 (TRPML1) channel plays critical roles in regulating multiple cellular functions, including synaptic plasticity, and dysregulation of TRPML1-mediated Ca²⁺ release has been increasingly linked to several major neurological disorders. Here we show that robust long-term depression (LTD) of synaptic transmission at the CA3-CA1 synapses of mice at postnatal day 17 (P17) is significantly reduced by a selective TRPML1 inhibitor, ML-SI1. Systemic injection of ML-SI1 in P17 mice before fear conditioning training significantly enhanced contextual fear memory recall 10 days post training. Furthermore, systemic ML-SI1 injection also significantly improved memory precision of P17 mice, as evidenced by significantly higher freezing time in the trained context than in an alternative context. Mechanistically, ML-SI1 treatment significantly increased the density of inhibitory synaptic terminals of parvalbumin (PV) neurons around the soma of CA1 pyramidal neurons. These results indicate that high TRPML1 activation in infantile mice, possibly due to reduced inhibition from LAMTOR1, enhances synaptic LTD and reduces memory consolidation and precision. These results also indicate that TRPML1 contributes to the regulation of PV interneurons and local circuitry maturation, which may play critical roles in hippocampal memory processing. Since the postnatal development of hippocampal circuits parallels that of

several mnemonic functions, including memory precision, emotional and spatial memory, these findings may have a significant impact on our understanding of cognitive and emotional health.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

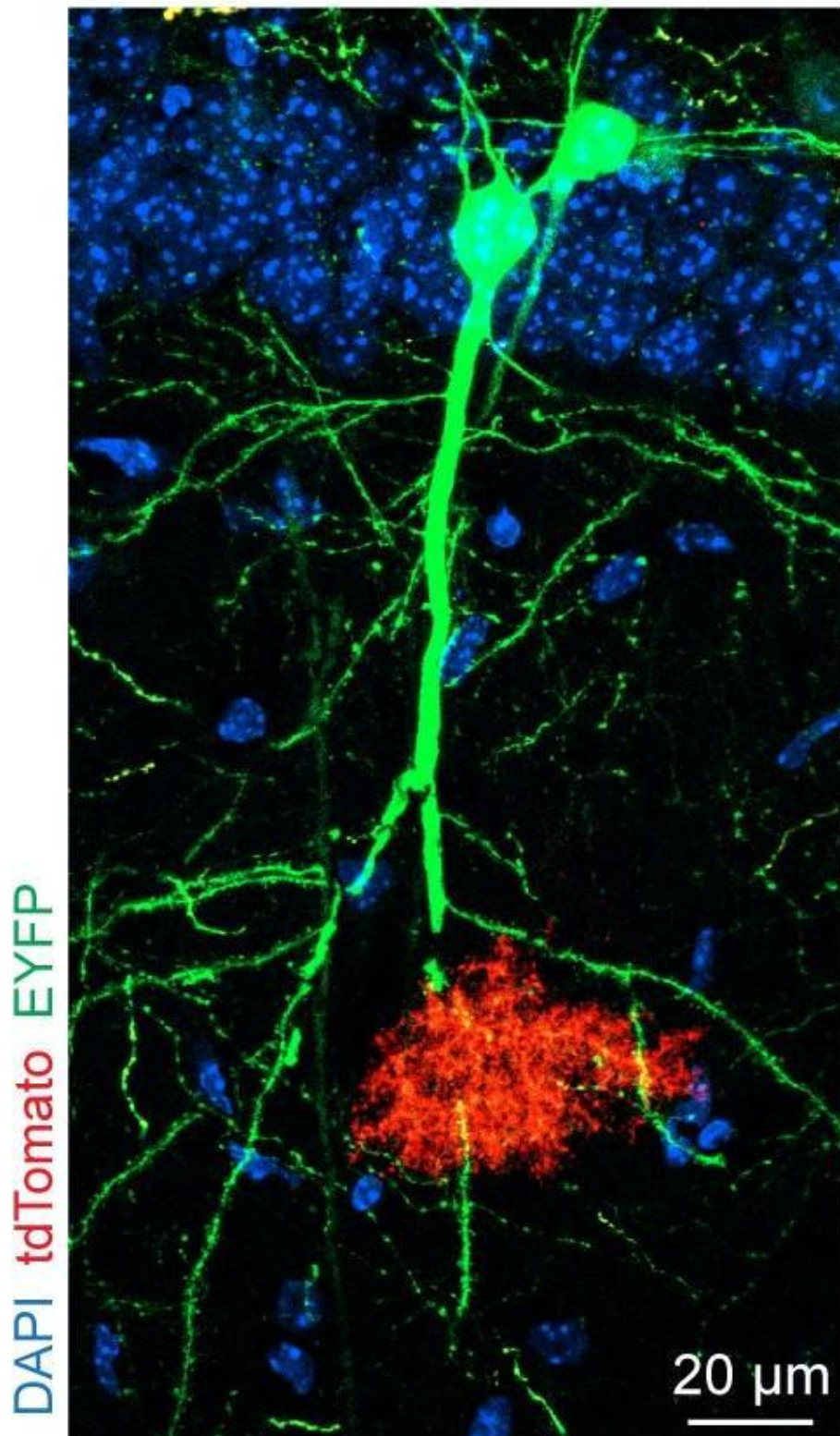
Program #/Poster #: PSTR361.07/RR6

Topic: I.08. Learning and Memory

Support: NIH Grant NS132230
NIH Grant MH134002
NIH Grant AG071687
AHA Grant 23POST1019413

Title: Learning-associated astrocyte ensembles regulate memory recall

Authors: ***M. R. WILLIAMSON**¹, U. KWON¹, J. WOO¹, Y. KO¹, E. MALEKI¹, K. YU¹, S. MURALI¹, D. SARDAR¹, B. DENEEN²;
²Neurosurg., ¹Baylor Col. of Med., Houston, TX



Abstract:

The cellular and molecular basis for memory formation and recall remain unresolved. Astrocytes modulate circuit function and plasticity. While ensembles of engram neurons whose activity is

necessary and sufficient for memory recall have been identified, it remains unknown whether there may be a cooperative ensemble of astrocytes that regulates learning and memory. We found that learning events induce expression of the immediate early gene c-Fos in a subset of astrocytes within the hippocampus of mice of both sexes. Furthermore, astrocytic c-Fos expression is required for memory formation. We subsequently generated a suite of viral tools to enable tagging and manipulation of learning-associated astrocyte ensembles. Using these new tools, we mapped engram neurons and learning-associated astrocytes after a single learning event and found that the two cellular ensembles were closely affiliated with one another (see accompanying image), suggesting that memory engrams may be composed of a set of interacting neuron-astrocyte ensembles. Furthermore, selective activation of learning-associated astrocytes was sufficient to induce fear memory recall, whereas perturbation of learning-associated astrocyte function impaired memory recall. By sequencing learning-activated astrocytes we also identified transcriptional changes underlying this form of experience-dependent astrocyte plasticity. These findings uncover new roles of astrocytes in circuit function and demonstrate the existence of distinct ensembles of astrocytes that regulate complex behavior.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Topic: I.08. Learning and Memory

Support: NRF-2021R1A2C109399112
KBRI-24-BR-01-02
RS-2024-00440824
IBS-R008-D1

Title: Exercise-induced Serpina1e as a hippocampal memory enhancer

Authors: ***H. KIM**, H. PARK;
Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of

Abstract: The exercise-induced enhancement of learning and memory is thought to be regulated not only by improved cardiometabolic and psychosocial health but also by body–brain interactions mediated by metabolites and proteins derived from peripheral organs. Given the prominent role of skeletal muscle during exercise, muscle-derived factors are believed to mediate the cognitive enhancements induced by exercise. However, the specific myokines that exert beneficial effects on cognitive functions remain to be elucidated. Here, we reveal that a novel myokine, Serpina1e, acts as a molecular mediator that directly supports long-term memory formation in the hippocampus. Using an in vivo myokine-labeling mouse model, proteomic

analysis revealed that the Serpina1 family of proteins are the myokines whose levels increased the most in plasma after 4 weeks of chronic aerobic exercise in male mice. Systemic delivery of recombinant Serpina1 or intramuscular overexpression of Serpina1 in sedentary male mice was sufficient to reproduce the enhancement of hippocampus-associated cognitive functions. Conversely, mice with a muscle-specific reduction in Serpina1 did not exhibit exercise-induced hippocampal memory enhancement, indicating a requirement of muscle-derived Serpina1 for exercise-induced cognitive enhancement. Moreover, our data showed that an increase in plasma Serpina1 level led to upregulated neurogenesis, brain-derived neurotrophic factor (BDNF), and neurite growth in the hippocampus, suggesting a positive role of exercise-induced Serpina1 in boosting hippocampal function. Because plasma Serpina1 can cross the blood-cerebrospinal fluid (CSF) and blood-brain barrier to reach the brain, our findings reveal that Serpina1 is a myokine that migrates to the brain and mediates exercise-induced memory enhancement by triggering neurotrophic growth signaling in the hippocampus. This discovery elucidates the molecular mechanisms underlying the beneficial effects of exercise on cognitive function and may have implications for the development of novel therapeutic interventions for alleviating cognitive disorders.

Disclosures: H. Kim: None. H. Park: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR361.09/RR8

Topic: I.08. Learning and Memory

Support: Departamento de Ciencias de la Salud

Title: Arc protein levels in the hippocampus of C57BL/6 mice exposed to high fat diet and environmental novelty

Authors: *S. GONZÁLEZ TREJO¹, B. X. DÁVILA-PÉREZ⁴, S. HERNÁNDEZ-RAMÍREZ², K. R. GUZMAN-RAMOS³;

¹Univ. Autónoma Metropolitana, Estado de México, Mexico; ²Fisiología y Neurodesarrollo, Univ. Autónoma Metropolitana, Lerma de Villada, Mexico; ³Ciencias de la Salud, Univ. Autónoma Metropolitana, Lerma, Mexico; ⁴Univ. Autónoma Metropolitana, Lerma, Mexico

Abstract: Obesity and diabetes mellitus are global epidemics increasingly associated with neurological dysfunction, including impaired synaptic plasticity and cognitive decline. While physical exercise is a cornerstone intervention, its long-term sustainability remains challenging. Environmental Novelty (EN) has emerged as a potential alternative to enhance neuroplasticity, but its effects under metabolic stress are incompletely understood. We investigated whether EN modulates hippocampal Arc protein levels—a critical mediator of synaptic plasticity—in C57BL/6 mice chronically exposed to high-fat diet (HFD), while exploring potential associations

between metabolic, behavioral, and molecular parameters. Male C57BL/6 mice (n=15 per group for metabolic/behavioral tests; n=5 per group for molecular analyses) were fed an HFD (60% kcal fat) or chow for six months post-weaning, with or without concurrent EN. Metabolic profiling included intraperitoneal glucose tolerance tests (IPGTT; 2 g/kg glucose after 6-hour fast; AUC analysis by two-way ANOVA). Body composition was assessed via epididymal fat percentage (final weight; two-way ANOVA). Arc protein expression was quantified via DAB immunohistochemistry (cell counts in Dorsal Hippocampus: DG, CA1, CA2, CA3; two-way ANOVA) using a subset of animals (n=5/group). Spatial learning was evaluated using the Morris Water Maze (two-way ANOVA for platform latency, target quadrant time, platform crossings and swim speed; repeated-measures ANOVA for learning curves). Six months of high-fat diet (HFD) exposure increased body weight, epididymal fat accumulation, and glucose intolerance compared to chow-fed controls. Environmental novelty (EN) intervention prevented HFD-induced cognitive decline, with HFD+EN animals performing similarly to chow controls in both spatial memory (Morris Water Maze) and social recognition tasks. Hippocampal analysis revealed region-specific Arc protein expression, with CA3 consistently showing the highest density of Arc-positive cells across all experimental groups. EN maintained near-normal Arc expression levels in HFD-exposed mice, particularly in the CA3 subregion.

Disclosures: S. González Trejo: None. B.X. Dávila-Pérez: None. S. Hernández-Ramírez: None. K.R. Guzman-Ramos: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR361.10/RR9

Topic: I.08. Learning and Memory

Support: NIH Grant R01MH108837
NIH Grant 1R01MH078064

Title: Neuroimmune regulation of ribosome biogenesis following learning

Authors: *E. WOOD¹, Z. PETROVIC², H. ZHANG², A. CICVARIC³, J. M. RADULOVIC⁴; ²Neurosci., ³Psychiatry, ¹Albert Einstein Col. of Med., Bronx, NY; ⁴Neurosci., Albert Einstein Col. of Med., New Rochelle, NY

Abstract: While neuroinflammation, or aberrant immune signaling, underlies psychiatric and neurodegenerative diseases, increasing evidence demonstrates essential roles for immune signaling pathways in memory formation. Our recent work delineated that toll-like receptor 9 (TLR9) signaling, classically known for its role in innate immunity, is essential for contextual fear learning in mice. We found that TLR9 coordinates the repair of persistent DNA breaks, preventing genomic instability, and facilitates essential cellular processes for memory storage. Depending on its localization, TLR9 activates either the NFκB subunit, RELA, or type 1

interferon (IFN1) signaling. While RELA knockout recapitulated the same effects as TLR9 knockout in dorsal hippocampal (DH) neurons, IFN1 receptor (IFNAR1) knockout did not, and the specific roles of these mediators remains unknown. In our current work, we observe that contextual fear conditioning (CFC) of mice induces localization of RELA to the nucleolus, the site of ribosome biogenesis, and that DH neuronal RELA knockout disrupts nucleolar morphology and function. Knockout of the type 1 interferon receptor (IFNAR1) in DH neurons leads to the CFC-induced accumulation of RELA in the nucleolus, suggesting that IFNAR1 may downregulate RELA signaling in response to learning. Furthermore, IFNAR1 knockout in DH neurons leads to increased cell death, suggesting that a balance between these pathways is essential for proper memory formation. Our results suggest that a network of immune signaling between TLR9, RELA, and IFNAR1 is necessary for memory formation and tightly regulates ribosome biogenesis and consequently proteostasis underlying learning. Our results point to a delicate balance between physiological and pathophysiological roles of immune mediators in the brain providing insight not only for basic knowledge of memory formation, but also hinting to more nuanced changes that may precede known pathologies like neurodegeneration.

Disclosures: E. Wood: None. Z. Petrovic: None. H. Zhang: None. A. Cicvaric: None. J.M. Radulovic: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR361.11/RR10

Topic: I.08. Learning and Memory

Support: NIA F31 AG087533
NIA R01 AG074041
Hevolution-AFAR HEV-NI23020

Title: Hippocampal engram coallocation necessary for successful memory updating

Authors: *C. BRUNSWICK, J. L. KWAPIS;
Pennsylvania State Univ., University Park, PA

Abstract: Memories are malleable and can be updated in response to new information. Deficits in memory updating are common with age-related cognitive decline and various dementias, but the mechanisms underlying these deficits are poorly understood. The field of behavioral neuroscience has overwhelmingly focused on understanding the formation of new memories with comparatively little work investigating how existing memories are updated. Previous work has demonstrated that related memories are coallocated (that is, stored in overlapping neuronal populations) within the dorsal hippocampus, and that this process is impaired with aging. We hypothesize that a similar mechanism might be occurring in memory updating: updated memories are stored in overlapping neuronal populations with their pre-update counterparts, but

older mice are failing to coallocate these updated memories, leading to updating impairments. To test this hypothesis, we modelled a memory update in young adult (3-m.o.) and old (18-m.o.) C57BL/6J mice using the Objects in Updated Locations (OUL) task. Using a viral TetTag approach administered to CA1 of the dorsal hippocampus, we tracked the activity of the original memory engram (neurons storing the initial training) during the update session. We found that while young adult and old mice recruit similar proportions of hippocampal neurons to store the initial training and the update, old mice exhibit reduced coallocation with fewer original-training neurons reengaged by the update session. Next, we explored if increasing coallocation would improve memory updating in old mice. To do so, we expressed an excitatory DREADD in a small proportion of neurons in CA1. Then, we activated these cells during the OUL training sessions and the update session. As excitable neurons are preferentially recruited to memory engrams, the DREADD-expressing neurons should be recruited to both the training and update engrams. We found that increasing coallocation via this approach alleviated memory updating impairments in old mice. Presently, we are using a bulk transcriptomics approach to investigate which genes are uniquely upregulated in the hippocampus following a memory update in young adult and old mice, hypothesizing that the transcriptional program required for proper neuronal coallocation is impaired in the aging hippocampus. We have already identified several genes (including *Npas4*, *Plekhf1*, and *Tent5a*) that are upregulated in response to a memory update in the young but not old hippocampus.

Disclosures: C. Brunswick: None. J.L. Kwapis: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR361.12/RR11

Topic: I.08. Learning and Memory

Support: NIH Grant R01AG074041

Title: Using Stress-Enhanced Fear Learning to Investigate Fear Sensitization in Male and Female Mice.

Authors: *H. M. BOYD¹, J. L. KWAPIS²;

¹Biol., Pennsylvania State Univ., University Park, PA; ²Dept. of Biology, Ctr. for Mol. Investigation of Neurolog. Disord, Penn State Univ., University Park, PA

Abstract: While fear is necessary for survival, aberrant or inappropriate fear can quickly become damaging. This is the case in post-traumatic stress disorder, in which individuals who have been exposed to a traumatic event can develop an exaggerated response to subsequent similar, milder stressors. Currently, we lack a clear understanding of the mechanisms that promote fear sensitization. To investigate how acute stress can induce exaggerated fear memory formation to future milder stressors, we used the stress-enhanced fear learning (SEFL) paradigm. In SEFL,

animals are first exposed to an acute stress event, a series of unpredictable foot shocks. Later, animals are exposed to weak fear conditioning in a distinctly new environment. First, we verified whether 10-footshock SEFL is sufficient to drive exaggerated fear memory in both male and female mice. We found that mice previously exposed to the 10-footshock acute stress event displayed exaggerated fear in response to a future weak fear conditioning event with no differences observed between the sexes. Next, we wanted to explore what molecular mechanisms could be driving fear sensitization in the SEFL paradigm. Histone deacetylase 3 (HDAC3) is a known negative memory regulator of fear memory which we hypothesize drives histone acetylation changes at key fear memory genes within the amygdala. We observed a significant reduction in HDAC3 expression in the amygdala 60 minutes after acute stress. Next, we measured gene expression changes at key fear learning genes: *cFos*, *BDNF* and *Grial* 60 minutes after weak fear conditioning. We found that *cFos* is significantly increased in both sexes in the amygdala following fear conditioning, regardless of a history of intense acute stress. Interestingly, we saw no expression differences for either *BDNF* or *Grial* regardless of experimental condition. We are currently using RNA sequencing to gain a more wholistic understanding of how a history of stress alters gene expression in the amygdala following similar milder stressors. Together these findings suggest that HDAC3 is altering histone acetylation within the amygdala following acute intense stress, which in turn likely alters chromatin structure at key fear memory genes, enabling excessive transcription following subsequent weak fear conditioning.

Disclosures: H.M. Boyd: None. J.L. Kwapis: None.

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR361.13/RR12

Topic: I.08. Learning and Memory

Support: NIA R00AG056596
NIA R21AG068444
Whitehall #2020-05-06
AFAR #A21105
NIH R01AG074041

Title: Tipping the scale: The sex-specific roles of Per1 and E2 in diurnal memory

Authors: *G. C. PIFER¹, L. BELLFY², H. M. BOYD³, J. C. BAEK⁴, J. L. KWAPIS⁵;
¹Biol., ³Neurosci., ²Pennsylvania State Univ., University Park, PA; ⁴Pennsylvania State Univ., State College, PA; ⁵Dept. of Biology, Ctr. for Mol. Investigation of Neurolog. Disord, Penn State Univ., University Park, PA

Abstract: Long-term memory persists over an extended period of time, operationally defined as 24+ hours following memory acquisition. Our lab has previously shown that long-term memory performance on the hippocampus-dependent Object Location Memory (OLM) task is different across the diurnal cycle, depending on age and sex. Additionally, we have shown that the circadian gene *Per1* oscillates in tandem with memory performance in young males and aged females, with the peak of expression occurring during the day when memory is best. Conversely, young females show good memory across the entirety of the diurnal cycle, and no significant induction of *Per1*. While this suggests that *Per1* may play a non-canonical role within the dorsal hippocampus in young male and aged female mice, it appears that young female mice may be engaging a divergent mechanism that enables their strong memory performance across the 24-hour day. One possible mechanism could be E2, a potent form of estradiol that is expressed highly in young female mice and drops precipitously with age. Supporting evidence from our lab shows that OLM performance is significantly worse during diestrus, the estrous phase with the lowest E2 concentration, and that young female mice who have undergone ovariectomies display oscillatory memory patterns matching those of young males. Additionally, preliminary data suggests that adolescent female mice lack the ability to perform OLM, which could be explained due to their pre-pubertal status. Together, our work suggests that E2 can overcome *Per1*'s ability to diurnally regulate memory, and disruption of estradiol enables *Per1* to re-exert this diurnal control. Further research is necessary to elucidate exactly how sex hormones and circadian genes are interacting to impact diurnal memory.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.14/RR13

Topic: I.08. Learning and Memory

Support: NIH Grant AG017628
NIH Grant AG062398
Roy J Carver Chair in Neuroscience
Dirk Warren Faculty Chair of STEM disciplines
John P. McNulty Foundation

Title: Sleep is enhanced in aged male mice that overexpress the protein calcium/calmodulin-dependent protein kinase IV

Authors: S. FEENEY¹, E. THRELFALL¹, J. BILBOA², **B. MAGEE**¹, N. KHOURY¹, C. C. ANGELAKOS³, M. E. WIMMER⁴, S. KIDA⁵, T. ABEL⁶, *J. C. TUDOR¹;
¹St. Joseph's Univ., Philadelphia, PA; ²St. Joseph's Univ., Philadelphia, PA; ³Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; ⁴Psychology, Temple Univ., Philadelphia, PA;

⁵Grad. Sch. of Agr. and Life Sci., The Univ. of Tokyo, Tokyo, Japan; ⁶Dept. of Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA

Abstract: The dysregulation of sleep-wake patterns that occurs during aging is well documented and coincides with changes in intracellular signaling pathways that regulate sleep, such as the calcium/calmodulin-dependent protein kinase (CaMKII)/cyclic-AMP response element-binding protein (CREB) pathway. However, much less is known about the relationship between other CREB-activating members of the CaMK family, such as calcium/calmodulin-dependent protein kinase IV (CaMKIV), and the regulation of sleep. Using 2- to 4-month-old (young adult) and 22- to 24-month-old (aged) male and female CaMKIV-overexpressing (CaMKIV-OE) mice, EEG/EMG data was scored for wake, non-rapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep by an experimenter blind to the traits of the mice. We observed that overexpression of CaMKIV in the forebrain decreased wakefulness and increased the amount of NREM and REM sleep in aged male mice, but not young adult male mice, in comparison to age- and sex-matched controls. Conversely, female mice overexpressing CaMKIV displayed no significant differences in the percentage of time spent in each vigilance state compared to their wild-type counterparts, regardless of age. While CaMKIV overexpression also led to more sleep-wake fragmentation in young adult and aged male mice, aged female mice displayed more consolidated NREM sleep. CaMKIV overexpression has previously been linked to memory improvements in young mice and memory restoration in aged mice. Our findings provide insights into the potential mechanism by which CaMKIV overexpression enhances memory and how that mechanism could be age and sex dependent.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.15/RR14

Topic: I.08. Learning and Memory

Support: NIH Grant R01NS128123
NIH Grant R01AG074041
NIH Grant T32GM152354

Title: Effects of 17 β -estradiol on object location learning-induced gene regulation in the dorsal hippocampus of ovariectomized female mice

Authors: H. M. BOYD¹, S. B. BEAMISH², A. W. FLEISCHER⁴, J. XU³, I. ALBERT⁵, A. SEBASTIAN⁵, J. L. KWAPIS⁶, *K. M. FRICK²;

¹Biol., Pennsylvania State Univ., University Park, PA; ²Psychological and Brain Sci., Univ. of

Wisconsin-Milwaukee, Milwaukee, WI; ³Psychological and Brain Sci., Univ. of Wisconsin-Milwaukee, Milwaukee, WI; ⁴Psychological and Brain Sci., Univ. of Wisconsin - Milwaukee, Milwaukee, WI; ⁵Dept. of Biochem. and Mol. Biol., The Pennsylvania State Univ., University Park, PA; ⁶Dept. of Biology, Ctr. for Mol. Investigation of Neurolog. Disord, Penn State Univ., University Park, PA

Abstract: The potent estrogen 17beta-estradiol (E2) regulates hippocampal gene expression and exogenous E2 treatment facilitates the consolidation of hippocampus-dependent object memories. However, it remains unclear how exogenous E2 influences learning-induced hippocampal gene expression to promote memory formation. Here, we used whole cell RNA sequencing (RNAseq) to determine effects of coincident learning and E2 treatment on dorsal hippocampus gene expression. Ovariectomized C57BL/6 mice remained in their home cage for the entire experiment (Home cage, HC) or were placed in a square white box with two identical objects and required to explore the objects for a total of 30 sec (Trained). Immediately after training, mice were injected (i.p.) with vehicle or 0.2 mg/kg E2; 60 min later, the dorsal hippocampus was collected for RNAseq. HC mice were also injected with vehicle or E2, resulting in 4 groups (n=6/group): HC+vehicle, HC+E2, Trained+vehicle, Trained+E2. RNA was extracted and submitted to RNAseq, which allowed for analysis of differentially expressed genes induced by E2 alone (in HC mice) or training alone (in vehicle-infused mice). Of particular interest were interactions between training and E2 (HC+E2 vs Trained+E2), most notably genes whose learning-induced expression was exaggerated by E2. Among genes uniquely upregulated in Trained+E2 relative to HC+E2 were 22 involved in protein folding and quality control, signal transduction, metabolism and homeostasis, transcriptional regulation, glycosylation and collagen synthesis, and mTOR signaling. The 6 uniquely downregulated genes were involved in cell adhesion and synapse formation, cartilage structure and function, membrane transport and signal transduction, and cardiovascular regulation. Of these, *ACAN* expression was selectively downregulated by E2 only in trained mice, as confirmed by qPCR. *ACAN* codes for aggrecan, a proteoglycan best known as part of the extracellular matrix in cartilage where it helps joints withstand compression. Aggrecan is also the primary functional constituent of the extracellular perineuronal nets (PNNs) in the brain that stabilize synapses by forming structural proteoglycan nets around neurons that restrict morphological remodeling. Aggrecan is expressed in hippocampal PNNs and conditional *ACAN* deletion ablates PNNs, increases synaptic plasticity, and enhances object recognition memory. Given that E2 promotes hippocampal pyramidal neuron dendritic spine density, synaptic plasticity, and memory, reduced *ACAN* expression in Trained+E2 mice reduce aggrecan expression and destabilize PNNs to allow for increased plasticity and memory consolidation.

Disclosures: H.M. Boyd: None. S.B. Beamish: None. A.W. Fleischer: None. J. Xu: None. I. Albert: None. A. Sebastian: None. J.L. Kwapis: None. K.M. Frick: 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.; Estrigenix Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix Therapeutics, Inc..

Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR361.16/RR15

Topic: I.08. Learning and Memory

Support: NIH Grant 1R01NS106056-01A1

Title: LINE-1 RNA regulates neuronal allocation to ensemble coding and memory

Authors: *N. POLITOWSKA¹, R. SCIORTINO², J. GAL TOTH², P. HAMOR², M. TOTH²;
¹Weill Cornell Med., New York, NY; ²Weill Cornell Med., New York, NY

Abstract: Experiences are encoded by small neuronal ensembles of activated cells in the hippocampus, a coding principle that provides large memory capacity and discrimination between similar memory traces to avoid interference. Despite their relative cellular homogeneity, only a 1-3% subset of neurons is “allocated” to coding and storing the memory. Deciphering the mechanism of neuronal allocation is fundamentally important to understand and possibly remediate deficits in encoding and memory in psychiatric and neurodegenerative disorders. A systematic epigenetic and transcriptomic analysis of hippocampal neurons in our laboratory revealed that while non-coding neurons have robust expression of full length LINE-1 (L1; long interspersed nuclear element) RNA, the small population of coding cells express significantly lower levels of this RNA. Knockdown (KD) of L1 RNA in the hippocampus by an antisense oligonucleotide (ASO) allocated more cells for coding, thus significantly increasing the size of the coding ensemble. L1 ASO infused mice demonstrated increased spatial working memory, indicating that the enlarged ensemble is functional in a cognitive task. Simultaneously, L1 ASO mice showed impaired discrimination between similar contexts, consistent with the requirement of sparse coding for pattern separation. Overall, our work has revealed L1 RNA as a key intrinsic regulator of neuronal ensemble size ensuring the coding population is large enough for efficient coding but small enough for pattern separation. Due to its powerful regulatory potential, manipulating L1 RNA levels can offer a novel therapeutic strategy to compensate the functional and structural neuronal loss in neurodegenerative, neurodevelopmental, and neuropsychiatric diseases.

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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

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Program #/Poster #: PSTR361.17/RR16

Topic: I.08. Learning and Memory

Support: RFINS126143

Title: The genetic signature of memory encoding across the human hippocampal axis

Authors: *M. DEHNAD¹, H. MOORE¹, A. A. KULKARNI⁴, T. TO¹, A. H. FREELIN², B. C. LEGA³, G. KONOPKA²;

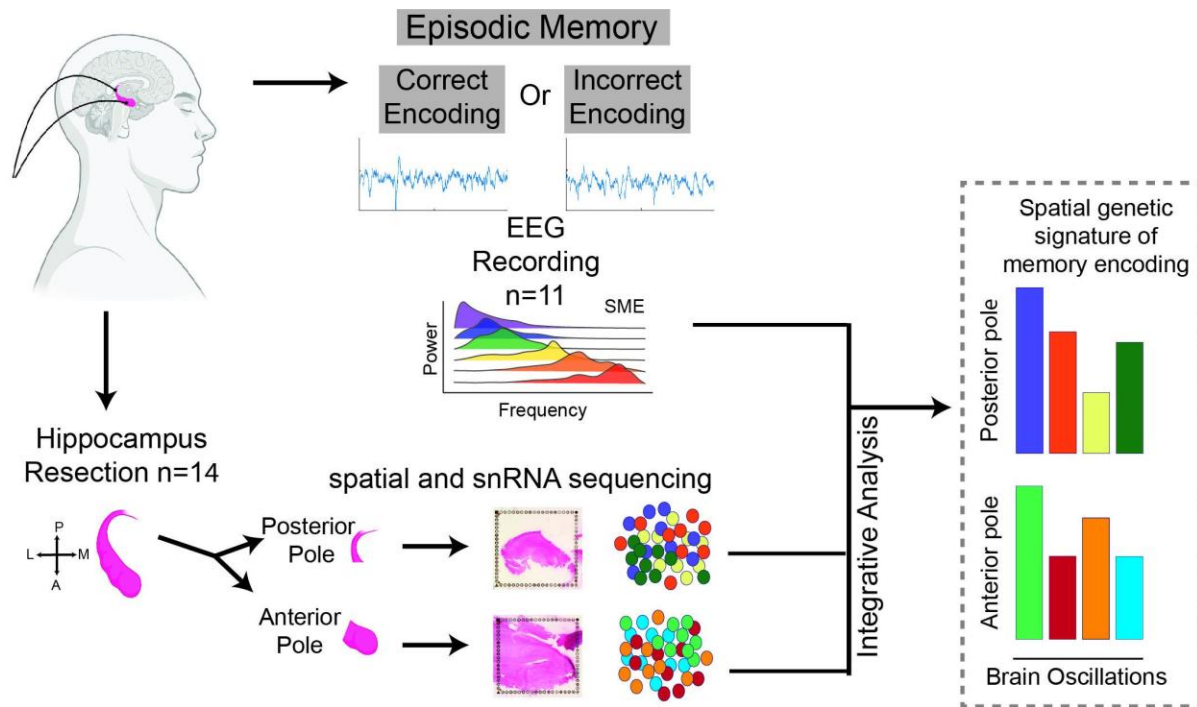
²Neurosci., ³Neurosurg., ¹UT Southwestern Med. Ctr., Dallas, TX; ⁴DEPARTMENT OF NEUROSCIENCE, UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER, Dallas, TX

Abstract: Episodic memory is central to human cognition, it underpins our capacity to recall personal experiences embedded in unique spatial temporal contexts. The hippocampus in particular, plays a central role in the formation and retrieval of episodic memory, binding elements of an experience into a coherent temporal framework. Subsequent-memory effects (SMEs) in the hippocampus are consistent oscillations predicting successful memory encoding and recall, yet the gene programs sustaining these rhythms are unclear.

Here we record iEEG from 61 anterior (aHC) and posterior (pHC) hippocampal depth-electrodes during verbal episodic memory tasks, in 11 patients, prior to resection. SMEs for multiple oscillatory frequency bands were quantified during the first 800ms of encoding. The aHC and pHC resection sites are profiled by snRNAseq (~106k nuclei, n=24, Scalebio) and VisiumHD spatial transcriptomics (~99k cells, n=4, 10Xgenomics). We link SME magnitude to cell-type and hippocampal pole specific gene expression and find oscillation specific gene networks across the hippocampal axis.

We find that the aHC gene-set is enriched for immediate-early and neuronal identity regulators. The pHC gene-set is enriched for regulators associated with structural maturation and metabolic regulation. We find that delta (2-4Hz) oscillation linked genes are enriched for zinc-finger homeodomain factors like ZEB3, ZNF746, and ZFX4, and activity dependent nuclear receptor NR4A1, whereas low-gamma (30-70Hz) oscillation features chromatin-modifying and architectural regulators like RFX3 and BAZ2B.

We conclude that integrating intracranial electrophysiology with single-nuclei and spatial genomics reveals that memory-relevant hippocampal oscillations engage region-, frequency-, and cell-type specific transcriptional programs along the antero-posterior axis. This molecular blueprint helps clarify hippocampal specialization and nominates possible gene networks for circuit-targeted interventions in studying memory and memory disorders.



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Poster

PSTR361: Learning and Memory: Genes and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR361.18/RR17

Topic: I.08. Learning and Memory

Title: Changes in feeding behavior and deficits in probabilistic learning in a mouse model of the schizophrenia-associated 3q29 deletion

Authors: R. M. POLLAK¹, B. A. MATIKAINEN-ANKNEY³, *J. G. MULLE²;

²Dept. of Psychiatry, ¹Rutgers, The State Univ. of New Jersey, Piscataway, NJ; ³Psychology, Rutgers Univ. Behavioral and Systems Neurosci., Highland Park, NJ

Abstract: Background The 3q29 deletion (3q29del) is associated with a range of neurodevelopmental and psychiatric phenotypes, including a >40-fold increased risk for schizophrenia. Prior work by our team has identified deficits in cognition and an increased incidence of feeding problems in humans with 3q29 deletion syndrome. Previously, we found behavioral deficits in our mouse model of 3q29del relevant to the human phenotype, including changes in sensorimotor gating and learning and memory. Here, we use an automated, high-throughput behavioral assay to identify novel feeding and learning phenotypes in the 3q29del

mouse model consistent with the human phenotype. **Methods** Adult male and female mice carrying the orthologous 3q29 deletion (3q29del) and wild type littermates (WT) (n=10 mice per sex/genotype) were used in the present study. Mice were singly housed with the home-cage operant pellet dispenser, Feeding Experimentation Device version 3 (FED3), containing grain pellets in the home cage. Mice were trained to use the FED3 with dual-poke paradigm for 3 days, then switched to a two-armed bandit task paradigm for 5 days wherein the successful choice switched after 30 pellets had been earned. All data were analyzed both jointly and stratified by sex to assess sex effects. **Results** During the training phase there was no difference in total pellets earned between WT and 3q29del mice. However, there were differences in feeding behaviors: male 3q29del mice consumed more pellets per meal than WT ($p<0.05$). During the bandit phase, 3q29del animals earned significantly fewer pellets than WT animals ($p=0.03$). A sex-stratified analysis revealed that this result was driven by females. Female 3q29del mice earned significantly fewer pellets in the bandit phase than WT females ($p=0.002$), with no difference in pellets earned between male WT and 3q29del mice. Female 3q29del mice also had significantly fewer reversal events during the bandit phase than WT ($p=0.002$). Finally, there were differences in feeding behavior during the bandit phase: 3q29del mice consumed fewer meals than WT ($p<0.05$), but 3q29del mice consumed more pellets per meal than WT ($p<0.05$). **Conclusions** 3q29del mice successfully learned how to use the FED3s at the same rate as WT but demonstrated altered feeding behaviors during the training and bandit phases. In the bandit phase, female 3q29del mice exhibited a learning deficit, earning significantly fewer pellets than WT controls and eliciting fewer probability reversals during the bandit task. Together, these data are the first report of changes in feeding behavior and deficits in probabilistic learning in a mouse model of the 3q29 deletion.

Disclosures: R.M. Pollak: None. B.A. Matikainen-Ankney: None. J.G. Mulle: None.

Poster

PSTR362: Human Navigation

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Program #/Poster #: PSTR362.01/RR18

Topic: I.09. Spatial Navigation

Support: SECIHTI CF-2023-I-1259

Title: Differential performance between children and adults in a vision-deprived maze spatial navigation task and exploration of the PPC tDCS impact on adult performance

Authors: *N. M. KEMP¹, H. GALVAN FREGOSO¹, A. MOLERO-CHAMIZO², M. A. NITSCHKE³, G. N. RIVERA-URBINA¹;

¹Fac. of Social and Administrative Sci., Univ. Autónoma de Baja California, Ensenada, Mexico;

²Univ. de Huelva, Huelva, Spain; ³Leibniz Res. Ctr. for Working Envrn., Dortmund, Germany

Abstract: Spatial navigation involves multiple sensory and cognitive processes, including the activation of visual, proprioceptive, vestibular, and somatosensory systems, as well as memory and movement planning mechanisms. These cognitive processes interact differentially depending on age. In order to explore the effectiveness of these interactions in different age groups, we compared the performance of children and adults in a double session spatial maze task under deprived vision. Thirty healthy participants were recruited for the children group (mean age 10.97 ± 0.55). The adult group included 30 healthy adults (mean age 21.16 ± 1.76). Results revealed a significantly higher number of correct actions during maze solving in children compared to adults. These findings suggest a lower reliance on visual inputs during spatial task resolution in children than in adults, whose cognitive processes related to spatial navigation might be more dependent on visual cues. In a second study with a different healthy adult group (mean age 21.23 ± 2.01), we applied excitatory anodal tDCS over the right posterior parietal cortex (PPC), a cortical region involved in spatial processing, to evaluate possible improvements in performance associated with this neuromodulation method. Compared to a sham group, the right PPC neuromodulation procedure did not significantly improve spatial task performance. Improved protocols with different types of spatial navigation tasks and optimized and personalized stimulation parameters are needed to further analyze the impact of PPC neuromodulation on spatial processing.

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Poster

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Title: Active, naturalistic navigation recruits a distributed network of functionally distinct regions in the human cerebral cortex

Authors: ***T. ZHANG**¹, E. X. MESCHKE², J. L. GALLANT³;
¹Univ. of California, Berkeley, Berkeley, CA; ²Univ. Of California, Berkeley Neurosci. Grad. Program, Berkeley, CA; ³UC Berkeley, Berkeley, CA

Abstract: Real-world navigation is a complex and dynamic task that requires a continuous perception-cognition-action loop. Multiple functional regions in the human cerebral cortex are known to be selective for navigation-relation information (Epstein et al. 2017). To date, most neuroimaging studies of human navigation have used controlled tasks and simplified stimuli to isolate specific navigation-related representations. However, because the brain is a nonlinear system, controlled tasks may recruit navigational systems differently than real-world navigation. Indeed, rodent studies have found that complex navigation tasks recruit more of the cortex than simpler navigation tasks (Pinto et al. 2019), that navigation-related representations are task-dependent (Fischer et al. 2020), and that navigation-related information is represented in overlapping spatial gradients that extend across functional regions (Minderer et al., 2019, Tseng et al., 2022). What regions in the human cerebral cortex are recruited by active, naturalistic navigation? What are their functional properties? Do they share the same organizational principles as those in the rodent cortex? To address these questions, we used fMRI to record brain activity from six participants (3 male, 3 female, age 25-32) performing an active taxi-driver task in a naturalistic virtual world (110-180 minutes of data per participant). From the experiment recordings, we extracted 28,161 features across 38 visual-, motor-, and navigation-related feature spaces, and used banded ridge regression to fit encoding models for all feature spaces simultaneously. We then used bootstrapped model connectivity to identify the network of cortical regions that specifically represent navigation-related information. Results show that during active navigation, navigation-related information is represented by a network of 11 distinct functional regions distributed across the visual, parietal, and prefrontal cortices. Each region represents a unique combination of features, suggesting each one mediates a different aspect of the perception-cognition-action loop that underpins active navigation. Analysis of the encoding model weights shows that functional tuning across the cortex is organized into broad gradients that extend across navigation-related regions. Our results suggest that the organizational principles underlying the representation of navigation-relation information in the cortex may be similar between humans and rodents. Furthermore, these results provide a detailed characterization of the functional network in the human cerebral cortex that mediates active, naturalistic navigation.

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Poster

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Lee Hyun Koo Brain Science Fellowship

Title: Cortical Representations of Hierarchical Goal-Directed Behavior in a Naturalistic Environment

Authors: *J. PARK^{1,2,3}, T. ZHANG⁴, J. L. GALLANT⁴, W. SHIM^{1,2,3};

¹Ctr. for Neurosci. Imaging Research, IBS, Suwon, Korea, Republic of; ²Dept. of Biomed. Engin., ³Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon, Korea, Republic of; ⁴Dept. of Neurosci., Univ. of California, Berkeley, Berkeley, CA

Abstract: Goal-directed behavior in dynamic environments requires the flexible coordination of perception, decision-making, and motor actions. To investigate how the brain supports this coordination in response to changing behavioral goals, we used fMRI to measure brain activity while participants performed a Minecraft-based “shepherding” task. The task consisted of three sequential subtasks: planning a path through a pasture, restructuring the environment to facilitate movement, and herding sheep to a target location while avoiding risks such as puddles and a fox. To explain brain activity elicited in each of the three subtasks, we first generated multiple feature spaces that reflect task-relevant information hypothesized to be represented in the brain. These included both shared feature spaces that reflect information that is likely to be represented in common across subtasks, and task-specific feature spaces that reflect information that is likely to be represented only in specific tasks. For example, the planning stage was modeled with 7 task-specific feature spaces related to future plans; the restructuring task with 3 feature spaces capturing the utility of environmental modifications; and the herding task with 9 feature spaces reflecting multi-agent dynamics. In addition, 15 shared feature spaces reflecting perceptual input, motor control, and spatial navigation were included. The voxelwise encoding model framework and banded ridge regression were used to fit these feature spaces separately to every voxel in every individual participant. Altogether, the joint encoding model comprised over 16,000 distinct features. All three subtasks engaged a distributed network of cortical regions, including visual, motor, attentional, and high-level cognitive areas. However, the planning task activated representations of spatial navigation and attention located in frontoparietal networks; the restructuring task activated representations of scene perception and elapsed time located in the retrosplenial cortex (RSC) and ventromedial prefrontal cortex (vmPFC); and the herding task activated representations of spatial relationships between agents (e.g., sheep-fox interactions) and agent-environmental risk located in the temporoparietal junction (TPJ). Taken together, our results suggest that complex tasks are not mediated by static cortical networks. Instead, the brain flexibly engages distinct functional representations optimized for each subtask, thus facilitating planning, environmental restructuring, and multi-agent coordination.

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Poster

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Title: Gaze while walking in dynamic natural environments

Authors: *N. V. POWELL¹, Y. OH², M. M. HAYHOE³;

¹Ctr. for Perceptual Systems, Univ. of Texas at Austin, Austin, TX; ²Ctr. for Perceptual Systems, Univ. of Texas at Austin, AUSTIN, TX; ³Ctr. for Perceptual Systems, Univ. Texas -Austin, Austin, TX

Abstract: In complex natural environments it is unclear how attention is directed to information needed to avoid independently moving obstacles that are largely unpredictable. While it is often assumed that attention to such objects is attracted by bottom up, stimulus-driven signals, familiarity with the probabilistic structure of natural environments might serve a more pro-active role. Little is known about how vision is used in real environments since it has been difficult to gather this kind of data. To examine visually guided behavior in such contexts we recorded gaze, head, and foot movements together with 3D scene data, while subjects walked through crowded sidewalks. Gaze was recorded using a Pupil Labs Core mobile eye tracker which was integrated with a head-mounted Intel RealSense 435i stereo camera used to extract depth relative to gaze location. Objects within the video feed of the RealSense depth camera were classified using a YoloV3 object detection algorithm. Gaze was broadly distributed across different distances, and far distances (>10m) occurred roughly 30% of the time. Average gaze distance was approximately 6 meters in both environments during fixations on pedestrians. Sharp changes in direction and speed were often observed shortly after a fixation of a pedestrian. These trajectory shifts often occurred when pedestrians were at a substantial distance from the walker. This suggests that subjects plan their movements well ahead of time. In the context of this strategy, the total time looking at pedestrians was quite short, even with high pedestrian density. Thus only short time periods of direct gaze on potential obstacles are necessary, and walkers spend most time looking in the direction of the future walking path. The effectiveness of this strategy indicates that the predictability of pedestrian behavior allows efficient use of gaze in these contexts. By remaining sensitive to the statistics of the natural world, subjects can allocate attention efficiently and minimize reliance on bottom-up mechanisms.

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Poster

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Support: National Natural Science Foundation of China, #32100839
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Title: A Unique Role for the Hippocampus in Cue Integration During Human Spatial Navigation

Authors: *Z. WEI¹, T. WOLBERS², X. CHEN¹;

¹Zhejiang Univ., Hangzhou, China; ²Aging, Cognition & Technol., German Ctr. For Neurodegenerative Dis., Magdeburg, Germany

Abstract: A central question in cognitive neuroscience is how the brain integrates distinct sensory and perceptual inputs to improve cognitive performance. This study investigated this question in the context of human spatial navigation by combining high-field 3T fMRI and desktop virtual reality. Participants encoded and retrieved spatial locations using either landmarks alone, visual self-motion cues alone (i.e., optic flow), or both cues together (combination condition). Participants behaviorally benefited from cue integration. fMRI analyses revealed a cue integration effect in the hippocampus, which displayed positional coding only in the combination condition, and only in participants who showed behavioral benefits from cue integration. Furthermore, hippocampal positional coding was more strongly associated with actual than self-reported locations, indicating it integrates sensory-perceptual inputs from spatial cues. Our findings suggest that the hippocampus's fundamental function is to integrate diverse sensory-perceptual inputs to support memory-guided navigation, rather than representing space per se, thereby challenging the dominant cognitive map theory in spatial navigation (O'Keefe & Nadel, 1978).

Significance Statement: The hippocampus is traditionally viewed as a cognitive map that represents spatial environments. However, our findings challenge this longstanding view by demonstrating that hippocampal involvement in spatial navigation lies in the integration of multiple sensory-perceptual inputs to enhance the precision of spatial representations, rather than spatial representation per se. Our findings also offers a unifying framework for understanding the hippocampal function across domains, including spatial navigation, cue integration, episodic memory, and relational memory.

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Support: Prebys Foundation
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Title: Memory and planning time influence performance on the Traveling Salesperson Problem

Authors: *R. E. BLASER, N. BUTCHER;
Univ. of San Diego, San Diego, CA

Abstract: The Traveling Salesperson Problem (TSP) is a combinatorial optimization problem in which participants seek the optimal route connecting an array of targets. As the number of targets increases, the number of possible solutions increases drastically. Humans perform extremely well on spatial versions of this task, despite its mathematical complexity, making it an appealing tool for the study of spatial cognition. Task performance in humans appears to involve both global and local visuospatial processing, with the precise contributions of each yet to be fully elucidated. We are interested in how aging and neurodegenerative disorders may differentially influence global and local spatial processing, as well as spatial memory. Previous results from our lab indicated surprisingly good performance on a version of the TSP requiring memorization of target locations, compared to a version that did not require memory. The current study was designed to test the hypothesis that planning time was responsible for this effect. We varied planning time in three versions of the TSP, one that involved a visual search (no memorization), one that allowed memorization using spatial cues, and one that required memorization with no spatial cues. Our results indicate that planning time improves performance in memorized versions of the TSP to a greater extent than in versions not requiring memory. Additionally, participants produce more efficient paths, but also produce more memory errors, when spatial cues are available compared to when they are unavailable during the memorization interval.

Disclosures: R.E. Blaser: None. N. Butcher: None.

Poster

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Title: Triple Dissociation of Information Processing in Dorsal Striatum, Ventral Striatum, and Hippocampus for Overlapping Spatial Memories During Route Learning

Authors: *T. I. BROWN, E. HERRERA;
Georgia Inst. of Technol., Atlanta, GA

Abstract: It is well established that daily life activities share overlapping memories (Brown et al., 2012, 2014). For instance, when learning our way to a new job we have to go through an artery road that branches off to the office or the grocery store. This overlap between memories can be a source of interference that can lead to errors, ending up in the grocery store when commuting to work. Previous research has shown that this conflict arises from two parallel memory systems: a more flexible goal-directed system (the hippocampus), and a more rigid system based on stimulus-response associations. However, recent work has questioned the classic notions of

striatal contributions to spatial cognition (Gahnstrom et al., 2020; Goodroe et al., 2018) and argued that it can also be involved in flexible spatial behaviors, but contributions of the different striatal subdivisions to human navigation remains elusive. To test this, we had humans navigate virtual mazes in fMRI. Critically, half of novel routes overlapped (OL) with previous familiar ones, requiring memory updating, which increases the demand of configural learning (i.e., memory integration) of the environment, whereas half of them were completely novel (NOL). We used machine learning to assess when and where the nucleus accumbens (NAcc), putamen, caudate, and hippocampus engagement dynamically change during the course of learning. We demonstrate that the hippocampus responds to task demands differently from dorsal and ventral striatum. OL task demands, specifically, drove a gradient from putamen to caudate to NAcc of increasing functional similarity with the hippocampus (whereas all striatal subdivisions differed to a similar degree from the hippocampus for NOL navigation). All three striatal subdivisions had dissociable spatiotemporal response patterns (in both OL and NOL), and among all striatal ROIs only the NAcc discriminated between OL and NOL, suggesting a unique sensitivity to the overlapping route memory demands.

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Poster

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Title: Decoding human behavior from fMRI using dynamical manifold embeddings

Authors: *A. CAMASSA¹, T. ZHANG², M. WAGNER³, J. PARK⁴, J. L. GALLANT⁵, T. J. SEJNOWSKI⁶, G. M. PAO⁷;

¹Salk Inst. for Biol. Studies, La Jolla, CA; ²Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; ³Bioengineering, Univ. of California San Diego, San Diego, CA; ⁴Biol. Nonlinear Dynamics Data Sci. Unit, Okinawa Inst. of Sci. and Technol. Grad. Univ., Okinawa, Japan; ⁵UC Berkeley, Berkeley, CA; ⁶Salk Inst., La Jolla, CA; ⁷Biol. nonlinear dynamics data science unit, Okinawa Inst. of Sci. and Technol., Kunigami district, Okinawa, Japan

Abstract: Understanding and predicting human behavior from neuroimaging data is a central challenge in neuroscience and artificial intelligence. Naturalistic tasks, such as real-world navigation, present complex, nonlinear neural dynamics that are often poorly captured by traditional linear models or deep learning approaches, especially when faced with limited and noisy datasets. To address these limitations, we introduce Manifold Dimensional Expansion with Cross-Validation (MDE-CV)—a novel, interpretable prediction framework grounded in dynamical systems theory. Based on the generalized Takens theorem and Simplex projection,

MDE-CV reconstructs latent neural dynamics directly from voxelwise fMRI time series, while integrating task-relevant feature selection with robust cross-validation. We apply MDE-CV to a naturalistic driving task, predicting acceleration, braking, and steering behaviors from human fMRI data. Unlike black-box models, MDE-CV offers interpretable, target-specific neural manifolds and identifies cortical voxels that are dynamically and causally linked to behavior. We uncover distinct functional networks associated with each behavioral output: motor-related regions for braking and acceleration, hand motor areas for steering, and visual attention areas such as the frontal eye field and intraparietal sulcus for braking. Additionally, prefrontal regions emerge as common predictors across behaviors, suggesting a broad, integrative role in naturalistic task performance. Comparative analyses reveal that while common methods like ridge regression show some predictive capacity, they lack the spatial specificity and causal interpretability of MDE-CV. Our findings underscore the power of dynamical embeddings for capturing behaviorally relevant neural processes and provide new insights into the distributed cortical networks supporting complex, real-world behaviors.

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Poster

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Title: Resting-state functional connectivity analysis of individual differences in spatial navigation

Authors: *N. KAUSHIK¹, M. HILAL¹, A. S. TU², E. R. CHRASTIL³;
²Neurobio. and Behavior, ³Neurobio. & Behavior, ¹Univ. of California, Irvine, Irvine, CA

Abstract: Individual differences in spatial navigation are linked to variations in brain structure and function in individual brain regions, yet how functional communication between regions relates to these differences remains unclear. Resting-state functional magnetic resonance imaging provides insight into intrinsic co-activation connectivity patterns and their relationship to behavior. This project examines how resting-state brain connectivity relates to navigational performance in two tasks. We analyzed resting-state fMRI data, in which participants only need to stay awake and let their minds wander without any task, using seed-based and network-based functional connectivity methods, generating correlation matrices to compare with behavioral performance metrics. In the loop closure task, participants are walked in a circular trajectory and they indicate when they think they have gone one time in the circle; accuracy is assessed via absolute angular error. In the dual solutions paradigm participants are asked to reach target

locations in a maze and can either take a well-learned route or a novel shortcut; strategy preference is quantified using the solution index, where higher values indicate greater shortcut use. We expect that functional connectivity between the parietal cortex, hippocampus, entorhinal cortex, and retrosplenial cortex will correlate with accuracy in a loop closure task, which assesses self-location tracking. Additionally, we predict that connectivity between the prefrontal cortex, posterior parietal cortex, striatum, and hippocampus will be associated with strategy selection in a dual solutions paradigm task. Preliminary analysis shows significant correlations between hippocampal/caudate nucleus/orbitofrontal cortex predicting navigation strategy in DSP; retrosplenial cortex/frontoparietal region connectivity predicting accuracy scores in Loop. Future steps will refine these results, providing deeper insight into how neural connectivity supports individual differences in navigation strategy and spatial decision-making.

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Topic: I.09. Spatial Navigation

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Title: Predicting Visuospatial Abilities from Structural MRI Using Convolutional Vision Transformers

Authors: *M. RASHIDI¹, E. R. CHRASTIL²;

²Neurobio. & Behavior, ¹Univ. of California, Irvine, Irvine, CA

Abstract: Visuospatial abilities, such as mental rotation and paper folding, rely on the capacity to mentally transform objects and perspectives. These abilities are essential for success in STEM fields and are often impaired in neurodegenerative disorders like Alzheimer's and Huntington's disease. Applied machine learning techniques have proven effective in diagnosing neurological conditions, but their use in modeling cognitive variation in healthy populations is still emerging. In this study, we aim to understand how individual differences in brain structure relate to visuospatial performance. We apply deep learning models—combining 3D convolutional neural networks (CNNs) with vision transformer (ViT) attention layers—to predict spatial task performance from T1-weighted MRI data. We collected structural MRI scans and task performance data from 112 healthy young adults across six standard visuospatial tasks, including five desktop-based VR tasks (mental rotation, paper folding, corsi blocks, road map, spatial orientation) and one immersive VR task (viewpoint transformation). Separate models were trained for each task using a hybrid CNN-ViT architecture. Our models achieved high accuracy and generalizability, with decreasing loss on both training and test sets. Using the Schaefer atlas, we identified the regions with the highest attention weights and calculated the proportion of

attended voxels within each anatomical ROI. Attention map visualizations revealed that the models consistently focused on brain regions previously associated with spatial cognition, including the precuneus, retrosplenial areas, posterior cingulate cortex, and somatomotor areas. These regions were consistent with our hypotheses and prior functional MRI findings, while also suggesting additional areas potentially involved in spatial ability. These results demonstrate the feasibility of using structural MRI and deep learning to model individual cognitive traits and to identify neuroanatomical correlates of visuospatial performance.

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Poster

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Title: Navigating Invisible Walls: The Stability and Context-Dependence of Grid-Like Signals in the Human Entorhinal and Perirhinal Cortices During Multi-Band fMRI

Authors: *O. ZEID¹, Q. HE², T. I. BROWN¹;

¹Sch. of Psychology, Georgia Inst. of Technol., Atlanta, GA; ²Col. for Health, Community and Policy, Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Navigating our surroundings competently is integral to human daily function. Conversely, impairments in spatial navigation can hinder many people's ability to live independently and their overall quality of life - creating an imperative to understand fully the neural circuits responsible for spatial awareness and traversal. In a previous single-band fMRI study, our lab has shown that BOLD activity in the Entorhinal Cortex (ERC) exhibits a "grid-like" representation with 6-fold symmetry relative to the angle traversed while navigating a virtual open field ("OF") but a representation with 4-fold symmetry when barriers were added - creating a hairpin maze ("Barr"). This demonstrated that the same neural region can represent different space morphologies with different codes. However, the question remains whether this BOLD pattern shift is due to subjects' awareness of impenetrable barriers or the altered visibility. In an ongoing study, we have collected data from 31 neurotypical young adults using whole-brain multi-band fMRI where we repeated the previous paradigm, but with the visible barriers replaced with invisible walls ("Invis"). Here, with both experimental conditions now being visually identical, during OF trials, the 6-fold symmetry was greatly reduced while the 4-fold symmetry we previously linked to the presence of barriers increased. This overall decrease in grid-like signal strength in OF trials cannot be attributed to a catastrophic reduction in the data's temporal Signal to Noise Ratio (tSNR) when compared to the previous study - which

remained well above acceptable levels. In addition to this anatomical ERC ROI analysis, we also located a functional ROI within the Perirhinal Cortex (PRC) which exhibited evidence of grid-like signal coding. In both our ERC ROI and this functional PRC ROI, we found an experience-dependent double dissociation in the expression of grid-like signals for (a) subjects that were given the OF trials before the Invis trials (here, the 4-fold signal was stronger than the 6-fold signal during OF trials) and (b) subjects given the OF trials after the Invis trials (here, a dominant 6-fold representation emerged while only the PRC's 4-fold signal weakened during OF trials.) It is clear from these results that mechanisms in these neural systems may be more context-dependent than previously hypothesized - here modulated by stimulus presentation order and neuroanatomical subdivision. In the future, this paradigm can be applied to groups with reduced navigational ability (e.g., older adults, people with anxiety disorders) to better understand how changes in neural circuitry in those groups lead to weaker grid representations.

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Center for Research and Education in Navigation

Title: Environment cues and geometry interact in shaping cognitive maps

Authors: *J. LONG¹, E. HERRERA¹, Y. LI², T. I. BROWN¹;

¹Georgia Inst. of Technol., Atlanta, GA; ²Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Environment cues are critical for human spatial navigation. Different cue types can interact to shape the cognitive maps upon which spatial memory relies. Previous research has detailed how environment cues and geometry may interact together to shape spatial memory, specifically across square and trapezoid-shaped environments. Prior literature suggests that local geometric cues can distort neural metrics which putatively support cognitive maps, reducing single-object spatial memory in trapezoid environments relative to square environments. However, this theoretical framework has not been examined relative to route-based navigation and as a function of interactions between different cues. Human participants completed an egocentric, virtual navigation task where they learned routes of six object locations in two square and two trapezoidal open-field environments. Participants' spatial memory of the object locations and sequences was then tested by navigating the same environments from a first-person and overhead perspective once again. In this testing, participants were instructed to place the objects in the learned locations and order as accurately as possible. Distance error estimation was

defined by the Euclidean distance between participants' remembered locations and true object locations. Importantly, this task contained two conditions that varied in cue availability: one version with local geometric differences and external environment cues, and one with only geometric and internal environment cues. Analyses revealed that across both conditions of cue availability: spatial memory was broadly worse in the narrow end of the trapezoid compared to both the wide end of the trapezoid and to objects in the square, consistent with the spatial metric distortion theoretical framework. However, an environment geometry effect on memory, collapsing across all objects within a route, was only observed in the absence of external orientation cues. Intriguingly, this effect opposed the metric distortion theoretical framework: in an overhead “map building” test of spatial memory, we observed facilitated spatial memory in the trapezoid compared to the square when only local geometric cues were available. These results highlight the importance of investigating complex route navigation scenarios as a function of cue availability, where geometric information encoding can have different influences on navigation outcomes.

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Poster

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Topic: I.09. Spatial Navigation

Support: CIHR #488505

Title: Spontaneous Navigation Strategies as a Predictor of Age-Related Cognitive Decline

Authors: J. TURCOTTE¹, M. IDRIS³, R. DAGENAIS⁴, V. D. BOHBOT⁵, *G. WEST²;
¹Univ. of Montreal, Montreal, QC, Canada; ²Univ. of Montreal, Montréal, QC, Canada; ³Med., McGill, Montreal, QC, Canada; ⁴Psychologie, Univ. de Montréal, Montréal, QC, Canada; ⁵Dept. of Psychiatry, McGill Univ., Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada

Abstract: Aim: In humans and rodents, the hippocampus (HPC) and caudate nuclei (CN)—part of the striatum—exhibit a reciprocal inhibitory relationship: increased reliance on one system often comes with decreased activation or structural integrity in the other. Both structures support their own memory systems that can, in navigation settings, express themselves through different strategies: the hippocampus underlies spatial (map-based) navigation, while the caudate nuclei support response-driven strategies. Further, atrophy specifically in the HPC is associated with cognitive decline. Because of this, we investigated whether the spontaneous navigation strategy used by older adults could serve as a behavioural marker of future cognitive decline.

Methods: Participants (N=93), twice (6 to 12 years apart) tested in two separate labs and aged 60 and above were administered tests of navigation strategy, a neuropsychological test battery and an MRI exam. The navigation tests, the 4-on-8 virtual maze (4/8VM) and Concurrent Spatial

Discrimination Learning Task (CSDLT), are computerized spatial memory tasks that require participant to remember and retrieve hidden objects in a radial maze. The 4/8VM selectively impairs participants who rely on spatial strategies by removing landmarks. The CSDLT disturbs response-based strategy' reliance by altering landmarks and paths. The test battery included the Montreal Cognitive Assessment (general cognition), the Rey Auditory Verbal Learning Test (RAVLT; verbal memory), the Rey-Osterrieth Complex Figure (visuospatial memory) and the Repeatable Battery for the Assessment of Neuropsychological Status (domain-specific cognition). MRI images were collected to assess changes in HPC and CN grey matter.

Results: In both cohorts, baseline response strategy users showed a significant decline ($p < 0.05$) in episodic memory (RAVLT) which was not observed in the spatial group. Further, response learners showed a significant decrease ($p < 0.01$) in HPC grey matter, but not in the CN. In contrast, the spatial learners showed a significant decrease ($p < 0.01$) in CN grey matter, not in the HPC. The loss in HPC grey matter in the response group was significantly correlated ($p < 0.05$) with decrease in episodic memory scores. The other endpoints were non-significant.

Conclusion: Elderly response strategy users exhibit episodic memory decline absent in spatial strategy users. This decline correlates with a significant loss in HPC grey matter. Only spatial learners show atrophy in CN grey matter, whilst no atrophy of HPC grey matter. As such, the results suggest that spontaneous navigation strategy may predict cognitive decline in aging.

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Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.15/TT2

Topic: I.09. Spatial Navigation

Support: NIH/NINDS 2R01NS076856

Title: Differentiating Hippocampal Contributions to Mental Simulation and Spatial Navigation in Familiar and Novel Environments

Authors: *C. PRECIADO, A. D. EKSTROM;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: The hippocampus plays a critical role in memory and spatial navigation, yet its precise function across mental simulation versus real-world action—and how this differs in familiar versus novel contexts—remains unclear. Competing frameworks, including Cognitive Map Theory (CMT) and Episodic Memory Theory (EMT), offer distinct predictions about hippocampal engagement during imagined and active navigation. This study examined how hippocampal subfields contribute to mental simulation and navigation across varying levels of environmental familiarity. Twenty-six adults (ages 18-35, 13 female) performed a virtual

navigation task during fMRI. Each participant completed 6 novel and 6 familiar blocks, each with a simulation phase followed by active navigation. Familiar routes were trained prior to scanning. Odd-even number judgments served as a baseline, and verbal recall was assessed post-scan. Imaging was acquired using a Siemens 3T Skyra (TR=2590 ms, 1.8 mm slices, 84 slices). Data were preprocessed in FSL with motion correction, slice timing correction, and skull stripping. Behavioral performance and neuroimaging contrasts were analyzed using nonparametric permutation testing (10,000 permutations) with Threshold-Free Cluster Enhancement (TFCE). Behaviorally, participants exhibited decreasing excess path over time for novel runs, consistent with spatial learning. Familiar runs were stable across blocks. Surprisingly, mental simulations of novel routes were temporally compressed relative to familiar ones, despite requiring more time for actual navigation—suggesting that imagined traversal was more abbreviated when planning unfamiliar paths. This pattern supports EMT in early runs and shifts toward CMT with increased familiarity. ROI-based analyses of hippocampal subfields (25% probability mask) are underway to assess simulation vs. navigation contrasts across familiarity conditions. Preliminary whole-brain results are being validated. Conclusion: These findings highlight dynamic shifts in hippocampal function as a function of experience. Early reliance on episodic-like simulation for novel routes gives way to more map-like representations with familiarity, offering a nuanced reconciliation of EMT and CMT.

Disclosures: C. Preciado: None. A.D. Ekstrom: None.

Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.16/TT3

Topic: I.09. Spatial Navigation

Support: NIH T32AG044402

Title: Ambulation improves memory for rotated scenes

Authors: *S. SROKOVA^{1,2}, M. WATSON³, M. GIN³, A. D. EKSTROM^{3,2};
²Evelyn F. McKnight Brain Inst., ³Psychology, ¹The Univ. of Arizona, Tucson, AZ

Abstract: In the real world, navigation and spatial memory operate through the interplay between visual, proprioceptive, and vestibular systems to give rise to multimodal spatial representations of the environment. However, numerous studies assess spatial memory abilities using desktop-based virtual reality (VR) paradigms which involve navigating virtual environments with a computer monitor, keyboard and/or a joystick. As a result, such studies rely almost entirely on visual input and omit natural body-based movement cues, providing only a partial approximation of real-world spatial memory. We examined how self-motion cues shape spatial memory across perspective changes by comparing participants' recall of object layouts after physically moving versus after display rotations without movement. We modeled our

design closely on a previous study by Simons and Wang (1998) that used a similar design except in a real-world room. We employed immersive and ambulatory VR technology which enabled us to study spatial memory in humans (N=26) as they freely navigated untethered in ecologically enriched environments. This setup also enabled us to capture additional metrics beyond simple memory accuracy, such as eye gaze data and pupillometry. Our analyses demonstrate across numerous measures that self-motion cues enhance spatial memory accuracy by shaping the temporal dynamics of decision making, pupillary responses, and eye movement behaviors. Specifically, diffusion modelling of reaction times revealed that when self-motion cues are available to support performance, participants' responding tendencies become faster, more confident, and less conservative. Additionally, the absence of self-motion signals was associated with increases in pupil size and changes in eye movement strategies which may reflect greater task difficulty. These findings suggest that incorporating real-world ambulation, such as walking to a new location within a room, can improve performance in VR-based spatial memory tasks.

Disclosures: S. Srokova: None. M. Watson: None. M. Gin: None. A.D. Ekstrom: None.

Poster

PSTR362: Human Navigation

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Program #/Poster #: PSTR362.17/TT4

Topic: I.09. Spatial Navigation

Support: NINDS Grant 5R01NS114913-05

Title: Is the human hippocampus necessary for space, time, or both?

Authors: *J. GARREN, M. GIN, M. GRILLI, A. D. EKSTROM;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: One hypothesis about the role of the hippocampus in cognition is that it is fundamental to episodic memory, particularly temporal processing. A competing hypothesis is that the hippocampus functions as the neural basis of the cognitive map, meaning that the hippocampus is more prominent in encoding spatial information. A third idea is that the hippocampus is most important for associative binding, particularly of space and time. We addressed these three hypotheses by testing amnesic patients with focal lesions to the medial temporal lobes. Participants navigated a hallway in desktop virtual reality in which objects appeared at different spatial locations and varying temporal intervals. Following encoding, participants were prompted to replace the objects in space, recreate the temporal duration of navigating prior to the object's appearance, or do both tasks for each object (referred to as "binding trials"). As a control, participants completed a sequencing block in which they navigated the hallway once and were presented with five objects all at varying locations and moments in time, with their task being to remember the sequence of objects. For spatial precision, we computed distance error in virtual meters by taking the actual object location in

Unity space and the selected location by participants and calculating the Euclidean distance. For temporal duration calculations, we took the response time and subtracted the actual onset time in seconds. For sequencing error, we computed the Kendall's Tau rank correlation coefficient. Preliminary analysis suggested that amnesiac subjects showed a tendency to overestimate distance and underestimate temporal interval to a greater extent than controls. These preliminary results suggest that the hippocampus might be necessary for both spatial and temporal coding.

Disclosures: J. Garren: None. M. Gin: None. M. Grilli: None. A.D. Ekstrom: None.

Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.18/TT5

Topic: I.09. Spatial Navigation

Support: NIH/NIMH Grant R21 MH134100
University of Arizona Sensor Lab

Title: Straight-line distance estimation during mismatched visual feedback is best predicted by Bayesian cue combination

Authors: *A. VISHWANATH¹, M. WATSON², M. GIN², Y. K. DU³, Y. HUANG³, A. D. EKSTROM², R. C. WILSON⁴;

¹The Univ. of Arizona, Tucson, AZ; ²Psychology, Univ. of Arizona, Tucson, AZ; ³Univ. of Arizona, Tucson, AZ; ⁴Univ. of Arizona, Tucson, AZ

Abstract: Navigation requires the ability to update and track one's location and heading from available multisensory information. Multisensory input comes in two prominent forms of spatial cues: body-based idiothetic cues and visual allothetic cues, usually from visual landmarks. Yet, how these two streams of information are combined together remains unresolved. We used highly controlled straight-line distance estimation ('Translation Task') and angular estimation ('Rotation Task') tasks in immersive virtual reality to investigate how idiothetic and allothetic spatial cues are integrated. In our Translation Task, participants reproduced a walked distance in the dark (path integration), with some trials involving misleading visual feedback showing a translated virtual room with an offset of up to 1.5 meters from the true distance. We used computational models to determine the effect of visual feedback offset on distance estimation behavior in participants. The three models we used were: pure path integration (ignoring the visual cue), pure landmark navigation (ignoring path integration estimate), and Bayesian cues combination (weighted cues combination based on feedback uncertainty). The model results showed that the behavior of most participants ($n = 24/30$) was best predicted by a Bayesian cue combination model that involved averaging the two spatial cues according to their perceived level of uncertainty. Our data showed considerable individual differences in the uncertainty estimates of participants which spanned almost uniformly from pure path integration to pure

landmark navigation. These findings provide evidence in favor of Bayesian cue combination strategy in distance reproduction with individual differences in navigation behavior dictated by perceived level of uncertainty. Future analysis will investigate the effects of mismatched visual feedback on distance and angular estimation with test retest reliability (tested ~1 week apart) to determine best fit strategies for Translation and Rotation behavior and their interdependence within subjects.

Disclosures: A. Vishwanath: None. M. Watson: None. M. Gin: None. Y.K. Du: None. Y. Huang: None. A.D. Ekstrom: None. R.C. Wilson: None.

Poster

PSTR362: Human Navigation

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Program #/Poster #: PSTR362.19/TT6

Topic: I.09. Spatial Navigation

Support: NIH Grant 1R21MH134100-01

Title: What happens when we cross our path

Authors: *S. HARADER¹, A. VISHWANATH³, M. GIN⁴, M. WATSON⁴, A. D. EKSTROM²; ²Psychology, ¹Univ. of Arizona, Tucson, AZ; ³Psychology, ⁴The Univ. of Arizona, Tucson, AZ

Abstract: Does crossing one's path result in greater error in path integration (tracking one's start position while moving in the absence of visual cues)? This is an important question because the location one "zeros out" on a path can greatly impact path integration accuracy. The present study addressed this issue by comparing performance on paths that crossed themselves to those that did not. Previous findings (e.g., Yamamoto et al., 2014) reported no effect of path crossing, but these may have been confounded by inadvertent variations in distance and turn angles between the crossed and uncrossed trajectories. To address this issue, the current study employed curved paths that were matched on these variables, allowing for a more complete comparison between the two conditions. To ensure participants only relied on self-motion cues, they were guided through the paths using vanishing poles in otherwise complete darkness. At the end of each path, they indicated the perceived starting location using a handheld controller. We controlled for the diameter of the loop to ensure that the position of the loop relative to the start and end location would not be a confounding factor. We have included 30 human participants thus far in our analysis, ages 18-35. Preliminary results suggested that crossing one's path led to decreased pointing accuracy compared to turns on a path that did not loop back on themselves ($t(29) = -4.6495, p < .001$). These findings suggest that crossing one's path may be a significant source of error in path integration. Additionally, these findings suggest that loop crossings and where to "zero out" are factors that should be considered in computational models of human path integration.

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Poster

PSTR362: Human Navigation

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Program #/Poster #: PSTR362.20/TT7

Topic: I.09. Spatial Navigation

Support: NIH/NINDS Grant K99NS126715
NIH/NINDS Grant R00NS126715

Title: Adaptive Neural Representations for Tracking Others in the Real World

Authors: *S. M. AHN¹, H. N. ZUBAIR², A. VENA¹, M. VALLEJO³, M. SEEBER³, U. TOPALOVIC⁵, S. HILLER³, D. ELIASHIV⁴, V. R. RAO⁶, I. FRIED⁷, J. D. ROLSTON⁸, S. D. TOBOCHNIK⁸, N. A. SUTHANA⁵, M. STANGL¹;

¹Boston Univ., Boston, MA; ²UCLA, Culver City, CA; ⁴Dept. of Neurol., ³UCLA, Los Angeles, CA; ⁵Duke Univ., Durham, NC; ⁶UCSF, San Francisco, CA; ⁷UCLA Sch. Med., Los Angeles, CA; ⁸Brigham and Women's Hosp., Newton, MA

Abstract: Spatial navigation is a complex, yet fundamental cognitive function used in everyday life. For instance, when in social environments, processing others' locations and movements with respect to oneself is required for successful navigation, such as avoiding collisions. Previous studies show examples of neural mechanisms to encode spatial information, such as place cells, grid cells, and the modulation of so-called theta oscillations. Though these mechanisms are thought to make up the brain's spatial representation system, the vast majority of these findings are limited to information about the self and not other individuals. In this study, we aimed to find evidence of neural mechanisms flexibly integrating behaviorally relevant spatial cues from another moving individual. For this, we recorded intracranial EEG (iEEG) from nine participants with chronically implanted neurostimulation systems (the NeuroPace RNS®) and depth electrodes in the medial temporal lobe (MTL) region. Participants completed a real-world navigation task to memorize certain locations in the room. Then, they were asked to observe another individual walking around the room, continuously focusing on one of three attributes: the speed of the individual, the distance to the individual, and the location of the individual. To analyze the iEEG data, we used a wavelet transform to calculate the oscillatory power of individual frequencies at each time point. From this, we found that theta oscillations (~3-8 Hz) in the MTL region were modulated by the dynamic spatial cues of another person in the room. Critically, this modulation reflected switching between different types of spatial representations as determined by the behavioral relevance of specific cues. For example, we discovered theta modulation by the relative position of the other individual when the participants were asked to monitor their location, but not when monitoring the speed or relative distance of the individual. The present results suggest that the human brain can flexibly switch between neural

representations reflecting specific attributes about another individual in a shared environment, and does so depending on task demands and behavioral relevance. These findings provide novel insights into the neural mechanisms of how humans integrate other individuals into their cognitive map of the environment.

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Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.21/TT8

Topic: I.09. Spatial Navigation

Support: NIH/NINDS Grant K99NS126715
NIH/NINDS Grant R00NS126715

Title: Theta-gamma coupling in the medial temporal lobe supports memory retrieval during real-world navigation

Authors: ***J. GONZALEZ-ASTUDILLO**¹, **D. JIANG**¹, **H. N. ZUBAIR**², **U. TOPALOVIC**⁴, **S. HILLER**³, **C. S. INMAN**⁵, **V. R. RAO**⁶, **C. HALPERN**⁷, **D. ELIASHIV**⁸, **M. ABDENNADHER**⁹, **P. ROHATGI**¹⁰, **J. W. HOLSAAPPLE**¹⁰, **I. FRIED**¹¹, **S. TOBOCHNIK**¹², **J. D. ROLSTON**¹², **N. A. SUTHANA**⁴, **M. STANGL**¹;

¹Dept. of Biomed. Engin., Boston Univ., Boston, MA; ²UCLA, Culver City, CA, ; ³UCLA, Los Angeles, CA, ; ⁴Duke Univ., Durham, NC, ; ⁵Univ. of Utah, Salt Lake City, UT, ; ⁶Univ. of California, San Francisco, San Francisco, CA, ; ⁷Univ. of Pennsylvania, Philadelphia, PA; ⁸Univ. of California Los Angeles, Los Angeles, CA; ⁹Dept. of Neurol., ¹⁰Dept. of Neurosurg., Boston Med. Center, Boston Univ. Chobanian & Avedisian Sch. of Med., Boston, MA; ¹¹DEPT NEUROSURGERY, UCLA Sch. Med., Los Angeles, CA, ; ¹²Brigham & Women's Hosp., Harvard Med. Sch., Boston, MA

Abstract: Spatial navigation and memory retrieval are fundamental cognitive functions supported by distinct neural oscillations within the medial temporal lobe (MTL), including low-frequency “theta” oscillations (2-8 Hz) and high-frequency “gamma” oscillations (30-90 Hz). These rhythms interact via phase-amplitude coupling (PAC), where the phase of slower oscillations modulates the amplitude of faster ones. Although theta-gamma coupling has been implicated in memory retrieval during stationary or virtual tasks, its role in real-world navigation remains unclear. To address this, we recorded intracranial EEG (iEEG) from five freely moving participants (ages range [31-52]; two women) with chronically implanted neurostimulation

systems (NeuroPace RNS System) and depth electrodes in MTL regions. Participants completed a real-world navigation task in which they alternated between two distinct conditions: (i) memory-guided navigation, where they walked to previously-learned invisible target locations, and (ii) visually-guided navigation, where they walked towards visible wall-mounted signs. PAC strength was quantified for each condition using the Modulation Index (MI), which involves estimating the average gamma amplitude within each theta phase bin and assessing whether the normalized gamma distribution significantly deviates from uniformity, thereby indicating PAC. This analysis revealed that memory-guided navigation was associated with a pronounced increase in PAC between low-theta (1-3 Hz) and low-gamma (35-45 Hz) oscillations in the MTL, compared to low or absent PAC during visually-guided navigation. Statistical significance was assessed via a non-parametric surrogate-based method (500 phase shuffles), considering effects significant at $p < 0.05$ and false discovery rate corrected for multiple frequency comparisons. These findings demonstrate that theta-gamma coupling in the human MTL is selectively enhanced during memory-based navigation in naturalistic settings, bridging the gap between laboratory-based studies and real-world cognition.

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Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR362.22/TT9

Topic: I.09. Spatial Navigation

Support: NIMH R61MH135106
NINDS U01NS117838
NIMH F30MH138086
David Geffen Scholarship
NIGMS T32GM008042

Title: Medial Temporal Lobe Oscillations Reflect Approach-Avoidance Conflict during Naturalistic Decision-Making in Freely Moving Humans

Authors: *H. N. ZUBAIR^{1,2}, M. SEEGER³, D. BATISTA⁷, U. TOPALOVIC⁷, S. HILLER⁴, R. MUSTAPHA⁴, M. VALLEJO⁷, C. ORAGWAM⁷, M. S. LARSON⁴, J. BAHAM⁴, M. STANGL⁹, A. ADHIKARI⁵, B. J. KNOWLTON⁵, A. BARI², V. RAO¹⁰, J.-P. LANGEVIN², I. FRIED², D. ELIASHIV⁶, N. A. SUTHANA⁸;

²Neurosurg., ³Semel Inst. for Neurosci. and Human Behavior, ⁴Psychiatry and Biobehavioral Sci., ⁵Psychology, ⁶Neurol., ¹UCLA, Los Angeles, CA; ⁷Neurosurg., ⁸Neurosurg. / Biomed.

Engin., Duke Univ., Durham, NC; ⁹Biomed. Engin., Boston Univ., Boston, MA; ¹⁰Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Real-world decision-making often involves simultaneously pursuing rewards and avoiding threats, an adaptive balance essential for daily tasks such as driving, navigating crowds, or playing sports. Disruptions to this balance, as seen in anxiety and post-traumatic stress disorder, can impair goal-directed behavior despite intact perception and motor function. To investigate the neural mechanisms underlying such behaviors, we studied decision-making under threat and reward uncertainty in freely moving humans. Using a naturalistic, augmented reality platform, we integrated motion capture, eye tracking, and intracranial EEG recordings from the hippocampus and amygdala. Nine participants with treatment-resistant epilepsy and implanted with Responsive Neurostimulation (RNS®) devices navigated a physical arena to collect target objects for monetary gain while avoiding a mobile experimenter who imposed monetary penalties if participants came within 1.5 meters. Trial types varied in reward-to-risk ratios to induce different levels of approach-avoidance conflict, with “High-conflict” trials defined by intermediate ratios. Behavioral results revealed systematic changes in target acquisition rates, collision frequency, trajectory detours, and spatial hesitation across trial types. Neural analyses showed that in the hippocampus, low-frequency (2-4 Hz) band power increased during “High-conflict” trials, while gamma (40-80 Hz) band power was elevated in zero-risk trials. In contrast, the amygdala exhibited low-frequency power during trials with the highest penalty risk. These findings reveal distinct roles of hippocampal and amygdalar oscillations in encoding context and conflict during real-world decision-making. This approach opens new avenues for developing naturalistic, biomarker-driven neuromodulation strategies for anxiety and affective disorders.

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Poster

PSTR362: Human Navigation

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.23/TT10

Topic: I.09. Spatial Navigation

Support: NIH U01NS117838

Title: Human hippocampal theta-informed scalp EEG signatures during real and imagined navigation

Authors: *M. SEEBER¹, M. STANGL³, M. VALLEJO¹, U. TOPALOVIC⁴, S. HILLER¹, C. H. HALPERN⁵, J.-P. LANGEVIN¹, V. R. RAO⁶, I. FRIED⁷, D. ELIASHIV¹, N. A. SUTHANA²;

²Neurosurg. / Psychiatry / Bioengineering, ¹UCLA, Los Angeles, CA; ³Biomed. Engin., Boston Univ., Boston, MA; ⁴Duke Univ., Durham, NC; ⁵Stanford Univ., Stanford, CA; ⁶Neurol., Univ. of California, San Francisco, San Francisco, CA; ⁷DEPT NEUROSURGERY, UCLA Sch. Med., Los Angeles, CA

Abstract: Hippocampal theta oscillations play a key role in spatial navigation and memory. In a previous real-world experiment using ambulatory neural recordings, we showed that theta power in the human hippocampus increases prior to key navigational instances such as turns and is reinstated during mental recollection of previously learned routes [1]. Here, we examine the scalp-level correlates of hippocampal theta oscillations (4-9 Hz) using simultaneous high-density scalp EEG (64 channels) and intracranial EEG (iEEG) recordings from the chronically implanted NeuroPace RNS system in epilepsy patients as they performed both real-world and imagined navigation tasks. We found increased hippocampal theta power associated with elevated global field power (GFP) across scalp EEG channels ($t=9.50$, $p<0.001$, mixed effect model). Transient hippocampal oscillatory theta bouts also coincided with increases in scalp GFP ($t=4.34$, $p<0.001$), suggesting that hippocampal theta is reflected in large-scale scalp signals. We further investigated the scalp EEG subspace that co-varied with intracranial signals. This approach aimed to constrain scalp EEG analyses while suppressing motion and muscular contamination of the scalp recordings in the real-world setup. When examining scalp topographies that span this functional subspace, we observed clear lateralization specific to the hippocampal electrode that informed the scalp EEG decomposition. Applying EEG source imaging to these topographies revealed that the reconstructed sources overlapped with the actual implantation site of the iEEG electrodes. In addition to these deep sources within the intracranial-informed subspace, we observed theta dynamics in the medial prefrontal cortex (PFC) during navigation and imagination. Theta power in the PFC was position-dependent and appeared consistently across navigational trials. However, these theta dynamics were moderately correlated with the hippocampal activities, showing more regularities within navigational segments. Therefore, scalp and intracranial EEG contain common and complementary information that, when combined, provide a more complete perspective of neural correlates during naturalistic tasks. These findings open new avenues for investigating subcortical-cortical interactions underlying naturalistic cognitive behaviors. Reference: [1] Seeber M, Stangl M, Vallejo M, Topalovic U, Hiller S, Halpern CH, Langevin JP, Rao VR, Fried I, Eliashiv D, Suthana N. (2025) Human neural dynamics of real-world and imagined navigation. *Nature Human Behaviour*. 9(4), 781-793

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Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.24/TT11

Topic: I.09. Spatial Navigation

Title: Phase-locked spatial coding in non-place cells

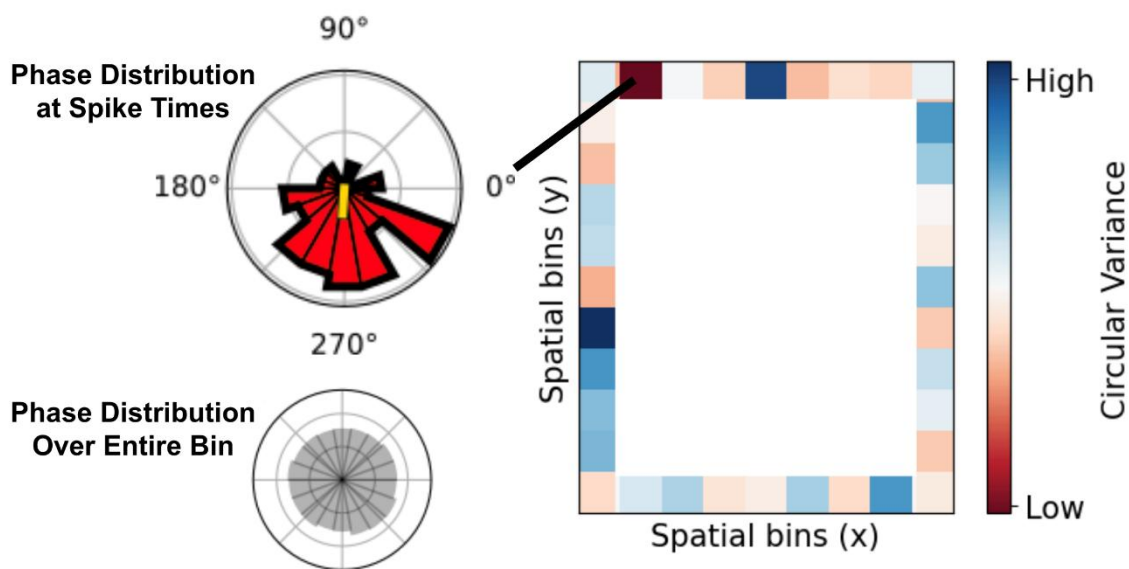
Authors: *L. D. KOLIBIUS¹, M. S. HERMILLER⁴, C. HOLMAN², M. F. KHAZALI⁵, M. J. KAHANA⁶, J. JACOBS³;

¹Columbia Univ., New York City, NY; ³Dept. of Biomed. Engin., ²Columbia Univ., New York, NY; ⁴Psychology, Florida State Univ., Tallahassee, FL; ⁵Neurozentrum, Freiburg Univ., Freiburg, Germany; ⁶Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Spatial navigation relies on hippocampal neurons that encode information through both firing rates and spike timing. Theta oscillations, a rhythmic activity in the 3-12 Hz range, are thought to organize this neural activity. One proposed mechanism is phase locking, where neurons fire preferentially at a particular phase of the theta rhythm depending on behavioral or spatial context.

In this study we investigated spatial memory encoding in the hippocampus of patients with refractory epilepsy using a virtual navigation task. Participants memorized six item locations in a rectangular arena. We concurrently recorded single-neuron activity. Traditional hippocampal spatial coding emphasizes place cells with elevated firing rates in specific fields. We explored the contribution of weaker-tuned neurons to spatial coding, focusing on spiking phases relative to theta oscillations.

Thirty of 64 non-place neurons fired at consistent theta phases when the participant occupied specific spatial locations, despite lacking strong rate modulation ($p = 0.012$; second-order permutation test). This discovery challenges the notion that spatial information is restricted to strongly rate-modulated neurons. Instead, it suggests that spatial information is encoded in spike timing, complementing rate-based coding. These results expand our view of hippocampal navigation and memory signals and may generalize to other cognitive domains.



Example phase-consistent neuron. A 2-D variance map shows a red hotspot on the top edge; spike phases there cluster at 270° with a long mean vector, whereas background theta phases are uniform, confirming neuron-specific phase locking.

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Poster

PSTR362: Human Navigation

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Program #/Poster #: PSTR362.25/TT12

Topic: I.05. Working Memory

Support: 5U01MH130447

Title: Activity in the striatum is modulated by continuous theta-burst stimulation to dlPFC during working memory

Authors: *D. F. GREGORY¹, I. SUN², M. PATEL³, H. GURA⁴, S. CHANDRASHEKAR¹, A. CASALVERA¹, M. TEFERI¹, N. L. BALDERSTON⁵;

¹Univ. of Pennsylvania, Philadelphia, PA; ²Univ. of Pennsylvania, Pennsylvania, PA; ³Univ. of Pennsylvania Dept. of Psychiatry, Philadelphia, PA; ⁴Univ. of Pennsylvania Neurosci. Grad. Group, Philadelphia, PA; ⁵Psychiatry, Univ. of Pennsylvania Department of Psychiatry, Philadelphia, PA

Abstract: Causal neuromodulation studies examining the effect of prefrontal stimulation on working memory are limited by a lack of individualized targeting and repeated-session designs. Further, disorders related to PTSD may benefit greatly in clinical targeted therapy and outcomes identifying stimulation sites related to cognitive deficits. Here, we present preliminary findings from an ongoing within-subjects, randomized trial assessing the neural consequences of intermittent (iTBS) and continuous (cTBS) theta-burst stimulation to the left dorsolateral prefrontal cortex (dlPFC). 100 participants (target n=200) completed six lab visits over two weeks, including MRI acquisition for targeting (T1, T2, resting state, and Sternberg working memory task), three TMS sessions, and three follow-up fMRI scans 24 hours post-stimulation. Stimulation was guided by individualized left dlPFC activity during the retention phase of a Sternberg task and optimized using SimNIBS-derived e-field modeling. Participants complete both “maintain” and “sort” conditions in the task and view a briefly presented letter series, the across retention interval respond to a probe question of a letter with a numbered location. In the sort condition, participants mentally alphabetize the letters during the delay. We conducted a whole brain voxelwise ANOVA comparing retention interval activity before and after stimulation for cTBS and iTBS and used cluster-based permutation testing to correct for multiple comparisons. A linear mixed-effects model revealed a significant interaction between stimulation group condition (iTBS vs. cTBS) and session, $\beta = -0.048$, $SE = 0.011$, $t(259) = -4.31$, $p < .001$, $d = 0.35$, 95% CI [0.28, 0.43]. Follow-up paired t-tests comparing activation between session 1 and session 2 within each stimulation group (activation during the retention interval from pre- to post-stimulation) found clusters showed deactivation during the retention interval that significantly attenuated post-stimulation in the cTBS group, $t(39) = -4.64$, $p < .001$, $d = 0.73$,

95% CI [-0.19, -0.07]. In contrast, the iTBS group showed a nonsignificant change, $t(46) = -1.81$, $p = .077$, $d = 0.26$, 95% CI [-0.10, 0.01]. These early results suggest that repeated cTBS to left dlPFC enhances striatal engagement during working memory maintenance of retention interval. These findings raise the possibility that cTBS modulates corticostriatal dynamics during working memory retention, potentially through disruption of top-down control mechanisms, with implications for targeting cognitive deficits in PTSD. Full analyses of behavioral performance and connectivity will be presented within the poster.

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Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.26/TT13

Topic: I.05. Working Memory

Support: DFG Grant SPP 2411
Machine Learning in Science, Excellence cluster Machine Learning,
Tuebingen

Title: Gamma-theta-spike interactions coordinate sequence representation in human MTL

Authors: *M. PRAKASH¹, J. NIEDIEK^{2,3}, R. SURGES³, F. MORMANN⁴, S. LIEBE^{1,5};
¹Univ. of Tuebingen, Tuebingen, Germany; ²Machine Learning Group, Technische Univ. Berlin, Berlin, Germany; ³Univ. Bonn Med. Ctr., Bonn, Germany; ⁴Univ. Clin. of Bonn, Bonn, Germany; ⁵Machine Learning in Sci., Excellence cluster Machine Learning, Tuebingen, Germany

Abstract: The medial temporal lobe (MTL) is essential for remembering the temporal order of events, yet the precise neural mechanisms supporting sequential memory in humans remain unclear. Theoretical models have proposed that hierarchical coupling between theta and gamma oscillations organizes neural activity to temporally structure information, but direct evidence linking these dynamics to single-unit activity during memory tasks has been lacking, specifically for gamma oscillations. To address this, we analyzed simultaneous microwire recordings of single-unit activity (SUA, N = 1417 units) and local field potentials (LFPs, N = 917 electrodes) from the MTL of epilepsy patients performing a sequence memory task. During encoding, gamma power represents stimulus information and is temporally linked to spike timing. Importantly, elevated firing rates similarly coincide with stimulus-specific gamma bursts during memory maintenance. Both gamma and SUA preferentially align to similar theta phases and exhibit theta-phase coding of sequence position. These findings demonstrate that theta-gamma-spike interactions support the encoding and maintenance of sequential information in the human

MTL, potentially constituting a general neural mechanism operating across memory systems and timescales to organize temporal information.

Disclosures: M. Prakash: None. J. Niediek: None. R. Surges: None. F. Mormann: None. S. Liebe: None.

Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.27/TT14

Topic: I.05. Working Memory

Support: R01 MH129042

Title: Flexible replanning dissociates internal and external attention for distraction-resilient behaviors in dynamic environments

Authors: *Z. ZHANG, J. A. LEWIS-PEACOCK;
Univ. of Texas, Austin, Austin, TX

Abstract: Navigating daily tasks requires working memory (WM) to dynamically allocate limited resources among competing goals based on agent states (e.g., prioritizing shopping items by proximity). In real-world scenarios, agent states are dynamic and are often disrupted by distractions such as promotions for new products. How people adapt to such perturbations remains unclear, as classical studies typically fix agent states. To investigate this, we developed a WM task (N=25) inspired by the arcade game Snake, where participants navigated an agent to collect memorized targets (apples) while EEG data and eye-tracking were recorded. On each trial, participants encoded 1 or 2 targets, maintained them during a delay period, then navigated a virtual snake to collect them. Crucially, in half of the trials, a distraction task appeared that forced the search for new special targets, resulting in the updates of the snake's position. Replicating our prior findings, participants flexibly prioritized targets nearest to the updated agent position rather than sticking to their original action plans. We tested whether these plan updates relied on dynamic object-vector representations (i.e., continuously updated agent-target representations) or static target coordinates. Representational similarity analysis of EEG data revealed dominant object-vector coding. Surprisingly, when two targets needed to be collected, the ultimate (further) target was more strongly represented in the EEG data than the immediate (closer) one, despite behavioral prioritization of closer targets. Eye-tracking results resolved this paradox, showing external attentional bias toward closer targets during the delay phase. This dissociation reveals a novel division of labor in multi-step planning: external attention prioritizes immediate subgoals, while internal attention in WM maintains stronger representations of future goals. Our findings demonstrate how dynamic neural coding and attentional mechanisms coordinate to support adaptive behavior in environments with complex and unpredictable demands.

Disclosures: Z. Zhang: None. J.A. Lewis-Peacock: None.

Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR362.28/TT15

Topic: I.05. Working Memory

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STI2030-Major Projects, No.2021ZD0203700/2021ZD0203705

Title: Attractor Dynamics of Working Memory Explain a Concurrent Evolution of Stimulus-specific and Decision-consistent Biases in Visual Estimation

Authors: *S.-H. LEE¹, S. LIM²;

¹Seoul Natl. Univ., Seoul, Korea, Republic of; ²Neural Sci., New York Univ. Shanghai, Shanghai city, China

Abstract: Sensory evidence is often fleeting and frequently unavailable when we need it to categorize or estimate the state of the world. Our brains utilize working memory to maintain representations of sensory information necessary for perceptual tasks. While it is crucial to keep these representations accurate during action, humans exhibit two primary biases: estimates are biased toward a few fixed stimuli (“stimulus-specific bias”) and prior decisions (“decision-consistent bias”). Integrative—particularly neural mechanistic—accounts of these biases are still limited. In this study, we identify drifting dynamics toward discrete attractors as a shared source of both biases in orientation estimation, with decisions further guiding memory states toward these attractors. Behavioral and neuroimaging data demonstrate how these biases evolve over time through the decision-steered attractor dynamics. Task-optimized recurrent neural networks indicate possible neural mechanisms that allow categorical decisions to arise from working memory for continuous stimuli while updating their trajectory, modulating decision-consistent biases under stimulus-specific drift.

Disclosures: S. Lee: None. S. Lim: None.

Poster

PSTR362: Human Navigation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR362.29/TT16

Topic: I.09. Spatial Navigation

Support: SNF Grant 320030- 182589
SNF Grant P1GEP3_184161

Title: Interactive effects of acute physical exercise and genetic risk for Alzheimer's disease on declarative and spatial memory

Authors: *K. IGLOI^{1,2}, B. MARIN BOSCH^{4,5}, C. F. DOELLER⁶, S. SCHWARTZ³;
¹Univ. of Geneva, Geneva, Switzerland; ³Neurosci., ²Univ. Hosp. of Geneva, Geneva, Switzerland; ⁴Fondation Campus Biotech Geneva, Genève, Switzerland; ⁵Fondation Campus Biotech Geneva, Geneva, Switzerland; ⁶Dept. of Psychology, Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: The $\epsilon 4$ allele of the *APOE* gene increases the risk of late-onset Alzheimer's disease and may be associated with early cognitive and neural specificities. Given the memory-enhancing effects of physical exercise—particularly through hippocampal plasticity mediated by endocannabinoid signalling—we investigated whether a single bout of exercise benefits memory and underlying neurophysiology in young $\epsilon 4$ carriers ($\epsilon 3/\epsilon 4$, risk group) compared to $\epsilon 3$ homozygotes (control group). Participants underwent fMRI during an associative memory task, followed by either moderate cycling or rest, and then completed two tasks: (i) associative memory recall and (ii) spatial memory formation involving grid-cell-like representations. Blood samples were collected to assess endocannabinoid levels. Behaviourally, the risk group showed reduced associative memory retention regardless of condition. Neuroimaging revealed elevated medial temporal lobe activity during retrieval in $\epsilon 4$ carriers across conditions, suggesting compensatory activation, while similar increases in the control group emerged only after exercise. In controls, exercise-induced increase in endocannabinoids correlated with hippocampal activation, a relationship that was absent in the risk group. For spatial memory, impaired grid-cell-like representations were observed in the risk group (replicating previous results) and were partially restored following exercise. These findings reveal that alterations in neural plasticity mechanisms affect memory performance in young, healthy $\epsilon 4$ carriers, and can be mitigated, in part, by acute physical exercise.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.01/TT17

Topic: I.10. Human Learning and Cognition

Title: Auditory processing fidelity, auditory learning, and behavioral rigidity in misophonia

Authors: *D. MAHMOUD¹, J. E. NORRIS¹, S. H. KIMBALL², B. ARLEDGE¹, L. ETHRIDGE¹;

¹Psychology, Univ. of Oklahoma, Norman, OK; ²Communication Sci. and Disorders, Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Misophonia is categorized by strong, negative reactions to idiosyncratic auditory stimuli called trigger sounds, which may significantly impact daily functioning. Research on cognition in misophonia is limited, though it shares characteristics with conditions with impacted cognition and perseverative behaviors (e.g. ASD, OCD). Given overlapping condition phenotypes, perseveration may also occur in misophonia. Perseveration has been minimally studied in misophonia and is typically assessed using the Wisconsin Card Sorting Test, a visual task. The current study tested perseveration and behavioral rigidity through an auditory learning task to target the sensory domain implicated in misophonia. Sixty-seven adult participants ($M\ age = 18.96$, $SD = 1.12$; 89% female; 32% with misophonia) completed a survey on severity of misophonia, an audiology workup, and behavioral tasks during dense-array EEG to correlate behavioral outcomes with neural markers of auditory sensory fidelity. Perseveration was quantified via an auditory learning task using auditory steady-state response (ASSR) with modulation frequency, carrier tone, and volume categories. Lag distribution analysis and perseveration probability analysis were used to examine perseveration. Inter-trial phase coherence (ITC) was calculated for ASSR to assess stimulus processing fidelity. Interestingly, lag distribution area under the curve (AUC) was positively correlated with 40 Hz ITC, $r(43) = 0.34$, $p = 0.03$. Trials to criterion for both 40 Hz and 80 Hz ASSR with 1000 Hz carrier tone were negatively correlated with perseveration probability AUC, $r(43) = -0.39$, $p = 0.01$, $r(43) = -0.35$, $p = 0.02$, respectively. Few participants met criteria for significant perseverative behavior, suggesting these correlations indicate that in the absence of significant perseveration, some patterned or repetitive behavior is beneficial to auditory learning. Misophonia participants did not differ from controls in behavioral measures of perseveration, however for misophonia only, 40 Hz ITC was negatively correlated with 40 Hz trials to criterion $r(18) = -0.48$, $p = 0.04$, suggesting that stimulus fidelity variations are more relevant to behavioral decision making for this group. These findings suggest that despite reports of cognitive impacts of auditory trigger exposure, auditory learning in general and related cognitive behaviors are not impacted in misophonia, however cognition in individuals with misophonia may be more sensitive to impacts of stimulus processing fidelity. Future studies in misophonia should explore impacts of trigger exposure on auditory neural processing and cognition.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.02/Web Only

Topic: I.10. Human Learning and Cognition

Title: Reinforcement-based learning styles as an effect of habitual video game practice

Authors: *A. QUEVEDO-CALDERON¹, S. QUIÑONES BELTRAN¹, A. Q. ANGULO-CHAVIRA², L. LLAMAS-ALONSO¹;

¹Univ. Autónoma de Baja California, Ensenada, Mexico; ²Univ. Nacional Autónoma de México, Ciudad de México, Mexico

Abstract: Habitual video game practice has been associated with anatomical and functional changes in the mesolimbic cortical system, whose dopaminergic activation supports reinforcement, motivation, and learning consolidation. This system allows organisms to associate actions with rewarding or aversive consequences, promoting adaptive behavior. Since video games rely on goals, immediate feedback, and reward structures, this study aimed to determine whether regular gaming influences reinforcement- or punishment-based learning patterns. Learning styles were assessed in video gamers (VG, n=36) and non-gamers (NVG, n=36) using a probabilistic paradigm across three phases: association with feedback, test without feedback, and a transfer phase with randomized stimuli. Group comparisons included the number of attempts, reward-based learning, punishment-based learning, and overall accuracy. No significant differences were found in reward accuracy ($t = -1.40$, $p = .16$) or punishment accuracy ($t = -1.61$, $p = .11$). However, video game players (VGs) performed better overall ($t = -2.26$, $p = .02$) and required fewer attempts ($t = 1.96$, $p = .05$), suggesting an enhanced ability to adjust behavior based on outcome history. While no specific bias toward reward or punishment was detected, results suggest that video game practice enhances general behavioral adaptation mechanisms, likely mediated by dopaminergic modulation of decision-making and error monitoring. The findings support the potential of video games as cognitive tools in educational and clinical settings to strengthen reinforcement-based learning and strategic behavior.

Disclosures: A. Quevedo-Calderon: None. S. Quiñones Beltran: None. A.Q. Angulo-Chavira: None. L. Llamas-Alonso: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.03/TT18

Topic: I.10. Human Learning and Cognition

Support: Innovative Science and Technology Initiative for Security Grant Number JPJ004596, ATLA, Japan

Title: Confidence about rules measured in a novel behavioral task and decoded from simultaneous EEG-fMRI

Authors: ***T. KURODA**¹, T. OGAWA², M. TSUTSUMI¹, T. KISHI¹, M. KAWANABE¹;
¹Advanced Telecommunications Res. Inst. Intl., Seika-cho Soraku-gun, Kyoto, Japan; ²ATR,
Advanced Telecommunications Res. Inst. Intl., Seika-cho, Soraku-gun, Japan

Abstract: In our project, we are developing a machine-learning model that decodes confidence from brain activities. To this end, we developed a novel behavioral task for measuring confidence about rules in an ambiguous situation and conducted simultaneous EEG-fMRI recordings during the task. In this task, each trial consisted of rule-, confidence-, and fruit-choice components in that order. In the rule-choice component, three icons appeared on a computer monitor, each representing a feature of “pacman” stimulus. These features included the color (red/green), line direction (vertical/horizontal), and mouth direction (left/right) of the pacman. This component ended upon selecting two of the three icons. Then two buttons appeared in the confidence-choice component, each representing high or low confidence about a combination of the two selected features. Participants selected one of the confidence buttons. Finally, a pacman and two kinds of fruits (e.g., apple and orange) appeared in the fruit-choice component. This pacman only had the two selected features, missing the third unselected feature. For instance, the pacman could be red and have a mouth on the left side without lines if icons for the color and mouth direction were selected. Participants had to answer which of the fruits the pacman preferred based on its appearance. If correct, they gained some points; otherwise, they lost some points. The fruit preference was determined only by two of the three features. Importantly, participants could gain points as long as the fruit choice was correct. Nonetheless, it was difficult for them to keep making correct answers if an irrelevant feature was selected. Moreover, an amount of point gain/loss depended on the level of confidence. A correct fruit choice after selecting a high- and low-confidence button resulted in +30 and +10 points, respectively. An incorrect choice after selecting those respective buttons resulted in -40 and -15 points. The rule being in effect was never presented and sometimes switched, thereby requiring re-learning. Most participants successfully learned the task in a screening test and 21 of them (10 males) in their 20s to 40s proceeded to simultaneous EEG-fMRI. An analysis of their behavioral data revealed that an increase in fruit-choice accuracy almost always preceded an increase in the level of confidence, suggesting that confidence be an intervening variable rather than a hypothetical construct explaining behavior. Decoding of the confidence level from EEG and fMRI each reached approximately 65% accuracy with a leave-one-subject-out validation. Abbreviations: EEG (electroencephalography); fMRI (functional MRI)

Disclosures: **T. Kuroda:** None. **T. Ogawa:** None. **M. Tsutsumi:** None. **T. Kishi:** None. **M. Kawanabe:** None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.04/UU1

Topic: I.10. Human Learning and Cognition

Title: Reduced conjunctive reinforcement learning in older adults

Authors: *D. OPPERMAN PEIXOTO¹, I. C. BALLARD²;

¹Univ. of California, Riverside, Riverside, CA; ²Psychology Dept., UC Riverside, Riverside, CA

Abstract: Tracking conjunctions of features is necessary for adaptive decision making. For example, although cinnamon is unpleasant on its own, when combined with rice pudding, it becomes part of a delicious dish. The hippocampus supports the formation of such conjunctions, and is required for their implementation in reinforcement learning. Age-related deficits in reinforcement learning have been largely attributed to striatal dysfunction. Since hippocampal loss of function accompanies healthy aging, we hypothesized that such decline leads to reduced conjunction-based reinforcement learning in old age. We tested younger (n=194) and older (n=196) male and female adults on a speeded target detection task in which four stimulus configurations differentially predicted the appearance of a target (70% for target-positive stimuli AB+ and B+ and 30% for target-negative stimuli AC- and B-). Because each feature alone (A, B, C) was not target-predictive, forming conjunctions (e.g., “AB”) was necessary to maximize task performance. We fit reinforcement learning models that capture conjunction-based learning and feature-based learning to reaction time and hit rate data. Older adults showed impaired conjunction-based learning; however, they demonstrated spared feature-based learning. This finding is consistent with a model in which age-related changes in hippocampal function reduces the ability to learn about conjunctive, but not individual feature representations. The differences in conjunctive learning were not attributed to specific deficits in the use of learned values to guide behavior, as both conjunctive and feature values were worse predictors of reaction times and hit rates in older adults. Collectively, these results suggest the presence of signatures of age-related differences in reinforcement learning that are linked to changes in the hippocampal learning system. Impairments in forming conjunctive representations could give rise to a profile of behavioral differences in reinforcement learning, memory and decision making in aging.

Disclosures: D. Oppermann Peixoto: None. I.C. Ballard: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.05/UU2

Topic: I.10. Human Learning and Cognition

Title: Machine and Human Recognition of Degraded Speech: a Dynamic Learning Perspective

Authors: *W. SUN, N. DING;

Zhejiang Univ., Hangzhou, Zhejiang, China

Abstract: How to reliably recognize speech despite various acoustic degradation is a primary challenge for automatic speech recognition (ASR). It is commonly believed that the human

speech recognition system is highly robust to acoustic degradation, and ASR systems should also have this property. Here, we challenge this view by showing that humans are highly sensitive to unfamiliar acoustic degradation, but they can achieve robust speech recognition through rapid adaptation. When first encountering a sentence that is subject to unfamiliar acoustic degradation, human speech recognition tends to be less robust than current ASR systems. Nevertheless, humans can quickly learn to recognize degraded speech, even without any supervision signal. After exposure to 10 degraded sentences, the human speech recognition rate can boost by 50% in some conditions. When the ASR systems are adapted to degraded speech through simple supervised and self-supervised procedures, they could achieve human-level speed of learning in most conditions. These results show that humans achieve robust speech recognition through quick adaptation, and continual adaptation is a plausible strategy to build more robust ASR systems.

Disclosures: W. Sun: None. N. Ding: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.06/UU3

Topic: I.10. Human Learning and Cognition

Support: NSTC 113-2314-B-002 -273 -MY3

Title: Reward learning impairment from punishment-associated exploration depends on dopaminergic signaling

Authors: *M.-T. TSENG¹, W. LIN²;

¹Natl. Taiwan Univ., Taipei, Taiwan; ²Grad. Inst. of Brain and Mind Sci., Grad. Inst. of Brain and Mind Sci., Zhongzheng Dist., Taipei City, Taiwan

Abstract: Effective reinforcement learning requires balancing exploration of uncertain options with exploitation of known outcomes. In real-world contexts, the same action may yield rewards in some situations and punishments in others, yet how these learning processes influence each other remains unclear. Here, we investigated the neurobiological mechanisms underlying how reward and punishment learning interact to guide adaptive behavior through functional magnetic resonance imaging (fMRI), computational modeling, and pharmacological manipulation across two experiments (N = 68). In the first experiment (N = 30), healthy participants engaged in an instrumental learning task with binary choices. Some options yielded only reward or punishment outcomes, while others were associated with both, allowing direct assessment of their interaction. Results showed that when both reward and punishment learning were concurrently engaged, the performance of reward learning was selectively impaired, while punishment learning remained intact. Computational modeling revealed that this impairment was predicted

by increased exploration linked to prior punishment associations in reward-learning choices, accompanied by enhanced blood oxygen level-dependent responses in prefrontal regions related to exploration behavior. In the second experiment ($N = 38$), we used a double-blind, between-subject design to investigate whether dopaminergic signaling mediates this interference. We found that pharmacological blockade of dopamine D2/3 receptor with amisulpride eliminated both the impairment in reward learning and the accompanying increase in exploration. These findings demonstrate that reward learning can be disrupted by punishment-driven exploration in a dopamine-dependent manner, offering a mechanistic account of how the brain resolves competing value signals and insights into dopamine-related learning disturbances in neuropsychiatric conditions.

Disclosures: M. Tseng: None. W. Lin: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.07/Web Only

Topic: I.10. Human Learning and Cognition

Title: Integrating Evaluation of Metacognitive Confidence May Improve Feedback Effectiveness of Intelligent Tutor Systems for ADHD Learners

Authors: *A. GEANA;
Providence Col., Providence, RI

Abstract: A crucial topic at the intersection of neuroscience, clinical practice, and education, ADHD impacts several aspects of learning, particularly learning from feedback. Individuals with ADHD are less able to detect errors in their work, less likely to adjust behavior after feedback, and more selectively responsive to feedback timing and valence. While positive feedback has been shown to elicit positive emotions and self-confidence and negative feedback to improve assessment of performance in healthy volunteers, ADHD groups may overfocus on negative feedback, and neither positive nor negative feedback are consistently associated with performance improvement. With recent developments in AI assistive technology, AI learning tutors have been suggested for implementation in an education-focused settings for ADHD learners; however, those that have moved beyond the theoretical level to implementation have found mixed success. Here, we report two experiments focusing on feedback structure as a critical aspect of intelligent tutors for ADHD and show results suggesting that monitoring metacognitive confidence optimizes feedback effectiveness in ADHD learners. 35 participants completed a behavioral time-estimation task that required them to measure time intervals and report their confidence in their performance while receiving intermittent positive, negative, or neutral feedback. 15 participants later completed a second eye-tracking experiment with a similar behavioral task. A computational model mixing behavioral and eye-tracking measures was found

to accurately predict metacognitive confidence and correctly identify trials in which feedback (positive or negative) was most likely to increase performance. Our findings showcase the utility of integrating an evaluation of confidence into the feedback structure of intelligent tutor systems for ADHD learners.

Disclosures: A. Geana: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.08/UU4

Topic: I.10. Human Learning and Cognition

Title: Selective attention in graph neural network underlies generalization of dynamic pursuit task

Authors: *D. KIM, S. M. YOO;
Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Humans exhibit remarkable generalization capabilities, often applying learned behaviors to novel but related tasks. In contrast, deep reinforcement learning (RL) agents typically struggle to adapt flexibly to unfamiliar environments. In this study, we propose that serial selective attention, which processes information sequentially, can serve as a key mechanism to improve generalization in RL agents. We trained deep RL agents on a virtual pursuit task in which an agent must catch a prey to receive a reward proportional to the prey's speed. This environment replicates a task previously performed by rhesus macaques. To implement selective attention, we used a Graph Convolutional Network (GCN) that represents all objects in the game as nodes, with edge weights defined by attention scores. The serial selective RL agents were trained in an environment with a screen resolution of 1280×720 pixels, slow-moving prey, and a single target. After training, agents were tested in a novel environment that matched the conditions used for rhesus macaques, including a larger screen resolution (1920×1080 pixels) and faster prey (twice the training velocity). The agents achieved a 97% success rate, identical to that of rhesus macaques. To assess behavioral similarity, we compared the agents' predicted trajectories to actual movements over six timesteps. The root mean square error (RMSE) was approximately 16.75 pixels, well below the agent's 30 pixel radius, indicating highly similar movement patterns. We further tested the agents in environments with multiple preys which were not part of the training conditions. Despite this, serial agents maintained strong performance: 95% success with two preys, and over 93% success with up to five preys. Moreover, like rhesus macaques, agents demonstrated a strong preference (93%) for targeting the higher-reward prey. In contrast, agents trained with parallel attention attending to all objects simultaneously, exhibited poor generalization. Their success rate dropped from 100% to 75% when screen size and prey speed increased, and fell below 50% as the

number of preys increased. They also showed reduced reward sensitivity, often choosing lower-value targets, indicating poor adaptation to novel conditions. Our findings demonstrate that serial selective attention enables RL agents to generalize effectively in a virtual pursuit task, mirroring both the performance and strategic preferences of rhesus macaques. These results highlight serial selective attention as a promising approach to address the generalization problem in reinforcement learning.

Disclosures: **D. Kim:** None. **S.M. Yoo:** None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.09/UU5

Topic: I.10. Human Learning and Cognition

Title: Modeling Prior-like HORs in DecNef with a Generative NERD Model

Authors: ***H. AZIMI ASRARI**¹, M. A. K. PETERS²;

¹Cognitive Sci., Univ. of California, Irvine, Irvine, CA; ²Cognitive Sci., UC Irvine, Irvine, CA

Abstract: Higher-order neural representations (HORs) encode information about first-order representations (FORs), such as their reliability, uncertainty, or structure, and appear critical for metacognition, learning, and consciousness. While FORs represent the external world, HORs represent internal processes such as reflection on or evaluation of one's own decisional uncertainty, yet their dimensionality, construction, and neural substrates are poorly understood. We propose that metacognitive estimates (confidence judgments) arise from a Bayesian-like process producing "posterior-like" HORs via integrating "likelihood-like" estimates of current FOR uncertainty with "prior-like" learned distributions of expected uncertainty. To isolate and investigate "prior-like" HORs, we analyzed neural data from a reinforcement learning task where subjects learn about representational noise in their own brains, decoded neurofeedback (DecNef). To mimic the brain's process of learning about its own noise, we developed the Noise Estimation through Reinforcement-based Diffusion (NERD) model, a reinforcement learning-based generative denoising diffusion model, to learn distributions of noise present in patterns of neural response. To confirm the hypothesis that humans solve DecNef through learning noise-distribution HORs, we applied NERD to multiple DecNef studies (Cortese et al., 2021) to model the denoising process human subjects undertake, focusing on patterns present in early visual cortex. In previous work, we showed that NERD demonstrates superior explanatory power for human behavior over a control model, showing that individual differences in noise expectation HORs can explain individual differences in humans' ability to do DecNef. Our new results from fitting the NERD model to data from two DecNef studies (Amano et al., 2016; Koizumi et al., 2016), both targeting the early visual cortex, also demonstrate NERD's power to capture task dynamics despite significant differences in experiment goals, one targeting fear reduction and the

other visual learning. Our approach provides a novel generative artificial intelligence driven theoretical framework to elucidate the estimation processes and neural correlates of HORs - especially “prior-like” HORs about expected uncertainty - advancing our understanding of how the brain represents and learns about its own ongoing representations.

Disclosures: H. Azimi Asrari: None. M.A.K. Peters: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.10/UU6

Topic: I.10. Human Learning and Cognition

Support: NSF GRFP Fellow (ES)
NIH/NINDS R01 NS-021135 (RTK)
NIMH Conte Center P50MH109429

Title: Human Single Neuron Correlates of Adaptive Precision of Interval Timing

Authors: *E. E. SANDOVAL¹, N. ACHARA², C. HOY³, D. R. QUIROGA-MARTINEZ⁴, J. ZHENG⁵, M. R. DEWEESE⁶, J. J. LIN⁸, R. T. KNIGHT⁷;

¹Univ. Of California, Berkeley, Berkeley, CA; ²Univ. of California, Berkeley, Berkeley, CA; ³Neurol., Univ. of California, San Francisco, San Francisco, CA; ⁴Psychology, Univ. of Copenhagen, København K, Denmark; ⁵Neurolog. Surgery and Biomed. Engin., Univ. of California Davis, Davis, CA; ⁶Physics & Neurosci., UC Berkeley, Berkeley, CA; ⁷Psychology and Neurosci., UC Berkeley, el cerrito, CA; ⁸Dept. of Neurol., Univ. of California, Davis, Sacramento, CA

Abstract: Reward prediction errors (RPE) are critical for goal-driven behavior and can be used to monitor performance on different tasks. In a previously designed and tested interval timing task with adaptive feedback using intracranial recordings in epileptic patients. We found that patients with intractable epilepsy rely on positive RPE (pRPEs) to guide behavior. Similarly, it was found that mesoscopic high frequency activity (HFA, 70-150 Hz) was a biased towards reflecting pRPEs over negative RPEs (nRPEs) in both dorsomedial prefrontal cortex and insula. It remains to be determined what single neurons in dorsomedial prefrontal cortex are doing. Using Behnke-Fried electrodes in a subset of these patients (n=12), we collected microwire local field potential (LFP) data and extracted single unit activities from anterior cingulate cortex, hippocampus, and orbitofrontal cortex. We found units in anterior cingulate cortex (ACC) that significantly encode expected reward at response time ($p=0.003$) and nRPEs at feedback ($p=0.005$), as well as others that encode either pRPEs ($p=0.003$), or the absolute value of RPEs at feedback time ($p=0.001$) using Poisson GLMs. These results confirm the heterogeneity of RPE encoding even at the single neuron level in humans.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

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Program #/Poster #: PSTR363.11/UU7

Topic: I.10. Human Learning and Cognition

Support: NH DA034685
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Title: Temporal fMRI Dynamics Map Dopamine Physiology

Authors: *I. C. BALLARD¹, I. PAPPAS², D. FURMAN³, A. S. BERRY⁴, B. FREDERICK⁵, R. L. WHITE⁶, A. S. KAYSER⁷, W. J. JAGUST⁸, M. D'ESPOSITO⁹;

¹UC Riverside, Riverside, CA; ²USC's Mark and Mary Stevens Neuroimaging and Informatics Inst., USC, Los Angeles, CA; ³Univ. of California San Francisco, San Francisco, CA; ⁴Brandeis Univ., Waltham, MA; ⁵Brain Imaging Ctr., McLean Hospital/Harvard Med. Sch., Belmont, MA; ⁶Washington Univ. in St. Louis, St. Louis, MO; ⁷Neurol., Univ. of California, San Francisco, San Francisco, CA; ⁸Helen Wills Neurosci Inst., Univ. of California, Berkeley, CA; ⁹Univ. of California, Berkeley, El Cerrito, CA

Abstract: Spatial variations in dopamine function are linked to cognition and substance use disorders but are challenging to characterize with current methods. Because dopamine influences blood vessel dilation, we hypothesized that hemodynamic latency, which reflects BOLD signal timing, could serve as an indirect marker of dopamine physiology. Across four datasets, we found a topography of hemodynamic latencies that precisely distinguished the nucleus accumbens, a dopaminergic region implicated in motivation and substance abuse, from other striatal regions in male and female subjects. Using PET (n = 44), genetics and pharmacology (n = 73), we found that hemodynamic latencies are robustly related to dopamine function and perseverative behavior. In males and females with cocaine use disorder (n = 40), we observed a spatial gradient of altered hemodynamic latencies in the striatum relative to controls (n = 39). This pattern independently predicted nicotine use, revealing a conserved physiological profile associated with addictive substance use. Hemodynamic latencies map regional, individual, and pathological differences linked to dopamine, opening new avenues for indirectly assessing the

role of dopamine in healthy cognition and disease. The preprint is available at:
<https://www.biorxiv.org/content/10.1101/2025.03.24.645022v1>

Disclosures: I.C. Ballard: None. I. Pappas: None. D. Furman: None. A.S. Berry: None. B. Frederick: None. R.L. White: None. A.S. Kayser: None. W.J. Jagust: None. M. D'Esposito: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.12/UU8

Topic: I.10. Human Learning and Cognition

Title: Cognitive factors affecting AI alignment.

Authors: *K. MOGI;

Sony Computer Sci. Labs., Shinagawa-Ku, Japan

Abstract: With the advancement of artificial intelligence systems, AI alignment (Ji et al. 2023) is becoming an important research issue. In aligning human cognition and activities with AI, limits and characteristics of human cognition, e.g. embodied cognition (Wilson & Golonka 2013), flow (Csikszentmihalyi 2013), communication (Noordzij et al. 2009, Mildner 2010), agency (Haggard 2017), and ikigai (Sone et al. 2008, Mogi 2017), becomes important constraints, making cognitive neuroscience an integral part of AI alignment. Here I study the cognitive factors involved in AI alignment, identifying several salient parameters that correlate with how human subjects perceive and adapt to artificial intelligence systems. 231 subjects (85 females, 136 males, and 10 other genders, with age 46.4 ± 12.9) participated in the study. The subjects were asked to rate various cognitive traits in 11 point (0 to 10) scales. Correlation analysis with the positive expectation for the future of generative AI revealed significant positive correlations for the experience of flow in childhood ($r=0.14$, $p=0.028$), ikigai ($r=0.13$, $p=0.046$), enjoyment of small talks ($r=0.19$, $p=0.0036$), and the tendency to perceive generative AIs as staples rather than snacks (Mendez-Torrijos et al. 2018) in the food metaphor scale ($r=0.25$, $p=0.000095$). There were no such significant correlations with the positive AI expectations for age, belief in free will, understanding of qualia, distribution of agency, social network size, enjoyment of works, creation of works, food eating and cooking. There was a significant difference in concerns for ikigai risk (Ziesche & Yampolskiy 2020) between subjects with opposite handedness ($t=0.012$), possibly reflecting various cultural and social contexts involved in embodied cognition of handedness. There was a significant positive correlation for the LLM use frequency ($r=0.13$, $p=0.049$) and food metaphor scale ($r=0.14$, $p=0.036$) with the word count of open-ended free questionnaire. These results indicate a network of cognitive abilities as humans cope with and adapt to the newly emerging artificial intelligence systems, and suggest

challenges that can help aligning AI with the human cognitive systems. Based on the results I discuss ways to make AI alignment cognitively robust.

Disclosures: K. Mogi: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.13/UU9

Topic: I.10. Human Learning and Cognition

Title: A Cross-Species Paradigm for Meta-Learning: Comparative Insights from Humans and Marmoset Monkeys

Authors: G. HUANG¹, W. LUAN¹, *X. WANG²;

¹Tsinghua Lab. of Brain and Intelligence (THBI), Tsinghua Univ., Beijing, China; ²Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Meta-learning—the ability to learn more efficiently when encountering new tasks by generalizing from previous experiences—has been demonstrated in both humans and some non-human animals. However, naturalistic, quantitative paradigms for directly comparing meta-learning capacities between humans and non-human primates are lacking. In this study, we developed parallel dynamic maze tasks for marmosets (physical mazes) and humans (virtual mazes), each with four levels of difficulty and identical learning rules. Subjects (marmosets or humans) were required to discover the optimal path at each difficulty level to receive a reward. Both species exhibited evidence of meta-learning, including clear instances of the “aha moment”, when subjects abruptly demonstrated understanding of the task rules. Specifically, 5 of 7 marmosets and 13 of 28 humans showed a significant negative linear relationship between difficulty level and the number of trials required to learn the rule (defined as successfully navigating the optimal path twice consecutively), indicating meta-learning behavior. Notably, all marmosets were able to recall and execute the optimal path at the highest difficulty level after a two-month delay in five or fewer trials—substantially fewer than required during initial learning—suggesting robust long-term memory for task structure. To further model learning dynamics, we employed Deep Q-Learning Networks (DQNs) with psychologically meaningful parameter groups: learning, reward estimation, exploration, and memory. Agent performance was optimized to maximize Spearman correlation with subject learning curves across difficulty levels, achieving a mean correlation of 0.959. Comparison of best-fit agent parameters revealed that humans exhibited a higher average future reward discount (i.e., stronger valuation of long-term outcomes), but lower learning rates and lower estimations of both end rewards and penalties compared to marmosets. These findings establish a novel, quantifiable paradigm for comparing meta-learning across species, and provide evolutionary insight into the cognitive mechanisms underlying adaptive learning in primates.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

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Program #/Poster #: PSTR363.14/UU10

Topic: I.10. Human Learning and Cognition

Support: NSTC 113-2314-B002-273-MY3

Title: Exploration driven by loss aversion impairs reward maximization under punishment history

Authors: *W. LIN¹, M.-T. TSENG²;

¹Grad. Inst. of Brain and Mind Sci., Zhongzheng Dist., Taipei City, Taiwan; ²Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Reward and punishment are key modulators of human behavior by reinforcing actions that maximize gains and minimize losses. Although much is known about how reward and punishment contribute to guiding our behavior independently, their interaction and underlying mechanisms remain incompletely understood. To address this issue, we designed a probabilistic instrumental learning task in which a single option was associated with rewarding and punishing outcomes on separate trials. Across two experiments ($n = 57$), we scanned healthy participants using 3 Tesla functional magnetic resonance imaging (fMRI) while they performed this task. The learning task involved binary choices, with monetary gains as reward stimuli, and either monetary losses or painful electrical stimuli as punishment stimuli. We found that when a choice involved only one type of learning, reward learning entailed less exploratory behavior than punishment learning, indicating a more exploitative strategy for reward maximization and a more exploratory approach for minimizing punishment. However, when both types of learning were involved, reward learning performance (but not punishment learning) declined, indicated by decreased correct rate. Furthermore, the extent of the decline in performance depended on the size of potential losses. Computational modeling capturing individual sensitivity to prior punishment, in combination with fMRI, revealed that the decline stemmed from increased exploration in reward-learning trials with a punishment history, accompanied by greater activation in exploration-related prefrontal regions. Moreover, individual loss aversion, assessed separately, predicted the extent of increased exploration. These findings suggest that when a choice involves both reward and punishment learning histories, increased exploration driven by punishment associations may disrupt the exploration-exploitation balance necessary for effective reward learning, leading to reward maximization failure. In this context, loss aversion plays a key role in shaping choice behavior.

Disclosures: W. Lin: None. M. Tseng: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

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Program #/Poster #: PSTR363.15/UU11

Topic: I.10. Human Learning and Cognition

Title: Noise Correlations in Feature Learning

Authors: *J. KIM¹, X. C. DAI², A. BHANDARI³, M. R. NASSAR⁴;

¹Brown Univ. Neurosci. Grad. Program, Providence, RI; ²Neurosci., ³Cognitive Linguistics and Psychological Sci., ⁴Metcalf Hall, Brown Univ., Providence, RI

Abstract: Learning to assign credit to task-relevant features is a formidable, high-dimensional problem. Recent theoretical work from our team has demonstrated that stimulus-independent covariation in trial-to-trial activity fluctuations can reflect a dimensionality reduction mechanism that facilitates faster and more robust learning. However, this remains a theoretical proposal which is yet to be empirically verified. We investigated the role of noise correlations in shaping perceptual discrimination learning in human subjects using computational modelling, functional magnetic resonance imaging, and behavioural measures. We utilized an experimental framework where subjects learn to discriminate multi-dimensional perceptual stimuli. Subjects learned a classification boundary in two-dimensional feature space (color & motion) that was translated occasionally and unpredictably, forcing them to learn continuously on one of two possible joint feature dimensions (color+motion; color-motion). We constructed a two-layer feedforward neural network, in which two input layer units respond to each of the options in the two feature categories (color or motion), a bias unit modulates learning, and each output unit produces a response consistent with a category perception (on either side of the classification boundary). Using this model, we found that enhancing the gain in task-relevant dimensions produced noise correlations aligned with the learning dimension and also enhanced learning. Human subjects were best fit by models with higher gains on task-relevant dimensions, supporting the idea that they focused preferentially on the dimensions most critical for learning. Finally, we found that the noise of the input unit activations of the models that were best fit to participant data correlate with their corresponding features in a task-dependent manner, raising the question as to whether noise correlations in neurons, or even voxel BOLD activations, might do the same. In line with this prediction, preliminary fMRI evidence suggests that noise correlations across motion and colour-sensitive regions are modulated across conditions (motion + color versus motion - color learning dimensions), supporting the idea that the brain is amplifying task-relevant dimensions to improve learning.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

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Topic: I.10. Human Learning and Cognition

Support: MCIN (PID2021-127924NB-I00)
CIBERNED (CB06/05/0066)
CSIC (202220I137 ID245014)

Title: Dendritic spines in human and mouse cortical pyramidal neurons

Authors: *R. BENAVIDES-PICCIONE;
Cajal Inst., Madrid, Spain

Abstract: Dendritic spines of pyramidal neurons are the targets of most excitatory synapses in the cerebral cortex and dendritic spine morphology directly reflects their function. However, there is scarce data available regarding both the detailed morphology of these structures for the human cerebral cortex and the extent to which they differ in comparison with other species. Thus, in the present study we used intracellular injections of Lucifer yellow to reconstruct—in three dimensions—the morphology of basal dendritic spines from pyramidal cells in the human and mouse CA1 hippocampal region and compared these spines with those of the human temporal and cingular cortex. We found that human hippocampal dendrites show lower spine density, larger volume and longer length of dendritic spines than mouse CA1 spines. Furthermore, human hippocampal dendrites show higher spine density, smaller spine volume and shorter length compared to dendritic spines from the human temporal and cingular cortex. This morphological diversity suggests an equally large variability of synaptic strength and learning rules across these brain regions in humans and between humans and mice. Nevertheless, a balance between size and density was found in all cases, which may be a cortical rule maintained across cortical areas and species.

Disclosures: R. Benavides-Piccione: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

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Program #/Poster #: PSTR363.17/UU13

Topic: I.10. Human Learning and Cognition

Support: CIFAR/Scialog PG13025

Title: Quantifying the emergence of metacognition in structure learning

Authors: ***R. KAPER**, M. A. K. PETERS;
Cognitive Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Humans learn not only via passive exposure, but also through predicting upcoming events and metacognitively evaluating their choices' and predictions' likely correctness. In fact, individuals with better metacognitive access (confidence well-calibrated to their predictions' accuracy) learn more quickly without external feedback. Neural evidence links metacognition-learning interactions to right inferior frontal gyrus, midcingulate cortex, dorsolateral/ventromedial prefrontal cortex, and ventral striatum—regions calculating valence and prediction error—showing a distinct role for metacognition over surprisal calculation. However, prior studies assume constant metacognitive access throughout learning. Here we ask, does metacognitive access change over time and influence learning? How might this affect how observers learn under uncertainty, and are there distinct neural bases for learning versus metacognitive access? Our exploratory behavioral study probes the interaction and separation of metacognition vs structure learning at 2 structure levels—probabilistic co-occurrences of item pairs (AA', BB'...) and sequences (AA'→BB'...)—to begin closing this gap. Across 4 training blocks, participants viewed probabilistic streams of item pairs and performed a cover task to assess implicit learning. After each training block, participants completed tests of explicit structure learning (forced-choice accuracy) and metacognitive access (accuracy-confidence correspondence). Participants completed 2 sessions in counterbalanced order: one with fractals, and the other with semantically meaningful line drawings. Preliminary data (n=5 drawings, n=7 fractals) reveal implicit learning across blocks for both stimuli sets via statistically significant changes in cover task accuracy, with drawings being easier to learn. We also saw explicit learning in both sets regardless of block, with sequence learning outpacing pair learning for fractals but the opposite for drawings. Confidence also significantly increased across blocks and stimuli, despite no change in metacognitive access. Our results reveal differences in how structure learning and metacognition unfold and interact across time for semantically meaningful versus abstract stimuli, motivating novel task designs that can assess (a) how uncertainty levels affect structure learning, (b) links between implicit and explicit learning, and (c) the emergence of metacognitive access throughout learning. Future work will use neuroimaging to identify generalizable neural correlates of structure learning and metacognition across stimuli and structure levels.

Disclosures: **R. Kaper:** None. **M.A.K. Peters:** None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

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Program #/Poster #: PSTR363.18/UU14

Topic: I.10. Human Learning and Cognition

Support: RS-2023-00217361
RS-2022-NR070240

Title: Context-dependent Reward Processing Reflected in Central Positivity

Authors: *J. JEONG¹, H. AN^{2,5}, S.-J. HONG^{2,3,4,5,6}, M.-S. KANG^{1,5};

¹Dept. of Psychology, Sungkyunkwan Univ., Seoul, Korea, Republic of; ²Dept. of Biomed. Engin., ³Dept. of Intelligent Precision Healthcare Convergence, ⁴Dept. of MetaBioHealth, Sungkyunkwan Univ., Suwon, Korea, Republic of; ⁵Ctr. for Neurosci. Imaging Research, Inst. for Basic Sci. (IBS), Suwon, Korea, Republic of; ⁶Ctr. for the Developing Brain, Child Mind Inst., New York, NY

Abstract: Reward processing enables flexible behavioral adjustments in response to dynamic environments. Previous research has identified frontal negativity and parietal positivity as neural markers of reward-related signals, typically using probabilistic choice tasks that induce prediction errors. However, such tasks often lack structured models that reflect real-world decision-making, where individuals rely on internal representations and adjust behavior based on contextual cues. To bridge this gap, the current study explores how reward processing operates when a stable task structure is preserved but the rewarding context changes. Participants first completed a Basic Association Task (association task), acquiring baseline stimulus-action-outcome associations through binary choices. This was followed by the Adaptive Contextual Choice Task (choice task), which introduced two distinct color-coded contexts (green and blue) with varying reward contingencies of associations. Each context was presented in two consecutive runs. Although accuracy of choice task initially declined sharply after context transitions, it quickly recovered, indicating efficient adaptation in associative learning. By the end of the first context, performance had plateaued, suggesting that associative learning was fully established. While the amplitude of central positivity following reward was greater in the first context and reduced in the second context, the reduction in its amplitude from the first to the second run within context was greater at the vertex electrode, which is more closely associated with error-related processing, than at the parietal electrode. These findings suggest that central positivity is associated with contextual reward processing.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

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Program #/Poster #: PSTR363.19/UU15

Topic: I.10. Human Learning and Cognition

Support: Proyectos de Investigación Plurianuales2021-2023-CONICET

Title: Reactivating aversive memories in humans: A EEG study of post-retrieval processes

Authors: *L. CAVALLINO, L. BAVASSI, M. E. PEDREIRA;

Inst. de Fisiología Biología Mol. y Neurociencias-UBA-CONICET, Buenos Aires, Argentina

Abstract: Although fear conditioning is one of the most widely used models to study anxiety in humans, it is underlying neural correlates remain poorly understood. It is known that the retrieval of consolidated memories can trigger modifications—weakening, strengthening, or updating the original memory—depending on the cues presented during the reminder. In this study, we employed a three-day threat conditioning protocol in which an angry face (conditioned stimulus, CS+) was paired with an aversive sound (unconditioned stimulus, US). Our main objective was to identify neural markers of post-retrieval processes triggered by the presentation of the CS+ alone, 24 hours after acquisition. Additionally, we aimed to assess cognitive biases by examining how the perception of the conditioned stimulus changes following conditioning. To this end, we recorded resting-state electroencephalographic (EEG) activity using a 30-channel system at a 256 Hz sampling rate. We analyzed four minutes of resting-state EEG following the presentation of the reminder cue, focusing on memory-related patterns in the frequency domain. Our results confirmed successful conditioning (CS+-US association) and memory retention 48 hours later. Moreover, participants rated the CS+ face as more aversive than the CS- face 48 hours after conditioning, compared to their pre-conditioning ratings. We compared neural activity in two groups: an experimental group exposed to threat conditioning on the first day (27 university students; mean age = 22.6 ± 0.6 years; 15 females, 12 males) and a control group with no prior conditioning (27 university students; mean age = 23.0 ± 0.8 years; 15 females, 12 males). Relative to the control group, the experimental group showed decreased beta-band activity (13-20 Hz) in central regions during the four minutes following the reminder (Cluster-based permutation test). Previous research has highlighted the role of reduced beta amplitude in the encoding and retrieval of episodic memories. Our findings provide initial evidence for neural correlates associated with post-retrieval processes, including decreased beta activity related to implicit memory and cognitive bias following threat conditioning.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

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Program #/Poster #: PSTR363.20/UU16

Topic: I.10. Human Learning and Cognition

Support: NCT05179083

Title: A Clinical Trial Investigating Exercise on Neuronal Regeneration and Cognitive Functioning in Epilepsy

Authors: *M. SHARIQ¹, A. N. CAYCE², M. A. BONAGUIDI³;

¹Stem Cell, university of southern california, Los angeles, CA; ²Neurosci., ³Stem Cell Biol. & Regenerative Med., USC, Los Angeles, CA

Abstract: Exercise has been shown to enhance neurogenesis and cognitive function in animal models of epilepsy, yet no regenerative interventions currently exist for restoring neurogenesis in human patients. Given the decline in neurogenesis with longer disease duration, we conducted a pilot clinical trial to assess whether a structured 12-week exercise intervention could improve fitness, cognition, and neurogenesis in patients with Mesial Temporal Lobe Epilepsy (MTLE) undergoing hippocampal resection. Pre- and post-intervention assessments included VO₂ max, blood pressure, 4- and 6-minute walk tests, seizure frequency, and neuropsychological tests (WHO-UCLA AVLT, WAIS-IV, and RCFT). Resected hippocampal tissue was analyzed using Dcx and Prox1 markers to quantify immature neurons. Preliminary results from two patients showed improvements in cardiovascular fitness (e.g., VO₂ max, walking distance) and cognitive performance (e.g., intelligence, verbal learning, and visual memory), without an increase in seizure frequency. However, neurogenesis levels were similar to those of historical controls matched for disease duration who had not received exercise intervention. These initial findings suggest that exercise may safely enhance cognition and fitness in MTLE patients, and ongoing recruitment will help determine its potential to modulate neurogenesis.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

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Program #/Poster #: PSTR363.21/UU17

Topic: I.10. Human Learning and Cognition

Support: WPI MEXT, IRCN-Daikin SCP

Title: Resting-state brain flexibility predicts creative potential: Insights from EEG energy landscape analysis

Authors: *A. ANUBHAV¹, T.-L. LIU², Y. LI^{2,1}, K. FUJIWARA^{2,1}, Z. C. CHAO², K. AIHARA²;

²IRCN, ¹The Univ. of Tokyo, Tokyo, Japan

Abstract: Creativity remains a complex cognitive phenomenon that is challenging to quantify and interpret at the neural level. This study presents novel evidence linking pre-task resting-state EEG brain dynamics to creative performance using an interpretable energy landscape framework. Participants completed two creativity tasks: the Alternative Uses Test (AUT), which was evaluated based on the novelty and feasibility of unconventional uses for everyday items, and the Fusion Innovation Test (FIT), which was assessed on the novelty, feasibility, and goal

attainment level of combining two given items to achieve a real-world goal. EEG data were recorded during an eyes-open 33-second resting-state period prior to each test question. To quantify creativity, we computed the overall creativity score as the geometric mean of the rated parameters for each response, generated by an automated GPT-based method, and applied a question-based normalization procedure to correct for difficulty biases. EEG signals were acquired from 19 dry electrodes positioned according to the international 10-10 system. To capture functional connectivity-based brain dynamics, electrodes were clustered into six anatomically informed groups (e.g., Fp1, F7, F3 for left frontal activity). Average signals from each cluster were used to construct energy landscapes via the pairwise Maximum Entropy Model (pMEM), fitted using maximum likelihood estimation. Two key energy landscape metrics were extracted: the number of local minima (interpreted as quasi-stable brain states) and the total number of transitions between them (reflecting state flexibility). Regression analyses revealed that the number of local minima positively correlated with creativity scores across both tasks (Spearman's $\rho = 0.08$, $p < 0.001$) and more strongly within the FIT task ($\rho = 0.13$, $p < 0.001$). In contrast, the number of transitions did not significantly correlate with creativity ($\rho = 0.02$ for both tasks, $\rho = 0.03$ for FIT). Our findings offer the first evidence that the structural complexity of pre-task brain states, specifically the number of quasi-attractors, can serve as a predictive neural marker of upcoming creative output across different creativity tasks. These results pave the way for future work on individualized brain-state profiles and their roles in higher-order cognition.

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Poster

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Topic: I.10. Human Learning and Cognition

Support: T32 NS007224
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Gruber Science Fellowship 2021

Title: Prior preferences interfere with the associative learning of food values

Authors: *A. RICH, S. KAPADIA, R. HENRY, O. DAN, I. LEVY;
Dept. of Comparative Med., Yale Univ., New Haven, CT

Abstract: Cognitive flexibility, vital for environmental adaptation, is often probed with monetary assignment to neutral cues. Food valuation reflects strong, diverse preferences beyond nutritional content that motivate food choices. Prior work finds intrinsic food values can be

changed, yet little is known about the flexibility of associative learning processes that drive such change. In a novel probabilistic reversal-learning task, participants ($n=279$) repeatedly predicted stimuli-coupled outcomes. The task used four food stimuli pre-rated by participants as (a) strongly disliked, (b) disliked, (c) liked, and (d) strongly liked. Outcome distributions alternated between "Congruent" blocks, where disliked foods (a, b) were mainly paired with negative outcomes ("punishments") and liked foods (c, d) with positive outcomes ("rewards"), and "Incongruent" blocks, where this was reversed. Four reinforcement learning models were fitted to determine whether behavior is consistent with value change or state detection. Food rating and block type interacted ($F(1,1108)=83.69, p<.001$) such that participants tracked changing associations less efficiently for liked foods ($ps<.001$). Participants predicted outcomes better for reward- than punishment-associated foods ($ps<.001$). Surprisingly, initial block identity had a significant impact on learning quality ($F(1,1108)=12.41, p<0.001$). Participants starting in a Congruent block, where food-value priors were reinforced, tracked stimulus values less efficiently, showing lower prediction accuracy on Incongruent trials ($p<.001$). Preliminary model comparisons indicate that a policy-based model outperformed valuation-based models across all participants, suggesting prioritization of environmental cues over intrinsic food values. However, the policy adherence model had lower accuracy than valuation-based models for Congruent-start participants. Our results indicate initial reinforcement of food-value priors potentiates learning inflexibility. Preliminary modeling suggests such priming may lead participants to rely more on intrinsic values than external cues in changing environments. The influence of food ratings on learning flexibility suggests it is easier to add relatively disliked items to a diet than remove liked ones. Highlighting rewarding food aspects, like health benefits, may more effectively influence learning than health consequences. Ongoing work probes the neural correlates of these findings. Inherent food values may drive disordered eating; understanding food-based associative learning can inform personalized dietary interventions for eating and weight disorders.

Disclosures: A. Rich: None. S. Kapadia: None. R. Henry: None. O. Dan: None. I. Levy: None.

Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.23/UU19

Topic: I.10. Human Learning and Cognition

Support: JSPS KAKENHI Grant Number Jp25K13298
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JSPS KAKENHI Grant Number Jp17K12065
JSPS KAKENHI Grant Number Jp2646319

Title: Associations between eye movement patterns, pupil dynamics, and radiographic interpretation in dental students: an eye-tracking study

Authors: *S. TANAKA¹, H. KARIBE³, A. OKAMOTO²;

¹Dept. of Pediatric Dent., ²Nippon Dent. Univ. Sch. of Life Dent. at Tokyo, Tokyo, Japan;

³Nippon Dent. Univ., Tokyo, Japan

Abstract: Accurate interpretation of panoramic radiographs during the mixed dentition period demands advanced visual processing skills, yet the cognitive strategies underlying successful diagnosis remain unclear. This study investigated the associations between diagnostic accuracy and scan path patterns, oculomotor behavior, and pupil dynamics in dental students. We hypothesized that students with higher diagnostic performance would exhibit (1) distinct scan paths, (2) longer viewing times, and (3) lower cognitive load, as reflected by smaller average pupil size. Fifty-four fifth-year dental students (41 males, 13 females) from Nippon Dental University participated. Eye movements were recorded using a non-contact eye tracker while participants viewed panoramic radiographs. Metrics included fixation count, total viewing time, mean angular velocity, fixation ratio and duration, spontaneous and voluntary blinks, and blink bursts. Pupil size was recorded during both full viewing and fixation periods, excluding saccades. Following observation, students identified diagnostic findings across five predefined regions; accuracy scores ranged from 0 to 5, based on the number of correct responses. The participants were then classified into high-scoring (HS, ≥ 3 ; $n = 21$) and low-scoring (LS, < 3 ; $n = 33$) groups. Gaze vectors were computed using cross-product methods, and scan paths were categorized into four types: R (clockwise), L (counterclockwise), F (fixation-dominant), and S (saccade-dominant). A chi-square test revealed a significant association between scan path type and diagnostic performance ($\chi^2(3) = 29.66$, $p < 0.001$, Cramer's $V = 0.741$). The R-type pattern was significantly more frequent in the HS group, suggesting a link between clockwise visual exploration and improved diagnostic accuracy. To further examine predictors of performance, ordinal logistic regression (Python 3.12.7; statsmodels) was conducted. The final model (AIC = 135.43; McFadden's $R^2 = 0.177$) identified several significant predictors ($p < 0.05$): scan path pattern ($\beta = 1.03$), pupil area during full viewing ($\beta = -0.65$), pupil area during fixation ($\beta = 0.53$), and voluntary blink count ($\beta = -0.33$). Viewing time was not a significant predictor ($\beta = 0.01$, $p = 0.36$). These findings suggest that clockwise scan paths and specific oculomotor and pupillary features are associated with higher diagnostic accuracy. A smaller pupil during full viewing may reflect lower cognitive load, whereas greater dilation during fixations may indicate focused attention. Eye-tracking and pupillometric data offer promising avenues for assessing and enhancing diagnostic training in dental education.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.24/UU20

Topic: I.06. Social Cognition

Support: NIH R21MH134217-01

Title: Disruptions in uncertainty processing and its transdiagnostic implications for learning under uncertainty

Authors: *X. FANG¹, P. PIRAY²;

¹Neurosci. Grad. Program, USC, Los Angeles, CA; ²Dept. of Psychology, USC, Los Angeles, CA

Abstract: Maladaptive uncertainty processing underlies cognitive dysfunction in psychiatric disorders like anxiety, behavioral addictions, depression, autism, and schizophrenia. Behavioral addiction, for example, involves attributing losses on bad luck, while anxiety is associated with intolerance of uncertainty. However, it is not clear what specific computations support this ambiguous notion of uncertainty, how they go wrong, and how these same computations influence many mental illnesses transdiagnostically. We addressed these questions by drawing on recent theoretical work identifying computational hypotheses about how uncertainty processes may go wrong when organisms face observations corrupted by two major types of noise: moment-to-moment stochasticity of observations, and volatility, i.e., how quickly they change. Dissociating the two noise sources is critical, as they have opposite effects on learning: while volatility requires faster learning, as the world is changing rapidly, stochasticity requires slower learning, as each individual outcome is less representative of the underlying contingency. Although human participants are statistically efficient at dissociating volatility and stochasticity by increasing their learning rate when facing volatility and decreasing it when facing stochasticity, psychopathologies may corrupt this adaptive process. Anxious individuals might be particularly stochasticity-maladaptive by taking unexpected outcomes caused by chance (stochasticity) as a signal of change (volatility). In contrast, individuals with impulse control disorders, such as gambling, might be volatility-maladaptive, erroneously attributing meaningful losses to bad luck, thereby systematically misattributing volatility as stochasticity. We tested these hypotheses using a large-scale online experiment (>1000 participants) with behavioral tasks that systematically manipulated volatility and stochasticity in a 2x2 factorial design. Participants also completed a battery of psychopathological instruments measuring anxiety, depression, behavioral addictions, and schizophrenia. We used factor analysis to identify two dimensions: one capturing anxiety and depression, and another reflecting behavioral addictions and impulsivity. Preliminary results confirm our hypothesis for the impulsivity dimension, which is linked to volatility-maladaptive behavior. In contrast, the anxiety dimension is associated with elevated learning rates regardless of volatility or stochasticity. These findings offer a computational framework for understanding transdiagnostic mechanisms of uncertainty processing.

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Poster

PSTR363: Mechanisms of Adaptive Learning: Reward, Metacognition, and Decision-Making

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR363.25/UU21

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

Title: Predicting Learning Ability in Alzheimer's Disease Using Resting-State EEG with a Meta-Diffusion Framework

Authors: *R. ZHANG;

Univ. of Southern California, Los Angeles, CA

Abstract: Abstract:

Traditional cognitive scales are widely used in the diagnosis of Alzheimer's disease (AD). However, task-based assessments suffer from limited real-time adaptability, reducing their utility in early-stage diagnosis. These tasks also introduce complexity, potential noise, and practical limitations—particularly for elderly or cognitively impaired individuals. To address these challenges, we propose a novel **meta-diffusion framework** that predicts learning ability from resting-state electroencephalography (EEG) via cross-domain knowledge transfer. We first leveraged 109 task-based EEG recordings from a publicly available dataset, where individual learning ability was quantified through linear fitting of learning curves to generate **Z-vectors** representing subject-specific learning profiles. A single-branch meta-diffusion model was initially trained to map testing-state EEG signals (from cortical regions) to these Z-vectors, incorporating subject-specific embeddings for personalized modeling. We then extended this to a two-branch architecture: one branch for transferring domain knowledge and the other for direct prediction of learning ability from resting-state EEG. The model employs a 1000-step diffusion process and subject-aware embeddings to robustly capture individual learning dynamics. We validated the framework on 13 held-out subjects from the same dataset, achieving high predictive accuracy. To further assess generalizability, we applied the model to an independent dataset comprising 36 AD patients and 29 age-matched healthy controls (aged 60+), with resting-state EEG recorded at 500 Hz. Despite substantial domain shifts and no fine-tuning, our framework demonstrated strong generalizability. Predicted Z-vectors revealed consistent group-level differences across three cognitive dimensions. Moreover, classification using anomaly detection achieved an AUC of 0.76, indicating clinically meaningful discrimination. These findings suggest that the proposed meta-diffusion framework effectively extracts disease-relevant neural signatures from resting-state EEG, offering a scalable and task-free solution for cognitive function assessment—particularly for tracking learning ability in Alzheimer's disease.

Disclosures: R. Zhang: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.01/UU22

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01NS107451

Title: Spatial transcriptomics and computational analyses to uncover novel genes related to recording quality and biocompatibility of the brain-electrode interface

Authors: *B. GUPTA¹, A. CHAKRABORTY², A. SAXENA¹, Q. WHITSITT¹, E. K. PURCELL³;

¹Biomed. Engin., ²Electrical and Computer Engin., ³Biomed. Engineering, Electrical and Computer Engin., Michigan State Univ., East Lansing, MI

Abstract: Implantable neurotechnology is increasingly used in research and clinical settings to study and treat a myriad of neurological conditions. Recent advances in neural prostheses rely on detecting and/or stimulating signals to drive therapeutic outcomes or assistive tools. Long-term, stable performance is a prevailing challenge for implanted electrodes, prompting deeper investigations of the device-tissue interface and associated foreign-body response (FBR). The innate FBR to neural implants has been implicated as a major contributor to poor electrode-tissue integration, signal instability over time, and overall device degradation or failure. Understanding the cellular and molecular changes taking place at the device-tissue interface can provide new information on genetic targets or pathways that influence the immune response to implanted devices in the brain.

In this work, we utilized the high-throughput technique of spatial transcriptomics to obtain genetic information from rats ($n = 10$ rats, 5 rats per time point) implanted with silicon microelectrode arrays for 1-week and 6-weeks. Differential gene expression analysis revealed: (1) a greater number of genes maintaining spatially consolidated expression are upregulated near the device implant ($< 300 \mu\text{m}$) at 6 weeks post implantation than previously reported, and (2) specific genes and groups of genes, such as chemokine signaling and apoptosis or cell survival genes, are more prominent and spatially consolidated near the device site at 6-weeks compared to 1-week post implantation. Additional computational network analyses of these large datasets revealed target genes, or biomarkers, that can be studied further as biological indicators of key cellular and molecular mechanisms taking place at the electrode-tissue interface. Further, we collected electrophysiological recordings from implanted devices ($n = 7$ rats) up to 6-weeks, and applied a computational model to relate gene expression at the device-tissue interface and recording quality of detected signals. Together, the findings reported here aim to explore and report novel biomarkers at the functional brain-electrode interface. These results provide potential genes of interest that can be studied in future investigations and therapeutic target studies involving implantable neurotechnology.

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Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.02/VV1

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Single-cell scale, spatiotemporal mapping of neurogenesis in developing mouse brain using Visium HD 3'

Authors: A. GUPTA¹, M. NAGENDRAN¹, S. MOHABBAT¹, **M. SERRA**¹, J. GU¹, D. CHITNIS¹, Z. MA¹, G. KAMATH¹, J. ARTHUR¹, *H. OH¹, S. OSINSKI¹, A. PATEL¹, D. SUKOVICH², A. M. TENTORI¹;

¹10x Genomics, Pleasanton, CA; ²10x Genomics, Pleasanton, CA

Abstract: Rationale: The developing brain exhibits a well-organized spatial architecture, where progenitors interact with spatially and temporally regulated molecular cues to commit to diverse neuronal fates. Recent advances in spatial transcriptomics have enabled profiling of these interactions to delineate boundaries between emerging cellular and functional domains in the brain. However, existing methods are limited in their ability to localize molecular and cellular events at single-cell resolution. Here, we utilized the Visium HD 3' Gene Expression assay to investigate the molecular mechanisms underlying the establishment and organization of neuronal cell types in the cortex and hippocampus of the developing mouse brain.

Methods: Visium HD 3' leverages an unbiased, poly(A) capture chemistry to enable single-cell scale, whole-transcriptome spatial profiling of H&E-stained fresh frozen tissue sections across a variety of species. The Visium HD slide features a continuous grid of oligonucleotides organized into 2 × 2 μm uniquely barcoded squares, and is optimized for use with the CytAssist instrument for precise transcript localization. A nuclei-based cell segmentation on H&E images can be incorporated to delineate individual cells and map gene expression to histologically defined structures. We applied this assay to profile gene expression in mouse brain from embryonic day 13.5 (E13.5), day 15.5 (E15.5), and postnatal day 1 (P1).

Results: The Visium HD 3' assay was used to map neurogenesis in E13.5, E15.5, and P1 neocortex (dorsal pallium) and hippocampal allocortex (medial pallium). In the neocortex, we recovered expected sequential progression of apical progenitors (*Pax6*, *Hes5*) close to the ventricle followed by intermediate progenitors (*Eomes*, *Neurog2*), and migrating excitatory immature neurons (*Neurod2*). We also detected localization of Cajal Retzius cells (*Reln*), astrocytes (*Slc1a3*), pericytes (*Pdgfra*), and endothelial cells (*Cldn5*). Focusing on hippocampal neurogenesis, we characterized the molecular progression of granule cell development. Specifically, we mapped neural progenitors (*Hopx*, *Hes5*) to be in close proximity of the ventricle at E15.5. This is sequentially followed by a zone of intermediate progenitor-like cells (*Eomes*, *Neurog2*) and a zone of newborn neurons expressing *Neurod1*. This molecular progression of granule cell differentiation became more apparent in the P1 mouse pup. Overall, our findings demonstrate the power of Visium HD 3' for high-resolution spatiotemporal mapping of neurogenesis in the developing mouse brain and highlight its potential to study development and disease disorders of the nervous system.

Disclosures: **A. Gupta:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **M. Nagendran:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **S. Mohabbat:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); 10x Genomics. **M. Serra:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **J. Gu:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **D. chitnis:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **Z. Ma:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **G. Kamath:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **J. Arthur:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **H. Oh:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **S. Osinski:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **A. Patel:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **D. Sukovich:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **A.M. Tentori:** A. Employment/Salary (full or part-time): 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.03/VV2

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: Wallenberg Scholar (2024-2029)
Hjärnfonden (2024-2027)
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Hjärnfonden PS2022-0036

Title: Establishing in vivo Perturb-seq to discern gene regulatory principles in the adolescent mouse brain

Authors: *E. GRUYTERS¹, E. KALLOU³, H. FRENCH², X. ZHENG⁴, X. JIN⁴, J. HJERLING LEFFLER²;

¹Dept. of Med. Biochem. and Biophysics, Karolinska Institutet, Solna, Sweden; ²Dept. of Med. Biochem. and Biophysics, Karolinska Institutet, Stockholm, Sweden; ³Dept. of Biol.

Applications and Technol., Univ. of Ioannina, Ioannina, Greece; ⁴Dept. of Neurosci. and Dorris Neurosci. Ctr., Scripps Res., La Jolla, CA

Abstract: CRISPR-based methodology has revolutionized our ability to perform large-scale functional genomics. In *in vitro* screens, perturbation of a single gene or a set of genes are introduced to assess the impact on for example cell fitness under specific conditions. A detailed dissection of the perturbation effect in single cells can be obtained by high-content read-out of the perturbations by single cell RNA-sequencing, an approach called Perturb-seq. By applying Perturb-seq *in vivo*, genes can be functionally assessed across different cell types and different developmental timepoints. We have established *in vivo* Perturb-seq to functionally assess 30 transcription factors, active during early neurodevelopment, in the adolescent mouse cortex. Previous applications of *in vivo* Perturb-seq using lentiviral guide RNA (gRNA) libraries have been limited by the low transduction rate. We therefore set up an approach based on Adeno Associated Virus (AAV) for delivery of gRNA libraries. Initial trials of intracranial delivery of an AAV-based gRNA library showed a limited spread of the virus across cortex, largely confined to the site of injection, as assessed by immunohistochemistry. Systemic delivery of AAV PHP.eB, through the tail vein or retro-orbitally, resulted in an even spread of the virus across cortex. High titer systemic injections of AAV PHP.eB gave clear populations of transduced cells through FACS-based enrichment of isolated cortical neurons. Our results show that a larger number of cells across cortex can be transduced through systemic delivery of a gRNA library as opposed to local delivery. Systemic delivery increases the number of perturbed cells obtained from the cortex of a single mouse, thereby reducing the number of animals required in *in vivo* Perturb-seq studies. An important aspect of *in vivo* Perturb-seq is to identify a viral titer that optimizes the proportion of transduced cells with a single perturbation. We are currently evaluating *in situ* detection of mRNA as a method for assessing viral titers and the extent of gRNA overlap in single cells. Being able to assess the viral titer based on the expression of subsets of gRNAs directly for the gRNA library, rather than theoretical multiplicity of infection ratios, could improve the proportion of cells with single perturbations. This is also important for reducing the number of animals required in *in vivo* Perturb-seq studies but also to maximize the number of cells with a single perturbation among the transduced cells. Our results outline a tractable approach to efficient functional genomic screens by *in vivo* Perturb-seq.

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Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.04/VV3

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH P30DA060810
NIH P50DA037844

Title: RATTACA: a genetic prediction service to facilitate genetic correlation and experimental design in outbred rats

Authors: ***B. B. JOHNSON**, R. M. VOGEL, T. MISSFELDT SANCHES, R. AVILA, N. SUZUKI, E. KEUNG, O. POLESSKAYA, A. A. PALMER;
Univ. of California San Diego, La Jolla, CA

Abstract: RATTACA (RAT Trait Ascertainment using Common Alleles) is a novel paradigm that uses existing experimental data to predict behavioral, physiological, and gene expression phenotypes in naive outbred Heterogeneous Stock (HS) rats. RATTACA leverages data from >20,000 HS rats that have been genotyped genome-wide and phenotyped for >700 traits to predict those same traits in experimentally naive rats. The method uses linear mixed models to produce polygenic scores from estimated SNP effects on phenotypes, with new predictions made for each cohort of ~500 colony-bred rats born every three months. These predictions are used to identify rats expected to display extreme phenotypes within the population. By selecting rats with extremely high and low predicted phenotypes, researchers can conveniently design experiments on phenotypically distinct samples without the need for inbred lines. RATTACA is a research service provided by the NIDA Center of Excellence for Genetics, Genomics, and Epigenetics of Substance Use Disorders in Outbred Rats at UC San Diego (<https://ratgenes.org/rattaca/>). With an extensive database of trait measurements related to substance use and addiction, the Center offers (1) predictions on a breadth of addiction-related traits and (2) experimental samples expected to display desired phenotypic values. RATTACA allows for experimental designs reminiscent of studies with selectively bred lines, however, unlike selective breeding, all RATTACA rats are drawn from a single population, which provides experimentalists with multiple benefits: (1) Using outbred rats eliminates the confounding effects of genetic drift on selected samples. (2) Selecting on predicted substance use allows experimentalists to measure addiction-related neurological or behavioral phenotypes without the need for drug exposure, disentangling the potential confounding effect of drug treatment on the measured trait of interest. (3) RATTACA does not require a sustained investment in selective breeding for multiple generations, providing a much more nimble system for exploring genetic correlations. Because RATTACA predictions are drawn from a single population, observed correlations between a predicted trait and a second measured trait suggest a genetic correlation between phenotypes, making RATTACA a useful tool for identifying neurobehavioral traits with a shared causal genetic architecture.

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Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.05/VV4

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: Aligning Science Across Parkinson's (ASAP-020519, ASAP-025193)
Michael J. Fox Foundation for Parkinson's Research (MJFF)

Title: Transcriptional and spatial mapping of the primate ventral striatum identifies RGS6+ neurons with rostro-caudal gradient of dopamine receptor co-expression.

Authors: ***G. ABDELHADY**¹, O. R. BRULL³, J. HE⁴, A. R. PFENNING², A. C. BOSTAN⁵, W. R. STAUFFER⁵;

¹Neurosci., ²Computat. Biol. Dept., Carnegie Mellon Univ., Pittsburgh, PA; ³Univ. of Pittsburgh, Pittsburgh, PA; ⁴Neurobio., Univ. of Pittsburgh, PITTSBURGH, PA; ⁵Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: With input from cortical and subcortical projections, the striatum functions as an integrative hub for inputs to the basal ganglia. These inputs arrive primarily on medium spiny neurons (MSNs). Single cell transcriptomic studies have revealed far greater medium spiny neuron subtype variation than predicted from existing functional models. However, such single-cell analyses have not yet been extended to the post-commissural striatum, leaving the rich cellular heterogeneity along the rostral-caudal axis largely unexplored. In this study, we integrate single-nucleus RNA sequencing and spatial transcriptomics to map spatially resolved molecular and cellular profiles on functionally distinct striatal regions. We focus on medium spiny neurons (MSNs), which we have previously stratified into nine different MSN subtypes in the rostral striatum of non-human primates (NHPs). Here, we sample the head of the caudate, the sensorimotor putamen, and both rostral and the caudal ventral putamen in five rhesus macaques. This reveals that the rostral-caudal axis exhibits higher transcriptional similarity over the dorsal-ventral axis. MSN subtypes in the dorsal caudal sensorimotor putamen resembled those from rostral associative regions, while MSN subtypes in the ventral caudal putamen resembled those from the rostral NAc. In the ventral striatum, we identify two novel MSNs subtypes marked by Regulator of G-protein Signaling 6 (RGS6), which co-localizes predominantly with DRD1-expressing cells rostrally and with DRD2-expressing cells caudally. In Parkinson's Disease (PD) macaque model, RGS6 expression was downregulated in the caudal ventral striatum of relative to control, suggesting depletion of the vulnerable RGS6+ population in PD. Jointly, we present a comprehensive molecular-cellular-anatomical map of the primate striatum. This work provides a roadmap for dissecting the cell-type architecture and contributions to striatal circuitry and dysfunction, paving the way for targeted therapeutic strategies.

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Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR364.06/VV5

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIDA 5U01DA043098
The Hope for Depression Research Foundation (HDRF)
Pritzker Neuropsychiatric Research Consortium

Title: Investigating the regulatory epigenome by relating chromatin accessibility to gene expression differences in the nucleus accumbens of a selectively bred rat model of individual vulnerability to psychiatric disorders and addiction

Authors: *E. K. HEBDA-BAUER¹, H. KHALIL¹, M. H. HAGENAUER¹, J. LI¹, S. J. WATSON, Jr¹, A. A. PALMER², H. AKIL¹;
¹Univ. of Michigan, Ann Arbor, MI; ²UCSD, La Jolla, CA

Abstract: Gene regulatory mechanisms are multifaceted and challenging to characterize. Although accessible (open) chromatin comprises a small portion of DNA, it captures most regions bound by transcription factors that modify gene activity. Pairing chromatin accessibility with gene expression data provides an avenue for delineating epigenetic interactions that may drive gene expression but poses bioinformatic analysis challenges including use of the rat genome. Chromatin accessibility varies in response to both external (e.g., stress) and internal (e.g., genetic, developmental) factors. To capitalize on genetic differences, we have used nucleus accumbens (NAcc) from selectively bred High Responder/Low Responder (bHR/bLR) rats, that display individual differences in vulnerability to psychiatric disorders, for generating paired ATACseq/RNAseq data. Bilateral punches from four 300mm slices of NAcc core/shell were obtained from 40 adult male and female bHR and bLR rats (F76). Nuclei were isolated for ATACseq and RNA extracted for RNAseq, followed by bulk ATACseq/RNAseq. Data analysis included differential gene expression and chromatin accessibility (peaks), genomic overlap identification of significant genes with peaks, functional enrichment analyses, and creation of gene regulatory networks. Chromatin accessibility showed greater bHR/bLR differences than with sex (9460 peaks, FDR<0.05 vs 319 peaks, FDR<0.05). The same is true for gene expression (bHR/bLR: 1820 genes, FDR<0.05 vs sex: 23 genes, FDR<0.05). 41% of differentially accessible peaks overlap with genomic features (introns, exons, promoters). We identified 408 sequence overlaps with 75% concordance between gene expression and chromatin accessibility: 182 concordant overlaps upregulated in bHRs; 124 upregulated in bLRs. Functional enrichments among concordant gene-peak matches include peroxisome; EF-hand, calcium binding motif; transit peptide; and metabolism. These gene-peak enrichments show consistency with gene expression enrichment analyses, highlighting which genes may be regulated via chromatin accessibility differences. Further data integration is underway via gene regulatory network analyses (including transcription factors). Our findings elucidate gene regulation differences in NAcc, a critical brain structure implicated in motivation, reward, and addiction, that are involved in shaping individual vulnerability to externalizing/internalizing behaviors inherent in psychiatric disorders. Next, we will obtain a more comprehensive picture by integrating these findings with spatial transcriptomics, single nuclei RNAseq, and neuroanatomical data.

Disclosures: E.K. Hebda-Bauer: None. H. Khalil: None. M.H. Hagenauer: None. J. Li: None. S.J. Watson: None. A.A. Palmer: None. H. Akil: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.07/VV6

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U01MH117072
NIA U01AG077227

Title: A rapid, volumetric platform for organ-scale multiplexed transcriptomic analysis

Authors: *N. TOWNSEND HAAS¹, C. SU², K. CHUNG²;
¹Chem. Engin., MIT, Morris Plains, NJ; ²MIT, Cambridge, MA

Abstract: Spatially-resolved transcriptomic profiling in intact tissues is essential for decoding brain function and dysfunction. While recent methods enable single-transcript resolution gene profiling, most remain largely constrained to thin tissue sections, compromising structural context and severely restricting organ-scale analysis. Scalable, whole-organ *in situ* transcriptomics remains technically challenging due to slow and incomplete probe penetration, low multiplexing capacity, and tissue damage from repeated, harsh chemical treatments, leading to transcript loss over multiple rounds of labeling. As a result, current transcriptomic analysis relies on sparse sampling, risking anatomical bias and incomplete biological insight. Here we introduce SEAFISH (Scalable Electrophoretically Accelerated Fluorescence *in situ* Hybridization), a platform for rapid, uniform, and multiplexed transcriptomic labeling across whole mouse brain hemispheres. SEAFISH enables fast and uniform whole-organ labeling within one day by employing active electrophoretic transport to drive probes deep into tissues and overcome the diffusion limits of conventional FISH. We couple this with a robust, cleavable signal amplification strategy which allows for rapid multi-round RNA detection while preserving transcript and structure integrity. Compared to conventional methods, SEAFISH drastically reduces processing time, supports efficient multiplexing, and achieves labeling uniformity in large tissue volumes. We applied SEAFISH to map mRNAs for cell-type and state markers across the mouse brain, enabling spatially resolved gene co-expression analysis and cell distribution mapping at the organ scale. SEAFISH enables the construction of comprehensive, three-dimensional molecular atlases, revealing spatial gene organization principles obscured in 2D thin-section analysis and unlocking new opportunities for brain-wide transcriptome mapping. SEAFISH offers a generalizable framework for rapid, scalable, and high-content spatial omics in neuroscience and beyond.

Disclosures: N. Townsend Haas: None. C. Su: None. K. Chung: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.08/VV7

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Next-generation HCR™ HiFi probes enable high-fidelity detection of short RNA targets via background-suppressed in situ hybridization

Authors: *A. ACHARYA;
Mol. Instruments, Los Angeles, CA

Abstract: Detection of short RNA species—including microRNAs, exon-exon junctions, and transcript isoforms—is critical for studying post-transcriptional regulation and RNA processing in the nervous system. However, these targets have remained largely inaccessible to traditional *in situ* hybridization (ISH) methods due to off-target signal and limited sequence complexity. Prior to this work, background-suppressed probe strategies had not been effectively demonstrated for RNA targets shorter than ~50-100 nucleotides. We present a next-generation HCR™ HiFi probe technology that enables background-suppressed detection of RNA targets as short as 18 nucleotides with high specificity and signal-to-background performance in intact brain tissue. This capability is made possible by a new class of rationally designed probes that incorporate proprietary background suppression chemistry tailored for short target sequences. The system is fully compatible with HCR™ Gold and HCR™ Pro detection reagents, requires no changes to existing workflows, and supports both manual and automated protocols. Additionally, these new short-target probes can be used simultaneously with first-generation HiFi probes, allowing seamless multiplexing of short and long RNA targets within a single assay. Core HCR™ advantages are preserved:

Quantitative signal amplification

Protease-free tissue processing

Subcellular spatial resolution

Minimal non-specific background

This platform extends high-fidelity ISH to short targets for the first time, enabling neuroscientists to visualize small regulatory RNAs and isoform-specific signals with confidence, expanding the molecular toolkit for understanding gene expression dynamics in the brain.

Disclosures: A. Acharya: A. Employment/Salary (full or part-time): Molecular Instruments. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Molecular Instruments.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.09/VV8

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: Papiit IN215725
INB Proteogenomic Unit

Title: Construction of the concatenated oligodendroglial GABA_A receptor $\alpha 3\beta 2\gamma 1$.

Authors: *J. MALDONADO^{1,2}, P. CRUZ LAZO^{1,2}, E. GARAY², R. ARELLANO²;
¹Univ. Nacional Autónoma de México, Juriquilla, Mexico; ²Inst. of Neurobiology-UNAM, Queretaro, Mexico

Abstract: GABA_A receptors are pentameric ion channels. In myelinating cell lineage of the nervous system, oligodendrocyte precursor cells (OPCs) and oligodendrocytes (OLs), the GABA_A receptor is mainly composed of $\alpha 3\beta 2\gamma 1$ subunits. This receptor has pharmacological characteristics that distinguish it from those more commonly expressed in neurons (i.e., $\alpha 1\beta 2\gamma 2$, $\alpha 1\alpha 2\beta 2\gamma 2$). For example, unlike neuronal receptors, the oligodendroglial receptor is positively modulated by β -carbolines such as β -CCB. Using *in vivo* models of demyelination, we have shown that systemic administration of β -CCB promotes robust remyelination. Heterologous expression of this receptor in model systems such as *Xenopus laevis* oocytes or HEK293 cells would allow us to characterize molecularly the binding sites of β -CCB and other drugs. Initially, we have used this strategy by independently expressing each of the subunits that make up the receptor, however, the results obtained vary due to differences in the expression levels of each subunit, or by differences between the heterologous expression systems. To avoid these variability sources, this work proposed the construction of a concatenated receptor with the subunits arranged $\gamma 1$ - $\beta 2$ - $\alpha 3$ - $\beta 2$ - $\alpha 3$. To this end, we designed oligonucleotides to generate by PCR assays coding sequences for each of the subunits, modified at their amino and carboxyl termini to bind them and promote proper folding and achieve a functional receptor. The oligonucleotide sequences included recognition sites for restriction enzymes that are naturally present in the coding sequence of some of the subunits. To eliminate the HindIII restriction site of the $\beta 2$ subunit, which could prevent directional cloning, site-directed mutagenesis was performed. This work presents the results of the mutagenesis and the partial construction of the concatenated oligodendroglial GABA_A receptor.

Disclosures: J. Maldonado: None. P. Cruz Lazo: None. E. Garay: None. R. Arellano: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.10/VV9

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Cisplatin induces immunosuppressive gene networks in the healthy rat brain: implications for neurotoxicity and glioma susceptibility

Authors: *O. TORRES-PINEDA¹, L. LOPEZ-MERAZ², C. MORGADO-VALLE³, M. MACÍAS-CARBALLO⁵, L. BELTRAN-PARRAZAL⁴;

¹Inst. de Investigaciones Cerebrales-Universidad Veracruzana, Xalapa, Veracruz, Mexico;

²INSTITUTO DE INVESTIGACIONES CEREBRALES, ³Univ. Veracruzana, Xalapa, Mexico;

⁴Univ. Veracruzana, Xalapa-Enriquez, Mexico; ⁵Dept. de clínicas, Univ. de Guadalajara, Tepatitlán de Morelos Jalisco, Mexico

Abstract: Cisplatin (CP), a widely used antineoplastic agent, causes dose-limiting neurotoxicity within the central nervous system (CNS). To characterize CP-induced transcriptomic alterations in the healthy brain, we administered a clinically relevant cumulative dose of 20 mg/kg CP to Wistar rats (n = 10/group) over 5 cycles (2 mg/kg/injection, 48-hour intervals) with saline-treated controls.

Gene expression profiling was performed using custom-printed 70-mer oligonucleotide microarrays (~5,000 *Rattus norvegicus* probes). Differential expression analysis identified 287 genes ($|Z\text{-score}| \geq 2$): 123 upregulated and 164 downregulated. Functional enrichment analysis (KEGG, Gene Ontology) revealed significant disruption of synaptic transmission (*CAMK4*, *PLCG1* downregulated 2.1-2.6 fold), neuroplasticity (*SH2B1*), and notably, immune surveillance pathways (*CXCR2*, *MAPK14* downregulated).

Integration with low-grade glioma (LGG) datasets (TISIDB, GEPIA2) demonstrated that CP-downregulated immune genes correlated with reduced CD8⁺ T cell, NK cell, and memory B cell infiltration in human gliomas and predicted reduced overall survival in LGG patients, suggesting CP may create a pre-neoplastic immunosuppressive brain environment.

These findings reveal that CP disrupts neuroimmune homeostasis in healthy brain tissue, potentially increasing CNS vulnerability to tumorigenesis while causing direct neurotoxicity. This underscores the need for rationally designed neuroprotective strategies during CP-based chemotherapy.

Disclosures: O. Torres-Pineda: None. L. Lopez-Meraz: None. C. Morgado-Valle: None. M. Macías-Carballo: None. L. Beltran-Parrazal: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.11/VV10

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Selecting Your Epigenomic Assay: A Guide to ChIP, CUT&RUN, and CUT&Tag

Authors: C. R. COMEAU¹, F. CHEN¹, *C. J. FRY²;

¹Res. and Develop., Cell Signaling Technol., Danvers, MA; ²Res. and Develop., Cell Signaling Technol., BEVERLY, MA

Abstract: For many years, chromatin immunoprecipitation (ChIP) has been a go-to assay for studying histone marks and epigenetic mechanisms in development and disease. However, two new versatile techniques have recently been developed that are faster, easier, and cheaper than the ChIP assay. Cleavage Under Targets & Tagmentation (CUT&Tag) and Cleavage Under Targets & Release Using Nuclease (CUT&RUN) can be used for probing histone marks and protein-DNA interactions within the natural chromatin context of the cell.

Both CUT&Tag and CUT&RUN can be combined with NG-seq to analyze histone modifications and binding of transcription factors, DNA replication factors, or DNA repair proteins at specific target genes or across the entire genome. CUT&Tag and CUT&RUN are rapid, robust, and true low cell number assays for the detection of protein-DNA interactions. Unlike ChIP, both assays are free from formaldehyde cross-linking, chromatin fragmentation, and immunoprecipitation. When compared to ChIP, CUT&RUN and CUT&Tag require fewer starting cells (as low as 5,000 - 20,000), have a much faster protocol (one day from cells to DNA), and generate lower background signal (require less sequencing depth). In addition, CUT&Tag generates PCR-ready DNA fragments, eliminating the need for adaptor ligation steps during library DNA preparation. We have developed CUT&Tag and CUT&RUN assay kits and internally validated a broad set of antibodies against histone modifications, transcription factors and cofactors that are compatible with each assay.

I will discuss the basics of the CUT&Tag and CUT&RUN assays and important factors to consider when selecting which assay to use for your experiment. In addition, I will provide comparison data showing how these new assays perform versus ChIP and ChIP-seq. Finally, I will discuss the versatility of these assay for mapping various histone modifications, transcription factor, and transcription cofactor binding across multiple sample types, including cells and tissues.

Disclosures: C.R. Comeau: None. F. Chen: None. C.J. Fry: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.12/VV11

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Optimisation of a subtraction-free microarray-based protocol for accurate 5hmC quantification

Authors: O. RUSHTON¹, N. DUNTON¹, *M. KRISTIANSEN²;

¹UCL Genomics, UCL Great Ormond Street Inst. of Child Health, Univ. Col. London, London, United Kingdom; ²UCL Genomics, Univ. Col. London, London, United Kingdom

Abstract: Previously thought as just an intermediate in the conversion of 5-methylcytosine (5mC) to unmethylated cytosine, it's been established 5-hydroxymethylcytosine (5hmC) levels are relatively enriched in the brain comparative to other tissues and display heterogenous profiles across different cell types. 5hmC levels in the brain are estimated to be 10-fold higher than that of some other tissues and embryonic stem cells (where 5hmC has been associated with stem-like features); in Purkinje cells of the cerebellum, 5hmC modifications have been shown to be 40% more abundant than 5mC. Furthermore, the dysregulation of 5hmC has been associated with neurological disease such Alzheimer's and schizophrenia. Hence, there is a growing field of research investigating the functional importance of 5hmC and its applicability as a biomarker of neurological disease. The current gold-standard method to quantify 5hmC is HPLC-MS. Although this method of quantification is precise and independent of sample quality, caveats include the expensive equipment and expertise required to conduct such a method. The most common methods to quantify genome-wide 5hmC are sequencing based technologies. Protocols differ by the chemical transformation of 5hmC prior to sequencing for example as performed in the CAPS protocol, with Tet-mediated conversion prior to bisulfite conversion in the TAB-seq protocol and with A3A-mediated conversion in the ACE-seq protocol. In general, microarrays are a cheaper alternative to sequencing, have a less complex protocol and require less bioinformatic expertise to analyse data. Microarrays necessitate genome-wide profiling of pre-defined genomic loci, making arrays an appropriate platform to characterise 5hmC. To our current knowledge existing array-based methods, such as TAB-array and a modified ACE-seq protocol incorporate bisulfite conversion of DNA post selective enzymatic transformation of 5mC and 5C sites. The incorporation of bisulfite conversion across both protocols necessitates higher input requirements of DNA and reduces sequence complexity post conversion. The ideal protocol selectively transforms 5hmC sites prior to detection, is sensitive enough to quantify 5hmC with high sensitivity, and avoids the use of bisulfite conversion to distinguish 5hmC sites and is subtraction-free. Here we present findings from a bisulfite conversion-free microarray-based protocol, to directly quantify 5hmC by adopting the principles from chemical/enzymatic transformation-based sequencing protocols and modifying them to make them applicable to microarray workflows.

Disclosures: O. Rushton: None. N. Dunton: None. M. Kristiansen: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.13/VV12

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Single-cell spatial transcriptomic atlas of the primate superior colliculus

Authors: *N. YUAN¹, Y. LEI², Z. SHEN³;

¹Inst. of Neuroscience, Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chine,

Shanghai, China; ²BGI-Research, Hangzhou, China; ³Inst. of Neuroscience, SIBS, CAS, Shanghai City, China

Abstract: The superior colliculus (SC), a layered midbrain structure critical for multisensory integration and orienting behaviors, exhibits complex cellular architecture that remains poorly characterized in primates. In this study we present a transcriptome-defined cell type atlas of the macaque SC using an integrated approach combining single-nucleus RNA sequencing (snRNAseq) and spatial transcriptomics (stereo-seq). By snRNAseq, we identified distinct cell populations within the macaque SC, establishing a high-resolution taxonomy of neuronal and non-neuronal cell types. Spatial transcriptomics analysis mapped these molecularly defined cell types to their precise anatomical locations, revealing layer-specific distribution patterns. Comparative analysis with mice SC uncovered differentially expressed genes between homologous cell types across species. This study provides the spatial transcriptomic characterization of cell types in the primate superior colliculus, establishing a foundation for understanding the cellular basis of SC function in primates and its evolutionary divergence from rodents.

Disclosures: N. Yuan: None. Y. Lei: None. Z. Shen: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR364.14/VV13

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: Knut and Alice Wallenberg Foundation

Title: Using spatial transcriptomics to define the regional and cellular transcriptome landscape of the adult human brain

Authors: *N. MITSIOS¹, E. GERRITS¹, E. SKARWAN², P. OKSVOLD³, E. HUSÉN¹, T. ZHENG¹, K. VON FEILITZEN³, E. SJOSTEDT¹, M. PALKOVITS⁴, T. G. HOKFELT¹, J. MULDER¹;

¹Karolinska Institutet, Solna, Sweden; ²Dept. of Protein Sci., Dept. of Protein Science, Sci. for Life Laboratory, KTH-Royal Inst. of Technology, Stockholm, Sweden, Stockholm, Sweden;

³Human Protein Atlas, Stockholm, Sweden; ⁴Semmelweis Univ., Budapest, Hungary

Abstract: The Human Protein Atlas (HPA; www.proteinatlas.org) is a public online database that provides an integrated overview of protein expression and distribution in all major human tissue types, including 200 subregions of the human brain. There, a comprehensive overview of gene and protein expression in the main anatomical structures of the mouse, pig and human brain is provided, by combining in-house generated RNA sequencing and publicly available single-cell transcriptomic data with immunohistochemistry. Following recent advances in RNA-sequencing technologies, the field of high-resolution spatial transcriptomics has emerged as a powerful

technique for studying single-cell transcriptomes over space and these methods have provided valuable tools for mapping the spatial distribution of mRNA transcripts in the brain. The major advantages of these novel approaches are the possibility to study rare cell types with a small population size and the ability to investigate cells within local tissue environments. In the newest HPA version, released in October 2025, we have added spatial transcriptomic data, generated on the stereo-seq platform, to map the spatial patterns of gene expression across the main cell types (neurons, astrocytes, oligodendrocytes, microglia and vascular cells) from cortical, hippocampal and cerebellar tissues of the adult human brain. This approach has enabled the identification and spatial localization of all main cell types and offers insights into the structural and functional organization of these regions. All data presented is open access and available in the brain section of version 25 of the Human Protein Atlas (HPA25).

Disclosures: N. Mitsios: None. E. Gerrits: None. E. Skarwan: None. P. Oksvold: None. E. Husén: None. T. Zheng: None. K. von Feilitzen: None. E. Sjostedt: None. M. Palkovits: None. T.G. Hokfelt: None. J. Mulder: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.15/VV14

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: UM1HG011966
Banting Postdoctoral Fellowship to T.A.M

Title: Large-scale discovery of neural enhancers for cis-regulation therapies

Authors: *T. A. MCDIARMID¹, N. PAGE², J. SHENDURE¹;

¹Genome Sci., Univ. of Washington, Howard Hughes Med. Institute, Seattle Hub for Synthetic Biol., Seattle, WA; ²UCSF, San Francisco, CA

Abstract: CRISPR-based gene activation (CRISPRa) has emerged as a promising therapeutic approach for haploinsufficient neurodevelopmental disorders (NDD). Yet, scaling this cis-regulation therapy approach will require intricate knowledge regarding: 1) which candidate cis-regulatory elements (cCREs) are active in human neurons; 2) which genes these active elements regulate; and 3) which of these regulatory elements can be successfully targeted with CRISPRa to yield specific and therapeutically meaningful levels of upregulation of intended target genes. Here, we combined Massively Parallel Reporter Assays (MPRAs) and multiplex single-cell CRISPRa screening to uncover functional human neural enhancers that can be targeted with CRISPRa to yield specific upregulation of NDD risk genes. From an MPRA of 5,425 candidate enhancers surrounding 337 risk genes, we uncovered 2,422 active enhancers active in human neurons. We then applied large-scale multiplex single cell CRISPRa screening to test all MPRA-prioritized cCREs (2,422 enhancers, 761 promoters) in their endogenous genomic context (n =

200,517 cells with an average of 10 gRNAs multiplexed per cell). We identified hundreds of promoter- and enhancer- targeting CRISPRa gRNAs capable of upregulating 200/337 intended target genes, including upregulations mediated by 91 novel enhancer-gene pairs. We further demonstrate that regulatory elements we identified can display conserved autonomous enhancer activity in the developing mouse central nervous system in vivo, and that CRISPRa gRNAs we identified for *SCN2A* and *CHD8* yield target gene upregulation when delivered virally to human cerebral organoids. These results provide an unprecedented set of validated and target-linked human neural enhancers that can be used in the development of cis-regulation therapies, and contribute to our understanding of the functional circuitry of neural gene regulation.

Disclosures: T.A. McDiarmid: None. N. Page: None. J. Shendure: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.16/VV15

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: U19MH114830

Title: Epigenetic Landscape of Cell Types in the Adult Mouse Brain

Authors: *M. J. HOOPER¹, C. LEE³, D. YUAN⁹, D.-W. KIM¹⁰, Y. GAO², N. JOHANSEN¹¹, D. DWIVEDI², A. MERRITT-JOHNSON², J. ROTH¹², J. GOLDBY⁴, A. OSTER¹², S. W. WAY⁵, B. THYAGARAJAN², J. T. TING⁶, B. P. LEVI⁶, N. DEE¹⁰, K. SMITH², S. YAO¹², J. WATERS⁷, Y. BEN-SIMON², C. VAN VELTHOVEN², H. ZENG¹⁰, Z. YAO¹⁰, B. TASIC⁸; ¹Mol. Genet., ²Allen Inst., Seattle, WA; ³Modeling Analysis and Theory, ⁴BiCore, ⁵Mol. Genet., ⁶Human Cell Types, ⁸Cell and Circuit Genet., ⁷Allen Inst. For Brain Sci., Seattle, WA; ⁹Allen Inst. for Brain, Bothell, WA; ¹¹Human Cell Types, ¹²Mol. Genet., ¹⁰Allen Inst. for Brain Sci., Seattle, WA

Abstract: Purpose

The mouse brain comprises over 5,000 transcriptomically defined cell types. These cell type definitions can be used as a common framework to study cell types. Single-cell multiome data enables joint analysis of single-nucleus RNA (snRNA) and single-nucleus ATAC (snATAC) data, allowing deeper characterization of cell types. Putative cis- regulatory elements can be identified from cell type-linked snATAC data and can be used in combination with adeno-associated virus (AAV) to target specific cell types. Here, we describe and analyze a whole mouse brain multiome dataset to advance these goals.

Methods

Samples were collected by targeted brain dissection, followed by 10x multiome library preparation and sequencing. Low-quality cells and doublets were filtered out. Cell types were annotated by aligning transcriptomic data to a reference atlas using scVI. ATAC-seq peaks were

called with SnapATAC2 and MACS3. Downstream analyses were conducted using SnapATAC2, scvi-tools, and CRESTed. Enhancer activity was examined in vivo by retroorbital injection of AAVs with enhancer-driven SYFP2.

Results

We present a dataset of over 2.8 million cells collected from the entire adult mouse brain. Transcriptomically defined cell types have distinct regions of open chromatin, enabling identification of putative cell type-specific cis-regulatory elements across class, subclass, and supertype levels of the integrated taxonomy. Leveraging both data modalities, we integrated our data with community snATAC datasets, and constructed gene regulatory networks. Finally, DNA sequence modeling reveals the importance of TF motifs in driving cell type-specific epigenetic features in their native context. We show this by comparing enhancer-AAV driven SYFP2 expression in vivo, using histology.

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Poster

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Program #/Poster #: PSTR364.17/VV16

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: UM1MH130981
U24MH130918
R01MH060974
F32MH138113

Title: Joint analysis of anatomical and functional MRI, single cell multiomics, and spatial transcriptomics in macaque neocortex

Authors: *B. Q. ROSEN¹, D. HU², S. C. SEEMAN³, F. YAZDANIBANAFSHEDARAGH², T. COALSON¹, J. HARWELL¹, M. HUANG³, A. D. GARCIA³, R. D. HODGE³, T. IKEDA⁴, T. HAYASHI⁴, L. NG³, S. MUFTI³, E. LEIN³, D. C. VAN ESSEN¹, W. FREIWALD², M. F. GLASSER¹;

¹Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO; ²Rockefeller Univ., New York, NY; ³Allen Inst. for Brain Sci., Seattle, WA; ⁴RIKEN Ctr. for Biosystems Dynamics Res., Kobe, Japan

Abstract: The primate cerebral neocortex supports an extraordinary array of complex behaviors, but the spatial organization of cell types and gene expression underpinning these functions is

poorly understood. Due to interindividual variability, cellular and molecular features are best compared to functional organization in the same brains. A key challenge is to accurately register antemortem anatomical/functional MRI data to postmortem tissue samples dissected for snRNAseq and snATACseq analyses or histologically sectioned for spatial transcriptomics, as significant deformation occurs during removal, slabbing, and mounting. These challenges are particularly acute in gyrencephalic primate neocortex due to the large size and complex folding of the neocortical sheet. We present here (1) an exquisitely well-characterized macaque case study with high-quality antemortem anatomical MRI and >40 hours of awake task and resting-state fMRI recordings denoised using state-of-the-art HCP-style pipelines, (2) an integrated pipeline for functionally guided postmortem multiomic tissue sampling and analysis demonstrated in the same individual, and (3) Stereo-seq spatial transcriptomic sections precisely registered to the anatomical and functional MRI. In this case study, functional MRI revealed an intriguing interdigitation of novel task-negative default mode network patches with visual category selective patches in inferotemporal cortex, and spatial transcriptomic analysis revealed a diversity of neocortical cell type distributions including both spatially restricted cell types and cell types with broad heterogeneous spatial distributions. In preliminary analyses, gradients in transcriptomic features enabled delineation of some areal boundaries but were not uniformly consistent with anatomical and functional MRI gradients. The demonstrated precise registration to MRI will allow multiomic and spatial transcriptomic data to be accurately registered between individuals and to common coordinate frameworks. Taken together, these findings provide a solid foundation for bridging the gap between the microscale cellular and molecular neuroscience of transcriptomics and mesoscale systems and cognitive neuroscience of brain imaging.

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Poster

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Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.18/VV17

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: UM1MH130981
Paul G Allen Family Foundation

Title: A Whole-Brain Rhesus Macaque Spatial Transcriptomic Atlas

Authors: *S. C. SEEMAN¹, B. Q. ROSEN², D. HU³, F. YAZDANIBANAFSHEDARAGH³, M. HEWITT¹, Z. YAO¹, D. YUAN¹, O. KANA¹, R. D. HODGE¹, N. JOHANSEN¹, J. QUON¹,

M. KUNST¹, M. HUANG¹, J. ARIZA TORRES¹, L. NG¹, T. IKEDA⁴, L. KRUSE¹, C. M. PAGAN¹, T. JARSKY¹, T. HAYASHI⁴, M. F. GLASSER², D. C. VAN ESSEN², W. FREIWALD³, E. LEIN¹, H. ZENG¹;

¹Allen Inst. for Brain Sci., Seattle, WA; ²Washington Univ. Sch. of Med., Saint Louis, MO;

³Rockefeller Univ., New York, NY; ⁴RIKEN Ctr. For Biosystems Dynamics Res., Kobe, Japan

Abstract: The rhesus macaque is a critical model for understanding primate brain architecture, function, and disease. However, comprehensive, spatially resolved molecular atlases are limited. Here, we present a whole-brain spatial transcriptomic atlas of the adult rhesus macaque, generated using the Stereo-seq platform. This atlas profiled the spatial gene expression of more than 23 million cells across the entire brain, including not only the cortex—registered to individual functional MRI data—but also subcortical areas, including the cerebellum and brainstem. Unlike existing, region-specific atlases, our data enables the localization of diverse cell types and the mapping of continuous gene expression gradients throughout the brain, facilitating systems-level approaches to understanding primate brain organization. For instance, regional specializations and boundaries across the brain are evident in cell type localization alone. The whole-transcriptome nature of Stereo-seq further allows for investigation of gene expression profiles beyond typical marker genes. By mapping to conserved cell types between human and primate brains, this atlas highlights the potential for cross-species comparisons, providing crucial insights into the evolutionary conservation and divergence of brain organization and cellular identity. Specifically, this dataset reproduces the spatial organization of major basal ganglia cell types from the recently established BICAN Basal Ganglia Cross-species taxonomy underscoring the robustness of cell type identification across diverse spatial transcriptomic platforms. Finally, this atlas establishes a foundational resource for comparative neuroscience research and provides essential molecular context for functional and anatomical studies in non-human primate models.

Disclosures: S.C. Seeman: None. B.Q. Rosen: None. D. Hu: None. F.

YazdaniBanafsheDaragh: None. M. Hewitt: None. Z. Yao: None. D. Yuan: None. O. Kana: None. R.D. Hodge: None. N. Johansen: None. J. Quon: None. M. Kunst: None. M. Huang: None. J. Ariza Torres: None. L. Ng: None. T. Ikeda: None. L. Kruse: None. C.M. Pagan: None. T. Jarsky: None. T. Hayashi: None. M.F. Glasser: None. D.C. Van Essen: None. W. Freiwald: None. E. Lein: None. H. Zeng: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.19/VV18

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Unlocking the molecular mechanism of pathogenic and non-pathogenic Th17 cells with InTraSeq

Authors: *S. SUBRAMANIAN, M. ARISS, R. POLAKIEWICZ;
Cell Signaling Technol., Danvers, MA

Abstract: Single-cell RNA sequencing (scRNA-seq) provides a partial view of cellular function. To address this, InTraSeq™ enables the simultaneous measurement of mRNA, surface, cytoplasmic, and nuclear proteins within individual cells. This multimodal approach offers a comprehensive view of cellular function by combining RNA and protein profiling from the same sample. InTraSeq™ allows researchers to expand their view of critical intracellular protein expression, including post-translational modifications (PTMs) and transcription factors. This is valuable for understanding cell population heterogeneity and identifying distinct functional states. We used InTraSeq™ to characterize Th17 cell differentiation, simultaneously profiling RNA and protein expression in over 85,000 cells. Our results revealed novel insights into Th17 cell differentiation, including the identification of key regulatory factors and their target genes. By simultaneously measuring mRNA, extra and intra-cellular proteins, signaling proteins, and PTMs, InTraSeq™ offers a comprehensive understanding of cellular processes and enables the identification of novel regulatory mechanisms that could be applied to brain infiltrating T-cells, as well as other neuroinflammatory cells like microglia and astrocytes.

Disclosures: **S. Subramanian:** A. Employment/Salary (full or part-time): Cell Signaling Technology. **M. Ariss:** A. Employment/Salary (full or part-time): Cell Signaling Technology. **R. Polakiewicz:** A. Employment/Salary (full or part-time): Cell Signaling Technology.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.20/VV19

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

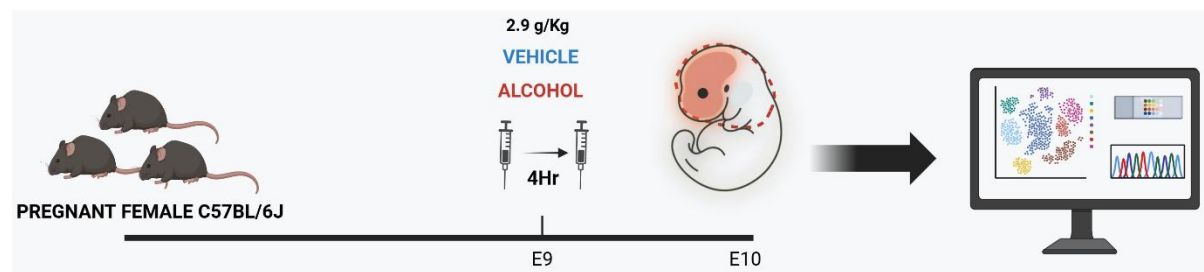
Support: NIAAA AA028273

Title: Fetal Alcohol Exposure Skews Neuroepithelial Trajectories via Chromatin and Transcriptional Rewiring

Authors: *K. BALACHANDRAN¹, H. SAWAL², S. FANG², K. BOSCHEN³;
¹Upstate Med. Univ., Syracuse, NY; ²Upstate Med. Univ., SYRACUSE, NY; ³Neurosci. and Physiol., Upstate Med. Univ., Syracuse, NY

Abstract: Investigating the impact of prenatal alcohol exposure (PAE) on the developing embryo at the genetic and epigenetics level is critical to understanding how alcohol disrupts craniofacial and brain development. To elucidate the transcriptomic alterations underlying alcohol-induced malformations, our current study examined gene expression and chromatin accessibility in tissue that will give rise to the brain and face following binge-like alcohol exposure during neurulation (embryonic day (E) 9 in mice, 3rd-4th week of pregnancy in humans). Our study used single-cell multiomic analysis to reveal disruptions to the

transcriptional and epigenetic landscape in the neuroepithelium and cranial neural crest cells. Integration of single-cell RNA-seq data identified cell type-specific transcriptional shifts, with the neuroepithelial and cranial neural crest cell populations showing the most pronounced divergence between PAE and vehicle-treated groups. Parallel chromatin accessibility analysis revealed overlapping differentially accessible regions (DAR) near these differentially expressed genes, suggesting coordinated transcriptional regulation. Correlation modeling demonstrated a weak but significant relationship between DARs and gene expression log2 fold changes. Pseudo-time trajectory analysis highlighted perturbed neurodevelopmental progression under PAE, while gene regulatory network inference revealed dampened activity of Tfap2b-centered regulons in PAE embryos. Enrichment analysis of differentially expressed and accessible loci implicated disrupted Wnt signaling, synaptic development, and neurogenesis pathways. Collectively, our results demonstrate that PAE induces spatiotemporal epigenetic priming of neurodevelopmental dysregulation during early embryogenesis.



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Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR364.21/VV20

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: 5R37DA054370-04

Title: Gene expression differences distinguish GABA neurons in the rostromedial tegmental nucleus and ventral tegmental area

Authors: *Z. J. HOUGH¹, N. SCHAFER², B. W. HUGHES³, C. W. COWAN⁴, E. J. NESTLER⁵, T. C. JHOU⁶;

¹Neurobio., Univ. of Maryland SOM, Baltimore, MD; ²Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; ³Friedman Brain Institute, Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ⁴Neurosci., Med. Univ. of South Carolina, Charleston, SC;

⁵Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; ⁶Neurosci., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD

Abstract: Recent studies have revealed significant diversity in the function and gene expression of ventral midbrain GABA neurons, which play a key role in modulating motivated behaviors. This highlights the need for a deeper understanding of the molecular differences among GABA neuron populations in the midbrain. Our research focused on two regions with a critical role in regulating motivated behavior: the ventral tegmental area (VTA) and the rostromedial tegmental nucleus (RMTg, also known as the tail of the VTA). Approximately one-third of VTA neurons are GABAergic and recognized for their heterogeneity. In contrast, RMTg neurons are primarily GABAergic, yet their diversity and potential overlap with VTA GABA subpopulations are not well understood. To investigate this, we performed single-nucleus RNA sequencing on VTA and RMTg tissue from two individual male and three pooled female 12-week-old Sprague Dawley rats and three pooled 12-week-old male C57Bl6/J mice. Each sample was multiplexed such that VTA and RMTg dissections were labeled prior to combining nuclei for library prep and sequencing, thus minimizing batch effects and enabling demultiplexing during analysis. Our analyses indicated that RMTg GABA neurons are genetically distinct from all VTA GABA neuron subtypes in both rats and mice, with *Foxp1* and *Sox14* among the top differentially expressed genes (DEGs) with elevated expression in the RMTg, consistent with previous findings. We further identified novel RMTg markers, including *Sulfl*, *Trpc4*, and *Cbln4*, which showed increased expression in RMTg GABA neurons compared to surrounding and VTA GABA neurons. Interestingly, *Tlll* was among the top DEGs in the RMTg in the rat but not mouse samples, while the other marker genes were consistent across both species. Using RNAscope, we assessed the spatial specificity of these markers in rat tissue, finding that *Foxp1*, *Sox14*, *Tlll*, *Sulfl*, *Cbln4*, and *Trpc4* were significantly elevated in the RMTg relative to adjacent cells and VTA GABA neurons. Notably, *Sox14* and *Tlll* exhibited the highest specificity for RMTg GABA neurons, though *Tlll* also had strong expression in dopamine neurons and is absent from RMTg GABA neurons in mice. Ongoing analyses, along with further spatial characterization using IHC, FISH, and evolving spatial transcriptomics methods, will further clarify distinct GABA neuron subtypes and their spatial distribution in these regions. In summary, our data indicate that RMTg and VTA GABA neurons have unique transcriptional profiles, suggesting different roles in regulating motivated behavior.

Disclosures: Z.J. Hough: None. N. Schaffer: None. B.W. Hughes: None. C.W. Cowan: None. E.J. Nestler: None. T.C. Jhou: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.22/VV21

Topic: J.02. Systems Biology and Bioinformatics

Title: Revolutionizing CNS tissue phenotyping with multiplexed primary antibody conjugates

Authors: L. MONTOYA¹, *A. CARTIER², S. CLARKE³, B. S. MANDAVILLI⁴;
¹Thermo Fisher Scientific, EUGENE, OR; ²Thermo Fisher Scientific, San Diego, CA; ⁴Cell.
Imaging and Analysis, ³Thermo Fisher Scientific, Eugene, OR

Abstract: Multiplex Immunohistochemistry (mIHC) is a powerful technique used to simultaneously detect and visualize multiple proteins within a single tissue section. By employing multiple antibodies, each conjugated to a unique fluorophore, mIHC provides a spatial map of tissue architecture, revealing intricate details about the spatial distribution, functional states, and interactions of various molecular markers and cell types within the native tissue environment. In this study, we utilize primary conjugated antibodies, which offer numerous advantages in mIHC. By eliminating the need for secondary antibodies as the end detection method, these ready-to-use conjugates streamline the staining protocol, making it more suitable for simpler, higher-throughput, and faster assay time. Using this new methodology, we explore detection of a range of protein markers across the central nervous system tissue types commonly used in biological research, employing a uniform labeling technique. To investigate the microenvironment of the neuroendocrine and observe how tumors infiltrate health tissue, we analyzed IHC labeling of neural biomarkers such as GFAP, PD-1, Olig 2, and NEFL in relevant tissues to provide insights into Grade 1 through Grade 4 tumors. Our findings reveal that PD-1 expression increases with tumor grade, and Olig2 plays a significant role in the progression of brain cancer stages. These markers allowed us to gain further insights into the intricate interactions between tumor cells and the surrounding healthy tissue. Furthermore, by staining all targets together on a single tissue slice, we obtained a comprehensive picture of the staining complexity at different time points of tumor growth, including tissue structure, tumor proliferation, and changes in expression levels over time. This poster demonstrates the effectiveness of our new mIHC methodology in providing detailed spatial information and highlights its potential for advancing our understanding of tumor biology and other complex tissue interactions.

Disclosures: L. Montoya: None. A. Cartier: None. S. Clarke: None. B.S. Mandavilli: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.23/VV22

Topic: J.02. Systems Biology and Bioinformatics

Title: Cell-type specialization and neural connectivity underlie gyri-sulci difference in the macaque cortex

Authors: Y. BAI, *Y. SUN;
Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci.,
SHANGHAI, China

Abstract: Gyri and sulci are key anatomical features of the primate cerebral cortex, distinguished by their structural and functional specialization. Here, we investigated their differences from a cell-type composition perspective. Using single-cell spatial transcriptomic data from adult macaque brains, we developed a customized deep learning model called gsModel to distinguish sulci from gyri based on local cell-type profiles. The gsModel method based on cell-type composition showed high accuracy (>90%) in predicting whether a region belongs to sulcus or gyrus, outperforming other machine learning models or anatomical features. By leveraging the model embeddings, we identified key cell types and their marker genes that likely underlie sulcus-gyrus distinctions, particularly emphasizing the role of upper-layer glutamatergic neurons and oligodendrocytes. Furthermore, with the integration of diffusion tensor imaging data, we found these compositional differences associated with variations in neural fiber projection patterns, suggesting a potential link between cortical cytoarchitecture and connectivity.

Disclosures: Y. Bai: None. Y. Sun: None.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.24/WW1

Topic: J.02. Systems Biology and Bioinformatics

Title: Advancing spatial discovery proteomics: Interrogation of Alzheimer's disease and Parkinson's disease neural tissue with a novel 1000+ plex Discovery Proteome Atlas

Authors: *A. HECK, A. LASLEY, G. ONG, T. THEISEN, M. VANDENBERG, C. WILLIAMS, M. HOANG, J. BEECHEM;
Bruker Spatial Biol., Seattle, WA

Abstract: Spatial proteomics is a transformative approach to resolve tissue heterogeneity in complex biological systems including the human brain. Whereas high-plex spatial transcriptomics has transformed tissue analyses, spatial proteomics has been limited by the low number of targets and limited proteome coverage, especially for targets critical to the latest neurobiology advances. Here we introduce the Human Discovery Proteome Atlas (DPA), a 1,100+ plex antibody-based proteomic assay for the GeoMx® Digital Spatial Profiler (DSP) platform. The GeoMx DPA assay is the highest spatial proteomics plex on the market with wide coverage of proteins that target critical immunology, oncology, and neurobiology processes. We profiled 32 ROIs (regions of interest) across cortical layers of the parietal and occipital lobes in brain sections from an Alzheimer's disease patient and compared them to similar regions in normal control tissue. We also profiled the spatial proteomic signatures in the postcentral gyrus, frontal lobe, temporal lobe, and cerebellum of Alzheimer's disease and normal tissues. We found differential expression of numerous protein targets between the disease and normal tissue that are spatially organized within brain regions. Additionally, we profiled thalamus tissue from a

Parkinson's disease patient and characterized the proteomic signature from multiple regions. We further characterized the specificity, sensitivity, and reproducibility of DPA in tumor tissue microarrays with 50+ tumor cases from various organs including the brain (e.g. atypical meningioma, malignant ependymoma of the right occipital lobe, anaplastic oligodendroglioma, glioblastoma). By capturing spatial information on over 1,100 antibody targets, DPA provides comprehensive insights into heterogeneous tissues with complex disease mechanisms, offering a discovery portal for novel therapeutic or biomarker identification.

Disclosures: **A. Heck:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **A. Lasley:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **G. Ong:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **T. Theisen:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **M. Vandenberg:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **C. Williams:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **M. Hoang:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **J. Beechem:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology.

Poster

PSTR364: Spatial and Multi-omics I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR364.25/WW2

Topic: J.02. Systems Biology and Bioinformatics

Title: Design and Development of the First Ready-to-Use Spatial Proteomics Panel for Human Neurobiology

Authors: *S. MILLER, Y. PEYMANFAR, R. MIHANI, M. POULIN;
Akoya Biosci., Marlborough, MA

Abstract: Spatial phenotyping has opened new frontiers in neuroscience by enabling high-dimensional, in situ profiling of the brain's cellular and molecular architecture. To meet the growing need for standardized tools in this space, we developed the PhenoCode™ Human Neurobiology Panel—the first ready-to-use, ultrahigh-plex antibody panel purpose-built for spatial proteomics in neuroscience research. Designed for compatibility with the PhenoCycler®-Fusion platform from Akoya Biosciences, this 40+ plex panel captures key markers across six neurobiological domains: • Neuronal cell types (e.g., NeuN, GABA, MAP2) • Neurodegenerative disease (e.g., amyloid-beta, phospho-tau isoforms) • Neuroinflammation and immune response (e.g., TMEM119, CD68, HLA-DR) • Neuroplasticity and remodeling (e.g., S100B, Ki67, MBP) • Blood-brain barrier and vascular integrity (e.g., Claudin-5, CD31, AQP4) • Astrocyte and endothelial interactions (e.g., GFAP, Vimentin) Marker selection was guided by literature-based relevance, expert-consultation, and technical performance in FFPE human brain tissue. For validation, the panel was tested on brain tissue from healthy individuals, Alzheimer's disease (AD) patients, and glioblastoma (GBM) cases, demonstrating its ability to resolve complex tissue architecture, identify disease-specific cellular states, and distinguish immune and glial activation patterns across conditions. This panel provides the neuroscience community with a scalable, standardized tool for spatial proteomics, accelerating discovery in neurodevelopment, neurodegeneration, and brain tumor biology.

Disclosures: **S. Miller:** A. Employment/Salary (full or part-time): Akoya Biosciences. **Y. Peymanfar:** A. Employment/Salary (full or part-time): Akoya Biosciences. **R. Mihani:** A. Employment/Salary (full or part-time): Akoya Biosciences. **M. Poulin:** A. Employment/Salary (full or part-time): Akoya Biosciences.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.01/WW3

Topic: J.04. Physiological Methods

Support: NIH Grant 2R01DC014420
NIH Grant 3R01DC018733

Title: A simple, inexpensive, battery-powered homeothermic warming pad for mice and rats

Authors: I. R. FLEITES, K. MORALES, ***S. D. ROPER**;
Physiol. & Biophysics, Miller Sch. of Medicine, Univ. of Miami, Miami, FL

Abstract: Anesthetics decrease core body temperature. This temperature drop can compromise the physiological status of an animal during a surgical procedure or an experiment. In particular, hypothermia alters many aspects of neural function. When recording nervous system activity in anesthetized animals, it is important keep their core temperature stabilized. Commercial heating pads for small animals are readily available but they are relatively expensive and can be

somewhat bulky. Further, commercially available warming pads often have power cords that must be plugged in to a 120/240V source. Consequently, transporting an anesthetized animal from one location (e.g., surgical suite) to another (e.g., imaging rig) involves moving power cords. Moreover, commercial devices are not always compatible with custom stereotaxic frames, microscope stages, or holding boxes. Here we describe an inexpensive, battery-powered, temperature-controlled heating pad for small mammals (e.g., mice, rats) and document its validity and utility. The device is portable, making it convenient for researchers who conduct procedures such as surgical preparations in one location and then relocate the anesthetized animal to another location for experimental recordings. Tests of the device in a cool room (20°) showed the apparatus keeps anesthetized mice normothermic $\pm 0.7^\circ$ for over 6 hours without supplemental heat (e.g., infrared lamp), despite $>15^\circ$ differential between ambient room temperature and animal's core body temperature. We demonstrate how the warming pad can be used for *in vivo* imaging of neuronal activity for a prolonged period in mice. In brief, the homeothermic heating pad provides a simple method for maintaining the core temperature of anesthetized small animals. It can be constructed in under 30 minutes, the components are readily available, and the cost is less than \$100. It is exceptionally useful for experiments on small mammals such as mice. The QR code below provides a description of this device.

Disclosures: K. Morales: None. S.D. Roper: None.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.02/WW4

Topic: J.04. Physiological Methods

Support: KAKENHI JP25H01156
KAKENHI JP24H01497
KAKENHI JP23K18485
KAKENHI JP24K03085
KAKENHI JP24K22259
KAKENHI JP23H03488
KAKENHI JP23K28178

Title: Quantitative large-scale voltage-sensitive dye imaging of intra- and interhemispheric connectivity in a Cuprizone-induced demyelination model at the anterior cingulate cortex

Authors: *T. TOMINAGA, K. TSUKUDA, M. TAKETOSHI, K. NAKASHIMA, Y. TOMINAGA;
Inst. of Neurosci., Tokushima Bunri Univ., Takamatsu, Japan

Abstract: Disruptions in both intra- and interhemispheric connectivity are key features of demyelinating disorders such as multiple sclerosis (MS). These disruptions affect the anterior

cingulate cortex (ACC), a critical hub for cortical communication. We quantitatively assessed functional connectivity within and between hemispheres via the ACC using wide-field voltage-sensitive dye imaging (VSDI) in a cuprizone (CPZ; bis-cyclohexanone-oxaldihydrazone)-induced demyelination and recovery model. VSDI's high temporal resolution and broad field of view make it ideal for mesoscale functional mapping in cortical slice preparations. Mice received a 0.3% CPZ diet for six weeks (CPZ group), followed by five weeks of recovery on standard feed (REC group). Control mice were fed powdered feed without CPZ. Coronal brain slices (350 μ m thick) containing the ACC and corpus callosum (CC) were prepared at defined bregma levels (1.34, 0.98, 0.62, and 0.26 mm) and stained with the voltage-sensitive dye Di-4-ANEPPS. Neural activity was evoked by electrical stimulation of layer II/III in the ipsilateral ACC, and fluorescence signals were recorded using a high-speed VSDI system (MiCAM03, Brainvision; 1 ms/frame; 256×256 pixels). Imaging was performed under ACSF perfusion with 1 μ M SR95531. Data were pooled from defined slice levels and analyzed using pixel-wise ANOVA. Slices were re-sectioned (12 μ m) and stained with FluoroMyelinTM Green for CC and anti-PLP antibodies for cortical myelin. Direct recording from the CC was hindered by background fluorescence from myelin, but both intrahemispheric spread and interhemispheric propagation via the ACC were robustly detected. Quantitative VSDI revealed significant impairments in interhemispheric connectivity in CPZ mice, with the most pronounced deficits at 0.62 mm from bregma (SL3), underscoring slice-specific vulnerability. In contrast, intrahemispheric signal propagation remained temporally intact, suggesting that CPZ-induced demyelination primarily reduced spatial signal extent rather than conduction speed. After the CPZ was stopped, the REC mice fully recovered, even though the histological analysis showed only partial remyelination. This dissociation between structural and functional recovery indicates that compensatory mechanisms support restored network function. These findings demonstrate the utility of quantitative wide-field VSDI for mesoscale functional mapping in demyelination models and contribute rare functional evidence on ACC-mediated interhemispheric communication via CC.

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Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.03/WW5

Topic: J.04. Physiological Methods

Support: NRF grant RS-2025-00562132
NRF grant RS-2025-02216928

Title: Optimizing a protocol for in vivo quantification of focused ultrasound-induced blood-brain barrier opening using two-photon fluorescence imaging in mice

Authors: *H. LEE¹, S. KIM²;

¹Dept. of Sci. in Korean Med., Kyung Hee Univ., Seoul, Korea, Republic of; ²Dept. of Physiol., Kyung Hee Univ. Col. of Korean Med., Seoul, Korea, Republic of

Abstract: The blood-brain barrier (BBB) is crucial for maintaining central nervous system (CNS) homeostasis, yet it prevents drug delivery to the brain. Focused ultrasound (FUS), when combined with microbubbles, offers a non-invasive method to transiently and locally open the BBB, presenting a promising strategy for delivering CNS therapeutics. However, existing imaging modalities often lack the resolution necessary to observe microscopic vascular changes in real time. This study employed in vivo two-photon (2P) microscopy to directly visualize and quantify FUS-induced BBB disruption in mice, with an optimized protocol to support consistent, high-resolution assessment. Adult mice (~30g) were anesthetized, and a cranial window was implanted over the somatosensory cortex. Fluorescent 10 kDa and 40 kDa dextran tracers were administered intravenously, and Lumason microbubbles were injected retro-orbitally before sonication. FUS was applied using a 1 MHz transducer (1 MPa, 10 ms bursts, 1 Hz, 120 s). Alignment was verified using anatomical landmarks and acoustic calibration. Two-photon imaging was performed immediately before and up to 30 minutes after FUS using a Ti:sapphire laser (800 nm). Z-stacks and time-lapse images captured tracer extravasation from cortical vessels. 3D image reconstruction and binary segmentation were performed using MATLAB to quantify leakage area, intensity, and spread. Our results showed rapid and localized dextran leakage from capillaries within minutes of FUS exposure, which was absent in control regions. Leakage increased for ~10-15 minutes and then stabilized. Tracer accumulation reflected the underlying vascular architecture, predominantly around capillaries and venules. The optimized protocol exhibited high reproducibility and facilitated the tracer leakage due to BBB opening. This study demonstrates that 2P microscopy provides precise, high-resolution visualization of FUS-induced BBB opening. It enables real-time assessment of microvascular dynamics and tracer leakage, thereby offering a robust tool for optimizing FUS-based drug delivery protocols and advancing neurotherapeutic applications.

Disclosures: H. Lee: None. S. Kim: None.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.04/WW6

Topic: J.04. Physiological Methods

Title: Studying reward encoding in auditory central processing pathway using two photon imaging in freely behaving mice

Authors: *Y. ZHANG¹, X. PENG², Q. XIONG³;

¹SBU Neurobio. & Behavior, Stony Brook, NY; ²SBU Neurobio. & Behavior, Stony Brook, NY;

³Neurobio. & Behavior, SUNY Stony Brook, Stony Brook, NY

Abstract: The sensory processing system exhibits remarkable plasticity, with neuronal activity in central pathways—such as the cortex and striatum—shaped not only by sensory input but also by task-related variables, particularly reward. However, it remains unclear whether the magnitude of reward associated with sensory cues modulates neural representations within these pathways, especially under freely behaving conditions. To address this, we implemented a newly developed dual-channel two-photon imaging system in freely behaving mice to investigate reward encoding in the central auditory pathway. In D1-Cre/Ai14 mice, we expressed GCaMP8f in the auditory cortex, enabling simultaneous imaging of D1-positive and D1-negative neurons during naturalistic behavior. We also extended our investigation to subcortical regions, auditory striatum, co-expressing CAG-jRGECO1a and GfaABC1D-GCaMP6f to monitor calcium dynamics in neurons and astrocytes, respectively. We quantified how reward magnitude modulates auditory responses in both cortical and striatal regions. This dual-channel, cell-type-specific imaging approach in freely moving animals provides a more comprehensive and ecologically valid view of how reward influences auditory processing across distributed brain circuits.

Disclosures: Y. Zhang: None. X. Peng: None. Q. Xiong: None.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.05/WW7

Topic: J.04. Physiological Methods

Title: Demonstration of awake and anesthetized in vivo hippocampal imaging using cranial cannula in mice

Authors: *Y. HWANG^{1,2}, J. YOON¹, S. LEE¹, Y. KANG¹, P. KIM²;

¹IVIM Technol., Daejeon, Korea, Republic of; ²KAIST, Daejeon, Korea, Republic of

Abstract: Deep brain imaging under awake conditions remains challenging but important for neural studies. Cranial imaging cannula systems have previously enabled such studies, providing access for confocal and two-photon microscopy. Here, we demonstrate an imaging protocol using a cranial imaging cannula and head-fixation system in Thy1-GCaMP6f transgenic mice and vascular tracer injection, to observe neuronal calcium signals, cellular morphology, and vascular responses in the hippocampus. Using a confocal and two-photon microscope system (>50 fps @512×512 pixels), we performed comparative demonstrations under awake and anesthetized (isoflurane, zoletil+xylazine) states. Our demonstrations enabled stable hippocampal imaging across both physiological conditions. We visualized neuronal structures and calcium dynamics in Thy1-GCaMP6f mice. Fluorescent-dextran injection outlined hippocampal vessels, enabling simultaneous neurovascular imaging. We observed neuronal activity patterns from the same mice under both awake and anesthetized states, while vascular imaging demonstrated vessel architecture under both conditions. The cranial cannula system

maintained optical access and mechanical stability throughout imaging sessions, suggesting its potential for chronic monitoring protocols. Image acquisition was possible in both states, but motion artifacts and signal stability differed between conditions. These differences are important for experimental design. While cranial cannula techniques for awake imaging are established, we found several technical factors that affect protocol success and imaging quality under different physiological states. This approach demonstrates the feasibility of dual-modality imaging through cranial cannula systems.

Disclosures: **Y. Hwang:** A. Employment/Salary (full or part-time): IVIM Technology. **J. Yoon:** A. Employment/Salary (full or part-time): IVIM Technology. **S. Lee:** A. Employment/Salary (full or part-time): IVIM Technology. **Y. Kang:** A. Employment/Salary (full or part-time): IVIM Technology. **P. Kim:** None.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.06/WW8

Topic: J.04. Physiological Methods

Support: NSF Grant # 2309589
NSF Grant # 2440448

Title: Agarose stamped device: a simple but effective immobilization method for zebrafish larvae through customizable 3D printed molds for danio rerio (zebrafish)

Authors: ***H. MEHRABI**, J. JUTOY, E. JUNG;
Mechanical and Industrial Engin., Univ. of Illinois Chicago, Chicago, IL

Abstract: Precise immobilization of zebrafish larvae is critical for high-resolution neural imaging and behavioral studies, yet conventional methods are often labor-intensive, low-throughput, and can induce stress. To address these challenges, we introduce the Agarose Stamped Device (ASD), a novel platform enabling efficient and gentle immobilization of zebrafish larvae for high-resolution imaging and behavioral assays. By employing a stamping approach to mold an array of consistent agarose wells, the ASD enables rapid, parallel, and reproducible positioning of larvae in a standardized orientation while minimizing stress and preserving viability. We validated ASD performance by comparing larval survival, heart rate, and imaging stability to conventional agarose embedding, finding no adverse effects on larval physiology and significantly enhanced imaging throughput and data consistency. Demonstrations in neuroscience and behavioral experiments underscore the device's versatility for diverse in vivo studies. The ASD offers a simple, cost-effective, and powerful tool for advancing zebrafish-based neuroscience and behavioral research.

Disclosures: **H. Mehrabi:** None. **J. Jutoy:** None. **E. Jung:** None.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.07/WW9

Topic: J.04. Physiological Methods

Title: Assessing cortical behavioral correlates with simultaneous EEG and widefield optical imaging

Authors: C. SCANNELL, S. FISCHBACH, S. LIPINSKI, *S. JAMES;
Psychology, Sacred Heart Univ., Fairfield, CT

Abstract: The interplay of cortical function and behavior holds the potential to be a critical investigative avenue for developmental, neurological, and behavioral disorders. Various dynamic components (neural, glial, and vascular) underly this interplay and are key targets for therapeutic development. We present a multimodal approach combining electroencephalography (EEG) and widefield optical imaging (WOI) to assess the cortical function of freely behaving rodents during behavioral assessment. This approach was developed to allow for the assessment of the spatiotemporal cortical correlates of behavior, both global and regional, that underly numerous disorders. We wirelessly measured cortical activity by creating a cranial window for WOI and implanting three electrodes into the skull for EEG. The recording device was mounted to the heads of the animals and transmitted data over Wi-Fi. While EEG provides high temporal resolution recordings of global cortical activity, WOI captured mesoscale neural dynamics via genetically encoded calcium indicators and vascular fluctuations through intrinsic optical properties. Behavior was analyzed using custom MATLAB scripts that are also tailored to correlate EEG spectral features with surface-level calcium and hemodynamic transients. Behavioral assessments included; open field, object recognition, elevated zero maze, and 3-chamber social tests. Our preliminary findings (n=3) demonstrate the utility of this approach by capturing an average increase of 28% (EEG), 8% (neural fluctuations), and 16% (hemodynamic response) during non-rest events across the behavioral assessments. This project aims to enhance the interpretability of the interplay between cortical activity and behavior and provide a detailed mapping of cortical state dynamics to support the development of novel therapeutics.

Disclosures: C. Scannell: None. S. Fischbach: None. S. Lipinski: None. S. James: None.

Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR365.08/WW10

Topic: J.04. Physiological Methods

Title: Comparison of Reference Standards Between Pulmotest Godart and Spirobank II in Respiratory Function Assessment in Conakry

Authors: *S. DIALLO^{1,2,3}, M. K. DIALLO⁵, M. DIALLO⁴;

¹Gamal Abdel Nasser Univ. of Conakry, Conakry, Guinea; ²Neurol., ³Neurophysiol., Hosp. Natl. Ignace Deen, Conakry, Guinea; ⁴Neurol., Hosp. Natl. Ignace Deen, CONAKRY, Guinea;

⁵Neurol., Carle Fndn. Hosp., Urbana, IL

Abstract: Introduction: Pulmonary function testing (PFT) is essential for diagnosing and monitoring chronic respiratory diseases such as asthma and chronic bronchitis. In Sub-Saharan Africa, particularly in Guinea, the mechanical Pulmotest Godart is commonly used, although its results lack precision. The modern portable Spirobank II provides more accurate data. This study aimed to compare the reference values from the Pulmotest Godart and the Knudson norms from the Spirobank II. Methods: A retrospective study conducted from January 1, 2019, to January 1, 2025, including 100 patients (50 males, 50 females), aged 8 to 95 years, who underwent PFTs at the Conakry physiology lab. The following parameters were measured and compared between devices: age, height, weight, SVC, FVC, FEV1, PEF, FEF25-75, FEF25, FEF75, MVV calculated, and MVV measured. Student's t-test was used for comparing means, with significance set at $p < 0.05$. Results: Significant differences were observed between most parameters from the two devices, except for PEF, where the results were similar (Pulmotest: 7.90 ± 1.34 ; Spirobank II: 7.69 ± 1.53 ; $p = 2.74 \times 10^{-8}$). Conclusion: The study demonstrated notable discrepancies between results from the Pulmotest Godart and Spirobank II. These differences may be attributed to device calibration, reference data used, and operator variability. The adoption of modern devices such as the Spirobank II could enhance the accuracy of PFTs in Guinea.

Disclosures: S. Diallo: None.

Poster

PSTR365: Optical Methodology: Application

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR365.09/WW11

Topic: J.04. Physiological Methods

Title: Non-genetic photostimulation of neurons using a flexible, organic polymer-based photovoltaic device

Authors: *H. CUI¹, X. HOU¹, C. N. YI², J. SHI³, G. HONG¹;

¹Materials Sci. and Engin., ²Bioengineering, ³Chem. Engin., Stanford Univ., STANFORD, CA

Abstract: Neural activity can be modulated using optogenetic and photothermal techniques, each providing distinct advantages for interfacing with neural circuits. While optogenetics offers

cell-type specificity, its reliance on gene delivery limits clinical applicability. Implantable optoelectronic systems provide a non-genetic alternative, enabling light-driven neuromodulation without the use of viral vectors. However, existing implantable optoelectronic systems often face limitations such as the need for tethered power or communication systems, which can hinder long-term biocompatibility in freely moving subjects.

By leveraging near-infrared (NIR)-responsive photovoltaic materials, photonic energy can penetrate deep tissue to wirelessly power implanted devices that electrically interface with the brain by leveraging reduced scattering of NIR photons. We present a flexible, organic semiconductor-based photovoltaic platform for non-genetic neural stimulation, utilizing a wirelessly powered NIR interface. Compared to silicon-based devices, our organic materials provide mechanical flexibility and biocompatibility, making them ideal for chronic implantation in freely moving subjects.

Candidate materials for our device's multilayer structure were identified, followed by optimization of the fabrication protocol focusing on solvent compatibility and aqueous stability. Our NIR-responsive photovoltaic device was characterized under illumination at 785 nm and 860 nm, which are optimized to afford maximum tissue penetration and minimum tissue absorption. Performance was validated through in vitro targeting of rodent cortical neurons using a confocal setup with calcium imaging and NIR laser stimulation.

This work demonstrates the potential of organic optoelectronic materials to expand the neuromodulation toolkit, particularly in clinical settings where genetic manipulation may not be feasible, thereby paving the way for advancements in neurotechnology.

Disclosures: H. Cui: None. X. Hou: None. C.N. Yi: None. J. Shi: None. G. Hong: None.

Poster

PSTR365: Optical Methodology: Application

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Topic: J.04. Physiological Methods

Support: Ministry of Science and ICT Korea 2021R1F1A1062265
Ministry of Science and ICT Korea DGIST R&D Grant 24-NT-01

Title: An optical imaging approach to simultaneously monitoring the human brain and spinal cord activity for movement control

Authors: *H. KIM;
DGIST, Daegu, Korea, Republic of

Abstract: Corticospinal interactions are essential to produce and modulate muscle force for our daily activities. Magnetic and electrical imaging approaches to simultaneous monitoring of the cortical brain and spinal cord activity are limited to conditions with little body movement and processing via batches of data. In this study, we propose an optical imaging approach based on

functional near-infrared spectroscopy that enables the simultaneous monitoring of hemodynamic activity in the cortical brain and spinal cord during body movement in real time. We show that the spatial activation patterns of cortical and spinal cord motor clusters identified during the hindlimb movement by this approach are comparable to those identified during intraoperative surgeries. Furthermore, our temporal analysis indicates that the motor clusters in the brain and the spinal cord associated with the hindlimb movement are synchronously activated and bidirectionally connected during the motor task. These results suggest that hemodynamic responses reflect synchronized brain and spinal cord neural activities for motor control of body movement.

Disclosures: H. Kim: None.

Poster

PSTR365: Optical Methodology: Application

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Program #/Poster #: PSTR365.11/WW12

Topic: J.04. Physiological Methods

Support: NIH 5R01DA029639-12

Title: Automated Quantification of Morphology in HEK Cells, N2A Cells and CD1 E15 Mouse Primary Neurons

Authors: *A. D. VANDELOO¹, K. STILLWAGON², N. MALTA², A. HOLBERTON², C. VAN ZYL², C. R. FOREST³;

¹BioEngineering, ³Mechanical Engin., ²Georgia Inst. of Technol., Atlanta, GA

Abstract: Primary neuron culturing requires years of hands-on experience to master and visually recognizing changes in morphology is a critical skill. Experts use brightfield microscopy to qualitatively assess cell culture health, differentiation, or stress. Quantitative validation of these cell attributes requires costly, sample-consuming methods such as immunocytochemistry or transcriptomics. We hypothesize that if trained scientists can qualitatively identify trends in cell morphology visually, then computer vision should be able to do so quantitatively—provided cells can be accurately segmented. **We present SamCell; an open-source tool for high-throughput segmentation and shape analysis of cells.** SamCell uses a fine-tuned version of Meta's Segment Anything Model with custom pre/post processing to extract cell features including area, circularity, aspect ratio, and other shape features. These metrics enable dynamic shape analyses for neurons with minimal user input. To train and test this model for neuroscience, we used images of a mouse neuroblastoma line (N2A cells) at different confluencies to mimic neurosphere and rounded cell morphologies. We quantified HEK cells to mimic astrocyte and immature neuron morphology. We used over 2,000 manually labeled cells for each group. We show that **SamCell performs well in the model neuron conditions**, mimicking primary neurons and achieving a Jaccard index of 0.7 on the N2A cells

and significantly outperforming existing tools such as Cellpose. Further, **preliminary studies show positive results on primary cortical neurons from CD1 E15 mice**. SamCell offers a noninvasive platform for quantitative morphologically guided training and discovery for neuronal cell culture.

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Poster

PSTR365: Optical Methodology: Application

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Program #/Poster #: PSTR365.12/WW13

Topic: J.04. Physiological Methods

Support: Philanthropic Consciousness Research Fund

Title: Peri-somatic Photo-modulatory Response Variation due to Unconsciousness and Conscious Mental Activity

Authors: *S. A. HELEKAR^{1,5}, D. GOTUR^{2,6}, A. DHALA^{8,7}, A. NGUYEN⁹, L. RAJESH KUMAR³, L. NGUYEN⁴, B. JOHN⁴;

²Med., ³Psychiatry, ⁴Translational Biomagnetics and Neurometry Program, ¹Houston Methodist Res. Inst., Houston, TX; ⁵Psychiatry, ⁶Med., ⁷Surgery, Weill Cornell Med. Col., New York, NY; ⁸Surgery, Houston Methodist Res. Inst., Houston, TX; ⁹Neurosurg., Houston Methodist Hosp., Houston, TX

Abstract: Real-time noninvasive recording of correlates of possible changes in consciousness and conscious mental activity on the millisecond timescale can presently only be attempted using electroencephalography and magnetoencephalography. The underlying assumption of this approach is that conscious events are a reflection of patterns of neuronal electrical activity. We have recently discovered that a new type of biophysical phenomenon produces noninvasively recordable millisecond-scale changes that vary with the state and level of consciousness, such as reduced response amplitude and rise-time in anesthetized mice. These changes appear to be neural in origin and extend into the peri-somatic space where they can modulate the intensity of diffracted light emerging from a low power laser diode. They can therefore easily and highly reproducibly be measured by a non-contact photo-electronic device called a Sentiometer, developed in our laboratory.

Here we present the results of two pilot studies designed to investigate further variations in the photo-modulatory sentiometric response (SR). We conducted 2 - 3 recordings of a 30-min SR at 1-cm peri-manual distance on different days in each of 8 patients being treated in the ICU. Three patients had acute primary brain impairments due to neurovascular or neurodegenerative conditions and were not sedated. Five other patients had hepatic failure and were administered varying doses of sedatives. Their results were compared with those obtained from 5 conscious

healthy adults under the same conditions in the ICU. The SR in neurological patients was significantly attenuated and showed a premature decline, despite persistent exposure of the hand to the device sensor. The amplitude of the SR also showed significant reduction in sedated patients with hepatic failure.

The second study tested whether focusing on playing a video game for 10 min 3 times during sentiometric recording with the device sensor attached to the left temporal region of the head causes a change in the response amplitude each time compared to matched control conditions. Analysis of the sentiometric data in 3 healthy subjects showed marked deviations of the recorded baseline corresponding to the anticipation and performance of the cognitive task. The deviations were significantly more pronounced in the test conditions compared to the control conditions. These findings suggest that the newly discovered photo-modulatory effect not only reflects alterations of the level of consciousness but also the complexity of the content of a conscious experience requiring attention, making the Sentiometer useful in both basic and clinical neuroscience.

Disclosures: **S.A. Helekar:** A. Employment/Salary (full or part-time): Houston Methodist Research Institute. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Houston Methodist Hospital. **D. Gotur:** A. Employment/Salary (full or part-time): Houston Methodist Hospital. **A. Dhala:** A. Employment/Salary (full or part-time): Houston Methodist Hospital. **A. Nguyen:** A. Employment/Salary (full or part-time): Houston Methodist Hospital. **L. Rajesh Kumar:** A. Employment/Salary (full or part-time): Houston Methodist Research Institute. **L. Nguyen:** A. Employment/Salary (full or part-time): Houston Methodist Research Institute. **B. John:** A. Employment/Salary (full or part-time): Houston Methodist Research Institute.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

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Topic: J.04. Physiological Methods

Support: NIH U01NS139877-01
NIH U01NS131914-02
NIH 1DP2EB030992-01

Title: Multimodal experiments to study neural activity and synchronization in cortical organoids and assembloids

Authors: ***M. WILSON**¹, J. YEH⁴, E. K. KHARITONOVA⁵, S. BEYENE⁶, S. HEMAIDAN², C. CRAIN⁴, J. LEE⁷, M. RAMEZANI³, I. SURUCU², E. CUBUKCU², F. MORTAZAVI⁸, M. THUNEMANN⁹, E. ZELDICH¹⁰, D. KUZUM¹¹;

¹Univ. of California San Diego, La Jolla, CA; ²Univ. of California San Diego, San Diego, CA;

³Electrical and Computer Engin., Univ. of California San Diego, La Jolla, CA; ⁴UCSD, La Jolla,

CA, ; ⁵Vassar Col., Somerville, MA, ; ⁶Boston Univ. Aram V. Chobanian & Edward Avedisian Sch. of Med., Boston, MA, ; ⁷UCSD Dept. of Neurosciences, La Jolla, CA, ; ⁸Anat and Neurobio, Sch. of Med., Boston, MA; ⁹Biomed. Engin., ¹⁰Boston Univ., Boston, MA; ¹¹UC San Diego, La Jolla, CA

Abstract: Cortical organoids are revolutionizing the ability to study neural networks in vitro. The higher complexity of organoid's 3D cellular structure compared to 2D cultures allows them to better recapitulate features of brain development. However, current methods to study organoid electrophysiology utilize flat, 2D electrode arrays. To gain a holistic view of organoids' activity, technologies which can conform to organoids' 3D organization are needed. Here, we create a flexible multielectrode array which deflects under the weight of organoids. The flexibility allows the array to measure cell activity in 3D space while keeping organoids suspended in media for longitudinal experiments without altering the natural growth and maintenance of the organoid. Brightfield microscopy and immunohistochemistry reveal that organoid cells make tight contact with the arrays, facilitating high-SNR recordings. The arrays allow combination of 2-photon imaging with simultaneous recordings of electrical activity. We developed computational methods to investigate evolution and synchronization of neural activity in single organoids and assembloids, generated through the organoids' fusion. This novel, multimodal system combined with computational methods will yield insights into organoid network assembly in longitudinal, in vitro experiments, furthering our understanding of brain development and disorders.

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Poster

PSTR366: Electrophysiology: Electrode Arrays II

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Topic: J.04. Physiological Methods

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Title: Multimodal investigation of cortical traveling waves using transparent graphene arrays and two-photon imaging

Authors: ***M. ASLIHAK**¹, **M. RAMEZANI**¹, **T. KOMIYAMA**², **T. P. COLEMAN**³, **L. E. MULLER**⁴, **D. KUZUM**⁵;

¹Electrical and Computer Engin., Univ. of California San Diego, La Jolla, CA; ²Ctr. for Neural Circuits and Behavior Rm 304, UCSD, La Jolla, CA; ³Bioengineering, Stanford Univ., Stanford, CA; ⁴Dept. of Mathematics, Western Univ., London, ON, Canada; ⁵Electrical and Computer Engin., UC San Diego, La Jolla, CA

Abstract: To understand complex, large-scale neural dynamics such as traveling waves, we need technologies that can access multiple brain areas with minimal tissue damage while providing high spatial and temporal resolution. Optically transparent graphene microelectrodes make it possible to record surface field potentials while imaging calcium fluorescence from deeper layers of the cortex simultaneously. In this work, we implanted transparent, flexible, high-density graphene microelectrode arrays over the cortex of transgenic mice and simultaneously performed two-photon calcium imaging across different layers (L1 and L2/3) of the visual cortex. Using the generalized phase method, we detected cortical traveling waves via a 64-channel graphene array spanning multiple brain areas, including the visual cortex, retrosplenial cortex, posterior parietal cortex, and somatosensory cortex. We analyzed the rate of wave occurrence, propagation speed, and direction before, during, and after a visual stimulus (drifting grating), as well as during spontaneous activity, to investigate how external stimuli modulate cortical waves and whether these waves influence visual perception. Leveraging the unique multimodal recordings, we further examined the calcium response from L1 and L2/3 neurons captured by two-photon microscopy to investigate whether activity from different layers correlates or contributes to the waves detected at the cortical surface. We also assessed the excitability of these neurons during wave events and explored whether the waves modulated their orientation and direction selectivity.

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Poster

PSTR366: Electrophysiology: Electrode Arrays II

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Title: Low-cost laser-induced graphene microelectrode arrays for electrophysiological interfacing with electrically excitable organoids

Authors: *C. CRAIN¹, M. WILSON², F. SUN³, M. RAMEZANI², E. CUBUKCU¹, D. KUZUM⁴;

¹UCSD, La Jolla, CA; ²Electrical and Computer Engin., ³Univ. of California San Diego, La Jolla, CA; ⁴UC San Diego, La Jolla, CA

Abstract: Organoids are three-dimensional, stem cell-derived tissue cultures that recapitulate key structural and functional features of human organs, offering powerful models for studying development, disease, and therapeutic responses. Electrically excitable organoids—such as

cortical and cardiac—are increasingly used to investigate network dynamics, electrophysiological properties, and responses to pharmacological interventions. Microelectrode arrays (MEAs) provide a non-invasive interface for recording spontaneous electrical activity and delivering controlled stimulation to organoids, enabling real-time functional assessment. However, traditional MEA platforms often rely on complex, cleanroom-based fabrication methods that are costly, time-intensive, and inaccessible to many research settings, limiting broader adoption and scalability. Here, we present a low-cost, cleanroom-free microelectrode array platform fabricated using direct laser printing and pyrolysis on polyimide film, designed to interface with electrically excitable organoids. This printable approach enables simultaneous electrophysiological recordings and electrical stimulation, providing a scalable and accessible tool for organoid research. This work demonstrates a reproducible, low-cost approach to organoid electrophysiology that supports broader adoption of functional studies in developmental neuroscience, disease modeling, and pharmacological screening.

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Poster

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Title: Smart Dura: Large-scale multimodal interface for neural recording and modulation in non-human primates

Authors: *N. HONG¹, S. MONTALVO VARGO², G. HATANAKA¹, Z. GONG¹, N. STANIS¹, J. ZHOU¹, T. BELLOIR¹, H. PAN¹, R. WANG¹, W. BAIR¹, M. CHAMANZAR², A. YAZDAN-SHAHMORAD¹;

¹Univ. of Washington, Seattle, WA; ²Carnegie Mellon Univ., Pittsburgh, PA

Abstract: A neural interface for multimodal recording and modulation is a powerful tool to understand brain function and dysfunction by enabling multiscale information integration with high spatiotemporal resolution. Despite significant recent efforts to develop multimodal interfaces, most demonstrations have been primarily in small animal models, and there is still a

strong need to scale up interfaces for applications to larger animals, such as non-human primates (NHPs). In this study, we present a large-scale neural interface for NHPs, named Smart Dura, which is a functional version of an artificial dura, providing electrophysiological recordings and high optical accessibility to large cortical areas. We demonstrate the multimodality of Smart Dura by combining it with several optical imaging and manipulation techniques. First, we used two- and three-photon microscopy to validate optical imaging capability and succeeded in obtaining images of cortical vasculature through the Smart Dura at depths of up to 200 and 550 μm , respectively. To explore the versatility of Smart Dura for optical intervention in addition to imaging, we employed photothrombotic lesioning that can induce ischemic stroke through photosensitive dye injection and localized light illumination. Before and after the stroke induction, we performed optical coherence tomography (OCT) angiography through the Smart Dura, showing the significant reduction in blood flow centered on the illuminated area. We also measure electrical signals using Smart Dura to assess stroke-induced changes in neural activity, showing a strong gamma power decrease in the stroke-induced region. To demonstrate simultaneous electrophysiological recording and optical functional imaging, we applied visual stimulation and imaged evoked responses in the primary visual cortex via intrinsic signal optical imaging (ISOI). We simultaneously recorded electrical signals from the same cortical area using Smart Dura and found consistent chromatic and ocular dominance patterns between the ISOI responses and high-gamma power from electrophysiology. Lastly, to show the applicability for optogenetic experiments, we tested Smart Dura in an animal that had widespread expression of an inhibitory opsin Jaws into the posterior parietal cortex. By delivering red-light stimuli through the Smart Dura and recording evoked responses, we observed a significant broadband power increase in the opsin-expressing region. We believe that this multimodal neural interface for NHPs will provide significant translational information and will further promote neurotechnology that will ultimately be applicable to humans.

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Poster

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Topic: J.04. Physiological Methods

Support: 2023 Adler Endowed Fund for Traumatic Brain Injury Research

Title: Covert speech comprehension measurements with standard clinical EEG

Authors: T. PRASANT¹, B. HA¹, K. E. WEAVER², S. HAKIMIAN³, J. COULT⁴, *P. J. SCHWAB^{5,3};

¹Data Sci., ²Radiology, ³Neurol., ⁴Med., ⁵Univ. of Washington, Seattle, WA

Abstract: Background: The detection of covert cortical processing (CCP) in critically ill comatose patients is a developing topic with significant clinical and ethical implications. Methods to detect covert language processing can predict neurologic outcome in traumatic brain injury patients (Sokoliuk 2021). Covert language processing methods have utilized high-density EEG (hdEEG), but hdEEG is not widely available in the clinical setting. By contrast, standard montage EEG (smEEG) is available in clinical settings and may offer potential to detect covert language processing and assess CCP. **Aim:** We sought to demonstrate proof-of-concept to use smEEG to detect covert language processing. **Methods:** We used a modified version of the passive auditory EEG protocol developed by Sokoliuk et al. (2021) to measure EEG oscillations and their coherence with an auditory stimulus consisting of streams of isochronous monosyllabic words organized into 2-word phrases and 4-word sentences. Four control patients were recruited from the Epilepsy Monitoring Unit. EEGs were recorded using smEEG and filtered to remove drift and noise. We applied independent component analysis to clean artifacts, and low-pass filtered the signal at 4 Hz to retain speech-relevant frequencies. We computed Inter-Trial Phase Coherence (ITPC) for each frequency to identify coherence peaks at ~3.1 Hz (word frequent), ~1.6 Hz (phrase frequency) and ~0.8 Hz (sentence frequency) in each individual electrode. **Results:** Two control subjects exhibited elevated ITPC around 3.1 Hz, suggesting that their EEG oscillations were phase-locked to the four-word sentences. Mean (standard deviation [SD]) ITPC at 3.1 Hz rose to 0.38 (0.02) during speech, compared with a pre-stimulus baseline average of 0.10. These peaks were most prominent in frontal and temporal electrodes (e.g. FT9 and T8), consistent with speech-entrainment. ITPC peaks at 1.6 Hz appeared in individual channels (e.g. Pz and P4), with mean (SD) ITPC values of 0.4 (0.02) versus a mean baseline of 0.15, and intermittent peaks at 0.8 Hz occurred in individual electrodes (e.g. F7, F4), in which mean peak ITPC values were 0.35 ± 0.02 during monosyllabic word presentations compared to a 0.15 baseline, possibly indicating entrainment to higher order linguistic structure (phrase and sentence, respectively). **Conclusions:** EEG-auditory stimulus coherence measurements may be possible using smEEG. To our knowledge, these are the first such measurements using smEEG, which is widely used in clinical settings. These results support the use of passive EEG-based evaluations of CCP to help predict neurologic outcome in comatose patients.

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Poster

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Program #/Poster #: PSTR366.06/XX3

Topic: J.04. Physiological Methods

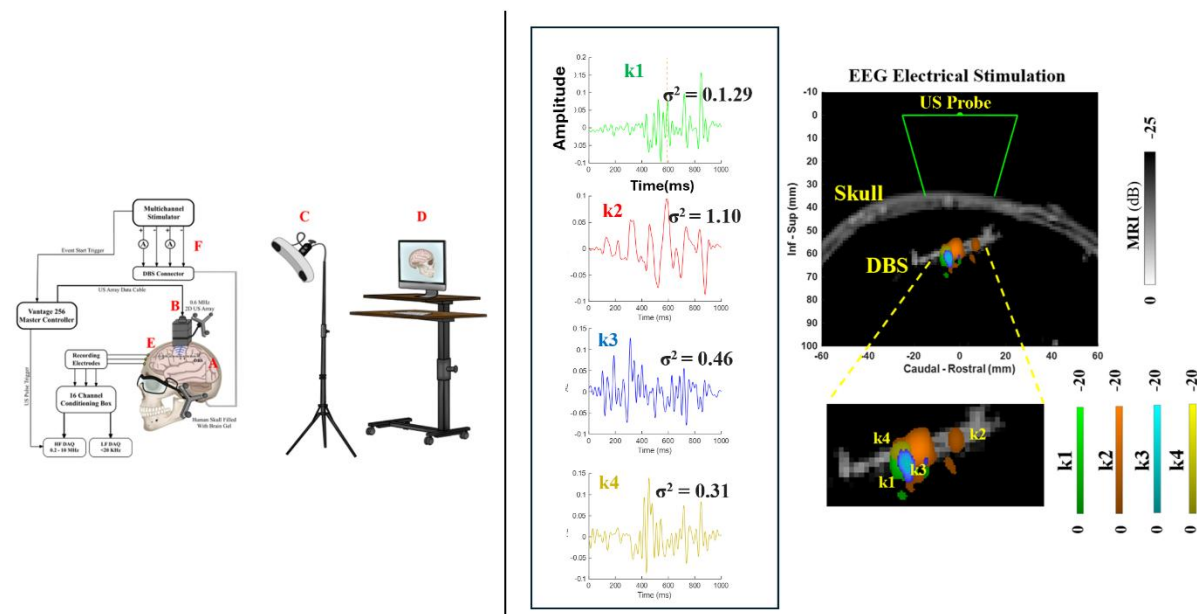
Support: NIH U01 EB028662
NIH Training Grant R25HD080811
NIH Training Grant T32GM132008
Flinn Foundation under Grant 23-06543

Title: Mapping Seizure Activity Using Transcranial Acoustoelectric Imaging in a Human Head Model with Neuronavigation

Authors: *N. ABU FARHA¹, R. S. WITTE²;

¹BME, ²Med. Imaging, Univ. of Arizona, Tucson, AZ

Abstract: Effective treatment of severe epilepsy relies on accurately localizing seizure onset zones. Traditional scalp EEG provides limited spatial resolution, while intracranial EEG, though more precise, is invasive. Transcranial Acoustoelectric Brain Imaging (tABI) offers a non-invasive alternative that uses focused ultrasound (US) probe to transiently modulate tissue resistivity, enabling detection of localized neuronal currents with high spatial and temporal resolution. However, detecting low-amplitude, seizure-related currents (<0.5 mA/cm²) remains challenging due to the weak acoustoelectric effect in biological tissues. This study demonstrates the feasibility and enhancement of tABI for mapping seizure-like activity using realistic current waveforms (0.15-10 mA) delivered via a clinical depth electrode array (DBS) implanted in an intact skull filled with conductive Agarose (Fig.A). A 0.6 MHz 2D ultrasound array (B), guided by neuronavigation (C & D), was electronically focused and steered to generate acoustoelectric (AE) signals, while gold cup electrodes recorded both high-frequency AE and low-frequency (LF) surface potentials (E), and LF stimulation currents were measured using a 1-ohm resistor in series for each anode (F). To overcome limitations in sensitivity and selectivity, we applied Wiener spatial filtering to suppress noise and Singular Value Decomposition (SVD) to isolate AE singular values (k) with maximized variance (σ^2) and with distinct spatial-temporal features (right panel). This approach enabled generation of color-coded AE images resolving overlapping normal and ictal activity patterns with sub-5 mm spatial resolution and signal-to-noise ratios of 46.8 dB (right panel). These results establish tABI as a potentially powerful, noninvasive technique for resolving seizure-related neuronal currents at less than 10 mA peak current with a detection threshold <100 uA at 1 MPa peak pressure (right panel).



Disclosures: N. Abu Farha: None. R.S. Witte: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.07/XX4

Topic: J.04. Physiological Methods

Title: Testing a new clip to correct racial bias in electroencephalography

Authors: *W. A. TORRENS¹, S. M. HAIGH²;

²Dept. of Psychology and Inst. for Neurosci., ¹Univ. of Nevada, Reno, Reno, NV

Abstract: There is a *racial* bias in electroencephalography (EEG) materials. EEG requires stable contact between the conductive gel, the electrodes, and the scalp. The caps traditionally used to hold the gel and electrodes in place securely are incompatible with curly and coiled hair textures, as the electrodes and gel often sit on the hair, not the scalp. Here, we test the effectiveness of an electrode clip we developed to address the electrode-to-scalp issue across hair textures. Thirty-three individuals (21 with straight hair and 12 with curly to coiled hair) listened to auditory clicks presented at 6 Hz and viewed grating patterns that reversed contrast at 6 Hz, separately, while we recorded EEG, first with the clips and then with the cap. Signal power was extracted from electrodes Fz (during auditory stimulation) and POz (during visual stimulation) at 6 Hz and the subsequent three harmonics. The root-mean-square signal-to-noise ratios (SNRs) were then calculated for the clip and cap, separately. At Fz during auditory stimulation, the clip recorded moderately higher signal power (17.4%) in participants with straight hair compared to the cap ($d=.50$). For participants with curly hair, the clip increased the signal power by 59.6% compared to the cap ($d=.85$). At POz during visual stimulation, the clip recorded slightly weaker signal power in participants with straight hair (-9.0%) compared to the cap ($d=-.30$), whereas the clip increased signal power in participants with curly hair by 29.7% compared to the cap ($d=.83$). Overall, the clips increased SNR, particularly in individuals with curly hair, with similar or even some increase in SNR in individuals with straight hair. This verifies that the clips method offers a step forward toward correcting the hair bias in EEG materials.

Disclosures: W.A. Torrens: None. S.M. Haigh: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR366.08/Web Only

Topic: J.04. Physiological Methods

Support: PAPIIT IA207824

Title: Neuropsychological and Electrophysiological Profile in Medical Students With and Without Depressive Symptoms

Authors: *D. P. GUÍZAR¹, A. MARTÍNEZ ZARRALUQUI², A. SÀNCHEZ MARTINEZ¹, A. A. HERNANDEZ CHAVEZ³, R. SAMPIERI¹;

¹Univ. Nacional Autónoma De México, Ciudad de México, Mexico; ²Univ. Anahuac, Ciudad de México, Mexico; ³Univ. Nacional Autónoma de México, Coyoacán, Mexico

Abstract: Introduction: The mental health of medical students represents a pressing global public health concern. Major depressive disorder (MDD) is particularly prevalent in this population and is frequently accompanied by cognitive impairments, including deficits in executive functions, attention, and processing speed. These symptoms are associated with academic underachievement, burnout, suicidal ideation, and impaired professional development. Objective: To compare the neuropsychological and electrophysiological profiles—specifically P300 event-related potentials—of medical students with and without depressive symptoms. Methods: A prospective, cross-sectional, and comparative study was conducted among medical students. Participants underwent assessment with the Patient Health Questionnaire-9 (PHQ-9), the Beck Depression Inventory (BDI), and a computerized neuropsychological battery (COGNIFIT). Electrophysiological data were collected using auditory event-related potentials (ERPs) during a standard two-tone auditory oddball paradigm to elicit P300 responses. Results: A total of 140 medical students (60% female; mean age 21 ± 1.6 years) were included and categorized into groups with and without depressive symptomatology. The groups were matched for age and sex to control potential confounding variables. Students with depressive symptoms exhibited significantly slower reaction times ($p < 0.01$), reduced processing speed ($p = 0.03$), and impairments in executive function ($p = 0.04$), memory ($p < 0.01$), attention ($p < 0.01$), and abstract reasoning ($p = 0.02$). Significant alterations in electrophysiological markers were also observed, including decreased amplitudes and prolonged latencies in N100, N200, and P300 components (all $p < 0.01$). Discussion: Neuropsychological and ERP (P300) parameters may serve as sensitive biomarkers of cognitive dysfunction associated with depressive symptoms in medical students. These findings support their potential utility as complementary tools in early screening and preventive mental health strategies within academic environments.

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Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.09/XX5

Topic: J.04. Physiological Methods

Support: Schmidt Science Fellows
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Searle Scholars Program

Title: High-density neural recordings of deep brain structures in humans during neurosurgery using Neuropixels 1.0-NHP 45 mm probes

Authors: *D. E. BROWN, II¹, E. OKOROKOVA², C. IACOBACCI³, B. F. COUGHLIN⁵, E. TRAUTMANN⁶, S. S. CASH⁷, A. C. PAULK⁸, D. BRANDMAN⁹, S. D. STAVISKY⁴;
¹UCSD, Sacramento, CA; ²Neurolog. Surgery, Univ. of California Davis, Davis, CA; ³Univ. of California, Davis, Pacifica, CA; ⁴Neurolog. Surgery, Univ. of California, Davis, Davis, CA; ⁵MGH, Cambridge, MA; ⁶UC Davis, New York, NY; ⁷Dept Neurol, Mass Genl Hosp, Boston, MA; ⁸Massachusetts Gen. Hosp., Boston, MA; ⁹Neurolog. Surgery, UC Davis Hlth., Sacramento, CA

Abstract: Recent advances in neural recording technology have enabled high-density electrophysiological recordings from the human brain, offering new insights into its function and pathology. Neuropixels probes—silicon-based devices with high channel count and recording-site density—are one such next-generation technology. These research-grade probes provide single-cell resolution recordings at a scale far beyond current clinically approved devices. While they have become widely adopted in systems neuroscience in small animal models such as rodents and songbirds, their use in humans remains nascent. Previous studies have successfully employed the 10 mm Neuropixels 1.0 to record from multiple brain regions simultaneously in small animals. However, in the human brain, the probe's relatively short length limits simultaneous multi-region recordings and precludes access to deep structures without resection of overlying cortex. To overcome these limitations, a new variant—Neuropixels 1.0-NHP Long—was developed to extend recording capabilities to larger brains. Designed initially for use in non-human primates, this probe features 4,416 recording sites distributed along a 45 mm shank, with 384 channels selectable for simultaneous recording. This design enables high-density, multi-region recordings spanning both superficial and deep brain structures, offering unprecedented access across the full depth of large-brained species. We report the first use of these new extended-length probes in six human participants undergoing intracranial procedures for clinical indications. To enable precise and safe deployment in the operating room, we designed custom, sterilizable 3D-printed surgical tools and refined insertion protocols to achieve submillimeter accuracy. Using these methods we successfully recorded intraoperative extracellular neural activity in all participants. Targets included the hand area of the postcentral gyrus, the posterior middle frontal gyrus, and the hippocampus. Recordings were obtained from participants both awake and under general anesthesia. To address the challenges of the intraoperative environment, we developed a dedicated signal processing pipeline to reduce operating room noise, correct for probe and tissue motion, and enable high-quality spike sorting. Our work establishes best practices for clinical deployment

and addresses key surgical and engineering challenges in translating Neuropixels probes to human neuroscience.

Disclosures: **D.E. Brown:** None. **E. Okorokova:** None. **C. Iacobacci:** None. **B.F. Coughlin:** None. **E. Trautmann:** None. **S.S. Cash:** None. **A.C. Paulk:** None. **D. Brandman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Brandman has patent applications related to speech BCI owned by the Regents of the University of California, including IP licensed to neurotech industry.. F. Consulting Fees (e.g., advisory boards); Brandman was a surgical consultant to Paradromics Inc. at the time of data collection, and is currently an advisor to Globus Medical. **S.D. Stavisky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stavisky is an inventor on intellectual property owned by Stanford University that has been licensed to Blackrock Neurotech and Neuralink Corp., Stavisky has patent applications related to speech BCI owned by the Regents of the University of California, including IP licensed to neurotech industry.. F. Consulting Fees (e.g., advisory boards); Stavisky is an advisor to Sonera and received equity..

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.10/XX6

Topic: J.04. Physiological Methods

Title: Fully printed 126 channel $\text{Ti}_3\text{C}_2\text{T}_x\text{MXene}$ micro ECoG array captures pathological and auditory evoked cortical activity in intraoperative recordings from human neurosurgical patients

Authors: ***S. AVERBECK**¹, **R. GARG**², **Z. WANG**³, **J. MICHIELS**⁴, **A. G. RICHARDSON**³, **H.-C. I. CHEN**³, **D. OSWALT**³, **M. BEAUCHAMP**³, **F. VITALE**²;
²Neurol., ³Neurosurg., ⁴Bioengineering, ¹Univ. of Pennsylvania, Philadelphia, PA

Abstract: Electrocorticography (ECoG) is a foundational tool in both clinical and research neuroscience, routinely used to localize epileptogenic zones and tumors during surgery and to enable brain-computer interface (BCI) applications. Despite its utility, conventional clinical ECoG arrays are limited by low spatial resolution, mechanical rigidity, and fabrication complexity - constraints that reduce their effectiveness for resolving cortical microcircuit dynamics critical to next-generation diagnostics and neuroprosthetics. To overcome these limitations, flexible thin-film arrays, often termed micro-ECoG arrays, have emerged as promising tools for high-density, surface-conforming cortical recording capable of capturing broadband neural signals with submillimeter precision. While many of these emerging technologies are produced via expensive and time-consuming photolithography, direct-ink-write (DIW) printing offers a low-cost, high-resolution alternative that enables rapid prototyping of microelectrode arrays using a wide range of conductive inks and substrates. Here, we present a

fully printed, 126-channel $\text{Ti}_3\text{C}_2\text{T}_x$ MXene-based ECoG array fabricated entirely through DIW. The array includes printable transparent low-curing temperature polyimide (PI) substrate and encapsulation layers, MXene-based conductive traces and contacts, and a top polydimethylsiloxane (PDMS) passivation layer for additional mechanical robustness. The 7×18 grid features $500 \mu\text{m}$ diameter contacts spaced 3 mm apart, with low electrochemical impedance at 1 kHz ($1.3 \pm 0.2 \text{ k}\Omega$) and excellent cortical conformity. The DIW process achieves $250 \mu\text{m}$ feature resolution across a 148 cm^3 workspace, allowing rapid iteration from design to deployment within a single day. The 126-channel micro-ECoG array was used intraoperatively in a human patient with drug-resistant epilepsy. During a 15-minute recording session, the array captured spontaneous epileptiform spiking. Auditory stimulation using a spoken-word podcast evoked robust, spatially distinct high-gamma activity consistent with the functional engagement of auditory cortex. The DIW-printed MXene arrays are also suitable for neural stimulation, as demonstrated in a rodent experiment in which hindlimb movements were elicited from the array placed over motor cortex. These results position DIW-printed MXene arrays as a next-generation neural interface technology - combining high-resolution neural sensing with customizable, scalable, low-cost fabrication. This platform shows potential for clinical mapping, seizure diagnostics, and high-density BCI applications.

Disclosures: S. Auerbeck: None. R. Garg: None. Z. Wang: None. J. Michiels: None. A.G. Richardson: None. H.I. Chen: None. D. Oswalt: None. M. Beauchamp: None. F. Vitale: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.11/XX7

Topic: J.04. Physiological Methods

Support: H2020 Framework Program of the European Commission, GA 964468

Title: The importance of high-density microelectrode arrays for recording multi-scale extracellular potential and label-free characterization of network dynamics in ipsc-derived neurons

Authors: *F. MODENA;
MaxWell Biosystems AG, Zurich, Switzerland

Abstract: Advances in microelectrode array (MEA) technology for in-vitro electrophysiological recordings have made it possible to study neuronal networks across multiple scales, from subcellular properties to network-level dynamics. These devices are essential for exploring the phenotypes of neurological disorders and accelerating drug discovery, offering unique insights into the behaviour of neuronal networks. Key factors such as electrode density, spacing, and size significantly impact signal quality, noise, and sensitivity. To exhaustively characterize neuronal

networks, MEAs must combine single-cell and subcellular resolution with high-throughput capabilities, maintaining sensitivity to small extracellular action potentials to capture the full range of network activity. In this study, the MaxOne and MaxTwo high-density (HD) MEA systems (MaxWell Biosystems, Switzerland) were utilized to record activity from induced pluripotent stem cell-derived neurons. These systems, with 26,400 electrodes per well, demonstrated the benefits of increased statistical power in longitudinal data collection. HD-MEA recordings were compared to simulated low-density recordings, where adjacent electrodes on HD-MEAs were clustered to mimic larger, lower-density electrodes. Additionally, the AxonTracking Assay, an automated tool for analysing individual axonal arbours from multiple neurons simultaneously, was used to evaluate axonal structures and network functionality in the recorded cultures. Results showed that higher electrode density and smaller electrode size enhanced sensitivity, allowing for the detection of smaller spikes and capturing the complete spectrum of network dynamics. The high-resolution analysis of network activity, combined with subcellular insights from the AxonTracking Assay, offers a robust platform for drug screening and disease modelling.

Disclosures: F. Modena: A. Employment/Salary (full or part-time): MaxWell Biosystems AG.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.12/XX8

Topic: J.04. Physiological Methods

Title: Multicellular profiling of human motor neuron co-cultures using the CytoTronics' Pixel

Authors: ***M. BOYD**, C. BANFILL, S. CHINTA, P. SURESH, S. SUTAR, S. CHITALE;
CytoTronics, Boston, MA

Abstract: Modeling the complex cellular environment of the central nervous system in vitro is essential for studying neuroinflammation, neurodegeneration, and therapeutic interventions. Traditional electrophysiological assays typically lack the spatial resolution or multimodal capacity to distinguish and track different cell types in mixed cultures. The CytoTronics' Pixel integrates high-density extracellular recordings with impedance-based electrical imaging, enabling real-time, label-free profiling of diverse cellular phenotypes within the same well. We cultured human iPSC-derived motor neurons, astrocytes, and microglia (BrainXell) in mono-, co-, and tri-culture formats on Pixel 96-well microplates pre-coated with poly-D-lysine and Geltrex. Neural activity and impedance-based electrical imaging were monitored longitudinally across 30 days. Pixel's >100,000 electrodes for impedance and 144 recording channels for electrophysiology enabled high-resolution spatial and temporal mapping of functional and structural features. High-dimensional analyses, including principal component analysis (PCA), were performed using over 20 orthogonal measurements to delineate cellular subtypes and track phenotypic evolution over time. Tri-cultures exhibited significantly increased spiking activity

and more organized morphological structures compared to mono- and co-cultures, reflecting enhanced network maturation. Electrical imaging revealed distinct impedance signatures for motor neurons, astrocytes, and microglia, enabling real-time differentiation of cell types within the same well. PCA of impedance parameters identified motility as a major driver of separation between the three cell types, supporting label-free classification based on dynamic structural features. These differences were stable and consistent across replicates, demonstrating the reproducibility and utility of the system for complex, multicellular models. The Pixel enables robust, label-free discrimination of multiple CNS cell types within multicellular cultures while simultaneously tracking functional network activity and morphological dynamics. This capability supports more physiologically relevant in vitro models for investigating cell-type-specific roles in health and disease and is well-suited for applications in disease modeling, mechanistic research, and drug discovery.

Disclosures: **M. Boyd:** A. Employment/Salary (full or part-time): CytoTronics, Inc. **C. Banfill:** A. Employment/Salary (full or part-time): CytoTronics, Inc. **S. Chinta:** A. Employment/Salary (full or part-time): CytoTronics, Inc. **P. Suresh:** A. Employment/Salary (full or part-time): CytoTronics, Inc. **S. Sutar:** A. Employment/Salary (full or part-time): CytoTronics, Inc. **S. Chitale:** A. Employment/Salary (full or part-time): CytoTronics, Inc..

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.13/XX9

Topic: J.04. Physiological Methods

Support: TUM Innovation Network Neurotechnology in Mental Health

Title: Long-term, stable microelectrode recordings with single-neuron resolution in human cortex after stroke

Authors: ***A. UTZSCHMID**^{1,2}, L. SCHIFFL^{1,2}, L. M. HELD^{1,2}, A. WAGNER¹, B. MEYER¹, J. GEMPT³, S. N. JACOB^{1,2};

¹Dept. of Neurosurg., TUM Univ. Hosp., Munich, Germany; ²Translational Neurotechnology Laboratory, Dept. of Neurosurg., TUM Univ. Hospital, Tech. Univ. of Munich, Munich, Germany; ³Neurosurg., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Intracortical single-unit recordings provide high-resolution insight into human brain functions. However, the feasibility of such recordings in structurally damaged cortex remains uncertain, a setting typically encountered e.g. in stroke patients for which brain-computer-interfaces to restore movement and communication are in high demand. Here, we present a unique case study of a patient with chronic language impairments (aphasia) following a large left-hemispheric stroke affecting frontal, temporal and parietal regions. Six years post-stroke, planar microelectrode arrays (Utah arrays) were implanted in four right-hemispheric regions of

the frontoparietal association cortex (angular gyrus, supramarginal gyrus, inferior frontal gyrus and middle frontal gyrus). Across 53 recording sessions spanning 275 days, 170 of 256 electrodes recorded spiking activity, yielding an average of 85 (SD: 14) well-isolated single units per session. To evaluate signal quality and recording stability, we determined spike amplitudes (mean: 72 μ V, SD: 11 μ V) and signal-to-noise ratio (mean: 20 dB, SD: 1 dB), both of which remained stable across sessions. To assess temporal integration at the single-unit level, we computed intrinsic timescales (IntTS), the decay constant of a unit's baseline spike autocorrelation. As a biologically grounded measure thought to reflect synaptic feedback mechanisms and intrinsic processing dynamics of individual neurons, IntTS is expected to remain stable over time - a prediction supported by our data. No significant differences were found in 43 out of 52 consecutive recording session comparisons. IntTS values were region-specific (medians: SMG 163 ms; AG 213 ms; IFG 271 ms; MFG 331 ms) and clustered within individual arrays (determined on arrays with $\geq 95\%$ active channels). Previously only reported in animal studies, IntTS has been shown to correlate with a myelination-based cortical hierarchy score derived from T1w/T2w MRI data. We were able to confirm this for the human cortex, with SMG showing the lowest hierarchy score, followed by AG, IFG and MFG. To explore the stability of single-unit measurements over time, we evaluated waveform shape, inter-spike interval histograms, firing rates and IntTS. Using these complementary features, we were able to successfully track individual units across multiple sessions. Our findings indicate that long-term, stable intracortical recordings with single-neuron resolution are feasible in patients with large brain lesions, supporting the broader applicability of high-resolution neuronal interfaces in this large clinical population.

Disclosures: A. Utzschmid: None. L. Schiff: None. L.M. Held: None. A. Wagner: None. B. Meyer: None. J. Gempt: None. S.N. Jacob: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

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Program #/Poster #: PSTR366.14/XX10

Topic: J.04. Physiological Methods

Support: NIH R01 NS105697

Title: Neurophysiology of resting state networks in monkeys.

Authors: *K. MANIKANDAN¹, N. S. CARD³, O. A. GHARBAWIE^{2,1};

¹Bioengineering, ²Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; ³Neurolog. Surgery, Univ. of California Davis, Davis, CA

Abstract: Resting state (RS) is widely used for inferring brain organization. Most RS analyses are predicated on measuring correlations in the infra-slow components (<0.1 Hz) of fMRI signals. But what is the relationship between these infra-slow time series and the millisecond

timescale of neural activity? To address this question, we used high spatial resolution tools to simultaneously record spontaneous hemodynamic and electrophysiological signals from sensorimotor cortex of two squirrel monkeys. Intrinsic signal optical imaging (ISOI) - an analog of fMRI - was conducted in a chronic cranial window (26x23 mm, 20 $\mu\text{m}/\text{pixel}$). Local field potentials and spiking activity were recorded from all cortical layers using a linear electrode array (32 channels, 100 μm inter-channel distance). Seed-based correlations on the rs-ISOI time series confirmed that functional connectivity matched known anatomical connections. Cross-correlations showed that rs-ISOI time series were coupled to the power of high-frequency electrophysiological signals. These temporal correlations were strongest for gamma power (30-90 Hz) at a ~ 2 s lag. We projected the cross-correlation results onto every pixel to generate cross-modal maps. Direct spatial comparison of the cross-modal and functional connectivity maps showed the strongest correspondence for maps generated from gamma power. In contrast, in all analyses, correlations were absent between rs-ISOI time series and alpha power. Cross-modal maps generated from three laminar compartments (superficial, middle, and deep) were surprisingly similar to one another, despite temporal offsets in gamma power across laminar compartments. Collectively, our results indicate that RSNs emerge from co-fluctuations in high-frequency (>13 Hz) oscillations across all cortical layers. Our multi-modal approach opens new opportunities for interrogating the neural basis of spontaneous cortical activity at spatial resolutions commensurate with cortical architecture.

Disclosures: K. Manikandan: None. N.S. Card: None. O.A. Gharbawie: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.15/XX11

Topic: J.04. Physiological Methods

Support: NSF CAREER Award EBMS-2145412
NSF DGE 2137420

Title: Longitudinal assessment of cortical LFP in center-out BCI task

Authors: *C. R. BARNETT¹, H.-Y. LU¹, Y. ZHAO¹, H. M. STEALEY², E. CONTRERAS-HERNANDEZ, Sr.³, S. R. SANTACRUZ⁴;

¹Biomed. Engin., Univ. of Texas at Austin, Austin, TX; ²Dept. of Biomed. Engin., Univ. of Texas At Austin, Austin, TX; ³NEUROPHYSIOLOGY, Univ. of Texas at Austin, Dept. of Biomed. Engin., coaclaco, Mexico; ⁴Biomed. Engin., The Univ. of Texas at Austin, Austin, TX

Abstract: Brain-computer interfaces (BCIs) have the remarkable capability of allowing subjects to control robotic limbs, computer cursors, and even voice synthesizers directly using their brain signals. Long-term BCI clinical trials have increased in number recently, and widespread clinical use of chronic BCIs is rapidly approaching. One important design consideration for chronic BCI

electrode technologies and mappings from neural space to the control space (“decoders”) is the stability of the neural signals which are decoded to infer the subject’s intent. The longitudinal dynamics of neural signals over months to years has not been well studied. The results presented here help to establish the balance between stability and non-stationarity of local field potential (LFP) signals and how they relate to chronic BCIs.

Two male rhesus macaques were implanted with chronic microelectrode arrays in their left motor cortex. Using sorted single unit activity, both subjects learned to control a biomimetic spike-based decoder to complete a standard center-out BCI task. They performed this BCI task across a period of months. Although the BCI decoder used spiking activity as the input signal, LFPs are another promising BCI control signal due to their robustness over long periods of time. Thus, it is critical to understand the longitudinal dynamics of LFPs. The power spectral density of LFP activity during each BCI trial was computed, and the power from physiologically relevant frequency bands was then extracted and characterized longitudinally. In addition, we examined the preferred direction (PD) associated with each frequency band and how the PD evolved across consecutive sessions. A machine learning classifier was also trained to decode target direction for each trial using the band powers from each LFP channel as features. In this work, we show that PD is not stable from session to session, and this indicates that certain properties of LFP and how it functionally relates to BCI tasks are non-stationary. However, we also show that the relative decoding power of each frequency band is consistent across many days, indicating that the way that BCI-task information is represented across the frequency bands is stable. Together, these results suggest that although the functional relationship between neural populations and BCI tasks is inherently dynamic, the way that these relationships are encoded may indeed be stable. This work helps to establish how neural populations balance stability and non-stationarity, providing key insights for long-term BCI decoder design.

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Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR366.16/Web Only

Topic: J.04. Physiological Methods

Support: Marie Skłodowska-Curie grant agreement No 956669

Title: Flexible Electrode Array Implantation Mechanism for Neural Recordings in the Non-Human Primate Brainstem

Authors: A. AARTS¹, I. TREPO^{1,2}, R. GUPTA⁴, R. VAN DAAL¹, A. BAKER⁵, A. BUCHERER², P. RUTHER³, T. WACHTLER⁶, S. N. BAKER⁵;

¹ATLAS Neuroengineering, Leuven, Belgium; ²Dept. of Microsystems Engin. (IMTEK), Univ. of Freiburg, Freiburg im Breisgau, Germany; ³Microsystems Engin. (IMTEK), Univ. of

Freiburg, Freiburg, Germany; ⁴Univ. of Munich, Munich, Germany; ⁵Newcastle Univ., Newcastle upon Tyne, United Kingdom; ⁶G-Node, Fac. of Biol., Univ. Muenchen, Planegg, Germany

Abstract: This study describes the development and validation of an ultra-flexible electrode array and respective insertion mechanism designed for chronic electrophysiological recordings of brain signals from the macaque brainstem over a 10-week-period. The flexible nature of the neural probe enhances compatibility with the monkey's dynamic lifestyle, while ensuring stable recordings and minimizing tissue damage. The insertion method was engineered to implant the electrode array at a target depth of 45 mm, overcoming the anatomical challenges to pierce the dura, as well as the deeper tentorium layer. The ultra-flexible 32-channel electrode array was produced using microfabrication techniques. The individual iridium oxide electrode had a diameter of 25 μm and showed an average impedance of 35 k Ω at 1 kHz. The probe shaft thickness was 12 μm . The flexible electrode array was delivered to the target region via an innovative implantation procedure based on a sharpened guide tube paired with a contoured rigid inner wire. The flexible probe is temporarily threaded onto the rigid wire while positioned outside the guide tube. The tube enables the system to traverse from the brain surface through 20 mm of brain tissue, penetrate the cerebellar tentorium, and reach the reticular formation 25 mm beyond, thus traveling in total 45 mm deep. After releasing the flexible electrode array at the targeted implantation depth, the insertion mechanism was carefully retracted with minimal displacement of the electrode array. This deep-brain-specific neural probe has been implanted chronically and successfully demonstrated its recording potential in vivo, sensing brain signals 10 weeks post implantation. This work paves the way for future chronic depth recordings, providing neuroscientists with instrumentation to gain new neural insights on subcortical areas.

Disclosures: A. Aarts: None. I. Trepo: None. R. Gupta: None. R. van Daal: None. A. Baker: None. A. Bucherer: None. P. Ruther: None. T. Wachtler: None. S.N. Baker: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.17/XX12

Topic: J.04. Physiological Methods

Title: Recording of identified cells from macaque motor cortex using 1024 channel SiNAPS probes

Authors: G. N. ANGOTZI¹, J. F. RIBEIRO², G. ORBAN³, M. VINCENZI⁴, A. BAKER⁵, S. N. BAKER⁵, *L. BERDONDINI⁶;

¹Inst. Italiano Di Tecnologia, Genova, Italy; ²Microtechnology for Neuroelectronics,

⁴Microtechnology for Neuroelectronics (NetS3), ³Inst. Italiano di Tecnologia, Genova, Italy;

⁵Newcastle Univ., Newcastle upon Tyne, United Kingdom; ⁶Fondazione Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Active dense CMOS probes offer a substantial increase in the number of recording channels, enabling high-resolution, large-scale neural recordings with reduced tissue disruption. This is particularly relevant to overcome challenges to record bioelectrical signals from large populations of single neurons in the macaque brain. Interestingly, whereas chronic implants sample from only a small (and fixed) region of cortex at a fixed depth, acutely inserted electrodes can sample from a wider region by making penetrations at different sites each day. However, this approach requires repeated penetrations through the dura. Here we report progress on the development and experimental use of an active dense CMOS probe for acute large-scale single unit intracortical macaque cortical recordings. A single-shank CMOS probe was specifically designed based on SiNAPS technology and the integration of additional multiplexing circuits to minimize outputs. It provides a regular array of 1024 electrode-pixels ($14 \times 14 \mu\text{m}^2$) arranged in 4 columns and an interelectrode pitch of $30 \mu\text{m}$. The probe is 10 mm in length, $155 \mu\text{m}$ in width, $50 \mu\text{m}$ in thickness, and samples neural activity from the whole array at 20kHz/channel. Experiments were performed in behaving macaques through multiple day insertions in the motor cortex. Penetration is easiest by making a small pilot hole and the optimized insertion procedure allows routine recordings in different sites. The probe configuration is sufficient to reach the anterior bank of the central sulcus, which contains many corticospinal cells that make direct connections to motoneurons in the spinal cord. Data analysis results show that high-resolution recordings allow to identify corticospinal cells by their antidromic responses to electrical stimulation. Overall, the results of this study showcase an important advance in the toolkit of primate neurophysiology.

Disclosures: **G.N. Angotzi:** A. Employment/Salary (full or part-time): Corticale Srl. **J.F. Ribeiro:** None. **G. Orban:** None. **M. Vincenzi:** None. **A. Baker:** None. **S.N. Baker:** None. **L. Berdondini:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Corticale Srl.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.18/YY1

Topic: E.08. Multisensory Integration

Title: Ultra-low power wearable EEG-SCG sensors for continuous neurological monitoring: validated clinical performance in elderly and neonatal care with edge-cloud computing integration

Authors: *Y. DU;
Vanderbilt Univ., Nashville, TN

Abstract: We present a clinically validated, wearable, ultra-low power EEG-SCG sensing platform designed for real-time, long-term neurological monitoring across a wide age range—

from preterm neonates (as early as 27 gestational weeks, 0.75 kg birth weight) to elderly patients. Validated against the gold-standard Natus EEG system, our sensors have been deployed at Northwestern Memorial Hospital in over 40 elderly individuals, capturing high-fidelity EEG and head movement data via integrated 3-axis IMU. For neonates, up to three EEG channels have been successfully used in more than 15 infants to screen for neurological disorders and seizures. In contrast to commercial wearable EEG systems like Epilog—which offer only 24-hour battery life, no real-time signal display, and no wireless charging—Our platform enables over 100 hours of continuous recording powered by a compact 120 mAh LiPo battery, featuring real-time signal visualization, wireless charging, an intuitive mobile app, and secure cloud-based data upload for remote monitoring and analysis. This enables both clinicians and caregivers to remotely monitor neural health over extended durations. We also developed a comprehensive signal analysis and feature engineering pipeline that extracts interpretable features in time, frequency, and nonlinear domains for accurate detection of various seizure types, including generalized and focal seizures. Our platform is highly versatile and applicable in both clinical and at-home settings, supporting early screening of neurological disorders, neurodevelopmental tracking, epilepsy monitoring, behavioral studies, sleep analysis, and real-world longitudinal neurophysiological monitoring. Its long battery life, wireless functionality, and AI-ready infrastructure represent a transformative step toward precision neurohealth and remote, continuous care.

Disclosures: Y. Du: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.19/YY2

Topic: J.04. Physiological Methods

Support: NIH Brain Initiative Grant U01-NS128612
Tianqiao & Chrissy Chen Institute
Foundation For OCD Research
Helene Houle Career Development Award

Title: Phase-velocity correlations to characterize coherence and cross-frequency interactions in human intracranial recordings

Authors: *K. J. MILLER;
Dept. of Neurosurg., Mayo Clin., Rochester, MN

Abstract: I present a new technique for quantifying interactions between oscillations in electrophysiological arrays. This technique begins by transforming the measured time series into a complex signal characterized by a time dependent amplitude and phase, within a centered frequency range. The instantaneous phase should vary at a constant rate for a pure sinusoid, but

interactions are determined when there is variation from this constant rate. Therefore, the derivative of the instantaneous phase is calculated and the constant rate is subtracted off to obtain a “phase velocity”. Phase velocity at the same center frequency between different measurement sites can be compared with one another and clustered to obtain distributed “domains” of coherence, using standard techniques such as principle component and independent component analyses. This circumvents the usual requirement of coherence quantification in a purely pairwise fashion. I show, in example human data from brain surface & depth recordings (electrocorticography - ECoG & stereoelectroencephalography SEEG), that identified domains for motor oscillations follow gyral boundaries, and that coherence varies as a function of movement. Phase velocity between oscillations centered at different frequencies, from both the same as well as different brain sites, can be easily characterized using simple correlation to quantify cross-frequency phase-phase coupling. Secondly, causal interactions between oscillations at different phases are explored by application of Granger causality. This simple approach allows for straightforward calculation of distributed coherence without the need for a “seed” site. Furthermore, interactions between oscillations of different center frequency are able to be qualified in a relatively intuitive manner. These findings are illustrated in implanted electrophysiological recordings from neurosurgical patients. Code to reproduce the findings, as well as to apply in one’s own data, are made freely available for open unrestricted use.

1. Create complex time-series $\tilde{V}_f(t)$

Apply a wavelet, band-pass filter, or Hilbert transform to the measured voltage $V(t)$ to obtain

$$\tilde{V}_f(t) = r_f(t) e^{i\phi_f(t)},$$

where $\tilde{V}_f(t)$ is a complex analytic signal centered at frequency f with instantaneous magnitude $r_f(t)$ and phase $\phi_f(t) \in [-\pi, \pi]$.

2. Compute the derivative of $\phi_f(t)$

First *unwrap* the phase to remove 2π discontinuities in phase, then differentiate:

$$\dot{\phi}_f(t) = \frac{d\phi_f(t)}{dt}.$$

For a pure sinusoid at frequency f , this derivative is constant, i.e. $\dot{\phi}_f(t) = 2\pi f$.

3. Identify spatial domains of coherence

Assign a channel index k to obtain $\dot{\phi}_f^{(k)}(t)$ for each site. Matrix decompositions of their pairwise interactions—e.g. principal-component analysis (PCA) or independent-component analysis (ICA)—reveal coherent spatial modes.

4. Quantify cross-frequency interactions

Motifs in the interaction between instantaneous phases at two frequencies f_1 and f_2 can be captured by the correlation

$$\rho(\dot{\phi}_{f_1}(t), \dot{\phi}_{f_2}(t)).$$

Directionalities between sites may then be inferred through application of lagged cross-correlations, or corrected lagged cross-correlation (e.g. Granger causality).

$$\rho(\tau) = \rho(\dot{\phi}_{f_1}(t), \dot{\phi}_{f_2}(t + \tau)).$$

Disclosures: K.J. Miller: None.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.20/YY3

Topic: J.04. Physiological Methods

Support: Shanghai Municipal Science and Technology Major Project grant 2018SHZDZX05,
Shanghai Municipal Science and Technology Major Project grant 2021SHZDZX
National Science and Technology Innovation 2030 Major Program grant 2022ZD0210300
National Natural Science Foundation of China grant 32200917
National Science and Technology Innovation 2030 Major Program grant 2021ZD0202202
Shanghai Municipal Science and Technology Major Project grant 2021SHZDZX

Title: Large scale single-neruon recording using the uFINE array in human cortex

Authors: *S. WU¹, Z. YAN², C. KONG¹, X. LI², Q. DONG², Y. QIAN³, G. CHEN¹, B. CHEN², C. REN¹, J. LU⁴, X. JIANG², Z. ZHAO¹, X. LI¹;

¹Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China; ²Dept. of Neurosurg., Xijing Hospital, Fourth Military Med. Univ., Xi'an, China; ³Dept. of Neurosurg., Huashan Hospital, Shanghai Med. College, Fudan Univ., shanghai, China; ⁴Dept. of Neurosurg., Huashan Hospital, Shanghai Med. College, Fudan Univ., Shanghai, China

Abstract: Introduction

Monitoring neural population activity at single-cell resolution is essential for driving fundamental research and clinical innovations. However, translating advanced recording techniques from animal models to humans remains a significant challenge. Flexible neural electrodes have recently emerged as powerful tools for large-scale single-unit recordings due to their superior biocompatibility and high recording density.

Methods

Here, we demonstrate reliable, high-density single-unit recordings during intraoperative procedures in human patients using ultra-Flexible Implantable Neural Electrode (uFINE) arrays. The uFINE array exhibited sufficient mechanical robustness to maintain structural integrity throughout surgical operations.

Results

We successfully recorded 616 single units from 10 patients, with up to 135 single units simultaneously recorded. The flexibility of uFINE array minimized signal disturbances from

brain pulsations, enabling stable single-unit detection. Stimulus and response tuning were observed at the level of individual neurons in awake patients.

Conclusion

This uFINE-based recording approach offers unique opportunities to investigate human-specific cognitive functions and develop next-generation brain-machine interfaces.

Disclosures: **S. Wu:** None. **Z. Yan:** None. **C. Kong:** None. **X. Li:** None. **Q. Dong:** None. **Y. qian:** None. **G. Chen:** None. **B. Chen:** None. **C. Ren:** None. **J. Lu:** None. **X. Jiang:** None. **Z. Zhao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder. **X. Li:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.21/YY4

Topic: J.04. Physiological Methods

Title: Mobile EEG in Dogs: Measuring Auditory Evoked Potentials Without Sedation or Restraint

Authors: ***L. SCHREINER**, C. GUGER, K. MAYR;
g.tec medical engineering GmbH, Schiedlberg, Austria

Abstract: This study presents a non-invasive methodology for recording electroencephalography (EEG) in freely moving, awake dogs using a miniature, wireless 8-channel system. EEG was recorded from four pet dogs of varying breeds during an auditory oddball paradigm without sedation or physical restraint. Gold cup electrodes were symmetrically positioned along the canine scalp, and signals were sampled at 250 Hz. Power spectral density analysis confirmed signal quality, and auditory evoked potentials (AEPs) reliably differentiated target from non-target tones. A clear N120 response to target stimuli was observed in all dogs, with strongest amplitudes found in 3 out of 4 dogs at the right lateral site (channel 6), suggesting lateralized auditory processing. The results validate a rapid, animal-friendly protocol for mobile EEG acquisition in naturalistic environments. This approach allows the investigation of canine brain activity in real-world contexts with minimal stress, opening new directions in comparative and translational neuroscience. Our findings support the feasibility of high-quality cognitive EEG studies in domestic dogs and highlight the potential of mobile neurotechnology for monitoring natural cognition across species.

Disclosures: **L. Schreiner:** A. Employment/Salary (full or part-time): g.tec medical engineering GmbH. **C. Guger:** A. Employment/Salary (full or part-time): g.tec medical engineering GmbH. **K. Mayr:** A. Employment/Salary (full or part-time): g.tec medical engineering GmbH.

Poster

PSTR366: Electrophysiology: Electrode Arrays II

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR366.22/YY5

Topic: J.04. Physiological Methods

Title: High-definition electrophysiological assessment of FUJIFILM human iPSC neuronal co-cultures on CorePlate HD-MEAs

Authors: ***J. E. SKEFOS**^{1,2}, I. VERDUCI², S. SCHACHTELE³, C. B. CARLSON³;
¹3Brain, Falls Church, VA; ²3Brain AG, Pfaffikon SZ, Switzerland; ³FUJIFILM Cell. Dynamics, Madison, WI

Abstract: 3Brain's CorePlate™ powered high-density microelectrode arrays (HD-MEAs) enable non-invasive, high-resolution electrophysiological recordings of neuronal activity and in depth analysis of network dynamics. Traditional low density in vitro MEA recordings offer few data points resulting in low acuity and resolution to adequately capture network maturation, limiting translational potential of human based induced pluripotent stem cell (iPSC) neuronal models. To address this gap, we developed a protocol to culture FUJIFILM Cellular Dynamics human iPSC neurons and astrocytes on 3Brain's HD-MEA (CorePlate™ 1W 30/60) to enable high resolution monitoring of the maturation of neuronal activity.

Human isogenic iPSC-derived glutamatergic (iCell® Induced Excitatory Neurons), inhibitory (iCell® GABANeurons), and glial cells (iCell® Astrocytes 2.0) from FUJIFILM Cellular Dynamics were co-cultured on 3Brain's CorePlate™ HD-MEA to support long-term culture and robust network development. Recordings were performed longitudinally to monitor the maturation of synchronous bursting activity across the culture.

Spontaneously active cultures displaying characteristic bursting patterns were observed after 25 DIV. HD-MEA recordings enabled robust quantification of activity changes over time, including an increase in firing rate, inter-spike interval (ISI) coefficient of variation, and peak-to-peak amplitude. Notably, there was a marked enhancement in both single-neuron bursting activity, shown by longer burst durations and a higher number of spikes per burst, and in network-level bursting properties. Within network bursts, network burst duration increased, the number of spikes per network burst increased and ISI decreased. The high spatiotemporal resolution of HD-MEA recordings facilitated detailed analyses of network connectivity, revealing a progressive increase in network complexity.

These findings support the use of 3Brain's CorePlate™ enabled HD-MEA platforms and FUJIFILM Cellular Dynamics iPSC-derived neuron and glial co-cultures as a scalable, physiologically relevant tool for studying neural networks. The system is well-suited for applications in neuropharmacology, offering a translationally relevant alternative to animal models.

Disclosures: **J.E. Skefos:** A. Employment/Salary (full or part-time): 3Brain US LLC. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FUJIFILM. **I.**

Verduci: A. Employment/Salary (full or part-time): 3Brain AG. **S. Schachtele:** A. Employment/Salary (full or part-time): FUJIFILM Cellular Dynamics. **C.B. Carlson:** A. Employment/Salary (full or part-time): FUJIFILM Cellular Dynamics.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.01/YY6

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Newly developed and validated in vitro co-culture myelination model with the use of lentivirus may offer new insights on therapeutic approaches in leukodystrophy

Authors: *K. HARADA;

JAPAN TOBACCO INC. Central Pharmaceut. Res. Inst., Osaka, Japan

Abstract: Leukodystrophies comprise a large group of rare genetic disorders primarily affecting CNS white matter and myelin loss. We have focused on Krabbe disease, an inherited lysosomal storage disease with leukodystrophy caused by a mutation in galactosylceramidase (GALC). Most patients show early onset form of Krabbe disease by cerebral demyelination with apoptotic oligodendrocyte (OL) death. However, the initial pathophysiological changes in developing OLs remain poorly understood. Therefore, to understand the mechanism as well as the pathology, we have developed an in vitro co-culture myelination model with the use of lentivirus mediated shRNA knock-down of Galc (Galc KD) that may offer new insight on the therapeutic approaches not only for Krabbe disease, but for leukodystrophy diseases as well. Our results presented that no myelination was revealed infected with the Galc KD. However, myelination was clearly visible with the scramble lentivirus (negative control, NC). Furthermore, FTY720 Phosphate, known to induce OL differentiation were used to assess myelination. As a result, increased myelinating oligodendrocytes were displayed compared to NC. Overall, our newly developed and validated *in vitro* co-culture myelination model with the use of lentivirus may offer new insights on therapeutic approaches in leukodystrophy.

Disclosures: K. Harada: None.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.02/YY7

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: CSIRO industry PhD program with Biosensis as the supporting industry partner

Title: First monoclonal antibodies for truncated BDNF enable isoform-specific detection via ELISA

Authors: *N. GRAHAM¹, L. M. FOSTER², R. RUSH, PHD³;

¹Adelaide Univ., Adelaide, Australia; ²Biosensis Pty Ltd & LMF Biosci., Oceanside, CA;

³Biosensis Pty Ltd, Adelaide, Australia

Abstract: Truncated brain-derived neurotrophic factor (tBDNF) is an understudied isomer of BDNF that has been implicated in neurological dysfunction, but its detection in biological fluids remains elusive due to its high sequence homology with other BDNF isoforms, proBDNF and mature BDNF (mBDNF). To enable specific detection of tBDNF in human plasma and saliva, we aimed to generate novel monoclonal antibodies targeting its unique N-terminal sequence. Two custom immunogenic peptides were selected based on sequence alignments that distinguish tBDNF from its isomers. Mouse immunisation and hybridoma generation were conducted by Monash Antibody Technologies to produce candidate antibodies. Screening against both immunogenic and epitope-specific peptides was employed to identify clones with high affinity and specificity for the tBDNF N-terminus. Of the antibodies harvested, 18 unique monoclonal antibodies demonstrated selective recognition of tBDNF without cross-reactivity to proBDNF or mBDNF in preliminary direct ELISA assays on purified tBDNF proteins. These are, to our knowledge, the first antibodies capable of discriminating tBDNF from its isoforms. Ongoing validation includes application of these antibodies in Western blot and two-site sandwich ELISA formats. Following purification using Biosensis' in-house protocol, the most robust candidates will be developed into an ELISA detection kit for clinical and research use. If successful, this tool will facilitate the quantification of circulating tBDNF and enable investigation of its potential role in neurological disease pathogenesis, addressing a major gap in current neurotrophic biomarker research.

Disclosures: N. Graham: A. Employment/Salary (full or part-time): CSIRO, Biosensis. L.M. Foster: A. Employment/Salary (full or part-time): Biosensis. R. Rush, PhD: A. Employment/Salary (full or part-time): Biosensis.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.03/YY8

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Using Next Generation Viruses to Interrogate iPSC-derived Neurons and Microglia

Authors: *M. THOMAS¹, K. BRAME¹, K. XU³, M. L. HENDRICKSON³, T. E. HUGHES²;

²Cell Biol. & Neurosci., ¹Montana Mol., Bozeman, MT; ³BrainXell Inc., Madison, WI

Abstract: Traditional viral vectors adeno-associated virus (AAV) and lentivirus, have a minimal cargo capacity. On the other hand, BacMam, a double stranded DNA insect virus, can carry up to 38 kilobases. This payload makes it possible to use large cell-type-specific promoters and large cargos. Our question was whether these promising vectors can be used to reliably express genes of interest in neurons and glial cells. We undertook this study using BrainXell's iPSC-derived cortical glutamatergic neurons, astrocytes, and microglia. We transduced with next-generation baculovirus engineered for high expression. These viruses were delivering genetically encoded biosensors as well as probes for mitochondria, lysosomes, omegasomes, cytoskeleton, nucleus, Golgi, and endosomes. We tested transduction on day 0, day 1, day 4, and day 7 in the two-week maturation protocol from BrainXell. We found the viruses produced robust expression without obvious toxicity if used on day 4 after seeding or later. Indeed, in mature cultures, as little as 24 hours was needed for strong expression. Regardless of expression level, the probes correctly decorated the specific organelles. The introduction of cADDiS, a cAMP sensor, made it possible to study endogenous receptor activation. Ultimately, the neuroscientist wants to study co-cultures of many different cell types. Therefore, we wanted to determine if we could use cell-type-specific promoters in BacMam. We transduced immature iPSC-derived cortical glutamatergic neurons with a synapsin-driven mNeongreen. This produced no fluorescence until the cells matured ~14 days later, upon which they became bright green. All work done had at least 3 biological replicates. Work is underway with other cell-type-specific promoters to be able to differentiate specific neuronal subpopulations as well as astrocytes and microglia.

Disclosures: **M. Thomas:** A. Employment/Salary (full or part-time): Montana Molecular. **K. Brame:** A. Employment/Salary (full or part-time): Montana Molecular. **K. Xu:** A. Employment/Salary (full or part-time): BrainXell. **M.L. Hendrickson:** A. Employment/Salary (full or part-time): BrainXell. **T.E. Hughes:** A. Employment/Salary (full or part-time): Montana Molecular.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.04/YY9

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NSTC 113-2923-B-002-005-MY2

Title: In vivo gene editing to rescue the aceruloplasminemia phenotype in mice

Authors: ***Y. CHIANG**¹, I.-S. YU², H. WANG³, C.-H. LIN⁴, S.-J. CHOU⁵, U. NENISKYTE⁶, Y.-T. CHEN¹;

¹Natl. Taiwan Univ., Taipei, Taiwan; ²Lab. Animal Ctr., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; ³Genome and Systems Biol. Degree Program, Academia Sinica and Natl. Taiwan Univ., Taipei, Taiwan; ⁴Dept. of Neurol., Natl. Taiwan Univ. Hosp., Taipei, Taiwan; ⁵Academia Sinica, Taipei, Taiwan; ⁶Vilnius Univ., Vilnius, Lithuania

Abstract: Aceruloplasminemia (ACP) is a rare, adult-onset, autosomal recessive disorder and one of the subtypes of Neurodegeneration with Brain Iron Accumulation (NBIA). It is caused by pathogenic variants in the *CP* gene, resulting in the absence or dysfunction of ceruloplasmin (CP), a serum ferroxidase that oxidizes toxic ferrous iron (Fe^{2+}) into non-toxic ferric iron form (Fe^{3+}). Loss of CP activity disrupts systemic iron homeostasis, leading to excessive iron deposition, particularly in the liver and brain, and clinically manifests as the characteristic triad of retinal degeneration, diabetes mellitus, and neurologic impairment. Current therapeutic strategies rely on oral or intravenous iron chelators, which can normalize serum ferritin levels, reduce hepatic iron accumulation, and improve systemic manifestations such as anemia and diabetes. However, their efficacy in alleviating neurological symptoms remains uncertain. To address this therapeutic limitation, we propose a gene editing-based approach targeting the underlying *CP* mutation. In this study, we focus on an ACP patient with a mutation at the *CP* c.607+1 delG site, identified by Dr. Chin-Hsien Lin at National Taiwan University Hospital. We generated a *Rosa26-hCP* c.607+1 delG minigene knock-in allele in an ACP (*Cp^{null}/Cp^{null}*) mouse. We aim to package prime editing components by virus-like particles (VLPs) to correct the pathogenic mutation *in vivo* and assess the restoration of ceruloplasmin expression and phenotypic rescue in the ACP mouse model.

Disclosures: Y. Chiang: None. I. Yu: None. H. Wang: None. C. Lin: None. S. Chou: None. U. Neniskyte: None. Y. Chen: None.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.05/YY10

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NSTC 113-2923-B-002-005-MY2

Title: Lipid nanoparticle-mediated delivery of CP mRNA to reverse aceruloplasminemia phenotype in a mouse model

Authors: *Y. LAI¹, I.-S. YU², C.-Y. KAO³, H. WANG⁵, C.-H. LIN⁴, S.-J. CHOU⁶, U. NENISKYTE⁷, Y.-T. CHEN¹;

¹Natl. Taiwan Univ., Taipei, Taiwan; ²Lab. Animal Ctr., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; ³NTUH Ctr. for Frontier Med., ⁴Dept. of Neurol., Natl. Taiwan Univ. Hosp., Taipei, Taiwan; ⁵Grad. Inst. of Genome and Systems Biol. Degree Program, Academia Sinica and Natl. Taiwan Univ., Taipei, Taiwan; ⁶Academia Sinica, Taipei, Taiwan; ⁷Vilnius Univ., Vilnius, Lithuania

Abstract: Aceruloplasminemia (ACP) is a rare autosomal recessive neurodegenerative disorder caused by CP mutants leading to ceruloplasmin deficiency. This deficiency disrupts systemic and cerebral iron homeostasis, which results in iron accumulation in multiple organs and subsequent

tissue damage, including neurological dysfunction. As a subtype of neurodegeneration with brain iron accumulation (NBIA), ACP is different from other NBIA subtypes due to systemic iron overload. The treatments for ACP primarily relies on oral iron chelators, such as deferasirox and deferiprone. However, these treatments are limited by their inability to effectively cross the blood-brain barrier and can cause side effects, including leukopenia and liver injuries. In addition, enzyme replacement therapy has been proposed as a potential treatment. However, its long-term efficacy is hindered by the development of anti-drug antibodies. In contrast, gene therapy presents a promising alternative by utilizing molecular biology techniques to deliver specific genetic material to patients, effectively targeting the underlying genetic defects. This study focuses on a CP c.607+1 delG mutation identified in an ACP patient at the National Taiwan University Hospital. We establish an ACP mouse model to evaluate the therapeutic effects of using a lipid nanoparticle (LNP)-delivered *CP* mRNA *in vivo*. We expect to see an exogenous ceruloplasmin expression to at least partially reverse the pathological and phenotypic manifestations of ACP.

Disclosures: Y. Lai: None. I. Yu: None. C. Kao: None. H. Wang: None. C. Lin: None. S. Chou: None. U. Neniskyte: None. Y. Chen: None.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.06/YY11

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Co-culture of frozen neural progenitors, neuron precursors, and astrocytes derived from the highly characterized reference scTi003-a line

Authors: A. MOOSA¹, K. WRIGHTSON¹, B. STREETER², *J. KEIL³, A. GAFFNEY¹, S. A. LOUIS¹, A. EAVES¹, E. KNOCK⁴;

¹STEMCELL Technologies Inc, Vancouver, BC, Canada; ²Axion Biosystems, Atlanta, GA;

³STEMCELL Technologies, Inc., Oak Park, IL; ⁴STEMCELL Technologies Inc., Vancouver, BC, Canada

Abstract: Cryopreserved cells can serve as a flexible and reproducible starting point for downstream differentiation. We developed highly pure cryopreserved neural progenitor cells (NPCs), neuron precursors, and astrocytes derived from the highly-characterized human induced pluripotent stem cell (hiPSC) line, SCTi003-A. SCTi003-A hiPSCs were seeded in complete STEMdiff™ Neural Induction Medium + SMADi and cultured for 21 days to generate NPCs. Day 21 NPCs were then cryopreserved in STEMdiff™ Neural Progenitor Freezing Medium at a density of 1.3×10^6 cells/mL. After thawing, NPCs were differentiated to forebrain neuron precursors and astrocytes using STEMdiff™ Forebrain Neuron Differentiation Kit and STEMdiff™ Astrocyte Serum-Free Differentiation and Maturation Kits, respectively. Forebrain neuron precursors were cryopreserved on day 7 and astrocytes were cryopreserved at day 54.

Forebrain precursors were thawed in STEMdiff™ Forebrain Maturation Medium for 24 hours, after which astrocytes were added to the culture and maintained in complete BrainPhys™ medium. The majority of thawed SCTi003-A-derived NPCs expressed PAX6 ($96.4 \pm 3.4\%$) and SOX1 ($91.5 \pm 2.4\%$), and exhibited little spontaneous neuronal differentiation ($3.5 \pm 1.3\%$ β IIIITUB+; mean $\pm$ SEM; n = 4). NPCs maintained in STEMdiff™ Neural Progenitor Medium and expanded for 5 passages displayed a 2.8 ± 0.5 -fold increase (mean $\pm$ SEM; n = 4) in cell number/passage while retaining NPC phenotype ($97.8 \pm 1.3\%$ PAX6+SOX1+). Forebrain precursor cells thawed into STEMdiff™ Forebrain Maturation medium and cultured for a further 14 days were 95.2% positive for neuronal marker β IIIITUB with only 3.4% off target differentiation to glial cells as marked by S100 β (n = 2). Microelectrode array analysis of the neural activity using the Maestro Pro™ MEA System showed an increasing mean firing rate reaching over 1.5 Hz with over 300 firing bursts and a synchronicity index over 0.06 by day 42 (n = 1). Astrocytes thawed into STEMdiff™ Astrocyte Serum-Free Maturation Medium and cultured for a further 7 days were positive for astrocyte markers S100 β ($84.3 \pm 4.7\%$) and GFAP ($79.0 \pm 4.4\%$) by immunocytochemistry with low levels of immature neuron marker doublecortin (DCX; $3.3 \pm 1.5\%$; n = 3). Co-cultures of neurons and astrocytes survived for at least 14 days and displayed the expected morphology. In summary, we have generated highly pure, hiPSC-derived NPCs, forebrain neuron precursors, and astrocytes to provide a standardized and reproducible tool box for performing disease modeling studies and large-scale neural research.

Disclosures: **A. Moosa:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **K. Wrightson:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **B. Streeter:** A. Employment/Salary (full or part-time): Axion Biosystems. **J. Keil:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **A. Gaffney:** 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, Simon Fraser University.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.07/YY12

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Optimized NSC proliferation, neural maturation, and maintenance of primary neurons in a xenofree system suitable for cell therapy development

Authors: ***R. SCOTT**¹, A. IBRAHIMI¹, K. LAHA², S. SHIN³, D. KUNINGER⁴;

¹Thermo Fisher Scientific, Frederick, MD; ²Thermo Fisher Scientific, Fitchburg, WI; ³Primary and Stem cell Systems, Thermo Fisher Scientific, Frederick, MD; ⁴Thermo Fisher Scientific, FREDERICK, MD

Abstract: Cell culture protocols targeted for clinical applications require growth media components that minimize the possibility of transmitting hazardous constituents to the culture. Animal and human derived media components represent a possible vector for these hazardous constituents, and minimizing their use is important in developing cell therapy protocols. Here we examine the performance of CTS™ B-27™ Xenofree Supplement, a formulation that has been modified from the research-use-only B-27™ Supplement, Xenofree using recombinant proteins that have been verified to help decrease raw material safety risk, to replace two of the components that were derived from human sources. Four production lots of CTS B-27 supplement were tested and compared to RUO B-27 Xenofree supplement formulation. Neural stem cells (NSCs) proliferated in CTS B-27 supplement showed equivalent or higher viable cell yields than NSCs proliferated in B-27 Xenofree supplement. Expression of SOX1 was not significantly different between groups. Neural maturation in CTS B-27 supplement showed significantly increased cell yield compared to cells matured in B-27 Xenofree supplement (ANOVA, $p < 0.001$). No significant difference was observed in Neurofilament expression of maturing neurons in the CTS B-27 supplement compared to the RUO xenofree supplement. In addition, primary human neurons were maintained for 10 days and analyzed using a high content analysis platform. There were no significant differences in the number of HuC/D positive cells per field between the CTS B-27 supplement and the B-27 Xenofree supplement. We conclude CTS B-27 Xenofree Supplement performs equivalently to B-27 Supplement, Xenofree. This makes CTS B-27 Xenofree Supplement a viable option for developing safe, effective, and regulatory-compliant cell therapy protocols.

Disclosures: **R. Scott:** A. Employment/Salary (full or part-time): Thermo Fisher Scientific. **A. Ibrahimi:** A. Employment/Salary (full or part-time): Thermo Fisher Scientific. **K. Laha:** A. Employment/Salary (full or part-time): Thermo Fisher Scientific. **S. Shin:** A. Employment/Salary (full or part-time): Thermo Fisher Scientific. **D. Kuninger:** A. Employment/Salary (full or part-time): Thermo Fisher Scientific.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.08/YY13

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH grant R01AG068581
NIH grant RF1AG076122
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FDOH Grant 23A04

Title: Nuclear Import Receptors as Modulators of Tau Aggregation and Pathology

Authors: *S. GHOSH¹, S. KONDAPALLI¹, C.-W. TSAI¹, J. GARCIA MORATO¹, B. KHALIL², J. H. LEE³, A. M. LY², W. ROSSOLL⁴;

²Neurosci., ¹Mayo Clin., Jacksonville, FL; ³Neurosci., Mayo Clin., St Augustine, FL; ⁴Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Title: Nuclear Import Receptors as Modulators of Tau Aggregation and Pathology

Authors: *Sumanta Ghosh, Ph.D., Sumanth Kondapalli, Chih-Wei Tsai, Jorge Garcia Morato, Ph.D., Jannifer H. Lee, Ph.D., Bilal Khalil, Ph.D., Anh M. Ly, and Wilfried Rossoll, Ph.D.*

Affiliation: Department of Neuroscience, Mayo Clinic, Jacksonville, FL The progressive accumulation of insoluble tau aggregates in neurons is a defining pathological feature of Alzheimer's disease (AD) and other tauopathies. The formation of neurofibrillary tangles (NFTs), composed primarily of hyperphosphorylated tau, correlates strongly with cognitive decline and remains a major therapeutic target. Tau pathology and TDP-43 proteinopathy are known to interfere with nuclear pore complex integrity and nucleocytoplasmic transport (NCT) function. Our group previously demonstrated that specific nuclear import receptors (importins) function as molecular chaperones to mitigate aberrant phase separation and aggregation of TDP-43. Motivated by these findings, we investigated whether importins similarly modulate tau pathology. Using a GFP-tagged tau construct with patient-derived mutations that promote its rapid pathological aggregation, we co-expressed importin constructs in cultured cells and assessed tau distribution, phosphorylation status, and amyloid formation using semi-quantitative fluorescence microscopy. Western blot analysis was used to quantify changes in soluble and insoluble tau fractions. We found that selected importins significantly reduced phospho-tau aggregation and promoted tau solubility in both overexpression and seed-induced aggregation models. Through a series of structure-function studies, we identified truncated importin variants that retained chaperone activity and enhanced tau solubility in both cell culture and organotypic brain slice culture models. These effects were replicated in Tau RD P301S biosensor cells seeded with AD brain-derived tau. Further, mutational analysis of cargo-binding domains revealed residues with a critical role for this protective activity. These findings demonstrate a novel function of importins in suppressing pathological tau aggregation and suggest that targeting nuclear transport pathways may offer a promising therapeutic approach for AD and related disorders.

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Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.09/YY14

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: In vitro models of OPC maturation and myelination for drug discovery

Authors: *B. HALL, S. GIACHETTI, T. MODEBADZE, E. MALAVASI;
Concept Life Sci., Edinburgh, United Kingdom

Abstract: Oligodendrocytes are the myelinating cells of the Central Nervous System (CNS). Intact myelin is required for the fast conduction of electrical signals along axons and provides critical metabolic and trophic support to neurons. Myelin integrity is essential for neuronal function and long-term viability. Disruption of, or loss of myelin (demyelination), is a pathological feature of several neurodegenerative and neuroinflammatory conditions, including Multiple Sclerosis, Alzheimer's Disease, and ischemic stroke. In these disorders, demyelination leads to impaired neuronal communication, axonal degeneration and neuronal death. Myelinating Oligodendrocytes originate from a pool of progenitors known as Oligodendrocyte Precursor Cells (OPCs). These cells persist throughout adulthood and typically remain in a quiescent state under homeostatic conditions. Upon CNS injury, OPCs respond by proliferating and migrating to the lesion site, where they differentiate into oligodendrocytes. This endogenous remyelination process is a vital component of CNS repair; however, in many pathological contexts, it is either insufficient, delayed, or fails. Consequently, therapeutic strategies aimed at enhancing the regenerative potential of OPCs, by stimulating their proliferation, migration, and differentiation, are the focus of considerable research efforts. As a result, robust translational *in vitro* assays that enable accurate and reproducible quantification of functional phenotypes in OPCs, are needed to support and accelerate drug discovery in neuro-regeneration. Here, we present data demonstrating that purified OPCs can be used to model key functional phenotypes *in vitro*, which can be predictably and reproducibly modified using known control compounds. OPCs are cultured under proliferative conditions and subsequently exposed to a defined differentiation medium, supplemented with either vehicle controls or reference substances. Over a 7-day treatment window, the impact of treatment is measured by quantifying the expression of Myelin Basic Protein (MBP) by both High Content Imaging and RT-qPCR. In addition to our OPC maturation assay validation data, we share single-cell transcriptomic snapshots of OPCs as they progress through the stages of *in vitro* maturation. We also introduce complementary data from our ongoing development of an *in vitro* myelination assay, allowing for the assessment of functional myelin deposition. Finally, we discuss how these therapeutic modality-agnostic and versatile models and assays can be utilised at multiple stages of the drug discovery process, from target validation through to candidate selection.

Disclosures: B. Hall: A. Employment/Salary (full or part-time): Concept Life Sciences Ltd. S. Giachetti: A. Employment/Salary (full or part-time): Concept Life Sciences Ltd. T. Modebadze: A. Employment/Salary (full or part-time): Concept Life Sciences Ltd. E. Malavasi: A. Employment/Salary (full or part-time): Concept Life Sciences Ltd.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.10/YY15

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R01AG072298
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CNSII, Inc

Title: Directed Evolution of AAV5 Combined with Glymphatic Delivery Results in Selective Targeting of Human Glial Progenitor Cells in vivo

Authors: *A. J. CONA¹, J. N. MARIANI², M. NEDERGAARD³, A. BENRAISS³, S. A. GOLDMAN²;

¹Biomed. Genet. and Genomics, Univ. of Rochester Med. Ctr., Rochester, NY; ²Dept of Neurol.,

³Ctr. for Translational Neuromedicine, Univ. Rochester Med. Ctr., Rochester, NY

Abstract: Many neurological disorders involve white matter atrophy, oligodendrocytic loss, and demyelination without remyelination despite a pool of resident glial progenitor cells (hGPCs). For therapeutic intervention, vectors are needed to deliver genetic payloads across the blood brain barrier to hGPCs without systemic transduction. Adeno-associated viruses (AAVs) are widely used but show low efficiency and high systemic transduction in humans, causing liver toxicity in clinical trials. To establish a means of efficiently transducing hGPCs in vivo with therapeutic transgenes, we targeted PDGFRA-driven Cre-recombinase expressing hGPCs in human glial chimeric mice with a library of capsid-modified recombination-reported AAVs. To maximize the intracerebral distribution of these viruses while minimizing their dosing and extracerebral spread, we paired their intracisternal delivery with systemic hypertonicity and modified prone Trendelenburg positioning. This method exploited intracerebral glymphatic flow to bypass the blood-barrier, delivering AAV directly into the brain parenchyma. A library created by using random, heptameric inserts at the AAV5 VP1 capsid Q574 was injected into 16-week-old Rag1^{-/-} mice neonatally chimerized with hESCs containing Cre under the PDGFRA promoter and were sacrificed one week later. PCR screening for gliotropic viral capsid sequences, filtered against visceral organs, identified a set of AAV5-based vectors that preferentially infected human GPCs and/or their derived astrocytes and oligodendrocytes in vivo, with minimal systemic infection. NGS identified the top capsid variants, which were tested in mice using CAG-EGFP viruses. Intracisternal delivery of AAV-EGFP with hypertonicity and prone Trendelenburg positioning enhanced brain-wide distribution. We investigated the human selectivity of CM1 using human glial chimeric *shiverer* mice (MBP^{shi/shi} x rag2^{-/-}), where over half of all Olig2-defined hGPCs and oligodendroglia are of human origin (52.2 ± 6.0%, n=3). Graft-derived Olig2-expressing cells were 6.7-fold more transduced than their mouse counterpart in the callosum; 87 ± 3.3% of infected cells were human, and 13 ± 3% mouse (n=3 mice/group; <0.0001 by t-test), indicating that this hGPC-evolved vector manifested strong selective tropism for human GPCs relative to their neighboring mouse GPCs. Glymphatic delivery of capsid-modified AAV5s, evolved on human GPCs in vivo, thus enables efficient, brain-wide transgene delivery to human glia and their progenitors in the adult brain, with minimal off-target transduction.

Disclosures: A.J. Cona: None. J.N. Mariani: None. M. Nedergaard: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Drs. Goldman and Nedergaard are co-founders and have equity in CNSII, Inc., which provided partial support for this project. A. Benraiss: None. S.A. Goldman: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Drs. Goldman and Nedergaard are co-founders and have equity in CNSII, Inc., which provided partial support for this project.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.12/YY16

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Tools for elucidation of GPR75 signalling and identification of GPR75 modulators

Authors: R. ALALWANY, A. KEMP, G. CHAN, P. COCCHIARA, ***L. GERRARD**;
Sygnature Discovery, Glasgow, United Kingdom

Abstract: G protein coupled receptors (GPCR) play vital roles in the regulation of metabolic processes, making them suitable therapeutic targets for metabolic associated disorders. These include conditions such as type 2 diabetes (T2D), obesity, and dyslipidaemia that together impose a major burden on healthcare systems worldwide. Orphan GPCR GPR75 has attracted a great deal of attention since its emergence as a potential druggable target for metabolic disorders. GPR75 is expressed in a range of tissues with high expression in the brain, where it is believed to play a key role in regulating appetite, body weight and energy metabolism. Chemokine CCL5 and 20-HETE have been reported to bind GPR75. However, challenges in reproducing consistent ligand responses across different cell-based systems have hindered target validation and drug discovery efforts. Advancing our understanding of GPR75 signalling is crucial to realise its therapeutic potential in neurometabolic disorders. To this end, a suite of GPR75 expressing cell lines and functional assays were generated to interrogate GPR75 signalling pathways and support the identification of novel ligands. These allow assessment of second messenger/G protein signalling, cell surface receptor expression, and β -arrestin interaction. β -arrestin recruitment and GPR75/CRE reporter assays were established to be suitable for detecting modulation of constitutive GPR75 activity as well as ligand-induced receptor signalling. These were optimised for high-throughput screening in 384-well plate format and validated for the detection of GPR75 interacting molecules. A screening workflow was created for compound testing that utilised the β -arrestin recruitment and GPR75/CRE reporter assays alongside GPR75 receptor surface/internalisation assays. Together, these tools form a solid foundation for the identification of GPR75 modulators and provide unique opportunities for understanding GPR75 signalling. Further development of these functional assays fulfils a critical need for HTS platforms that target metabolic GPCRs and can lead to the discovery of therapeutic molecules for metabolic associated disorders.

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Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.13/YY17

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: The HCR™ HiFi Encoder: Rapid, Purification-Free Functionalization of Primary Antibodies for Background-Suppressed Signal Amplification

Authors: *C. EARL, A. ACHARYA;
Mol. Instruments, Los Angeles, CA

Abstract: Multiplex antibody-based imaging is a powerful tool for probing protein expression in the nervous system, but current approaches for multiplex immunofluorescence often rely on antibody-oligonucleotide conjugation, which can introduce nonspecific background, involve cumbersome protocols, and result in inconsistent labeling. To address these challenges, we developed the **HCR™ HiFi Encoder**, a novel reagent that enables **rapid, purification-free functionalization of unmodified primary antibodies** for *in situ* detection using HCR™ Gold or HCR™ Pro amplification. The Encoder consists of two domains: A **species-specific docking domain** that binds the constant region of IgG antibodies with defined stoichiometry and no observable impact on antigen binding. A **background-suppressed initiator domain** compatible with HCR™ amplifiers X1-X10, leveraging second-generation HiFi probe technology for minimal non-specific signal. Upon simple incubation, the Encoder forms a **1:1 initiator-to-antibody complex** in just 10 minutes without chemical conjugation or purification. Importantly, the resulting encoded antibodies exhibit **no detectable non-specific binding** in tissue, effectively avoiding the high background typically associated with antibody-oligonucleotide conjugates. This is enabled by the incorporation of second-generation HiFi probe background suppression chemistry. **Key technical features:** Works with any unmodified primary antibody. Maintains antigen-binding performance. Supports both manual and automated workflows. Preserves tissue and epitope integrity (no protease digestion). Enables highly specific multiplex protein detection using HCR™ amplification. The HCR™ HiFi Encoder enables efficient and background-free antibody functionalization for quantitative *in situ* protein imaging in brain tissue, expanding access to high-plex, high-resolution molecular mapping for neuroscience research.

Disclosures: C. Earl: A. Employment/Salary (full or part-time): Molecular Instruments, Inc. A. Acharya: A. Employment/Salary (full or part-time): Molecular Instruments, Inc.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.14/YY18

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant R01NS070267

Title: Preliminary report: Augmenting functional recovery of decentralized bladders using somatic nerve transfer and administration of an AAV construct expressing BDNF in bladder wall

Authors: ***T. HANLON**¹, E. E. TULLY¹, S. SABU¹, L. BRIGHT-ROWE¹, I. TAMAS¹, B. A. HILLIARD¹, M. KOLPAKOV¹, E. TIWARI², M. G. VAN DER BAS³, J. M. BROWN⁴, M. F. BARBE¹;

¹Aging + Cardiovasc. Discovery Ctr., Lewis Katz Sch. of Med. of Temple Univ., Philadelphia, PA; ²Urology, Albert Einstein Col. of Med., Brooklyn, NY; ³Aging + Cardiovasc. Discovery Ctr., Lewis Katz Sch. of Medicine, Temple Univ., Philadelphia, PA; ⁴Dept of Neurosurg., Massachusetts Gen. Hospital, of Harvard Univ., Boston, MA

Abstract: Previous patient surveys have shown that patients with spinal cord or cauda equina injuries prioritize recovery of bladder function. Previously, we found that surgical transfer of a portion of the obturator nerve to distal vesical nerves innervating the bladder can partially restore motor function in dogs after bladder decentralization, and that higher bladder contractility after this somatic nerve transfer was associated with higher BDNF expression in bladder nerves and intramural ganglia. We seek now to improve the beneficial effects of nerve transfer to decentralized bladders via BDNF administration. This project was first approved by the university animal care and use committee. Thirty-seven canines were included. Under anesthesia, bladder decentralization was performed in 22 animals by transecting the vesical nerves to the bladder intra-abdominally. Thirteen of these underwent immediate obturator-to-pelvic nerve transfers (a portion of the obturator nerve was transferred to the bladder and sutured end-on-end to the vesical nerves, ObNT), while still under anesthesia. ObNT animals were divided into 3 groups that received either: 1) injections into the bladder wall of adeno-associated virus encoding brain derived neurotrophic factor (ObNT-AAV-BDNF, n=5; 100 ul/site, 10 sites in dome and mid bladder); 2) AAV encoding green fluorescent protein (ObNT-AAV-GFP, n=4, similarly injected), or 3) no injections (ObNT, n=5). Nine animals were left decentralized (Decentralized). Remaining were sham Controls. Bladder function was examined using awake urodynamics longitudinally, and electrophysiological stimulation of vesical branch pre-surgery and at final testing. Bladder, neural tissues, and organs were collected. To date, data from 3 ObNT-AAV-BDNF, 2 ObNT-AAV-GFP, 9 ObNT and 15 Controls have been collected and analyzed. Stimulation of repaired vesical nerves in AAV-BDNF injected dog induced bladder pressures equivalent to Controls, and higher bladder pressures than Decentralized and ObNT groups. cMyc staining of AAV promoter was observed in intramural neurons, nerves, myofibers, and arterial walls around sites of injections. Although preliminary in nature, these data suggest that AAV-BDNF injection to the bladder wall could positively augment bladder reinnervation by a somatic nerve (the obturator nerve). These results support the need for further experimental investigation.

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Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.15/YY19

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A Streamlined Approach to Multigene Vector Design for Simplified Development of Complex Therapeutics

Authors: Z. HERMSEN¹, J. LEE¹, *A. D. CLAUSEN¹, S. GLICKSTEIN², T. REED²;
¹Zymo Res. Corp., Irvine, CA; ²OspreyBioScience, Frederick, MD

Abstract: Molecular therapies have transformed personalized medicine by offering long-lasting, low-risk alternatives to conventional treatments. Many neurodegenerative conditions, like Alzheimer's, Parkinson's, and ALS, are characterized by complex regulatory pathways. As a result, effective treatments often require multigenic approaches rather than traditional monogenic therapies. However, constructing multigenic vectors is hindered by high costs, limited precision, off-target effects, and the difficulty of troubleshooting increasingly complex designs. In addition, the size and complexity of multigene vectors are challenging for modern DNA synthesis methods, necessitating labor-intensive and time-consuming cloning procedures. To overcome these barriers, Osprey Bio developed the Bird of Prey vector system, a standardized, cost-effective platform for customizable multigene vector assembly using modular genetic units. In this study, we utilized the Bird of Prey system along with Zymo Research's highly efficient cloning tools to build a four-gene reporter vector containing *rLUC*, *fLUC*, *GFP*, and *RFP*. Restriction-digested DNA inserts were purified using a gel extraction method with micro-elution spin columns, yielding concentrated, ultra-pure fragments ready for ligation. Recombinant plasmid was transformed into competent *E. coli* cells that do not require heat shock, enabling efficient plasmid propagation. Highly concentrated, endotoxin-free plasmid DNA was then isolated using a rapid spin-column method, ensuring suitability for downstream transfections. To evaluate functionality and cloning success, monogenic, bigenic, and quadgenic constructs were transfected into HEK293T cells. Fluorescence and luciferase assays revealed that all constructs exhibited successful reporter gene expression. Additionally, *rLUC* expression was consistent across mono-, bi- and quadgene constructs, and fluorescence microscopy verified colocalization of *GFP* and *RFP* expression. These results demonstrate the efficiency and flexibility of the Bird of Prey system when used with Zymo Research's robust cloning technology for the construction of multigenic vectors. This streamlined approach accelerates the development of multigene therapeutics, enabling rapid progress in neurodegenerative disease research.

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Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR367.16/YY20

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Systematic Evaluation of AAVs in Two Non-Human Primate Models Following IVT Injection Identifies AAV(Eye) as a Promising Candidate for Further Capsid Engineering

Authors: B. WANG¹, C. XU², *Q. WANG³, D. YU²;

¹AAVnerGene Inc., Rockville, MD; ²AAVnerGene Inc, ROCKVILLE, MD; ³Wang, Rockville, MD

Abstract: Gene therapy holds great promise for the treatment of eye diseases. AAVs are widely used for retinal gene delivery. AAV2 is predominantly employed for intravitreal injection (IVT), while AAV5 and AAV8 are commonly used for subretinal injection (SRI). IVT represents a safe and promising approach for ocular gene therapy. However, the inner limiting membrane (ILM) poses a significant barrier, restricting the penetration of natural AAV serotypes into deeper retinal layers beyond the GCL. To overcome these limitations, engineered AAV2 variants such as AAV2-YFs, AAV2-7M8, and AAV-4D-R100 have been developed through directed evolution, enabling improved delivery to the outer retina. However, significant challenges remain in translating these advancements to large-animal retinas, which differ substantially from rodent models. Large-animal retinas, such as those in dogs and non-human primates (NHPs), possess specialized high-acuity, a thicker vitreous, and a more robust ILM. Consequently, AAV2 may not be the optimal serotype for retina-targeting capsid engineering in NHPs via the IVT route. To identify more suitable AAV capsids for further development, we systematically evaluated commonly used AAV serotypes and a series of AAV2 variants in the whole retina of Rhesus macaques using barcoded vector technology. Among the natural serotypes, AAV2 showed the highest retinal DNA levels, followed by AAV5, AAV9, AAV8, and AAV6. AAV2-YF variants provided a modest increase in DNA levels. Notably, AAV2-7M8—evolved in large-animal models—achieved an approximately 10-fold increase in DNA levels compared to AAV2, whereas mouse-derived variants (AAV2-NN and AAV2-GL) yielded comparable or reduced levels. At the RNA level, AAV6 exhibited approximately an 8-fold increase in expression compared to AAV2. Remarkably, AAV2-7M8 achieved a several-hundred-fold increase in RNA expression. We further identified a novel AAV capsid, AAV(Eye), which demonstrated a 3.4-fold increase in DNA levels and a 47-fold increase in RNA expression relative to AAV2, along with enhanced retinal specificity compared to AAV2-7M8. In Cynomolgus monkeys, AAV(Eye) showed approximately a 12-fold increase in RNA expression over AAV2. An evolved derivative, AAV(Eye)-1, achieved a 77-fold increase in RNA expression. In summary, AAV(Eye) and its derivatives show strong potential as next-generation AAV capsids for retinal gene delivery, particularly in NHP models. These capsids represent promising platforms for future capsid engineering efforts aimed at improving the efficiency and specificity of intravitreal gene therapies.

Disclosures: B. Wang: None. C. Xu: None. Q. Wang: None. D. Yu: None.

Poster

PSTR367: Biotechniques: Gene, Protein, or Cell Based Approaches

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Program #/Poster #: PSTR367.17/YY21

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Automated 4-Plex Workflow Enables High-Throughput, Flexible Immunofluorescence Analysis Across Neuro-Oncology Models

Authors: *J. HEATH, S. LINDSAY, R. JONES;
Leica Biosystems, Newcastle, United Kingdom

Abstract: Background: Multiplex immunofluorescence (mIF) holds immense promise for neuro-oncology research, yet adoption is often limited by challenges in throughput, assay optimization, and workflow complexity. Traditional mIF approaches require extensive manual intervention and technical expertise, creating barriers for broader use in discovery studies. To address these limitations, we are developing a ready-to-use 4-plex mIF designed for use on the BOND RX research staining platform by Leica Biosystems. This system provides an automated, streamlined solution for simultaneous visualization of tumor, immune, and neural markers in formalin-fixed, paraffin-embedded (FFPE) tissues from preclinical models of brain tumors, for research purposes. Methods:

A 4-plex mIF detection kit was applied to FFPE tissue sections from murine neuro-oncology models and fully automated on the BOND RX platform. Marker panels under evaluation included combinations of neural lineage and tumor markers, immune markers, and neuroanatomical references. Slides were scanned using the new Aperio FL scanning system. Image analysis workflows are being developed in partnership with Indica Labs to quantify co-expression, spatial relationships, and regional biomarker distribution within the tumor microenvironment and surrounding brain tissue. Preliminary Results and Planned Applications: Early results in murine models show strong, specific signal across all four markers. Staining is consistent with expected biological patterns and requires minimal panel re-optimization across different targets and tissue types. Upcoming studies will apply the research workflow across diverse neuro-oncology models, including genetically engineered mouse models (GEMMs), with a focus on analyzing target engagement, immune modulation, and regional marker heterogeneity. Conclusions:

The 4-plex mIF assays highlighted here, coupled with automated staining on the BOND RX research platform, address key barriers in spatial biomarker research: limited throughput, complex assay development, and reproducibility. This research workflow enables high-resolution, multiplexed analysis of tumor and immune biology in neuro-oncology models and supports a scalable, user-friendly solution for exploratory biomarker discovery in neuroscience research.

Disclosures: **J. Heath:** A. Employment/Salary (full or part-time): Leica Biosystems. **S. Lindsay:** A. Employment/Salary (full or part-time): Leica Biosystems. **R. Jones:** A. Employment/Salary (full or part-time): Leica Biosystems.

Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.01/YY22

Topic: E.05. Auditory and Vestibular Systems

Support: NIH R01 DC018650
NIH R00 DC015014
JHU Kavli NDI Distinguished Graduate Fellowship

Title: Multi-area cortical mechanisms underlying continual learning

Authors: ***Z. ZHU**¹, **A. CHARLES**³, **K. KUCHIBHOTLA**²;
²Psychological and Brain Sciences, Neurosci. and Biomed. Engin., ¹Johns Hopkins Univ., Baltimore, MD; ³Johns Hopkins Univ. - Homewood Campus, Baltimore, MD

Abstract: Humans and other animals can learn and execute many different tasks throughout their lifespan, a process known as continual learning. However, this biological ability challenges many artificial neural networks that suffer catastrophic forgetting, unless these networks are regularized or expanded. Specifically, unique information about new tasks can be encoded through expansion (adding ‘neurons’ into the network for a new task), while shared information between old and new tasks can be integrated into shared representations. Here, we aimed to test how the biological brain naturally solves this problem. We trained mice on a class of tasks involving the learning of multiple, related, sensorimotor associations, specifically multiple distinct auditory two-choice tasks using a moveable wheel. We exploited a sequential training curriculum where mice expertly performed both tasks in a block-based manner at the final stage of training. We tracked neural activity of L2/3 pyramidal cells in the auditory cortex (AC) and the posterior parietal cortex (PPC) using multi-area two-photon mesoscopic calcium imaging, which allowed us to longitudinally track expansion and integration of neural representation at single-cell resolution throughout different stages of multi-task learning. Surprisingly, a sub-area in PPC showed both reliable auditory responses even in naive animals and dynamic response patterns during learning, indicating its importance in learning multiple auditory tasks. Together, our behavioral and neural approach promises to help us better understand the precise computations used by biological neural networks for continual learning and how this depends on the learning curriculum.

Disclosures: **Z. Zhu:** None. **A. Charles:** None. **K. Kuchibhotla:** None.

Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.02/ZZ1

Topic: J.06. Computation, Modeling, and Simulation

Support: Gonzaga Science Research Program
ERDF-Project Brain dynamics, No. CZ.02.01.01/22_008/0004643
NIH Grant 1R01NS109553
NIH Grant 1R01MH125557
NIH Grant R01NS132913
NSF Grant 2223839
NSF Grant 2209874

Title: Neuron simulation code for modeling slow waves, spindles, and STDP during sleep

Authors: *C. G. FINK;
Gonzaga Univ., Spokane, WA

Abstract: Here we present a NEURON implementation of a well-established computational model of thalamocortical dynamics during sleep [1,2]. Implementation in NEURON not only makes this model more accessible to the neuroscientific community, but also enables more streamlined development of further features. For example, we present results demonstrating spindle-slow wave coupling [3] and sequence replay based on spike-timing dependent plasticity. This implementation also calculates local field potential from biophysical first principles. The code for this model is freely available online [4] and will be a valuable resource for anyone interested in computationally modeling sleep.

1. Bazhenov, Maxim, et al. "Model of thalamocortical slow-wave sleep oscillations and transitions to activated states." *Journal of Neuroscience* 22.19 (2002): 8691-8704.
2. Krishnan, Giri P., et al. "Cellular and neurochemical basis of sleep stages in the thalamocortical network." *eLife* 5 (2016): e18607.
3. Mushtaq, Muhammad, et al. "Differential thalamocortical interactions in slow and fast spindle generation: A computational model." *PLOS One* 17.12 (2022): e0277772.
4. Fink, Christian G., et al. "Python/NEURON code for simulating biophysically realistic thalamocortical dynamics during sleep." *Software Impacts* 21 (2024): 100667.

Disclosures: C.G. Fink: None.

Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.03/ZZ2

Topic: J.06. Computation, Modeling, and Simulation

Title: Annotated community structure of the adult *Drosophila* connectome

Authors: ***R. BETZEL**¹, M. PUXEDDU², C. LYNN³, B. TERVO-CLEMMENS¹, B. LARSEN¹, L. M. PARKES⁴, O. SPORNS², S. DVALI³, B. MISIC⁵;

¹Univ. of Minnesota, Minneapolis, MN; ²Psychological and Brain Sci., Indiana Univ., Bloomington, IN; ³Physics, Princeton Univ., Princeton, NJ; ⁴Sch. of Psychological Sci., Monash Univ., Melbourne, Australia; ⁵Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Connectomes are maps of nervous system wiring. Understanding the principles by which they are organized and contribute to brain function is a central aim of neuroscience. Connectomes can be modeled as graphs and analyzed using tools from network science. In these models, neural elements and their pairwise interactions are represented as nodes and edges, respectively. One key architectural feature is so-called “community structure”. Communities represent sub-networks whose nodes exhibit statistical regularities in their connectivity patterns. Community structure supports specialization of function, adaptability and evolvability, and cost-efficient spatial embedding.

However, most studies of community structure have been limited to meso-scale networks (nodes as regions) and use techniques blind to certain classes of communities, e.g. core-periphery and bipartite structure. Here, we examine the community structure of the whole-brain adult *Drosophila* connectome using a nested stochastic blockmodel (SBM). The connectome is weighted and directed and reconstructed at neuron resolution (N=138639 neurons) and the SBM, unlike other popular community detection methods, can detect generalized community configurations.

Briefly, we find evidence supporting the hypothesis that the *Drosophila* connectome’s community structure is organized hierarchically (communities within communities; 11 non-trivial hierarchical levels). We find that most communities interact assortatively—i.e. are segregated—though a small subset of communities comprised of neurons associated with processing of visual information are non-assortative. Additionally, we compare community boundaries to expert annotations of neurons and find that annotations tend to concentrate within communities, rarely crossing community boundaries. This observation suggests that neuronal function, class, and cell-type are highly determined by connectivity (and *vice versa*). Finally, we derive a set of features for communities based on their connectivity motifs, input/output annotations, and the distribution of neurotransmitters across their input/output synapses. Using data-driven clustering, we identify a set of archetypical multivariate feature vectors onto which communities load (along with their constituent neurons). This observation suggests that fine-scale communities may be viewed as “functional primitives,” that get combined into larger communities with increasingly diverse and heterogeneous functional profiles.

Our work joins other recent efforts to map and interpret the functional relevance of community structure in nano-scale connectome data.

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Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.04/ZZ3

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant MH124004
NIH Grant S10OD020039
NIH Grant 2024462

Title: Within-individual precision mapping of brain networks using task data and its application in identifying thalamic networks

Authors: *J. DU¹, V. TRIPATHI¹, M. ELLIOTT¹, J. LADOPOULOU¹, M. ELDAIEF², R. L. BUCKNER¹;

¹Harvard Univ., Cambridge, MA; ²Neurol. and Psychiatry, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Within-individual precision network mapping is a widely used tool that prevalently relies on functional connectivity analysis of resting-state data. Here, we investigated whether networks can be precisely estimated solely using data acquired during active task paradigms. We found that functional correlation matrices derived from task data were highly similar to those from resting-state fixation data, with the largest factor affecting network similarity being the amount of data. Networks estimated within individuals from task data showed strong spatial overlap with those estimated from resting-state fixation data and predicted the same triple functional dissociation across language, social, and episodic processing domains in independent data. These results suggest that there is an underlying, stable network architecture that is idiosyncratic to the individual and persists across task states. Critically, this finding implies that previously acquired task data can be reanalyzed and pooled in novel ways. To demonstrate the utility of this approach, we explored the functional organization of the human thalamus - a region that is notoriously difficult to map due to its small size and anatomical variability across individuals. Our analyses revealed that five distinct association networks remained segregated (or partially segregated) along the thalamic midline, including anterior, mediodorsal, and medial pulvinar. Seed regions from these thalamic subregions recapitulate spatially distinct cerebral association networks. In addition, our results showed that task-based data can be simultaneously used to estimate within-individual networks (from the residualized data) and the task response itself from the individualized regions, yielding between-subject estimates that are highly correlated to those that utilize independent resting-state data. This finding suggests that future data collections might exclusively acquire task data and use that task data to both localize networks and quantify the task response from regions within those networks. Overall, our results offer several key implications: 1) existing task data can be reanalyzed to estimate within-individual network organization, 2) resting-state fixation and task data can be pooled to increase

statistical power, and 3) future studies can exclusively acquire task data to both estimate networks and extract task responses.

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Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.05/ZZ4

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH R01NS117405

Title: Neuron-specific recruitment curves and activation differences in a motor cortex network model due to transcranial magnetic stimulation across stimulus orientations

Authors: *G. J. YU¹, F. RANIERI³, V. DI LAZZARO⁴, M. A. SOMMER², W. M. GRILL², A. V. PETERCHEV¹;

¹Psychiatry & Behavioral Sci., ²Biomed. Engin., Duke Univ., Durham, NC; ³Neurosci., Univ. of Verona, Verona, Italy; ⁴Med. and Surgery, Univ. Campus Bio-Medico di Roma, Roma, Italy

Abstract: Transcranial magnetic stimulation (TMS) is a versatile tool for treating neuropsychiatric disorders and providing diagnostic information. However, the strong TMS artifact prevents recording of the cortical neuronal response immediately after a pulse, so the acute neuronal and microcircuit responses to TMS remain largely unknown. In lieu of direct empirical evidence, computational models are used to explore hypotheses on the cortical response to TMS.

Recordings from the corticospinal tract during TMS of the motor cortex allow the response of pyramidal tract neurons (PTNs), that send axons directly to the spinal cord, to be separated from the artifact. We optimized spiking neuronal network models to reproduce the activity of motor cortex, as reflected in epidural recordings from cervical spine, in response to single pulses of TMS in the posterior-anterior (PA) and lateral-medial (LM) orientations across multiple stimulus intensities. Data from eight human subjects were used, and models were optimized on a subject-specific basis.

The network model represented a macrocolumn of human motor cortex and included layers 2/3, 5, and 6. Each layer contained excitatory intratelencephalic neurons (ITs) and inhibitory basket cells (BCs). Additionally, layer 5 contained PTNs. Each cell type received spiking input from extracolumnar afferents, and neurons were represented as leaky-integrate-and-fire models. Cell-to-cell connectivity was derived from anatomical studies. Activation was modeled using generalized logistic functions that reflected the probability of activation at a given stimulus intensity. Particle swarm optimization was used to identify synaptic weight factors and TMS activation parameters that reproduced the experimental responses.

Response types were divided into LM, PA with a large D-wave (D+), and PA without a D-wave (D-). Linear mixed effects models were computed with model activations as observations, response types as the fixed effect, and subject as the random factor. Statistically significant differences ($p < 0.05$) indicated that PA D- responses resulted from higher activation of superficial layers (L2/3 IT and BC, L5 IT, L5 PTN afferents) while LM responses had less superficial activation and more direct L5 PTN activation. PA D+ activations were intermediate between these two.

Using these data-driven network models, we identified functional distributions of activations within motor cortex that underly many types of corticospinal responses to TMS. Beyond motor cortex, these activations may also underlie the extratelencephalic responses that are communicated from other cortical TMS targets to their connected brain regions.

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Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.06/ZZ5

Topic: J.06. Computation, Modeling, and Simulation

Support: DIRP, NIMH, USA, ZIAMH002797

Title: Emergence of “gliders” in cellular-automata-based balanced E-I networks

Authors: *M. BELLUSCIO, K. SRINIVASAN, S. PAJEVIC, D. PLENZ;
Sect Critical Brain Dynamics, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD

Abstract: Synchronous neural activity in the cortex, as measured by local field potentials (LFPs), often follows a power-law distribution defined as neuronal avalanches. Within this avalanche activity, a subset has been observed to propagate across cortical regions while precisely preserving their original waveform, defined as Coherence Potentials. These propagating, network-level events are thought to support high-dimensional information transfer across large-scale neural circuits. Intriguingly, their stable, self-replicating spatiotemporal dynamics bear a striking resemblance to gliders in cellular automata, identified as localized patterns that maintain their structure while traveling through space and time. To explore this

analogy, we constructed a simplified neural network model consisting of an excitatory and inhibitory (E-I) layer with local within- and across-layer connectivity. Using a custom glider detection algorithm, we investigated the emergence and behavior of gliders under different network configurations. By systematically varying excitatory and inhibitory synaptic strengths and initiating activity through random stimulation, we observed the spontaneous formation of gliders in a narrow regime near the E-I balance—a transition zone between excitation- and inhibition-dominated dynamics. A single glider type emerged as dominant, enabling us to study its dynamics and reintroduce it artificially into the network. Simulated glider-glider collisions revealed a rich repertoire of interactions, including complex chain reactions capable of sweeping across the entire network even when the network remains critically balanced. Interestingly, glider formation was preceded by localized asymmetries in activity and synchronized E-I oscillations across glider generations, suggesting key prerequisites for their emergence. Our findings highlight a dynamical parallel between gliders and coherence potentials. If coherence potentials in the brain share these glider-like properties, they may support similarly complex, self-organizing dynamics—offering new insights into cortical information processing and the conditions required for select and stable propagation in neural systems.

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Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.07/ZZ6

Topic: J.06. Computation, Modeling, and Simulation

Support: Shanahan Family Foundation
AccelNet: International network for brain-inspired computation, NSF 2019976

Title: Learning using switching synaptic plasticity rules

Authors: *D. TURCU^{1,2}, R. POGODIN^{4,5,6}, J. CORNFORD⁸, B. A. RICHARDS^{4,7,5,6,9}, S. DORKENWALD³, S. MIHALAS²;

¹Allen Inst., Seattle, WA; ²Studio D3, ³Allen Inst. for Brain Sci., Seattle, WA; ⁴Mila - Quebec Artificial Intelligence Inst., Montreal, QC, Canada; ⁵Dept. of Neurol. & Neurosurg., ⁶Montreal Neurolog. Inst., ⁷Sch. of Computer Sci., McGill Univ., Montreal, QC, Canada; ⁸Sch. of Computer Science, Univ. of Leeds, Leeds, United Kingdom; ⁹CIFAR Learning in Machines and Brains Program, Toronto, ON, Canada

Abstract: Considering recent observations from the MICrONS datasets, we explore computational benefits of neural networks whose synapses can independently and dynamically change the governing plasticity rule during training. The mouse connectome data revealed that synapse strengths between cortical synapses across multiple cortical layers are well described by

a binary state variable, such that the synaptic weight distribution is bi-modal. The two log-normal modes were found to correspond to strong synapses having developed the spine apparatus and weak synapses lacking it. It is believed that the spine apparatus, effectively a calcium reservoir within the synapse that can affect synaptic dynamics, plays a significant role in plasticity and learning, though its exact function is not fully understood. While the connectome data is static, it is also known that synapses can gain or, slightly less likely, lose the spine apparatus. Here, we simplify these observations by assuming that synapses of a network can switch between one of two learning rules: a weak, pre-post-only rule governing weak synapses (e.g., Hebbian), and a strong, credit-assignment-based rule governing strong synapses (e.g., backpropagation). We explore the benefits and drawbacks of such a system in working memory tasks using recurrent neural networks. We focus on individual NeuroGym tasks, that require both long-term memory in the form of learning, and short-term, within-trial memory to store and reproduce the stimuli. We contrast our switching-rule with typical backpropagation-only trained recurrent neural networks. Observations from our simulations suggest that networks trained with our switching rule learn faster than backpropagation-only networks. While still exploring the mechanisms of these observations, our current intuition is that the Hebbian synapses in the switching networks are prone to create memory attractors faster than backpropagation alone can, and the backpropagation synapses in the switching rule can readily use those attractors to solve the task.

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Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.08/ZZ7

Topic: J.06. Computation, Modeling, and Simulation

Title: Emergence of Periodic Spike Sequences in a Computational Model of Layer II Medial Entorhinal Cortex

Authors: *D. SEDERMAN¹, D. Z. JIN²;

¹Penn State Univ., State College, PA; ²Physics, Penn State Univ., University Park, PA

Abstract: Long periodic sequences of neural activity in the medial entorhinal cortex (MEC) of mice are thought to be critical for episodic memory formation (Cogno et al., Nature, 2024). Layer II of MEC consists of two broad excitatory cell types, stellate and pyramidal cells. Stellate cells are integral in grid activity for spatial coding, while pyramidal cells provide timing information to the hippocampus. Here we propose that pyramidal neurons form a recurrent network producing long periodic sequences. We expand on a theoretical model of sequence formation in randomly-wired, pulse-coupled leaky integrate-and-fire neurons (Jin, PRL, 2002). Our network consists of sparse connections between pyramidal neurons, simulated with a multi-

compartment conductance-based model. Pyramidal neurons are driven by high-frequency cortical inputs to the distal dendritic compartment. They inhibit each other through feedforward inhibition mediated by fast-spiking inhibitory neurons. Axonal delay times allow for inhibition to act more quickly than direct excitation between pyramidal neurons. This follows experimental measurements of axon path lengths, diameters, and myelination (Schmidt et al., Nature, 2017). Both excitatory and inhibitory inputs act at the proximal dendritic compartment. Pyramidal cells form patches with roughly 100 neurons per patch. These patches are organized across MEC on a hexagonal grid (Ray et al., Science, 2014). Model pyramidal neurons are placed on a layer II MEC-sized, two-dimensional hexagonal grid with stellate cells and interneurons distributed randomly in between patches. In this network, periodic sequences of pyramidal activity emerge with periods of tens of seconds. We suggest that pyramidal neurons are critical to the formation of the experimentally observed long periodic sequences in MEC.

Disclosures: D. Sederman: None. D.Z. Jin: None.

Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.09/ZZ8

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant R01EB029813
NIH Grant R01NS122742
NIH Grant U24NS124001
NIH Grant U01MH130907

Title: From point neurons to biophysically detailed networks: A data-driven framework for multi-scale modeling of brain circuits

Authors: *B. HERRERA¹, S. ITO¹, X.-P. LIU², D. HAUFLER¹, K. DAI¹, B. E. KALMBACH³, A. ARKHIPOV¹;

¹Allen Inst., Seattle, WA; ²Allen Inst. for Brain Sci., Seattle, WA; ³Human Cell Types, Allen Inst. For Brain Sci., Seattle, WA

Abstract: The Patch-seq technique connects transcriptomics, morphology, and electrophysiology in individual neurons. Recent work at the Allen Institute has yielded a comprehensive database of mouse and human neurons, featuring Patch-seq data for diverse cell types. In this study, we conduct a large-scale optimization of point-neuron generalized leaky integrate-and-fire (GLIF) and Izhikevich models based on these Patch-seq data for thousands of cells. Furthermore, in anticipation of applying models of diverse cell types for network simulations at multiple levels of resolution, we propose a strategy to convert point-neuron network models into detailed biophysical models. Point-neuron models were derived from the Patch-seq electrophysiology recordings using a hybrid optimization method that combines

Particle Swarm Optimization with Gradient Descent. We quantified the optimization performance for different types of current injection stimuli and compared it with an earlier approach [1]. We find that optimizing GLIF models using long-square step current stimuli generalizes better to noise stimuli than vice versa. With this approach, we obtained GLIF models for a total of 6,460 cells from various mouse and human glutamatergic and GABAergic interneurons [3-6]. Finally, we tested our GLIF-to-biophysical network conversion on our V1 point-neuron model [2]. The strategy involved (1) mapping each GLIF model to a biophysical neuron model; (2) replacing GLIF network parameters with corresponding biophysical parameters [2]; (3) estimating conversion factors to translate current-based synaptic weights to conductance-based weights for each source-target pair; and (4) constructing the biophysical network model using the scaling factors from (3). We simulated responses to spike train stimuli and calculated synaptic weight factors to match GLIF firing rates. We applied this approach to convert the point-neuron mouse V1 model into a biophysical network model, fine-tuning weights to align with recordings and validating it against in vivo Neuropixels data. Our work lays the foundation for advanced brain network simulations. By developing models for various cell types and utilizing transcriptomic data from Patch-Seq experiments, we offer insights into gene-morpho-electrophysiological relationships. Additionally, our method for converting point-neuron network models into detailed biophysical models facilitates the optimization of complex models while maintaining computational efficiency.

[1] Teeter et al. Nat Commun 2018. [2] Billeh et al. Neuron 2020. [3] Berg et al. Nat 2021. [4] Gouwens et al. Cell 2020. [5] Chartrand et al. Sci 2023. [6] Lee et al. Sci 2023

Disclosures: B. Herrera: None. S. Ito: None. X. Liu: None. D. Haufler: None. K. Dai: None. B.E. Kalmbach: None. A. Arkhipov: None.

Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.10/ZZ9

Topic: J.06. Computation, Modeling, and Simulation

Support: Villa Montallegro, “I progetti di ricerca: la ricerca che cura”
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University and Research (MUR), National Recovery and Resilience Plan
(NRRP), project MNESYS (PE0000006) – (DN. 1553 11.10.2022)

Title: Neuromorphic Twins recapitulate electrophysiological signatures of in vivo cortical networks

Authors: M. CARÈ¹, S. ZAHEDI GHASABEI², T. LAMBRESA², D. VECCHIA², T. LEVI³, F. BARBAN^{2,1}, *M. CHIAPPALONE^{2,1};

¹IRCCS Ospedale Policlinico San Martino, Genova, Italy; ²Univ. of Genova, Genova, Italy;

³IMS, Univ. of Bordeaux, Bordeaux, France

Abstract: Introduction. Neuromorphic engineering was originally conceived as an interdisciplinary field, aiming at building hardware systems by replicating the intricate biophysics of real neurons and synapses. Recent advancements have expanded its horizons, including novel computational principles, materials, and applications. In this context, we introduce the concept of ‘Neuromorphic Twins’: biomimetic Spiking Neural Networks (SNNs) embedded in neuromorphic platforms, designed to emulate the functional dynamics of biological brain circuits. **Objective.** This study aims at (i) introducing the novel concept of ‘Neuromorphic Twin’; (ii) extracting key electrophysiological signatures from in vivo cortical recordings; (iii) applying an automated optimization algorithm to tune SNN parameters to match these features; (iv) comparing the behavior of the optimized SNN with respect to the original biological networks. **Methods.** Neuronal activity was recorded from the rostral forelimb area (i.e. the analogous of the premotor cortex in the rat) of six anesthetized healthy rats. Key electrophysiological descriptors related to firing and bursting activity were computed. In parallel, we implemented the SNN on BioemuS (*Beaubois et al., Nat Comms 2024*), an FPGA-based platform comprising 1024 Hodgkin-Huxley single-compartment neurons and 2^{20} configurable synapses. A multi-objective genetic algorithm (Indicator Based Evolutionary Algorithm - IBEA as described in *Masoli et al., Front. Cell. Neurosci, 2017*) was used to automatically tune the SNN parameters to align its output with the in vivo metrics. **Results.** The optimized SNN successfully reproduced electrophysiological patterns in terms of firing rate, bursting rate, burst duration and Inter Spike Interval (ISI) as observed in the biological recordings. The computed metrics closely matched those derived from the in vivo cortical data, demonstrating the system’s capacity to emulate realistic cortical dynamics through automatic tuning. **Conclusions.** Neuromorphic Twins represent a powerful new approach for modeling brain activity in silico. Their ability to reproduce biological network behavior in real time positions them as promising tools for advancing brain-computer interfaces, neuroprosthetics and electroceutical therapies, particularly by addressing current limitations in personalization and adaptability.

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Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.11/ZZ10

Topic: J.06. Computation, Modeling, and Simulation

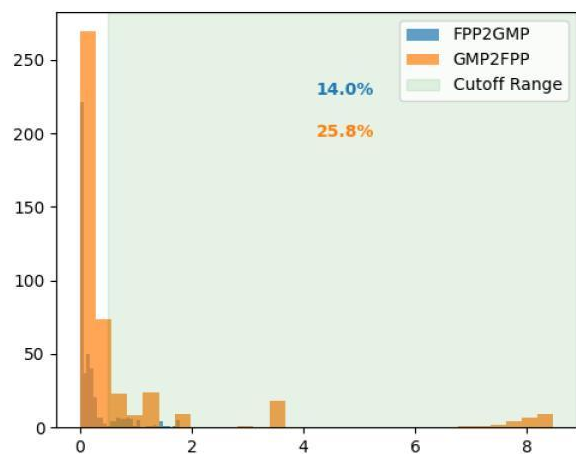
Support: AFOSR Grant FA9550-19-1-0306
NINDS Grant R01NS135850

Title: Spatial Representations Emerge in a Model Linking Global Maps to First-Person Perspectives

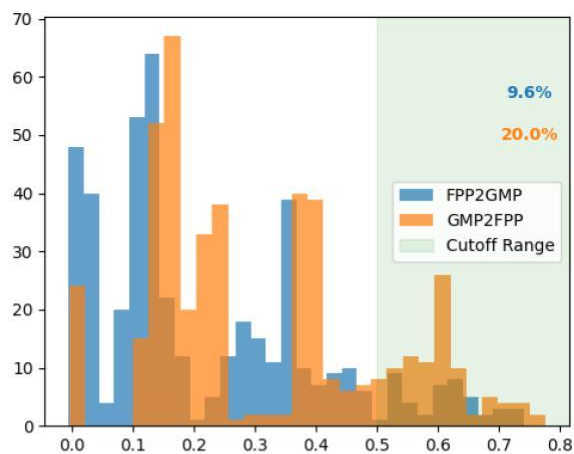
Authors: *H. LUI¹, E. R. CHRASTIL², D. A. NITZ⁴, J. L. KRICHMAR³;

¹Computer Sci., Univ. of California Irvine, Irvine, CA; ²Neurobio. & Behavior, ³COGNITIVE SCIENCES, Univ. of California, Irvine, Irvine, CA; ⁴Univ. of California San Diego, San Diego, CA

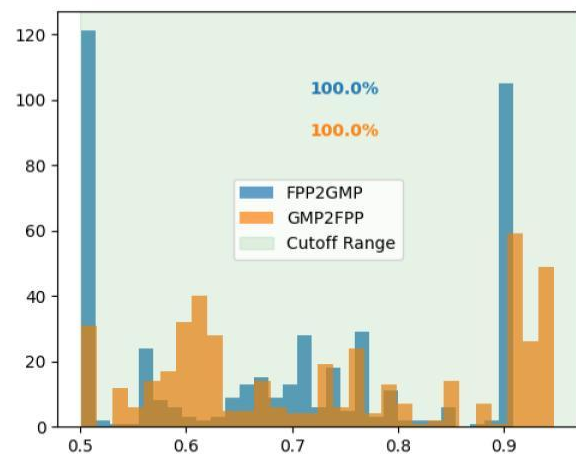
Abstract: Humans and many animals possess the remarkable ability to navigate environments by seamlessly switching between first-person perspectives (FPP) and global map perspectives (GMP). However, the neural mechanisms that underlie this transformation remain poorly understood. In this study, we developed a variational autoencoder (VAE) model, enhanced with recurrent neural networks (RNNs), to investigate the computational principles behind perspective transformations. We collected 8,000 training and 2,000 testing FPP and GMP image pairs per environment using a Khepera robot in Webots simulations across three environments, with each environment repeated across 5 random seeds. Our results reveal that temporal sequence modeling is crucial for maintaining spatial continuity and improving transformation accuracy when switching between FPPs and GMPs. The model's latent variables spontaneously develop spatial representations similar to those seen in the distributed cognitive maps of the mammalian brain, such as head direction cells, place cells, corner cells and border cells - but notably not grid cells. Evaluation against rodent spatial cell criteria showed the GMP to FPP direction consistently produced stronger spatial representations, with 25.8% of variables meeting spatial information thresholds, 20.0% meeting spatial coherence criteria, 100% meeting borderiness scores, and 24.0% meeting head direction criteria. This suggests that perspective transformation engages multiple brain regions beyond the hippocampus and entorhinal cortex. Furthermore, our findings demonstrate that landmark encoding, particularly proximal environmental cues such as boundaries and objects, play a critical role in enabling successful perspective shifts, whereas distal cues are less influential. These insights provide a new computational framework for understanding spatial cognition and offer valuable directions for future studies. This work highlights the contributions of temporal sequences, distributed representations, and proximal cues for navigating complex environments.



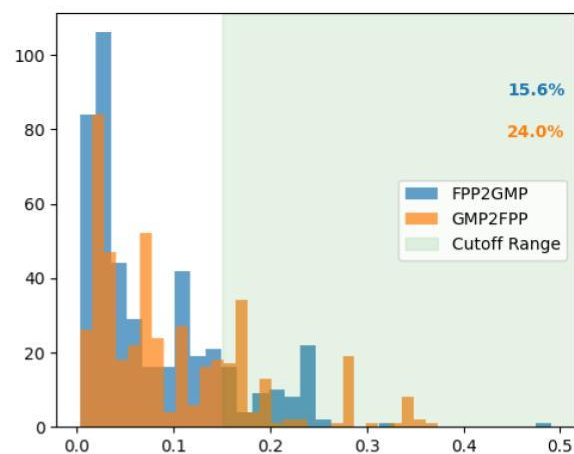
a) Spatial Information (bit)



b) Spatial Coherence



c) Borderiness



f) Rayleigh Score

Disclosures: H. Lui: None. E.R. Chrastil: None. D.A. Nitz: None. J.L. Krichmar: None.

Poster

PSTR368: Network Models I

Location: SDCC Halls B-H

Time: Tuesday, November 18, 2025, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR368.12/ZZ11

Topic: J.06. Computation, Modeling, and Simulation

Support: AFOSR Grant FA9550-22-1-0532

Title: A flexible binding-centric model in visual working memory via dendritic bistability

Authors: *J. XU;
Dartmouth Col., Lebanon, NH

Abstract: To understand the mechanisms underlying visual working memory, many experimental and modeling studies focus on capacity—specifically, how many items can be maintained. Binding is mostly used as a probe. For example, after memorizing an item with a location-color binding, subjects are asked during the test period to recall the color bound to the location. The response performance is then used to assess capacity.

However, these studies do not primarily focus on binding itself, but rather use it as a tool to investigate capacity. Binding is used in working memory to flexibly link features. For example, we can maintain a red square and a blue circle without confusing them with a blue square and a red circle. Another example is memorizing a directional mental path: turning left upon seeing a red sign, then turning right upon seeing a blue sign. Although the brain can flexibly bind features either bidirectionally or unidirectionally, the underlying mechanisms remain poorly understood. In this study, we propose a network model to investigate flexible binding in visual working memory. The network is fully connected with homogeneous weights. Each neuron has multiple dendrites, each receiving a separate recurrent input from other neurons. Each dendrite follows a bistable input-output relation, which can be implemented through conductance-based dynamics involving NMDA receptors (a spiking version of the model is also available). Functionally, each dendrite exhibits fast Hebbian-like behavior based on its activity, without requiring actual synaptic weight changes.

Using this model, we investigate how flexible binding can be achieved. With features A, B, C, D, and E represented in the network, we demonstrate the maintenance of two items ('A+C' and 'B+D'), two overlapping items ('A+C+E' and 'B+D+E'), and a sequence (A to B to C). The memory is robust to noise and perturbations, while remaining flexibly erasable or updatable in a palimpsest-like manner. Noise-induced interference reproduces the swap errors observed experimentally. We further present predictions of this model and demonstrate by simulation that how binding can limit working memory capacity.

Disclosures: J. Xu: None.

Poster

PSTR368: Network Models I

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Program #/Poster #: PSTR368.13/ZZ12

Topic: J.06. Computation, Modeling, and Simulation

Support: NRF-2022R1A2C3008991
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Title: One-shot initial alignment guides resilient learning without weight transport

Authors: *J. BAE, J. CHEON, S.-B. PAIK;
Brain and Cognitive Sci., KAIST, Daejeon, Korea, Republic of

Abstract: Learning, in both biological and artificial systems, relies on feedback signals to correct errors. Early-stage feedback, particularly its directional precision, critically influences learning accuracy and efficiency. Backpropagation (BP), the foundation of modern machine learning, enables accurate weight updates using precisely computed gradients. Yet this method enforces strict symmetry between forward and backward connections throughout training, incurring high memory demands, synchronization overhead, and vulnerability to noise and adversarial perturbations. Feedback alignment (FA) replaces exact weight transport with fixed random feedback connections, enabling approximate error correction at significantly lower computational cost (Lillicrap, 2016). However, this simplification sacrifices early guidance, leading to slower, less stable convergence and reduced accuracy. Here, to bridge this gap, we propose Initial Feedback Alignment (IFA) — a one-time soft alignment between forward and backward weights at network initialization. We evaluated IFA's impact by comparing learning dynamics under three rules: BP, FA, and IFA, using multi-layer feedforward neural networks on a natural image classification task. Across these experiments, IFA achieved fewer epoch-to-convergence and achieved performance comparable to BP, whereas FA lagged significantly behind. To further investigate these improvements, we examined training trajectories within loss landscape, using principal component analysis for visualization. Specifically, IFA's single preconditioning step establishes a reliable initial teaching signal that corrects the noisy and misdirected trajectories seen in FA, thereby driving rapid, stable error minimization. Subsequent spectral analyses of the loss Hessian reveal that such pre-alignment smooths error flow and promotes convergence toward flatter minima, thereby enhancing both trainability and generalization. Additionally, as training progresses, IFA permits gradual relaxation from its initial alignment, enabling adaptive learning dynamics that improve robustness to input corruption and resistance to adversarial attacks. These findings demonstrate that a single soft alignment at the outset leverages early guidance to efficiently shape the entire learning trajectory and achieves stable, resilient performance in a biologically plausible manner.

Disclosures: J. Bae: None. J. Cheon: None. S. Paik: None.

Poster

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Support: ZIAMH002783

Title: Characterizing the events that drive resting state fMRI functional connectivity

Authors: *J. FASKOWITZ¹, P. BANDETTINI², J. GONZALEZ-CASTILLO^{2,3},

¹NIH, BETHESDA, MD; ²Section on Functional Imaging Methods, NIMH, NIH, Bethesda, MD;

³Basque Ctr. on Cognition, Brain and Language, San Sebastian, Spain

Abstract: Distinct parts of the mammalian brain activate in a synchronized manner, even in the absence of external stimuli, forming what is referred to as intrinsic “functional connectivity” (FC; Biswal, 2010). Using fMRI-derived neurovascular signals, these relationships have often been characterized as low-frequency phenomena unfolding over the course of tens of seconds. However, prior research has shown that functional connectivity is driven by disparate discrete synchronized events of high-amplitude activity (Tagliazucchi, 2012). Here we temporally resolve FC at each acquisition and at the level of network edges, termed edge time series (Faskowitz, 2020), to identify salient neuronal information from event characteristics, that can potentially yield information beyond correlation. For this investigation we analyzed minimally preprocessed resting state data (15 min, TR=0.72) from a low-motion subset (N=352) of the Human Connection Project (Van Essen, 2013). Data were denoised (Behzadi, 2007) and parcellated into 200 activity traces (Schaefer, 2018). Edge time series were extracted, capturing frame-by-frame connectivity between all pairs of regions. Supra-threshold connectivity at each edge was recorded, forming distinct “edge events” with associated above-threshold durations. The group-average edge event count matrix is nearly identical to group-average FC (Spearman’s $\rho=0.96$), showing that simply counting edge events produces nearly the same information as measuring the Pearson correlation between regions. This relationship holds for within-subject comparisons as well (mean $\rho: 0.84 \pm 0.04$). The average edge event duration matrix (mean event duration 2.2 sec., ± 0.25 sec) is correlated but not redundant with FC ($\rho=0.73$, at the level of edges), suggesting that different relationships are punctuated by events with longer or shorter durations on average (range: 1.44-3.3 sec). Areas having the longest edge events include central visual and somatomotor areas, providing support for the hypothesis that contextual feedback into primary sensory regions shows more sustained activation (Cooper, 2023). The map of event duration is not redundant with other cortical maps, such as those of node-wise mean FC ($\rho=0.51$) or temporal signal-to-noise ratio ($\rho=-0.21$) and is not confounded by data quality as measured by node-average DVARS ($\rho=-0.35$). Here we show that FC can be effectively cast as a series of moments of high-amplitude connectivity, implying discrete, neuronal-information rich activity previously uncharacterized with fMRI. This opens up avenues of research into the timescale of connectivity between areas.

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