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Nanosymposium

NANO001: Synapses, Stress, and Systems: Modulators of Neural Plasticity and Dysfunction: Synapses

Location: SDCC Rm 24A

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO001.01

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01MH119347 (ED)
NIH Grant R21NS112749 (DJS, ED)
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Title: The adhesion G-protein coupled receptor Brain-specific angiogenesis inhibitor 2 (BAI2/ADGRB2) mediates synaptic nanostructure in excitatory hippocampal synapses

Authors: *B. H. LEE[1,2], C. MEYER[1,3], D. J. SPECA[1], E. DIAZ[1];
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Abstract: Specific nanoscale alignment of proteins across the presynaptic active zone and postsynaptic density (PSD) is critical for robust and efficient neurotransmission. Synaptic nanostructure is determined, in part, by the activity of a group of proteins referred to as trans-synaptic adhesion molecules (TSAMs). One such TSAM, the adhesion G-protein-coupled receptor Brain-specific Angiogenesis Inhibitor 2 (BAI2/ADGRB2), has been implicated in the development of excitatory synapses. We have recently published that knockout (KO) of full-length BAI2/ADGRB2 results in deficits in excitatory synapse density and a decrease of mushroom spines in primary hippocampal cultures, with no observed effect on inhibitory GABAergic synapses. However, the nanostructural alterations to excitatory synapses, and whether changes in synapse density and structure are also observed *in vivo*, are untested in BAI2/ADGRB2 KO mice. Here, we investigate the role of BAI2/ADGRB2 in regulating excitatory synaptic nanostructure in cultured hippocampal neurons and in *ex vivo* hippocampal slices. Using stimulated emission depletion (STED) microscopy, we demonstrate altered synaptic nanocolumn alignment in primary hippocampal cultures from BAI2/ADGRB2 KO mice compared to wildtype (WT), measured by surface AMPA receptor subunit 1 (sGluA1) and Rab-interacting molecules 1/2 (RIM1/2) colocalization. *In vitro* deficits also included a decrease in the percent volume of F-actin within dendritic spines. To further assess the role of BAI2/ADGRB2 in *ex vivo* tissue samples, we examined synaptic ultrastructure in the hippocampus using transmission electron microscopy (TEM) on the CA1 stratum radiatum dendritic field of 3-month-old wildtype WT and KO mice. TEM micrographs were used to quantify PSD thickness, PSD length, and the distance between the pre- and postsynaptic terminals. These findings suggest that BAI2/ADGRB2 influences excitatory synapse

development at the nanostructural level. Future studies will investigate the physiological consequences of altered synaptic nanoalignment in BAI2/ADGRB2 KO mice and the molecular mechanisms by which BAI2/ADGRB2 functions.

Disclosures: B.H. Lee: None. **C. Meyer:** None. **D.J. Speca:** None. **E. Diaz:** None.

Nanosymposium

NANO001: Synapses, Stress, and Systems: Modulators of Neural Plasticity and Dysfunction: Synapses

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Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO001.02

Topic: B.05. Synaptic Plasticity

Title: Quantifying the precision and storage capacity from structural correlates of synaptic strengths in the rat hippocampus

Authors: *M. SAMAVAT[1], T. BARTOL[2], T. J. SEJNOWSKI[3];
[1]The Salk Inst., Sunnyvale, CA; [2]Salk Inst. for Biol. Studies, La Jolla, CA; [3]Salk Inst., La Jolla, CA

Abstract: Synapses are the fundamental units of information storage in neural circuits, with their structure and strength modulated by synaptic plasticity. Investigating the structural variability and precision of synaptic strength correlates is essential for elucidating the mechanisms of learning and memory, advancing agentic artificial intelligence algorithms, and guiding the development of neuromorphic computing systems.Previous work (Samavat et al., Neural Computation, 2024; Samavat et al., bioRxiv, 2023) quantified the precision of synaptic plasticity and the information storage capacity of spine head volumes in the adult rat hippocampus. In the present study, we extend and compare this analysis to additional structural correlates of synaptic strength (spine head volumes)—including postsynaptic density (PSD) area, spine neck diameter (weak correlation), and the number of docked vesicles—using densely reconstructed neuropil from the CA1 region of the rat hippocampus. The correlations between these measures and spine head volumes were illustrated in Bartol et al., eLife, 2015.Synapses with the same axon onto the same dendrite (SDSA pairs) have a common history of coactivation and have nearly the same spine head volumes (Bartol et al, eLife, 2015; Samavat et al., Neural Computation, 2024; Samavat et al., bioRxiv, 2023) , suggesting that synapse function precisely modulates structure. To quantify the precision of additional structural correlates, we first computed the coefficient of variation (CV) for each measure across 10 SDSA pairs. The median CV for each measure was then used to define the bin width—representing the precision level—for binning the full distribution of that measure in the complete dataset reconstructed from the CA1 neuropil volume.Our analysis revealed that PSD area (12 bins), spine neck diameter (11 bins), and number of docked vesicles (8 bins) each exhibit fewer than half the number of distinguishable bins compared to spine head volumes (24 bins) estimated in Samavat et al., Neural Computation, 2024. These findings underscore the need for a multimodal structural approach to better

understand how structural correlates of synaptic weight evolve during plasticity and contribute to information encoding in connectomics.

Disclosures: M. Samavat: None. **T. Bartol:** None. **T.J. Sejnowski:** None.

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NANO001: Synapses, Stress, and Systems: Modulators of Neural Plasticity and Dysfunction: Synapses

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Presentation Number: NANO001.03

Topic: B.05. Synaptic Plasticity

Title: Therapeutic potential of Astaxanthin in long-term vascular dementia: modulation of oxidative stress and neuroinflammation in rats

Authors: *T. CHAO;
Inst. of Vet. Medicine, Natl. Chung Hsing Univ., South Dist., Taichung City, Taiwan

Abstract: The mortality rate associated with dementia has shown a continuous upward trend in recent years. Among its subtypes, vascular dementia is characterized by a relatively higher and more acute mortality rate. The proposed pathophysiological mechanism involves a reduction in cerebral blood flow, which exacerbates oxidative stress within brain tissues and induces region-specific neuronal damage and neuroinflammation, particularly within vulnerable brain nuclei. Astaxanthin is currently a drug under clinical development, functioning as an antioxidant. It is known to directly cross the blood-brain barrier and regulate the oxidation and phosphorylation processes of the mitochondrial electron transport chain. However, the mechanism of Astaxanthin in vascular dementia is not clear, so this study used permanent bilateral common carotid artery occlusion (BCCAO) to induce vascular dementia. In this study, we used Sprague-Dawley (SD) rats as experimental animals and induced vascular dementia through bilateral common carotid artery occlusion (BCCAO). Astaxanthin was administered via subcutaneous injection. Through behavioral testing, immunohistochemistry, and intracellular dye injection techniques, we analyzed the effects of ATX treatment. In the results, short- to long-term BCCAO induction exerts varying impacts on the physiological mechanisms of the animals. In the short term, the expression of pro-inflammatory cytokines, microglia, and astrocytes is elevated, but these levels tend to stabilize over the long term. In contrast, oxidative stress, as indicated by ROS levels, shows a progressively accumulating trend over time. Rats with ATX treatment in the third month can improve animal behavioral performance, increase synaptic connections in the hippocampus area, decrease oxidative stress, and regulate the protein expression of oxidative stress-related proteins. In a nutshell, rats with ATX treatment can improve spatial memory ability and neuroplasticity. This also provides more feasibility and evidence for using ATX to treat vascular dementia.

Disclosures: T. Chao: None.

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Topic: B.05. Synaptic Plasticity

Support: National Sci-Tech Innovation-2030 Major Projects 2021ZD0203100
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Title: Dysfunctional S1P/S1PR1 signaling in the dentate gyrus drives vulnerability of chronic pain-related memory impairment

Authors: *M. CUI;
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Abstract: Memory impairment in chronic pain patients is substantial and common, and few therapeutic strategies are available. Chronic pain-related memory impairment has susceptible and unsusceptible features. Therefore, exploring the underlying mechanisms of its vulnerability is essential for developing effective treatments. Here, combining two spatial memory tests (Y-maze test and Morris water maze), we segregated chronic pain mice into memory impairment-susceptible and -unsusceptible subpopulations in a chronic neuropathic pain model induced by chronic constrictive injury of the sciatic nerve. RNA-Seq analysis and gain/loss-of-function study revealed that S1P/S1PR1 signaling is a determinant for vulnerability to chronic pain-related memory impairment. Knockdown of the S1PR1 in the dentate gyrus (DG) promoted a susceptible phenotype and led to structural plasticity changes of reduced excitatory synapse formation and abnormal spine morphology as observed in susceptible mice, while overexpression of the S1PR1 and pharmacological administration of S1PR1 agonist in the DG promoted an unsusceptible phenotype and prevented the occurrence of memory impairment, and rescued the morphological abnormality. Finally, the Gene Ontology (GO) enrichment analysis and biochemical evidence indicated that downregulation of S1PR1 in susceptible mice may impair DG structural plasticity via interaction with actin cytoskeleton rearrangement-related signaling pathways including Itga2 and its downstream Rac1/Cdc42 signaling and Arp2/3

cascade. These results reveal a novel mechanism and provide a promising preventive and therapeutic molecular target for vulnerability to chronic pain-related memory impairment.

Disclosures: M. Cui: None.

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Title: Osteoarthritis accelerates brain aging and cognitive vulnerability via the bone-brain axis

Authors: *Y. TANG[1,2], L. ZHAO[1,2], Y. TU[1,2];
[1]State Key Lab. of Cognitive Sci. and Mental Hlth., Inst. of Psychology, Chinese Acad. of Sci., Beijing, China; [2]Dept. of Psychiatry, Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Osteoarthritis (OA) is a prevalent articular disorder primarily characterized by chronic pain. Although traditionally viewed as a localized peripheral disease, its frequent co-occurrence with neuropsychiatric conditions has prompted a paradigm shift toward recognizing the critical role of the brain in OA-related systemic effects. However, how OA affects brain function and the mechanisms underlying this bone-brain interaction remains poorly understood, underscoring the need for an integrative investigation spanning phenotypic, genetic, and molecular domains. In this study, we investigated the impact of OA on the brain and the potential role of the bone-brain axis by integrating structural neuroimaging and genetic data from the UK Biobank. We first developed an MRI-based brain age prediction model to quantify the difference between predicted brain age and chronological age. Individuals with knee OA (KOA) exhibited significantly increased predicted age difference, suggesting accelerated brain aging. This pattern was associated with hippocampal atrophy and predicted subsequent memory decline and a higher incidence of dementia. To uncover the mechanisms underlying this brain vulnerability, we examined the broader relationship between bone and brain phenotypes and their shared genetic basis. We identified widespread associations between bone and brain imaging measures, which

varied by anatomical region and aligned with cortical neurotransmitter receptor distribution, cell-type composition, and functional evolutionary hierarchy. We then conducted genome-wide association studies of bone measures across multiple anatomical locations and discovered 40 novel loci. Multiscale genetic analyses at genome-wide, pathway, locus, and single-variant levels revealed location-specific genetic overlaps and causal relationships between bone measures and various brain-related phenotypes and disorders. Notably, cross-trait genetic analyses highlighted pleiotropic effects between KOA and brain-aging acceleration. Regional contributions to brain-aging patterns were enriched for genes involved in synaptic structure and neurodevelopmental processes and were highly expressed in microglia and astrocytes. Finally, we highlight the role of the canonical Wnt signaling pathway in mediating bone-brain associations and identify potential drug repurposing opportunities targeting this pathway. Together, this study provides a comprehensive view of how OA may modulate brain function through coordinated phenotypic and genetic pathways, laying the groundwork for future mechanistic and translational research into the bone-brain axis.

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NANO001: Synapses, Stress, and Systems: Modulators of Neural Plasticity and Dysfunction: Synapses

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Presentation Number: NANO001.06

Topic: B.05. Synaptic Plasticity

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Title: Plasticity enhanced by pharmacologically-augmented VNS: leveraging noradrenergic mechanisms for motor and sensory rehabilitation

Authors: *C. L. NEIFERT[1], T. T. DANAPHONGSE[1], M. P. KILGARD[2], C. A. THORN[2], S. A. HAYS[3];
[2]Neurosci., [1]Univ. of Texas at Dallas, Richardson, TX; [3]Bioengineering, Univ. of Texas at Dallas, Dallas, TX

Abstract: Vagus nerve stimulation (VNS) paired with rehabilitative training has emerged as a therapy that facilitates recovery of motor and sensory function after stroke and other neurological injuries, and is linked to a plastic structural reorganization of neurons. To inform and improve therapies, studies have developed a systemic understanding of the effect of VNS on structures throughout the brain. Norepinephrine (NE) is critical for VNS-dependent plasticity and recovery, but the dynamics of this relationship are not fully understood. Increasing VNS intensity raises the activity of noradrenergic neurons in the locus coeruleus, however, the degree of VNS-driven

cortical plasticity follows an inverted-U function corresponding to increased VNS intensity. This suggests a plasticity-stabilizing effect of high NE activity in the cortex. Identifying the contribution of NE to VNS-dependent plasticity holds promise to guide further improvements of VNS-driven therapies.
The goal of this presentation is to compile the field's current understanding of the noradrenergic mechanism of VNS-mediated plasticity and to emphasize our recent breakthrough findings that identify a plasticity-regulating role of adrenergic receptors (ARs). The primary hypothesis of this work was that β-ARs are critical for modulating VNS-dependent plasticity, and can be manipulated to further enhance plasticity. To test this hypothesis, rats performed a simple behavioral task: VNS was paired with jaw muscle activation (chewing). For five days, each rat received 200 pairings of VNS delivered at a frequency of 30Hz, at various intensities, and with or without a β-blocker drug injection. 8 groups were defined by the following drug and intensity parameters: injection of Propranolol or saline injections; and VNS at 1.6 mA (High VNS), 0.8 mA (Moderate VNS), or Sham stimulation. Two more groups received Nadolol injections with High VNS or Sham to identify any peripheral contributions. Following each rat's final behavioral session, intracortical microstimulation (ICMS) was used to assess movement representations in the motor cortex.
Our results show that High VNS with Propranolol produces greater levels of VNS-driven plasticity than our group has ever reported. This suggests that β-ARs are a limiting factor of VNS efficacy, and that blocking β-ARs may further improve VNS therapeutic strategies. This study further elucidates the neuromodulatory mechanism of VNS-mediated plasticity. Our findings also form the basis for pharmacologically-augmented VNS therapy for neurological injury, currently being tested in an ongoing study to improve post-injury motor and sensory rehabilitation.

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Presentation Number: NANO001.07

Topic: B.05. Synaptic Plasticity

Support: NIH 1 R01 NS123074

Title: Does activation of alpha-2a noradrenergic receptors enhance VNS-induced motor cortical plasticity?

Authors: *N. Y. KOPCHENKO[1], T. DANAPHONGSE[2], M. RAJENDRAN[3], C. A. THORN[1]; [1]Neurosci., Univ. of Texas at Dallas, Richardson, TX; [2]Univ. of Texas, Dallas, Plano, TX; [3]Neurosci., The Univ. of Texas at Dallas, Lewisville, TX

Abstract: Vagus nerve stimulation (VNS) has been an FDA-approved treatment for upper limb motor rehabilitation in stroke patients since 2019. When paired with a specific motor task, VNS can drive task-relevant motor cortical plasticity and improve functional recovery after stroke. While the mechanisms underlying VNS-mediated plasticity are not fully understood, it has been shown that noradrenaline, specifically activation of alpha-2 adrenergic receptors (A2-AR) is necessary for VNS-mediated cortical plasticity to occur. Pharmacological activation of A2-ARs has been shown to improve cognitive function by increasing cortical neuronal excitability. In the current study, we hypothesized that administration of guanfacine, a selective agonist of A2a adrenergic receptors (A2a-ARs), should increase excitability in the motor cortex (M1), resulting in enhanced VNS-driven cortical plasticity, especially at lower stimulation amplitudes that are not typically effective. To test the effects A2a-AR agonism on VNS-mediated plasticity, we trained male and female Long Evans rats to perform a skilled reaching lever press task to receive a food reward. Following task mastery, rats were implanted with stimulating cuff electrodes targeting the left cervical vagus nerve. After surgical and behavioral recovery, animals received 5 days of lever press training in which correct task performance was paired with standard VNS (0.8 mA), attenuated VNS (0.4 mA), or sham stimulation (0.0 mA). Thirty minutes prior to each stimulation-paired training session, rats received intraperitoneal injections of guanfacine (0.2 mg/kg) or drug-free vehicle. Within 24 hours of the last treatment-paired behavioral session, intracortical microstimulation mapping of the motor cortex (M1) was performed to quantify the cortical representation of task relevant musculature (proximal forelimb). As in previous studies, standard VNS paired with lever press training increased the proximal forelimb representation in M1. Preliminary data further suggest that guanfacine does not dramatically enhance VNS-driven cortical plasticity in rats given attenuated VNS or sham treatment, and the drug may block VNS efficacy at standard VNS intensities. The results from this study will provide useful insight into the underlying noradrenergic mechanisms driving VNS-mediated motor cortical plasticity.

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Topic: B.05. Synaptic Plasticity

Support: Center on Compulsive Behaviors, NIH
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Title: Amygdala choreographs pain in times of stress

Authors: *N. ROY[1], S. SINGH[2], J. GARCIA[2], S. LEE[2], D. L. MENÉNDEZ ESCALERA[2], Y. CARRASQUILLO[2];
[2]Natl. Ctr. for Complementary and Integrative Hlth., [1]NIH, Bethesda, MD

Abstract: Pain is often studied in isolation, neglecting the interplay of systems shaping pain behaviors, including stress. Acute stress can result in robust analgesia. Despite critical contribution of a forebrain limbic structure, the central amygdala (CeA), in pain processing and stress responses, we know little on how stress-induced changes in CeA cells and circuits contribute to stress-induced changes in pain processing. We started addressing this gap in knowledge by studying stress-induced analgesia (SIA) in a mouse model. CeA, comprising of heterogenous group of neurons, includes somatostatin-expressing cells (CeA-Som) that once activated drives analgesia. At the circuit level, CeA is well affiliated with periaqueductal gray (PAG). In this study, we hypothesized that stress increases excitability in CeA-Som and PAG-projecting CeA neurons, enhancing inhibitory synaptic inputs into GABAergic PAG neurons, leading to SIA. Combining forced swim with the formalin test model of inflammatory pain, we validated SIA by observing delayed and reduced formalin-induced nociceptive behaviors post-forced swim. We also measured the serum corticosterone concentration at 2, 20, 60, and 120 mins. Our preliminary results show that forced-swim increases serum corticosterone concentration at all the time-points, compared to baseline corticosterone concentration. Ongoing experiments evaluate the co-expression of the pain plasticity marker pERK and the neuronal activity marker cFos with various CeA markers in mice injected with retrograde tracers in the PAG to anatomically and genetically characterize activated CeA neurons during SIA. Using optogenetic-assisted circuit mapping we first show that ChR2 injection in CeA resulted in terminal labeling within PAG, confirming CeA inputs into PAG. Using whole-cell patch-clamp electrophysiology in acute mouse brain slices, we confirmed functional channelrhodopsin (ChR2) expression by recording optically evoked currents and action potential firing in transduced CeA neurons. Optically evoked postsynaptic responses occurred in the presence of TTX and 4-AP, demonstrating that the inputs from the CeA to the PAG are monosynaptic. Ongoing circuit mapping electrophysiology experiments assess changes in the strength of the input and transmitter release probability in VGAT+ and VGAT- PAG neurons during SIA. Using chemogenetic approach and the mouse model of SIA, we are also investigating a causal link between CeA neuron activation with stress-induced pain suppression. Together our results will provide a new avenue for comprehending neural circuitry associated with behaviors that lie at the intersection of stress and pain.

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Topic: B.05. Synaptic Plasticity

Support: NIH Grant R15DA038092
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Title: Cocaine Blocks a Novel Form of Inhibitory iLTD, but not iLTP of VTA GABA Neurons

Authors: *S. HOFFMAN[1], B. WU[2], R. BONNEY[1], T. FULLER[1], J. G. EDWARDS[3];
[2]Cell, [3]Cell Biol. and Physiol., [1]Brigham Young Univ., Provo, UT

Abstract: The ventral tegmental area (VTA) is part of the reward system that under maladaptive conditions facilitates drug seeking and dependence behaviors. Synaptic plasticity, underlying normal reward learning, is altered by abused drugs. Our research extends the understanding of inhibitory VTA network synaptic plasticity. We previously examined iLTP and identified a novel iLTD at GABAergic inputs to VTA GABA cells, illustrating bipotential plasticity. The iLTP was only partially NMDAR-dependent hinting at the existence of additional mechanisms mediating iLTP induction. We determined that iLTP is not cholecystokinin (CCK)-dependent as bath application of CCK2R antagonist LY225910 (1μM) did not affect the observed iLTP or iLTD (iLTP: $p<0.0001$, compared to baseline here and throughout, ANOVA, n=7; iLTD: $p<0.0001$ ANOVA, n=6), unlike iLTP of VTA dopamine cells. In addition, while we previously noted neuronal nitric oxide synthase (nNOS) inhibitor L-NAME did not block iLTP nor iLTD, suggesting iLTP is nNOS-independent; the NO donor SNAP potentiated some inhibitory inputs to GABA cells and interestingly occluded iLTP ($p=0.092$, ANOVA, n=4), suggesting a shared common pathway of nNOS and iLTP. Since VTA GABA neurons receive inhibitory input from inside and outside the VTA, we hypothesized plasticity type was input specific. We therefore optogenetically activated three different GABAergic axonal inputs to the VTA, the Lateral Hypothalamus (LH), the rostromedial tegmental nucleus (RMTg) and VTA interneurons. To accomplish this, VGAT-Cre mice were injected with AAV-ChR2 into these select brain regions. Immunohistochemistry examples demonstrated correct viral infection location with adequate transfection rates in VGAT-Cre/GAD67-GFP positive cells of 58.7% in LH, 64.3% in RMTg, and 42.1% in VTA. Activation of GABAergic LH and RMTg terminals induces only iLTP in response to optical 5Hz stimulus (iLTP, LH to VTA: $P<0.0001$, n=14; iLTP, RMTg to VTA: $p<0.0001$, n=6; ANOVA), while activation of the VTA interneurons induced only iLTD (iLTD, VTA to VTA, $P<0.0001$, ANOVA, n=12). Additionally, we observed both acute and chronic cocaine exposure eliminated iLTD (Chronic: no plasticity group: $p=0.1937$, ANOVA, n=7, and $p<0.0001$ compared to control iLTD; iLTP: $p<0.0001$, ANOVA, n=7. Acute: no plasticity group: $p=0.8362$, ANOVA, n=7, and $P<0.0001$ compared to control iLTD; iLTP: $p<0.0001$, ANOVA, n=9), while leaving iLTP intact. This study provides insights into GABAergic neurons unique bipotential plasticity types, and selective impact of cocaine eliminating iLTD while leaving iLTP intact, elucidating the impact of cocaine on the VTA GABAergic circuit.

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Topic: B.05. Synaptic Plasticity

Support: NIH Grant DA049260

Title: Bipotential plasticity of VTA GABA neurons is partially output specific

Authors: S. HOFFMAN[1], ***R. BONNEY**[1], T. FULLER[2], M. DAHL[2], J. G. EDWARDS[2]; [1]Brigham Young Univ., Provo, UT; [2]Brigham Young Univ., Provo, UT.

Abstract: Synaptic plasticity within the ventral tegmental area (VTA) is a process facilitating reward learning and memory. Understanding synaptic plasticity within the inhibitory networks of the VTA is essential to build a complete foundational knowledge of reward system functions under normal physiological conditions. Previously, our lab identified that a 5Hz stimulus induced either inhibitory long-term potentiation (iLTP) or inhibitory long-term depression (iLTD) of GABAergic inputs to VTA GABA cells in mice. VTA GABA cell electrophysiology recordings revealed that iLTP is present in ~50% of recorded cells and iLTD in the other ~50%. While iLTD is $GABA_B$ receptor-dependent, and iLTP is partially N-methyl-D-aspartic acid receptor (NMDAR) dependent, it is unknown if other mechanisms are driving the induction of iLTP or iLTD. In preliminary prior studies, we illustrated that plasticity of these VTA GABA cells is input selective, GABA neurons that receive inhibitory input from the lateral hypothalamus (LH) and rostromedial tegmental nucleus (RMTg) exhibiting iLTP and local VTA GABAergic inputs to VTA GABA cells exhibiting iLTD. Furthermore, since VTA GABA neurons innervate local dopamine (DA) cells and/or regions outside the VTA including the nucleus accumbens (NAc), we hypothesize that plasticity type could be explained by unique VTA GABA cell outputs. To examine VTA interneurons we employed synaptic tracer rabies virus. First, Cre-inducible helper virus was injected into the VTA of DAT-CrexGAD67GFP+ mice, later we injected a replication deficient rabies/mCherry virus requiring the helper virus for expression. Next, we recorded GFP+/mCherry GABA interneurons using electrophysiology, which exhibited iLTP in response to 5Hz stimulus (iLTP, VTA GABA to VTA DA: $p<0.0001$ compared to baseline, ANOVA, n=13). Next, we examined projection GABA neurons by injecting red Retrobeads into the NAc of GAD67GFP+ mice to label and record VTA GABA neurons projecting to the NAc. These cells also exhibited iLTP in response to 5Hz stimulus (iLTP, VTA GABA to NAc: $p<0.0001$ compared to baseline, ANOVA, n=12). Alternatively, plasticity is also determined by cell subtype. Preliminary single cell PCR data revealing subtypes of recorded VTA GABA cells suggests that variable plasticity can potentially be explained by circuit dynamics and subtype classification. Our study illustrates VTA GABA cells innervating DA cells may facilitate increased dopamine release via iLTP, and GABA neurons innervating NAc could modulate reward or aversion through iLTP. Additional experiments examining VTA GABA cells innervating other brain regions could reveal circuits that exhibit iLTD.

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Presentation Number: NANO001.11

Topic: B.05. Synaptic Plasticity

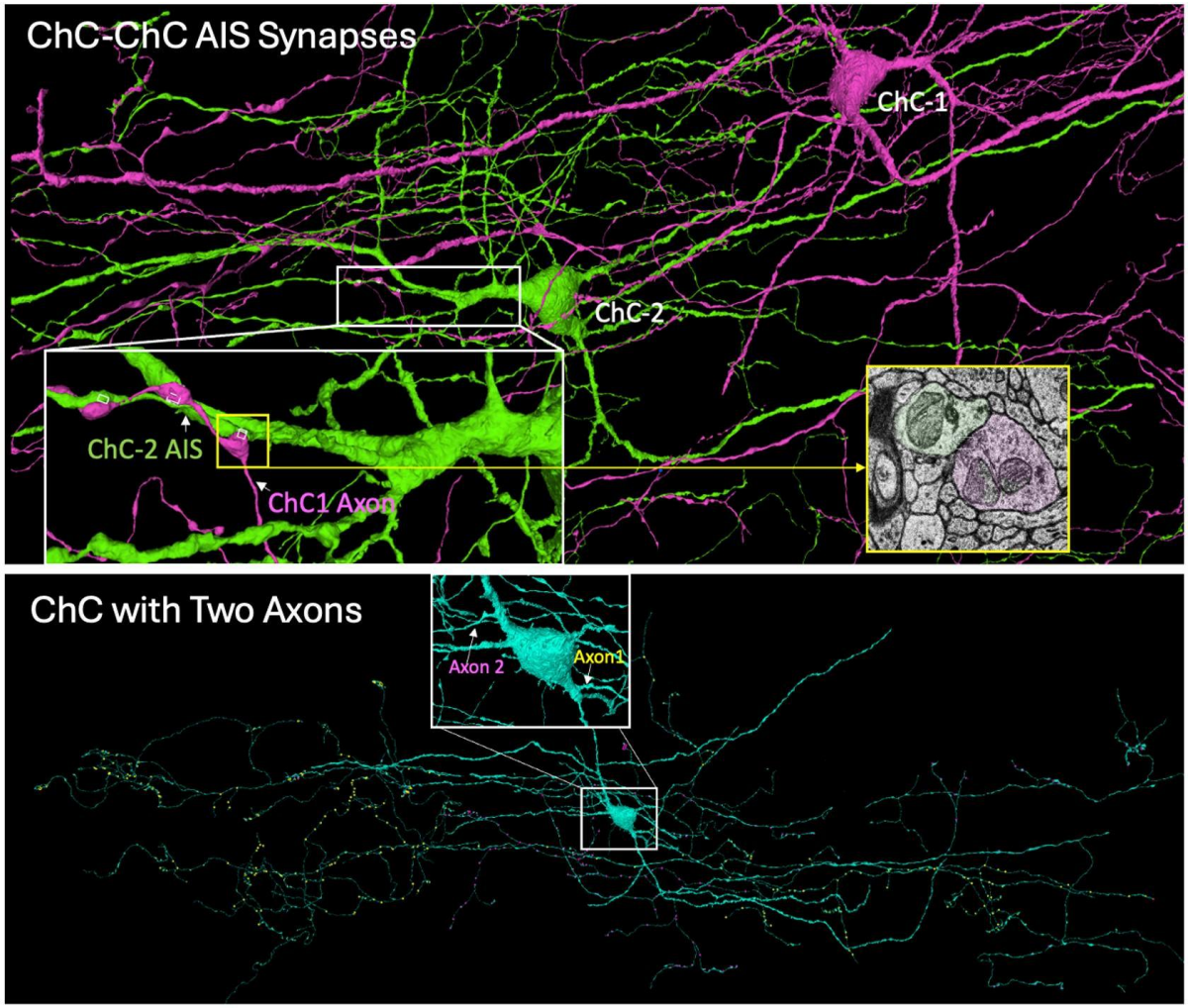
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Stanley Center at Broad Institute

Title: Unusual Behavior of Chandelier Axons in the Temporal Lobe of Human Cerebral Cortex

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Abstract: Chandelier cells (ChCs) are inhibitory neurons with unique ornate organization of their axonal terminals. These cells are known for their proclivity to make multiple synapses on axon initial segments (AIS) of pyramidal cells (Py). Given the location of their synaptic terminals at the spike initiation zone of Py cells, they are ideally suited to be master regulators of Py excitability (Woodruff et al., 2010). To better understand the organization of the output of ChCs, multiple ChC axons were reconstructed in our connectomics dataset, collected from temporal cortex of a patient with epilepsy (Shapson-Coe et al., 2024). Axons of 14 ChCs across cortical layers 2-6 whose somata were within the volume were proofread using CAVE (Dorkenwald et al., 2025), and their outgoing synaptic connections and postsynaptic partners were identified. The synaptic output of ChC axons was not limited to making axo-axonic synapses with Py AIS sites. Rather, they also innervated AISs and dendrites of both excitatory and inhibitory neurons. For example, a stellate cell's dendritic spine was co-innervated by a ChC bouton and an excitatory input. Surprisingly, AISs of most of these 14 ChCs, were innervated by other ChC axons. For example, one ChC (cell A) established 9 synapses on the AIS of another ChC (cell B), and ChC B established 8 synapses on ChC A. In total 9 of the 14 (64%) identified ChCs were innervated by one (or more) of the other identified ChCs. In addition, other likely ChC axons (i.e. that innervated AISs of Py cells, but whose somata were not in volume) also innervated the identified ChCs. Only 3 of the 14 ChCs did not have AIS innervation. The function of these reciprocal ChC connections is unknown. Another surprise was that 2 of the 14 ChCs were bi-axonal. These axons projected to non-overlapping regions but established synapses on Py cell AISs. The number of synapses established by these bi-axonal ChCs was not greater

than that of ChCs with a single axon. These results raise more questions than they answer, and we are seeking to discover whether this is related to the patient's condition or is a feature of human ChC axons.



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Nanosymposium

NANO002: Glial Mechanisms of Alzheimer's Disease

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Presentation Number: NANO002.01

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

Title: Astrocyte-derived cholesterol in neuroinflammation: biophysical insights from in vitro, in vivo and super-resolution imaging study

Authors: *Y. YU[1], H. WANG[2], H. A. FERRIS[3], S. B. HANSEN[4];
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Abstract: Neurodegeneration diseases are associated with inflammation and an accumulation of cholesterol. In the brain, cholesterol is synthesized by astrocytes and transported to adjacent cells via apolipoprotein E mediated mechanisms. Studies have demonstrated elevated cholesterol promotes inflammation in peripheral tissues, but whether cholesterol can drive neuroinflammation and the mechanism how inflammatory receptors react to high and low cholesterol environment in the brain remain unclear. The present study is to explore the relationship between astrocyte-derived cholesterol and neuroinflammation. The interaction between cholesterol and primary astrocytes was identified by multiplex cytokine assay for pro-inflammatory markers and later tested on Alzheimer's disease mouse model. Furthermore, the observation of localization and movement of inflammatory factors was done by super resolution imaging. Results shown 1) Elevated cholesterol levels induce cytokine upregulation, while a high-cytokine milieu with immune cells stimulates astrocyte cholesterol synthesis, establishing a self-reinforcing feedback loop. 2) In AD mouse model, a knockdown in astrocyte cholesterol decreases the activation of microglia and macrophages. 3) cholesterol and LPS cluster inflammatory receptors into the GM1 lipids and activate inflammatory signaling. In conclusion, astrocyte-derived cholesterol drives neuroinflammation.

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

Support: NIH R01 NS120488

Title: Differential vulnerability of glial subtypes in tauopathy

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Abstract: Disease progression involves non-cell autonomous interactions among multiple cell types, including neurons and glia. Pathogenic tau accumulation is the hallmark of a wide range of neurodegenerative diseases named tauopathies, which can be modeled in *Drosophila*

melanogaster. These diseases exhibit cell type-specific neuronal vulnerability, but little is known about how various subtypes of glia contribute to disease progression. *Drosophila* has five distinct glial subtypes: perineurial- and subperineurial glia, which line the blood-brain barrier; cortex glia, and neuropil glia, comprising astrocyte-like glia and ensheathing glia. We examined the impact of expressing the human familial tauR406W mutation in these different glial subtypes. Tau pathology and gliosis were more pronounced in astrocyte-like and ensheathing glia than in other glial subtypes, and shortened lifespan when expressed in these two glial subtypes ($p<0.0001$). To uncover the underlying mechanisms of why certain glial subtypes are more vulnerable to tau pathology, we are conducting single nuclear RNA sequencing and proteomics. These studies will provide valuable insight into tau pathogenesis and may identify therapeutic targets to slow the progression of tauopathies.

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Presentation Number: NANO002.03

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

Support: Fl DoH 22A06
NIA R01AG061796

Title: Profiling microglia from Alzheimer’s disease rare risk variant carriers reveal protective and risk signatures

Authors: *Ö. IS[1], J. TAN[1], X. WANG[2], J. BERGMAN[1], F. TUTOR-NEW[1], M. HECKMAN[2], W. TSAI[1], Z. QUICKSALL[2], M. ATIK[1], J. S. REDDY[2], Y. MIN[1], J. GAO[1], K. SOTELO[1], T. NGUYEN[1], T. KANEKIYO[1], D. W. DICKSON[1], M. ALLEN[1], N. ERTEKIN-TANER[1]; [1]Neurosci., [2]Quantitative Hlth. Sci., Mayo Clin., Jacksonville, FL

Abstract: Introduction: Alzheimer's disease (AD) impacts all brain cells and involves intricate changes at the genomic and immune levels. Prior studies have identified missense variants in microglial risk genes like *PLCG2* and *ABI3*, which can either impair or enhance microglial function in the context of AD. This study’s goal is to identify the molecular signatures in microglia associated with these protective and risk variants, aiming to clarify their influence on different microglial subtypes and activation states in AD by analyzing various human single-cell (sc) and single-nucleus (sn) RNAseq datasets. **Methods:** We generated microglia-enriched snRNAseq data from 30 donors carrying *ABI3* or *PLCG2* missense mutations, or neither. After quality control, we performed differential expression analysis using MAST to compare microglial profiles between variant carriers and non-carriers. A “protective signature” was defined as genes upregulated with the *PLCG2* variant and downregulated with the protective *ABI3* allele, while the inverse defined a “risk signature.” We assessed the conservation of these

signatures across multiple datasets, including both internal and external snRNAseq data that characterize stages of AD based on pathological phenotypes, as well as scRNAseq datasets from iPSC-derived microglia. To relate these signatures to microglial phenotypes, we compiled subtype markers from ten recent microglia-focused studies and grouped them into seven categories. Subtype and signature enrichment was assessed using AUCell. **Results:** We generated snRNAseq profiles from 54,000 frozen human brain nuclei, including 35,000 microglia from individuals carrying AD-associated variants. We identified 227 protective and 293 risk signature genes linked to these variants. Through integration of multiple internal and external datasets, we refined the protective signature to 69 genes that are downregulated in early AD and pathologic aging (PA), but upregulated in late-stage AD relative to PA and early AD. In contrast, 25 risk genes were upregulated in both early and late AD compared to PA. We observed that cells with protective signatures show a relative alignment with homeostatic microglial subtypes, whereas risk signatures appear more closely associated with damage associated profiles. Finally, we evaluated whether protective and risk gene expression signatures are modulated by *PLCG2* variant load and Aβ exposure using scRNAseq from iPSC microglia. **Conclusion:** Our study identifies microglia-specific protective and risk gene signatures linked to distinct stages of AD. These findings highlight novel genes with potential as biomarkers and therapeutic candidates.

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

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Title: The Effect of Vitamin E on Microglial Dysfunction in a Rat Model of Alzheimer’s Disease

Authors: *C. J. FINNO[1], K. ROBERTS[2,3], A. E. VALENZUELA[5], B. DURBIN-JOHNSON[6,4], P. ANDREW[7,9], A. MAGANA[8,3], P. J. LEIN[10];
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Abstract: Single-cell transcriptomics have identified that Alzheimer's disease (AD) pathology is associated with a shift from neuroprotective to neurotoxic signatures of microglial activation. Vitamin E, an antioxidant known for its neuroprotective properties, has the potential to modulate microglial activation. Over 93% of Americans consume half of the recommended dietary intake of vitamin E. We hypothesized that decreased dietary vitamin E intake would accelerate AD progression in a transgenic rat model of AD by potentiating microglial dysfunction. We profiled hippocampal microglial transcriptomes in the TgF344-AD rat, which overexpresses human mutant *APPSW* and *presenilin 1* (*PS1ΔE9).* Female transgenic rats were maintained on diets of varying vitamin E concentrations: adequate (35 IU/kg feed), deficient (<10) and high-dose supplemented (200, ~6x requirement), over a 9-month period and compared to wild-type rats on an adequate diet. Serum and hepatic vitamin E concentrations were significantly altered over time in accordance with each dietary intervention. Eosinophilic plaques developed in the hippocampus and entorhinal cortex in all transgenic rats regardless of diet. Vitamin E deficiency potentiated hippocampal microglial dysfunction, with over 5,000 differentially expressed genes, including upregulation of MHC Class II genes. Surprisingly, high-dose vitamin E supplementation led to the upregulation of similar genes, suggesting a therapeutic window for vitamin E intake to maintain microglial health. Monitoring and adjusting vitamin E concentrations in individuals at risk for AD may be a simple way to promote microglial health.

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Nanosymposium

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Presentation Number: NANO002.05

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

Support: AG085666
AG072905

Title: Transcriptional analysis of atypical astrocytes during healthy and pathological aging

Authors: *P. TIRJA[1], M. SOMMER[2], M. ARMBRUSTER[3], E. CHAREST[5], M. GOOD[6], A. K. MAURO[7], A. TAI[4], G. TESCO[3], S. ROBEL[8], C. G. DULLA[3];
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Abstract: Astrocytes, the most abundant glial cell type in the central nervous system (CNS), are essential for glutamate and K^+uptake, neuronal metabolic support, amyloid-beta (Aβ) clearance, and blood-brain barrier (BBB) maintenance. Astrocyte dysfunction contributes to aging-related

brain decline and numerous neurological disorders, including Alzheimer's disease (AD). When considering astrocytes, most AD research has focused on reactive astrocytes potentially overlooking the contributions of a previously unrecognized, molecularly distinct astrocyte subpopulation known as atypical astrocytes (AtAs). AtAs first identified after mild traumatic brain injury (mTBI), are a novel subpopulation lacking key functional proteins like GLT1, GLAST, AQP4, and others critical for Aβ clearance and CNS homeostasis. Our preliminary data show that AtAs also emerge during healthy aging in a region- and age-dependent manner, with pronounced accumulation in specific cortical areas and the hippocampus. Using the EAAT2-tdTomato reporter mouse, we identified AtAs as tdTomato+ (tdT+) astrocytes lacking GLT1 and other astrocytic proteins as well as GFAP, distinguishing them from reactive astrocytes, consistent with the non-reactive phenotype observed in mTBI. Functional deficits in glutamate uptake were confirmed in AtA-enriched regions using the iGluSnFR sensor and electrophysiological recording of glutamate transporter currents, highlighting impaired glutamate clearance. To assess their transcriptional profile, we isolated putative AtAs (tdT+/GLT1-) and typical astrocytes from aged WT mice via fluorescence-activated cell sorting for qPCR and bulk RNA sequencing. The results revealed significant downregulation of key astrocytic and other cell type-specific transcripts, and enrichment of genes associated with cellular senescence, metabolic dysfunction, glial differentiation, and BBB dysfunction. Given the non-reactive nature of AtAs and that most AD research focuses on GFAP+ astrocytes, we investigated their presence in AD. Using APP^{NL-G-F} mice crossed with EAAT2-tdTomato mice confirmed the existence of AtAs (tdT+, GFAP-, GLT1-) in the cortex and hippocampus of mice with AD. APP^{NL-G-F} mice show earlier and regionally distinct AtA accumulation with increased densities in the hippocampus and altered cortical dynamics over time, suggesting that AD pathology affects the regional progression of AtAs. Our findings highlight a distinct astrocyte population with functional deficits and potential involvement in early AD and aging-related pathology. Targeting this novel population may open new therapeutic avenues for modifying disease progression.

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Nanosymposium

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

Support: NIH R01AG069447

Title: Age-dependent cognitive rescue via microglial Myd88 knockout

Authors: *A. X. VALLEJOS[1,2], R. RONG[5], C. HOLAS[3], J. YANG[6], K.-I. FUKUCHI[4]; [1]Univ. of Illinois Chicago, Peoria, IL; [3]Cancer Biol. and Pharmacol., [4]Cancer Biololgy and

Pharmacol., [2]Univ. of Illinois Col. of Med. Peoria, Peoria, IL; [5]Univ. of Illinois Col. of Med. - Peoria, Peoia, IL; [6]Cancer Biol. & Pharmacol., Univ. of Illinois Col. of Med. At Peoria, Peoria, IL

Abstract: Alzheimer's disease (AD) progression may be driven by a self-reinforcing cycle of neuroinflammation, where amyloid-β (Aβ) activates microglia through TLR4 and IL-1β receptors. These pathways signal through MyD88 to prime the NF-κB/NLRP3 inflammasome, amplifying IL-1β release and ASC speck formation, which in turn promotes further Aβ aggregation. To investigate the role of MyD88 in this inflammatory loop, we used tamoxifen-inducible Cre recombinase under the TMEM119 promoter to selectively knock out MyD88 in microglia of TgAPP/PS1 mice. Four genotypes were studied: Cre:MyD88 fl/fl TgAPP/PS1, MyD88 fl/fl TgAPP/PS1, Cre:MyD88 fl/fl, and MyD88 fl/fl. All mice received tamoxifen at two months of age. Two independent cohorts were tested: one at 5-6 months and one at 12 months. Behavioral assessments included open field and Morris water maze. In this study, we aimed to determine how microglial MyD88 signaling shapes cognitive performance at distinct disease stages in vivo. At 5-6 months, microglial MyD88 knockout reduced hyperactivity ($F(1,37)=7.34$, $p=0.01$), increased anxiety-like behavior, and significantly improved spatial learning ($F(4,148)=3.88$, $p=0.013$) and memory ($F(3,37)=4.685$, $p=0.007$). In the 12-month cohort, the APP/PS1 genotype increased locomotion ($F(1,49)=4.688$, $p=0.035$), while MyD88 knockout had no effect on open field activity. In the Morris water maze, knockout showed a trend toward improved learning (escape latency $F(1,49)=3.068$, $p=0.086$; swim distance $F(4,196)=2.308$, $p=0.068$), though memory deficits persisted (probe trial $p=0.004$). Together, these findings provide behavioral evidence that microglial MyD88 signaling contributes to both learning and memory dysfunction in AD, with its impact diminishing as disease pathology advances. We propose that dampening MyD88-mediated inflammation slows cognitive deterioration in early stages, but cannot fully counteract late-stage damage driven by Aβ toxicity and possible redundant immune pathways. These results identify microglial MyD88 as a critical node in AD progression and a potential therapeutic target for interrupting inflammatory cascades that underlie cognitive decline. Our data highlight the need for therapeutic strategies that target inflammation before irreversible circuit-level dysfunction takes hold.

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Nanosymposium

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

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Title: Examination of Twin Astrocytes in the Hippocampus Along the Normal Aging to Severe Alzheimer's Disease Continuum

Authors: N. RABANEDA-LOMBARTE[1], C. R. BIANCO[2], *A. SERRANO-POZO[1]; [1]Massachusetts Gen. Hosp. & Harvard Med. Sch., Boston, MA; [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: Background: Over a century ago, Cajal described "twin astrocytes" as astrocytes with closely apposed, similarly sized, and shaped cell bodies, and hypothesized that they might be proliferating cells. Despite this early observation, the prevalence and biological significance of twin astrocytes in aging and neurodegenerative diseases remain poorly understood. This study aimed to characterize the frequency, distribution, and molecular features of twin astrocytes in the human hippocampus in health and Alzheimer's disease (AD). **Methods:** We conducted multiplex fluorescent immunohistochemistry on formalin-fixed, paraffin-embedded hippocampal sections obtained from neurotypical controls and individuals with AD, sourced from the Massachusetts Alzheimer's Disease Research Center Neuropathology Core Brain Bank. Twin astrocytes were defined by juxtaposed cell bodies identified via ALDH1L1 immunostaining. We quantified twin astrocytes across hippocampal subfields and evaluated their expression of both homeostatic (glutamine synthetase, EAAT2/GLT-1, Kir4.1) and reactive (GFAP, vimentin, MAO-B, YKL-40) markers, as well as their spatial relationship to the two AD neuropathological hallmarks, amyloid-β (Aβ) plaques and tau neurofibrillary tangles (NFTs). **Results:** Twin astrocytes were present throughout all hippocampal subregions, although most conspicuous and abundant in the dentate gyrus and CA1. Their frequency did not significantly differ between controls (Braak NFT stages 0/I/II) and AD (Braak NFT stages V/VI) donors, although a group with intermediate AD neuropathological changes (Braak NFT stages III/IV) showed a trend toward higher counts in dentate gyrus and CA1. Twin astrocytes could co-express markers of both homeostatic and reactive states but did not express the proliferative marker Ki-67 and showed no consistent spatial association with Aβ plaques or NFTs. **Conclusions:** Twin astrocytes are a conserved feature of the hippocampal cytoarchitecture, most prominently in the dentate gyrus and CA1, and are present across the spectrum of normal aging to severe AD neuropathology. Their expression of both homeostatic and reactive markers, lack of spatial association with AD lesions, and absence of expression of the proliferation marker Ki-67 suggest they are neither a feature of AD-related reactive astrogliosis nor a hallmark of ongoing astrogliogenesis. Further investigation is warranted to elucidate their origin and function.

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

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Title: Microglial gene expression is altered in human individuals resilient to Alzheimer's disease

Authors: N. E. KARAGAS[1], C. S. C. JOHNSON[1], A. COCHOIT[2], R. BLAINE[1], A. REID[7], S. MAMDE[8], C. D. KEENE[3], T. J. GRABOWSKI, Jr.[9], C. S. LATIMER[4], K. Z. LIN[5], S. JAYADEV[10], ***K. E. PRATER**[6];
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Abstract: Alzheimer’s disease (AD) is the most common form of dementia, affecting millions of individuals worldwide. Despite the enormous impact on patients and their care partners, limited therapeutic options are available. One research avenue with therapeutic potential is understanding the biology underlying “resilience” to AD. Resilient individuals display amyloid beta plaques and phosphorylated tau tangles neuropathologically, but lack the cognitive decline associated with dementia. Studies in both postmortem human brain and animal models suggest that inflammation is lower in resilient individuals than in affected individuals with pathology and dementia. Microglial cells are key actors in inflammation processes in the brain. We hypothesized that the microglial response to pathology may differ in resilient individuals compared to affected individuals. Here we report a cohort of 33 individuals with an average age over 80 years and average post-mortem interval less than 8 hours. Tissue was obtained from the dorsolateral prefrontal cortex (DLPFC). The single-nucleus RNA-seq (snRNA-seq) cohort contains 13 resilient individuals (9 female and 4 male, non-cognitively impaired with a cognitive assessment less than 12mo from death, average Alzheimer’s disease neuropathic change score (avg. ADNC)=2.23), 10 “resistant” aged controls (5 female, 5 male, avg. ADNC=0.7), and 10 “affected” demented individuals (6 female, 4 male, avg. ADNC=2.9). We performed snRNA-seq on nuclei enriched for microglia. We also assessed microglial morphology using immunohistochemistry for Iba-1 along with antibodies for amyloid beta (pan-amyloid-beta) and phosphorylated tau (AT8) in a cohort of 29 individuals (13 resilient, 6 resistant, 10 affected) that largely overlap with the snRNA-seq cohort. The density of microglia in the distal plaque area was decreased in resilient individuals compared to affected while the number of microglia in the peri-plaque area was unchanged. We also replicated the finding that resilient individuals display less phosphorylated tau burden in the DLPFC. In the snRNA-seq dataset, we observed multiple microglial states as expected. Across microglial states, including homeostatic states, we observe lower inflammatory gene expression and shifts in DNA repair processes in resilient individuals

compared to affected individuals. These findings suggest that although microglial morphology overall may not be shifting, gene expression and potentially biological functions may be shifted in specific microglial states. Targeting these specific microglial states may further new therapeutic development.

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

Title: Receptor-mediated delivery of dgat2 inhibitor to lipid droplet-laden microglia to combat alzheimer’s disease

Authors: *G. YANG[1], Y. CHEN[2], V. WENDT[4], M. PATIL[2], J. RAJPOOT[3], K. P. SHARMA[2], K. BISHT[2], S. VIRANI[2], R. YU[2], K. JETHAVA[2], A. HOSSAIN[2], G. CHOPRA[1];
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Abstract: Microglial phagocytosis plays a critical role in Alzheimer’s disease (AD) by clearing amyloid β (Aβ) plaques. Deficiency in microglial phagocytic function contributes to AD pathogenesis. In our previous study, we found that microglia near Aβ plaques exhibit increased lipid droplet (LD) accumulation and reduced phagocytic ability. Lipidomic profiling showed elevated triacylglycerol (TAG) levels and upregulation of Diacylglycerol O-Acyltransferase 2 (DGAT2), a key enzyme in TAG synthesis. Ex vivo treatment with a DGAT2 inhibitor reduced LD accumulation and restored Aβ phagocytosis in LD-laden microglia from both 5xFAD mice and human AD brains. However, in vivo administration of the inhibitor via osmotic pump failed to reduce LDs in 5xFAD mice, likely due to insufficient drug concentration at microglia surrounding plaques. To address the gap, we developed a microglia-targeted small-molecule based drug delivery strategy. Tissue staining in 5xFAD mice revealed selective overexpression of a receptor (R1) on microglia adjacent to plaques. We also found on the microglia cell line receptor R1 is upregulated upon Aβ treatment despite a downregulation of the mRNA. Based on these findings, we synthesized a bifunctional molecule (L1-D2i) by conjugating a DGAT2 inhibitor (D2i) to a ligand (L1) targeting R1. *In vitro* studies in Aβ-induced LD-laden microglia showed that L1-D2i retained a comparable LD reduction effect compared to free D2i, indicating preserved activity after conjugation. To demonstrate receptor-mediated uptake, we synthesized a ligand-dye conjugate by tethering L1 to a pH-sensitive fluorophore. Following intracerebral injection, confocal imaging confirmed colocalization of the ligand-dye with plaque-associated microglia. Specifically, the L1-dye induced internalization of R1, which recycled back to the

surface over time, suggesting ligand-driven endocytosis. To validate receptor dependency, we performed siRNA-mediated knockdown. Microglia lacking the receptor showed significantly less LD-reduction effects of L1-D2i and internalization of L1-dye, indicating receptor-mediated uptake is required for efficacy. Finally, we evaluated our cell-targeted DGAT2 inhibitor in vivo for LD load, microglial phagocytosis, Aβ plaque burden and neuronal health in aged 5xFAD mice. We believe that microglia-specific delivery could offer a promising strategy for modulating microglial lipid metabolism and function in AD by enabling precise delivery of therapeutics to disease-relevant cells.

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Nanosymposium

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

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Title: Targeted degradation of dgat2 in plaque-associated microglia mitigates lipid droplet burden and alzheimer's pathology

Authors: *V. WENDT[1], J. RAJPOOT[2], G. YANG[3], A. HOSSAIN[3], Y. CHEN[3], G. CHOPRA[4], P. SAKLANI[3];
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Abstract: Alzheimer's disease (AD) is characterized by the accumulation of amyloid-beta (Aβ) plaques, where dysfunctional microglia with impaired phagocytic activity contribute to disease pathogenesis. Our previous work revealed that exposure to Aβ alone can induce the formation of lipid droplets (LDs)—dynamic, fat-storing organelles—in microglia. We observed an increased LD burden in plaque-associated microglia from both postmortem human AD brains and the 5xFAD mouse model. These LD-laden microglia exhibit compromised phagocytosis and a distinct lipid profile marked by reduced free fatty acids (FFAs) and elevated triacylglycerols (TGs). Importantly, we identified diacylglycerol acyltransferase 2 (DGAT2) as a key enzyme

catalyzing the conversion of FFAs to TGs, with DGAT2 protein levels elevated in both human AD and 5xFAD brains. While pharmacological inhibition of DGAT2 failed to reduce LD burden in aged microglia, a PROTAC designed to specifically degrade DGAT2 significantly decreased LD content and restored microglial phagocytic function, leading to reductions in plaque burden and neuritic dystrophy in 12-24-month-old 5xFAD mice. In our current study, we show that the spatial accumulation of DGAT2 closely parallels that of LDs, with an increased expression of DGAT2 in plaque-associated microglia in aged 5xFAD mice. Therefore, to mitigate any potential off-target effects of non-targeted DGAT2 degradation, we designed and synthesized a novel microglia-targeted PROTAC—a trifunctional small molecule that selectively degrades DGAT2 in microglia, leveraging a receptor (R1) enriched in plaque-proximal microglia. We confirmed in vivo selectivity using a rhodamine-conjugated R1 ligand, which preferentially labeled plaque-associated microglia. Treatment with the R1-targeted DGAT2 degrader led to significantly greater reductions in lipid droplet content, Aβ plaque burden, and neuritic dystrophy than the non-targeted degrader. These findings identify a functionally distinct, disease-relevant microglial subset and offer a novel, cell-specific strategy to therapeutically modulate neuroinflammation via targeted metabolic reprogramming.

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Nanosymposium

NANO002: Glial Mechanisms of Alzheimer's Disease

Location: SDCC Rm 33

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO002.11

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

Title: Disease associated microglia activation is protective against LSD1-induced neurodegeneration

Authors: *Y. BAI[1], C. FIX[2], S. GOSRANI[2], D. J. KATZ[1];
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Abstract: The H3K4me1/2 demethylase LSD1/KDM1A, functions to repress gene expression. Our lab found that: 1) the inducible deletion of LSD1 in adult mice leads to terminal paralysis and severe neurodegeneration in the hippocampus and cortex, 2) LSD1 mislocalizes to cytoplasmic pathological tau aggregates in human Alzheimer’s disease (AD) cases and Tau P301S mice, and 3) LSD1 modulates neurodegeneration in Tau P301S mice. Together, these results indicate that LSD1 may function in the pathological tau pathway and that the *Lsd1* inducible deletion mice serve as an excellent model of late-stage tau-mediated neurodegeneration. Consequently, understanding how the loss of LSD1 leads to

neurodegeneration in mice is of particular interest. In the hippocampus of *Lsd1* inducible deletion mice, we observed the upregulation of microglial and immune genes that are also misregulated in **l**ate-**o**nset **A**lzheimer's **d**isease (LOAD) cases. Therefore, we sought to investigate whether this immune response contributes to the severe neurodegeneration observed in *Lsd1* mice. To address this question, we depleted microglia and blocked disease associated microglia activation in our *Lsd1* inducible mouse model. Depleting microglia through the chemical inhibitor PLX3397 resulted in a more severe neurodegeneration phenotype with significantly increased neuronal cell death. Similarly, blocking microglia activation through homozygous deletion of the **T**riggering **R**eceptor **E**xpressed on **M**yeloid Cells 2 (TREM2) gene also resulted in a more severe neurodegeneration phenotype with significantly reduced survival. These findings suggest that disease associated microglia activation is protective against LSD1-mediated neurodegeneration and late-stage tau-mediated neurodegeneration.

Disclosures: Y. Bai: None. **C. Fix:** None. **S. Gosrani:** None. **D.J. Katz:** None.

Nanosymposium

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Location: SDCC Rm 33

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO002.12

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

Support: UK DRI grant UKDRI-4204

Title: Astrocyte-neuron interactions are protective against synapse loss in an ex vivo model of early-stage of Alzheimer's disease

Authors: *F. GOBBO[1], D. KING[1], J. H. TULLOCH[1], D. GOBBO[3], J. ROSE[1], C. SMITH[2], C. S. DURRANT[1], T. L. SPIRES-JONES[1];
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Abstract: Synapse loss begins early in Alzheimer's disease (AD) and strongly correlates with cognitive decline. Data from human AD brains and ex vivo models show increased phagocytosis of synapses from astrocytes. It is unclear, however, whether this is a protective mechanism by removing dysfunctional synapses or a detrimental process, either by targeting connections essential for normal function or by reducing the pool of synapses available to compensate for pathological synapse loss. Here, we address whether astrocytes ingest functionally active synapses in living brain slices using multiphoton fluorescence imaging. To model the response of healthy tissue to toxic Aβ species, we challenge organotypic mouse brain slices with Aβ-immunodepleted (17.3pM Aβ40, <0.9pM Aβ42) or mock-immunodepleted (52.5pM Aβ40, 31.5pM Aβ42) human AD brain homogenate (ADBH). Synaptic structure and activity are monitored with mScarlet/jGCaMP7b expression in CA1 pyramidal neurons, while astrocytes

express a blue fluorescent reporter (ECFP). Data are analysed with generalised linear mixed models (GLMM) with animals and slices included as a random effect to factor in repeated measures. Calcium data is processed with ImageJ and is analysed blind with CaImAn/OASIS in Python. We demonstrate that Aβ+ ADBH induces a significant loss of synapses compared to ACSF or Aβ- ADBH treatment. Aβ+ ADBH induces a significant increase in the frequency of synaptic calcium events (GLMM, Aβ:F=5.5,p=0.049 Aβ*Treatment Time:F=6.9,p=0.008, Tukey post-hoc ($A\beta^{+}$|POST-PRE):z=2.6,p=0.008). Furthermore, we observe a significant difference between the rate of change in synaptic activity of surviving versus lost synapses at 24h (GLMM, Survival:F=5.1,p=0.02, Aβ:F=3.2,p=0.08, Tukey post-hoc: ($A\beta^{+}$|Survived-Eliminated):z=-3.3,p=0.001. Astrocyte contact had a minimal, non-significant effect on the rate of change in synaptic activity at 2h. Conversely, we found that synapses contacted by astrocytes were significantly more likely to survive at 24h after Aβ+ ADBH challenge (GLMM, Aβ:F=17.0,p=0.0002 Astrocyte:F=18.2,p=0.0001 Aβ *Astrocyte:F=6.0,p=0.18, Tukey post-hoc comparison ($A\beta^{+}$|Astrocyte):p<0.0001). Our findings suggest that our brain slice model effectively reproduces key features of early AD, including synapse loss and hyperexcitability. Furthermore, they indicate that astrocytes play a protective role in maintaining synapses, particularly under conditions of short-term exposure to low concentrations of toxic forms of Aβ. Further work will elucidate the role of synapse phagocytosis by astrocytes due to continued presence of Aβ species.

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Nanosymposium

NANO002: Glial Mechanisms of Alzheimer's Disease

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Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO002.13

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

Support: Italian Ministry of University and Research - PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE - 2020AMLXHH
Italian Ministry of Health - Ricerca Corrente - Oasi Research Institute IRCCS

Title: Astrocytic α7 nicotinic receptor dysfunction disrupts glutamate and memory flexibility in a prodromal Alzheimer’s disease-like phenotype

Authors: *M. TROPEA[1], M. MELONE[3,5], R. PIACENTINI[6,9], A. DI SPIEZIO[10], M. TORE[11], R. TROVATO[1], V. VACANTI[1], S. BEN ABID[12], M. ZONTA[10], C. RIPOLI[7,9], G. LOSI[11,13], F. CONTI[4,5], C. GRASSI[8,9], D. PUZZO[2,14];
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Ancona, Italy; [6]Inst. of Human Physiol., Univ. Cattolica del Sacro Cuore, Rome, Italy; [7]Inst. of Human Physiol., Univ. Cattolica del Sacro Cuore, Roma, Italy; [8]Dept. of Neurosci., Univ. Cattolica del Sacro Cuore, Rome, Italy; [9]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [10]Natl. Res. Council, Padova, Italy; [11]Inst. Nanoscienze, Consiglio Nazionale delle Ricerche (CNR-NANO), Modena, Italy; [12]Inst. Nanoscienze - Consiglio Nazionale delle Ricerche (CNR-NANO), Modena, Italy; [13]Univ. of Modena and Reggio Emilia, Modena, Italy; [14]Oasi Res. Institute-IRCCS, Troina, Italy

Abstract: Alzheimer's disease (AD) is the leading cause of dementia in the elderly, yet its complex pathogenesis has limited the development of effective treatments. Beyond amyloid beta (Aβ) and tau pathology, cholinergic dysfunction is increasingly recognized as a critical event contributing to disease progression. We previously demonstrated that genetic deletion of the α7 nicotinic acetylcholine receptor (α7nAChR) disrupts Aβ physiological signaling and leads to an age-dependent AD-like phenotype in 12-month-old α7nAChR knockout (α7KO) mice, characterized by synaptic and memory deficits, Aβ and tau accumulation, neuronal loss, and astrocytosis. However, the early mechanisms driving this phenotype remain poorly understood.Here, we used a multidisciplinary approach including electron microscopy, glutamate, calcium, and sodium imaging, electrophysiology, and behavioral assays to investigate the role of astrocytic α7nAChR dysfunction in subtle initial alterations in 3-6-month-old α7KO mice, preceding the full manifestation of AD phenotype.Electron microscopy revealed high expression of α7nAChRs in astrocytic processes at excitatory synapses in the hippocampal CA1 region. In α7KO mice, both spontaneous and evoked astrocytic Ca^{2+} transients were diminished, and glutamate uptake was impaired, resulting in altered glutamate dynamics and its synaptic accumulation after repeated stimulation. GLT-1 transporter spatial distribution was also disrupted, with reduced astrocytic expression and altered subcellular localization. This was associated with a displacement of astrocytic processes from synaptic sites, likely contributing to the observed glutamate uptake deficits. These alterations correlated with impaired synaptic vesicle recycling and reduced long-term depression at CA3-CA1 synapses. Deficits in spatial memory flexibility were also observed in α7KO mice, evaluated through a modified Morris water maze task. Remarkably, AAV-mediated astrocyte-specific re-expression of α7nAChRs rescued calcium signaling, glutamate dynamics, synaptic plasticity, and memory flexibility. Similarly, treatment with ceftriaxone, a GLT-1 enhancer, ameliorated synaptic and behavioral impairments.These findings position astrocytic α7nAChR dysfunction as an early and potentially causative event in the development of an AD-like phenotype. By revealing measurable synaptic and cognitive alterations that precede Aβ and tau accumulation, this work offers new insights into the mechanisms of the disease onset and highlights novel potential biomarkers and targets for early therapeutic intervention.

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Nanosymposium

NANO002: Glial Mechanisms of Alzheimer's Disease

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Presentation Number: NANO002.14

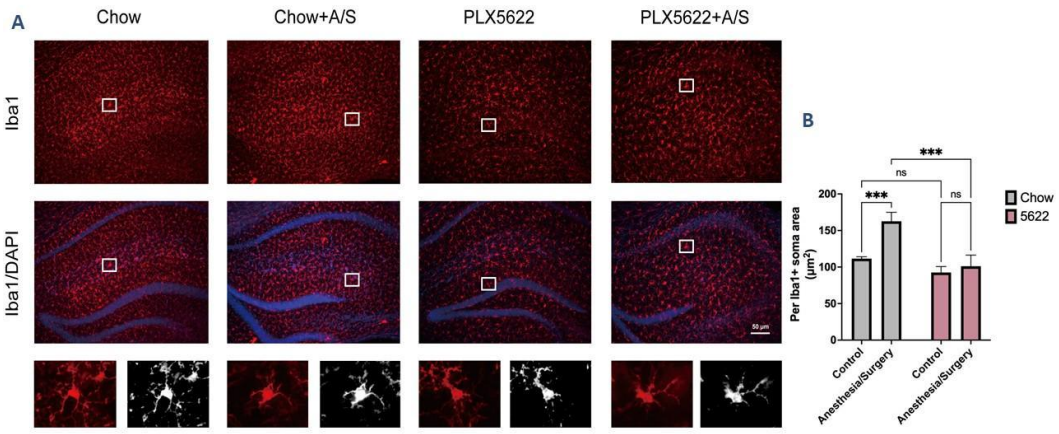
Topic: C.02. Alzheimer's Disease and Other Dementias

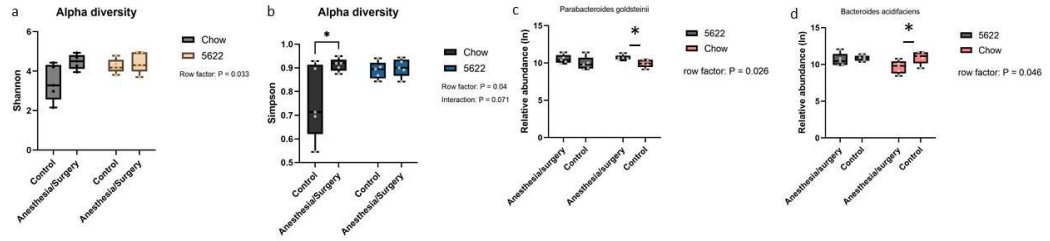
Support: NIH/NIA1 R21 AG065606-01A1
NIH/NIA1 R21 5R21AG081763

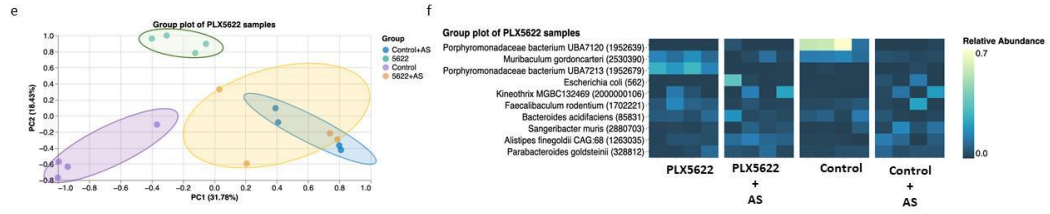
Title: Effects of Microglia-associated Gut Microbiota Changes on Surgery induced Neuroinflammation

Authors: W. QI[1], **J. K. MATSUBARA**[2], Z. XIE[3], *Y. ZHANG[1];
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Abstract: Introduction: PLX5622, a CSF1R inhibitor, is used to study microglia, though its mechanisms remain unclear. Anesthesia and surgery had been associated with neuroinflammation via the microbiota-gut-brain axis. We propose that PLX5622 attenuates anesthesia and surgery-induced microglial activation via this axis. **Methods**: PLX5622 600mg/kg/day was used for 6 days before anesthesia/surgery. 18-month-old mice brain tissues were extracted and used for immunostaining. Tissues were immersed in 4% PFA (4°C, 12h) sectioned using a vibrotome, incubated with anti-Iba1 (1:1000, 018-28523, Fujifilm), and counterstained using DAPI. Mice fecal samples were collected before anesthesia/surgery, and after 6/7 days after anesthesia/surgery for 16s rRNA gene sequencing. Data were analyzed and plotted using two-way ANOVA in RStudio and Prism. **Results**: Immunofluorescence showed that PLX5622 reduced anesthesia-induced microglia activation. Moreover, PLX5622 reversed anesthesia and surgery-induced gut microbiota changes, including in Parabacteroides goldsteinii and Bacteroides acidifaciens. **Conclusions**: PLX5622 reduces microglial activation and neuroinflammation while reversing anesthesia and surgery-induced microbiota changes. These findings suggest a protective role via modulation of the microbiota-gut-brain axis, though further validation is needed.







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Nanosymposium

NANO003: Insights From the Machine Intelligence From Cortical Networks (MICrONS) Project

Location: SDCC Rm 30

Time: Saturday, November 15, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO003.01

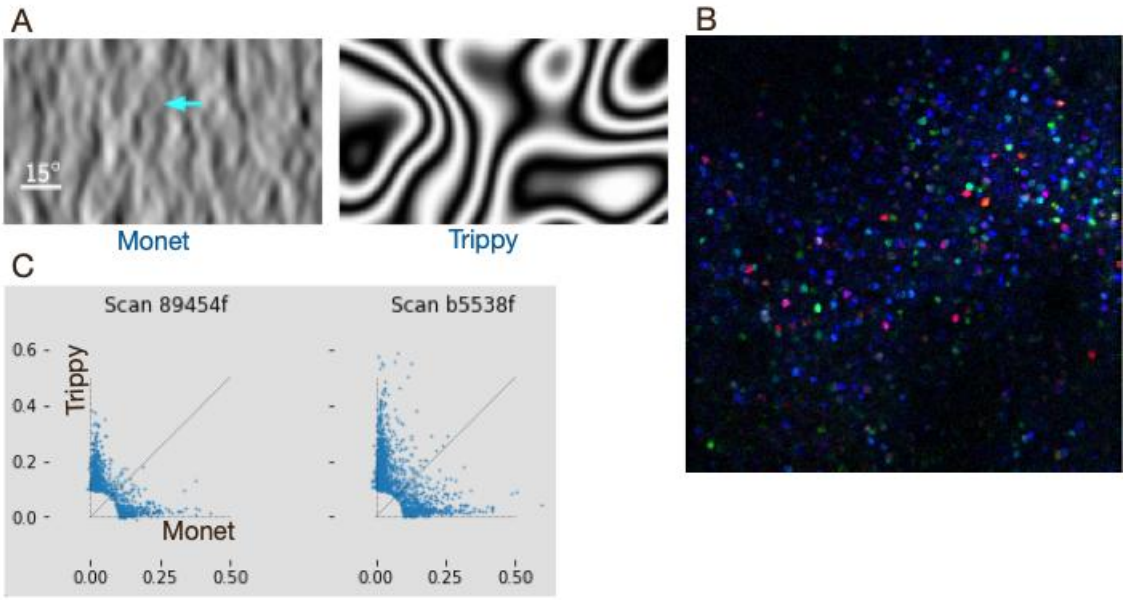
Topic: E.06. Vision

Support: U24NS116470
R24MH117295

Title: Parametric Stimuli Reveal Functional Subcircuits in Visual Cortex

Authors: *D. YATSENKO[1], P. FAHEY[2], T. MUHAMMAD[3], M. SHAKIBA[4], R. ROKNI[4], M. MOHAMMADI[4], N. DEHGHANI[5], A. S. TOLIAS[2];
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Abstract: The MICRONS project introduced two parametric stimuli with distinct spatial structures: **"Monet"** (coherent global motion) and **"Trippy"** (localized contour motion). Building on initial observations that these stimuli activate separate neurons, this study was designed to precisely test and quantify this differential engagement of neuronal subpopulations in mouse primary visual cortex (VISp). We used two-photon calcium imaging in six GCaMP6s-expressing mice during stimulus presentation. Analysis of activity across 14 scans revealed distinct, non-overlapping subpopulations of excitatory neurons preferentially responsive to either Monet or Trippy. As shown in response maps, these populations are strikingly segregated, with cells responsive to Monet (red) or Trippy (green) showing minimal spatial overlap. Cell-wise analysis confirms this: scatter plots show a profound deficit of neurons responsive to both stimuli ($p<10^{-9}$, permutation test), with cells clustering along axes of preference for one stimulus type.This differential activation provides clear evidence for a functional subdivision in the mouse visual system. The significance of this finding is the identification of distinct subcircuits using precise parametric characterizations. The full MICRONS dataset now allows us to relate these functional streams to their underlying circuit connectivity, anatomical organization by area and layer, and cell morphology.



Synthetic stimuli divide visual cortex neurons into two functional subcircuits. A: Monet and Trippy stimuli are designed to have similar spatiotemporal spectra but distinct spatial structures. B: Pixelwise analysis indicates distinct responsiveness to natural movies (blue), Monet (red), and Trippy (green), with little overlap between Trippy and Monet (no yellow). C: Cell-wise analysis indicates a striking separation of responsiveness with a clear deficit of cells responsive to both stimulus types ($p<10^{-9}$, permutation test) in each of 14 scans.

Disclosures: D. Yatsenko: None. **P. Fahey:** None. **T. Muhammad:** None. **M. Shakiba:** None. **R. Rokni:** None. **M. Mohammadi:** None. **N. Dehghani:** None. **A.S. Tolias:** None.

Nanosymposium

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Presentation Number: NANO003.02

Topic: E.06. Vision

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DoI/IBC D16PC00004
DoI/IBC D16PC00005
NSF CAREER grant IOS-1552868
NSF NeuroNex grant 1707400
NSF NeuroNex grant 1707400
RF1 MH130416

Title: Functional connectomics reveals general wiring rule in mouse visual cortex

Authors: *Z. DING[1], P. FAHEY[1], S. PAPADOPOULOS[1], E. Y. WANG[3], B. CELII[6], C. PAPADOPOULOS[4], A. CHANG[3], A. B. KUNIN[7], D. TRAN[4], J. FU[8], Z. DING[3], S. S. PATEL[5],

L. NTANAVARA[1], R. FROEBE[1], K. PONDER[4], T. MUHAMMAD[9], D. YATSENKO[10], E. FROUDARAKIS[11], F. H. SINZ[12], K. JOSIC[13], R. ROSENBAUM[14], H. SEUNG[15], F. C. COLLMAN[16], N. M. DA COSTA[16], R. REID[17], E. Y. WALKER[18], X. PITKOW[19], J. REIMER[3], A. S. TOLIAS[2];
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Abstract: Understanding the relationship between circuit connectivity and function is crucial for uncovering how the brain computes. In mouse primary visual cortex, excitatory neurons with similar response properties are more likely to be synaptically connected; however, broader connectivity rules remain unknown. Here we leverage the millimetre-scale MICrONS dataset to analyse synaptic connectivity and functional properties of neurons across cortical layers and areas. Our results reveal that neurons with similar response properties are preferentially connected within and across layers and areas—including feedback connections—supporting the universality of 'like-to-like' connectivity across the visual hierarchy. Using a validated digital twin model, we separated neuronal tuning into feature (what neurons respond to) and spatial (receptive field location) components. We found that only the feature component predicts fine-scale synaptic connections beyond what could be explained by the proximity of axons and dendrites. We also discovered a higher-order rule whereby postsynaptic neuron cohorts downstream of presynaptic cells show greater functional similarity than predicted by a pairwise like-to-like rule. Recurrent neural networks trained on a simple classification task develop connectivity patterns that mirror both pairwise and higher-order rules, with magnitudes similar to those in MICrONS data. Ablation studies in these recurrent neural networks reveal that disrupting like-to-like connections impairs performance more than disrupting random connections. These findings suggest that these connectivity principles may have a functional role in sensory processing and learning, highlighting shared principles between biological and artificial systems.

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Nanosymposium

NANO003: Insights From the Machine Intelligence From Cortical Networks (MICrONS) Project

Location: SDCC Rm 30

Time: Saturday, November 15, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO003.03

Topic: E.06. Vision

Support: Paul G Allen grant

Title: Cell type-specific microcircuits of the primary visual cortex

Authors: *C. ZHANG, C. M. SCHNEIDER-MIZELL, B. P. DANSKIN, L. ELABBADY, E. JOYCE, B. D. PEDIGO, R. REID, F. C. COLLMAN, N. M. DA COSTA;
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Abstract: Excitatory neurons in layers (L) 2/3 and 5 are principal sources of intra-telencephalic (IT) projections. Both populations target neurons within their respective and each other's layers. This suggests direct influence between IT pathways through monosynaptic connections, which potentially gates cortical information flow. However, the detailed intra- and interlaminar connectivity remains largely unknown. Large-volume serial section transmission electron microscopy (ssTEM) provides unprecedented opportunities to uncover the anatomical underpinnings of these cortical circuits. Using the MICrONS ssTEM dataset of mouse primary visual cortex, we reconstructed and analyzed the connectivity of IT neurons in L2/3 and L5. Specifically, we focused on their direct synaptic targets and disynaptic networks via inhibitory neurons in L2/3 and L5. Additionally, the connectivity of other involved cell types across cortical layers was also explored. We observed that L5a neurons heavily invested their output in L5, forming recurrent excitatory and inhibitory circuits among their own type and with specific types of inhibitory cells. In contrast, L5b neurons formed sparse connections in L5 and predominantly projected axons into L2/3, driving direct excitation and disynaptic feedforward inhibition onto L2/3 IT neurons. Furthermore, the L5b population possessed another level of diversity in their morphology and connectivity, where we identified L5b-1 and L5b-2 subtypes. These two subtypes were distributed at different depths of L5 and innervated different populations of L2/3 neurons, with L5b-1 neurons targeting upper L2/3 whereas L5b-2 neurons targeting lower L2/3. L2/3 IT neurons also exhibited heterogeneous connectivity. Upper L2/3 IT neurons avoided synapsing with L4 neurons, whereas lower L2/3 IT neurons innervated neurons across layers. L2/3 IT neurons located at similar cortical depths were more likely to interconnect through direct synapses or via inhibitory interneurons. Interestingly, all the L2/3 IT neurons selectively targeted L5 extra-telencephalic (ET) neurons, which barely contact L2/3 IT neurons, instead of L5 IT neurons that abundantly co-existed with ET neurons. In conclusion, this study demonstrates intricate interconnections between L2/3 and L5 IT neurons and their associated inhibitory cells in an asymmetric pattern: L5 IT neurons largely innervate L2/3 neurons, whereas L2/3 IT neurons preferentially target subcortically projecting L5 ET neurons. Moreover, distinct excitatory cell types in layer 5 exhibit different connectivity patterns within layer 5 and across cortical layers.

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Nanosymposium

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Presentation Number: NANO003.04

Topic: E.06. Vision

Support: NIH Grant 5U24NS120053-02
NIH Grant 5UM1NS132253-02

Title: Mapping millions of postsynaptic structures in a large electron microscopy volume of mouse visual cortex reveals cell-type-specific spine targeting patterns

Authors: *B. D. PEDIGO, B. P. DANSKIN, R. SWANSTROM, E. NEACE, N. M. DA COSTA, C. M. SCHNEIDER-MIZELL, F. C. COLLMAN;
Allen Inst., Seattle, WA

Abstract: Neurons display remarkable sub-cellular specificity in their synaptic targeting, which can vary by cell type—for example, excitatory neurons classically prefer to target the spines of other excitatory cells. Modern dense neuroanatomy data, such as large volumetric electron microscopy connectomes, enable the study of these structures and their context in a circuit at unprecedented scale and resolution. However, this scale has also made it challenging to create accurate and efficient methods for classifying and segmenting fine cell components (including spines) across entire volumes. Here, we present a cost-efficient computational pipeline for classifying postsynaptic targets, as well as segmenting structures such as spines. Our method relies only on having a mesh representation of a neuron (which can easily be derived from a voxel-based segmentation) and avoids processing imaging data directly. Instead, we leverage tools from geometry processing to create features from the intrinsic geometry of a neuron's surface. We couple this core technique with tools for object simplification, overlapping subdivision, eigendecomposition acceleration, and robust surface operators to create a pipeline which can be deployed reliably over hundreds of thousands of neurons in the commercial cloud for a few hundred dollars. We then show how a simple but accurate classifier can use these mesh-based features to classify synapses as targeting somas, dendritic shafts, or spines (macro F1 score 96%). Using this pipeline, we present a map of the postsynaptic structures present at over 200 million synapses in the MICrONS mouse visual cortex dataset. Our analysis reveals previously under-characterized cell type targeting patterns such as strong basket cell input onto other inhibitory cell types, layer 6 corticothalamic cells targeting the dendritic shafts of other excitatory neurons, and layer 5 extratelencephalic cells targeting Martinotti cell spines. We also examine spines receiving more than one input, finding an enrichment of multiply-innervated spines on inhibitory neurons, which often receive input from multiple excitatory cells. We make our postsynaptic target predictions available for study, as well as the code for the computational pipeline and commercial cloud deployment. More generally, our work demonstrates how

representations derived from neuronal meshes can be a powerful primitive for describing neural morphologies without extensive training data.

Disclosures: B.D. Pedigo: None. **B.P. Danskin:** None. **R. Swanstrom:** None. **E. Neace:** None. **N.M. da Costa:** None. **C.M. Schneider-Mizell:** None. **F.C. Collman:** None.

Nanosymposium

NANO003: Insights From the Machine Intelligence From Cortical Networks (MICrONS) Project

Location: SDCC Rm 30

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Topic: E.06. Vision

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NIH NINDS U24NS120053
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Title: The arteriole-to-capillary transition zone has an increased axon-to-dendrite ratio and fewer synapses

Authors: *J. J. HOW[1,2,3], B. P. DANSKIN[4], B. D. PEDIGO[4], E. MEMKE[5], D. KLEINFELD[6,7], A. Y. SHIH[5,8];
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Abstract: Cerebral blood flow regulation involves constant communication between active neurons and vascular endothelial and mural cells to control the size and stiffness of vessels that source blood. However, the physical interactions and ultrastructural anatomy that support this cell-to-cell communication remains largely uncharacterized. We made use of the Machine Intelligence from Cortical Networks (MICrONS) dataset, a cubic millimeter volume electron microscopy (EM) reconstruction of mouse visual cortex, to address this question. We found that 1) more axons than dendrites fill the neuropil volume around blood vessels and 2) classical synapses are less common than expected near blood vessels. Furthermore, these features are pronounced at branches of the vascular network at the transition between penetrating arterioles, the source of blood flow into the cerebral cortex, and the capillary network (i.e., the arteriole-capillary transition zone, or ACT zone). We also found that axons at the ACT zone appear to bundle more tightly into ‘micro-tracts’ than axons at other parts of the vasculature. Since potassium (K+) release from active neurons and astrocytes is a potent vasodilator (Longden et

al., 2017) and propagating action potentials release K+, we speculate that the preponderance of nearby axonal micro-tracts at the ACT zones contributes to dilation during neurovascular coupling. Current efforts are focused on exhaustively analyzing all ACT zones, and a subset of other vascular zones for comparative analysis, in the MICrONS dataset.

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Nanosymposium

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Topic: E.06. Vision

Support: NIH Grant U01NS137250
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Title: Method for fiducial-less automated functional co-registration validated on IARPA MICrONS data

Authors: *J. JOYCE[1], S. PAPADOPOULOS[2], D. XENES[1], P. FAHEY[3], F. C. COLLMAN[4], C. A. BISHOP[1], B. CELII[1], N. M. DA COSTA[4], J. REIMER[5], A. S. TOLIAS[3], B. A. WESTER[1]; [1]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [2]Dept. of Neurosci., [3]Ophthalmology, Stanford Univ., Stanford, CA; [4]Allen Inst. For Brain Sci., Seattle, WA; [5]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Under the IARPA MICrONS Program, several large-scale connectomics datasets were generated, including the Minnie dataset which covers a cubic millimeter of mouse visual cortex. This dataset contains functional activity for 75,000 neurons obtained via two-photon (2P) imaging and a reconstruction of a serial electron microscopy (EM) imaging volume of the same functionally-imaged tissue that consists of over 200,000 cells. While these two image volumes are overlapping on the same tissue, there are non-linear deformations that make volumetric co-registration and matching of neurons challenging using both manual and automated methods, especially given the scale. However, co-registration of structural and functional data is critical to enabling researchers to better model and explain the organization and behavior of excitatory neurons within and across cortical areas. Thus, our team has developed a new automated and scalable technique to register functional units to their structural cell counterparts for this and other petascale image volumes.
Our method uses vasculature data from both the 2P and EM datasets to co-register the volumes without the use of fiducials. We first increase the signal-to-noise ratio of the 2P vessels by applying a normalizing filter. Then, both the 2P and EM vessels are skeletonized. The final co-registration is computed using SimpleITK's volumetric B-spline algorithm, treating the EM

volume as the 'moving' volume. The B-spline transformation was performed across multiple scales, progressing from coarse grids with strong smoothing to finer grids with minimal smoothing. After co-registration, matching between neurons across the 2P and EM datasets was performed with minimum weight matching for bipartite graphs using the soma centroids. Following the completion of our new co-registration workflow, our team has generated 34,712 total neuron matches with 84% precision compared to manual assigned matches. When filtering to higher confidence matches, we achieve 28,233 neurons with 90% precision. The automated set of matches generated by our workflow is available in the Minnie65 CAVE annotation framework, which includes the corresponding IDs from the structural and functional datasets and the residual distances between them. This co-registration method not only reduces reliance on fiducials, but also significantly extends the number of accurately matched cells beyond what is feasible with manual annotation, enabling scalable registration across large, multi-modal datasets.

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Topic: E.06. Vision

Support: NIH Grant U24NS120053

Title: Connectome analysis for the community

Authors: *B. P. DANSKIN[1], C. DAVID[2], E. NEACE[1], R. SWANSTROM[1], C. ZHANG[1], F. C. COLLMAN[1], N. M. DA COSTA[1], C. M. SCHNEIDER-MIZELL[1], R. REID[1], H. SEUNG[2]; [1]Allen Inst. for Brain Sci., Seattle, WA; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: The MICrONS project produced a massively dense reconstruction of the anatomical and functional connections of a millimeter volume of mouse visual cortex, all of which is available to the scientific community. The Virtual Observatory of the Cortex (VORTEX) is our ongoing program to assist scientists in analyzing the data for their own research interests, across scales from organelles to cells, circuits, and functional dynamics.
The cubic millimeter volumetric electron microscopy reconstruction of the mouse visual cortex contains more than 200,000 cells and half a billion synapses. Dense two-photon calcium imaging recorded the activity of 75,000 neurons while the mouse viewed natural movies and synthetic stimuli. The original consortium effort resulted in ten high-profile primary publications, and the

dataset has been used in dozens of additional studies from labs across the world.
We believe we have only scratched the surface of the scientific findings in this dataset. With the cooperation and engagement of the neuroscience community, we aim to grow the value of this dataset for years to come.
In response to requests from the scientific community thus far, VORTEX has doubled the number of proofread, high-confidence connections from 880,000 to 1,716,000 synapses, representing 1.2 million unique connections. We have tripled the number of synapses between functionally characterized cells from 35,000 to 116,000. We have proofread the axon of every neuron in the 100 micron spanning 'V1 column' (1355 neurons, 140,000 synapses between known neurons), a subset of the dataset representing a complete functional unit. This sample contains, to our knowledge, an example of every major excitatory cell type in cortex, and forms a valuable reference on connectivity of local cortical connections.
In addition, we have proofread 120 astrocytes in the same visual column, providing the most detailed reconstruction of neuron-glia-vasculature interaction to date. We constructed 25 putative thalamocortical axons making 100,000 synapses and 80,000 unique connections. Further manual annotations produced by VORTEX are released as ground truth, ready to train classification of synapse types, myelination, nodes of Ranvier, microglia lysosomes, and dense core vesicles.
With VORTEX, you can guide where we spend our resources. Our team of expert proofreaders support further refinement and annotation of the dataset, directed by the needs of the scientific community. We are committed to contributing to the advancement of science, and are actively reviewing and accepting requests. Access the data portal and submit a research request at: microns-explorer.org/vortex

Disclosures: B.P. Danskin: None. **C. David:** None. **E. Neace:** None. **R. Swanstrom:** None. **C. Zhang:** None. **F.C. Collman:** None. **N.M. da Costa:** None. **C.M. Schneider-Mizell:** None. **R. Reid:** None. **H. Seung:** A. Employment/Salary (full or part-time):; ZettaAI.

Nanosymposium

NANO004: Higher Visual Areas: Processing in the Ventral Visual Stream

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NDSEG Grant

Title: Parametric neural control identifies the deep encoding models with causal alignment to biological feature tuning

Authors: *B. WANG[1], A. V. JAGADEESH[4], J. PRINCE[2], G. A. ALVAREZ[2], T. A. KONKLE[3], M. S. LIVINGSTONE[4];
[1]Harvard Univ., Boston, MA; [2]Psychology, [3]Psychology Dept, Harvard Univ., Cambridge, MA; [4]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Deep neural networks have become the leading models for predicting neural responses in mid-to-higher visual areas of macaques and humans. However, near-ceiling prediction accuracy can mask whether these models truly capture the neuronal feature tuning or merely exploit spurious correlations. To address this, we leveraged *feature accentuation*, a technique allowing an encoding model to modify seed images to amplify the features it uses. By synthesizing images predicted to drive a neuron at specified levels, we tested whether encoding models could "control" neural activity across its full dynamic range—an essential criterion for a true "digital twin."In the first session of our closed-loop paradigm, we recorded responses from five macaques to ~1,000 natural images in *rapid serial visual presentation*. Neurons in V3, V4, or anterior IT were recorded via floating microelectrode arrays or Neuropixel probes. Overnight, we fit encoding models by ridge regression on hidden-layer activations from ten pre-trained backbones (AlexNet, ResNet, and vision transformers (ViT), including adversarially trained versions). After evaluating accuracy on held-out images, we selected each channel's best-predicting layer. For the most reliable channels, each model generated feature-accentuated (FA) images at 11 target activation levels—from below to beyond natural-image responses—using ten seed images.In a second recording session, we presented these FA images back and measured actual neural responses, comparing them to model predictions. Although most models (except AlexNet) had similar prediction accuracy on held-out natural images, their FA images differed substantially across backbones, reflecting diverse features used for prediction. Critically, adversarially trained ResNet and ViT consistently produced FA images that modulated real neurons in the intended parametric manner, with correlation for certain channels beyond 0.8. The FA images appeared more "shape-like," whereas those from other models were more "texture-like," suggesting that adversarial training promotes features better aligned with cortical neurons.Overall, our results demonstrate that standard fitting and evaluation procedure can yield models with equivalent predictive scores yet rely on different—and sometimes spurious—features. Closed-loop control is a more stringent test, revealing that adversarial training encourages shape-based representations that better match true neural tuning. Our comparison paves the way for developing foundation models of visual cortex and predicting causal interventions for neural control.

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Nanosymposium

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Presentation Number: NANO004.02

Topic: E.06. Vision

Support: NIH Grant R01EY032085
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Title: Traveling wave dynamics reflect perceptual events during bistable visual perception

Authors: *D. HASEGAN[1,2], R. HARDSTONE[3], B. J. HE[2,4,5];
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Abstract: Visual perception involves more than simple sensory processing as it actively generates the visual experience. Internal brain dynamics trigger spontaneous perceptual changes when viewing ambiguous bistable images, such as the Rubin face-vase illusion. However, the neural mechanisms driving spontaneous perceptual changes remain unclear, and the role of traveling waves (TWs) has not been explored. We investigate the spatiotemporal dynamics of neural activity during bistable visual perception by analyzing the TW properties and their coordination with perceptual events. We used an intracranial EEG (iEEG) dataset of 16 human subjects with extensive coverage of electrodes over the cortex. To extract and analyze TWs in iEEG data, we adapted an existing methodology: clustering nearby electrodes with similar oscillatory peaks, fitting the phase at each time point based on spatial spread, and comparing the fit with a permuted location-shuffled distribution. This process detects short segments (63 ms) with TW dynamics and their properties: speed, frequency, and propagation direction. Applying the methods on the iEEG dataset, we identified 122 electrode clusters with oscillatory frequencies between 2.6 and 20 Hz. Out of those, 39 clusters show significant differences in their TW directions when subjects view the bistable images, as compared to viewing unambiguous images or non-visual control (blank screen with fixation cross). Within the parietal lobe, a region associated with perceptual switching, six bilateral clusters show significant differences in TW patterns between the perceptual switching window (0.25 to 1.5 seconds before button press) and perceptual maintenance. During perceptual maintenance, different perceptual contents (e.g., face vs vase) are associated with significantly different TW patterns in an electrode cluster spreading over the temporal pole. Lastly, we observe that the duration of perceptual maintenance positively correlates with the occurrence of TW segments in the right parietal lobe for two electrode clusters. Interestingly, when selecting specific directions of propagation, TW occurrence in many more clusters correlates with perceptual duration (14 clusters from bilateral frontal, parietal, and temporal lobes, with frequencies from 4 to 18 Hz). These findings suggest that traveling waves from broad cortical regions and oscillatory frequencies are involved in maintaining conscious perception when viewing bistable images. Our results provide a deeper understanding of how neural dynamics coordinate across the cortex to resolve perceptual ambiguity.

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Nanosymposium

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Google Asia Pacific
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Title: Encoding of mass in monkey visual and motor cortex after interaction with real objects

Authors: *S. SAHA, P. PURKAIT, S. P. ARUN;
Indian Inst. of Sci., Bangalore, India

Abstract: We understand physical properties of objects, such as mass, after we interact with them. However, little is known regarding if, how and where in the brain, knowledge of physical properties is integrated into their representations. To address this fundamental question, we performed wireless brain recordings from inferior temporal (IT) and premotor/prefrontal (PMv/PFC) cortex of two monkeys. We created custom sipper bottles, each painted with a specific color and loaded with a specific mass (100-500g), so that mass was uncorrelated with color. We recorded brain activity in three phases. In Phase 1, we presented photographs of these bottles in a random sequence on a screen when monkeys passively fixated on them. In Phase 2, each monkey was given these bottles loaded with identical juice reward so that they could sip juice and experience the bottle mass. In Phase 3, monkeys repeated the passive fixation task while viewing the same bottle images as before. Our main findings are as follows: (1) In both IT and PMv/vlPFC, dissimilarity between the multiunit neural population responses to the bottle images was more strongly correlated with mass dissimilarity after experience with object mass compared to before; (2) During the real-world interaction with the bottles, monkeys interacted with all bottles to drink juice but showed clear behavioral differences in the way they interacted with heavier compared to lighter bottles, suggesting that they were sensitive to the bottle mass; (3) IT neurons showed increased firing just before monkeys lifted a bottle whereas PMv/vlPFC neurons fired more after bottles were lifted; (4) Importantly, both IT and PMv/vlPFC neural dissimilarities became increasingly correlated with mass dissimilarity as monkeys gained more experience with bottles over the course of the experiment. In the early trials, IT neurons encoded bottle mass only after monkeys lifted them, but in later trials, they did so before bottles were lifted. PMv/vlPFC neurons only encoded mass around the time of lift. Taken together, our results show for the first time that higher order visual and motor areas dynamically incorporate object mass into the underlying object representations after real-world interactions.

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Nanosymposium

NANO004: Higher Visual Areas: Processing in the Ventral Visual Stream

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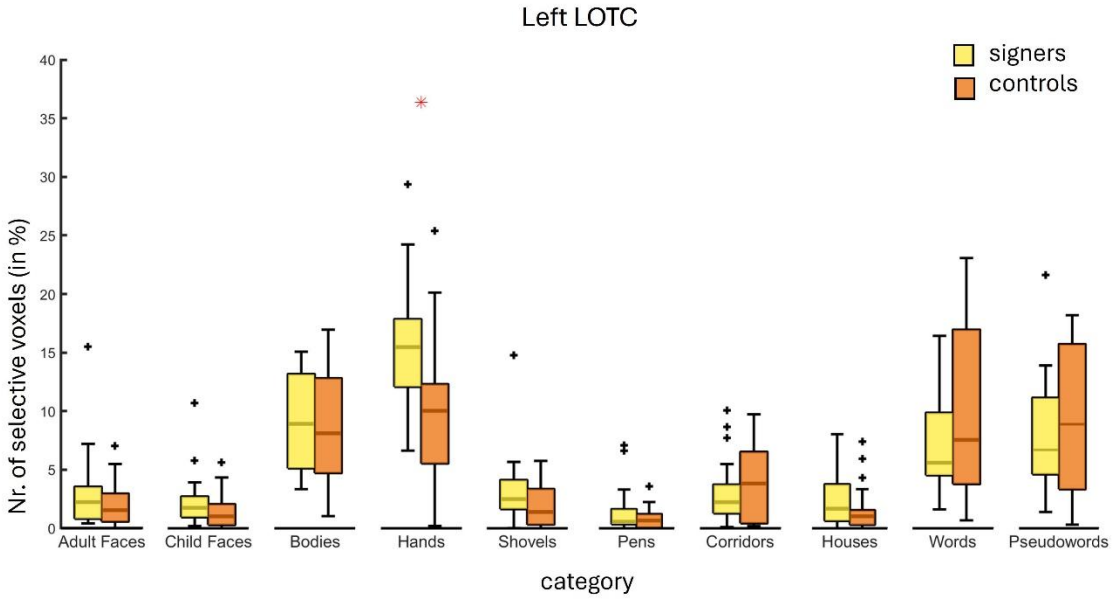
Topic: E.06. Vision

Support: Fellowship funded by the Federal Ministry of Education and Research (BMBF) and the Ministry of Culture and Science of the German State of North-Rhine-Westphalia (MKW) under the Excellence Strategy of the Federal Government awarded to Marisa Nordt

Title: Communication with sign language shapes representations for hands in high-level visual cortex

Authors: *L. KAHLER[1], M. NORDT[1,2];
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Abstract: High-level visual cortex is involved in the recognition and perception of categories like faces or words. In addition to category-selective regions that reside in this part of cortex, categories are also represented in distributed responses patterns. How are these category representations shaped by experience and are they still malleable in adulthood? While it is known that learning to read results in the formation of word-selective regions in ventral temporal cortex (VTC), much less is known about the effects of experience on the representation for other categories. Here, we test, if sign language, which relies on visual information conveyed through hands and facial expressions, influences category representations in high-level visual cortex. We collected functional magnetic resonance imaging (MRI) data of 20 adults without hearing impairments (mean age M=34.8, SD= 13.1) who use sign language extensively (mean sign language use per week M=20.1 h, SD=14.7) and 20 control participants (mean age M= 35.1 years, SD = 11.6). In the scanner, participants watched images of 10 categories including faces and hands while performing an oddball-task. For each category we assessed (i) the number of selective voxels and (ii) the distinctiveness based on multivariate response patterns. Analyses were performed in anatomically defined regions-of-interest (ROIs; medial and lateral VTC and the lateral occipitotemporal cortex; LOTC). Our results reveal differences across the groups that were specific to hands and faces: Signing participants compared to non-signing participants exhibited (i) higher distinctiveness for hands in left lateral VTC as well as left and right LOTC, (ii) more hand-selective voxels in the same ROIs (Fig. 1), and (iii) higher distinctiveness for faces in left medial VTC. Importantly, all effects, except for those in right LOTC, were also visible in a subset of participants, who acquired sign language in early adulthood. These findings enhance our understanding of how high-level visual regions are shaped by experience and provide evidence for their prolonged malleability.



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Nanosymposium

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Presentation Number: NANO004.05

Topic: E.06. Vision

Title: Electrophysiological dynamics of access and disappearance of face stimuli from visual awareness

Authors: *A. BORRIERO[1], A. PEROTTI[3], M. TAMIETTO[4], L. PIA[2], T. CIORLI[1];
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Abstract: Breaking and Reverse Continuous Flash Suppression (bCFS and revCFS) are valuable tools for exploring the dynamics of visual awareness. This study investigated the electrophysiological underpinnings of access to (bCFS) and disappearance from (revCFS) consciousness of face stimuli. In 24 participants, we analyzed the response-locked activity by contrasting the raw potentials and the time-frequency spectra related to access vs. disappearance, and by applying a time-resolved explainable Multivariate Pattern Analysis (xMVPA) to assess the relevance of each electrode in differentiating the two tasks.Results showed distinct neural patterns emerging when contrasting the dynamics of access and disappearance. In the time window preceding the two ‘awareness’ transitions, activity over the parieto-occipital regions

differentiates the two dynamics. Congruently, the time-frequency analysis showed that the access is characterized by enhanced delta and theta frequencies, whereas disappearance exhibits a more time-distributed enhancement of high-frequency activity. Moreover, time-frequency decomposition revealed differences in signal reversibility between the two awareness states: in bCFS, activity was largely irreversible, with power spectra differing between conscious and unconscious perception after conscious access. By contrast, revCFS showed a more reversible dynamic, as conscious and unconscious activity did not substantially alter the signal's temporal properties. xMVPA results showed that the classifier achieves above-chance performance across time when trained on the electrode's mean activity, and the electrode-wise explanations confirm the parieto-occipital differences observed. When the model is trained on the signal's variability, performance improves, highlighting a crucial role of frontal regions, as a complementary frontal contribution for distinguishing access from disappearance. Our findings identify distinct temporal dynamics of access and disappearance from awareness, indicating that early visual areas and frontal regions modulate awareness with different temporal and spectral dynamics.

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Nanosymposium

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Topic: E.06. Vision

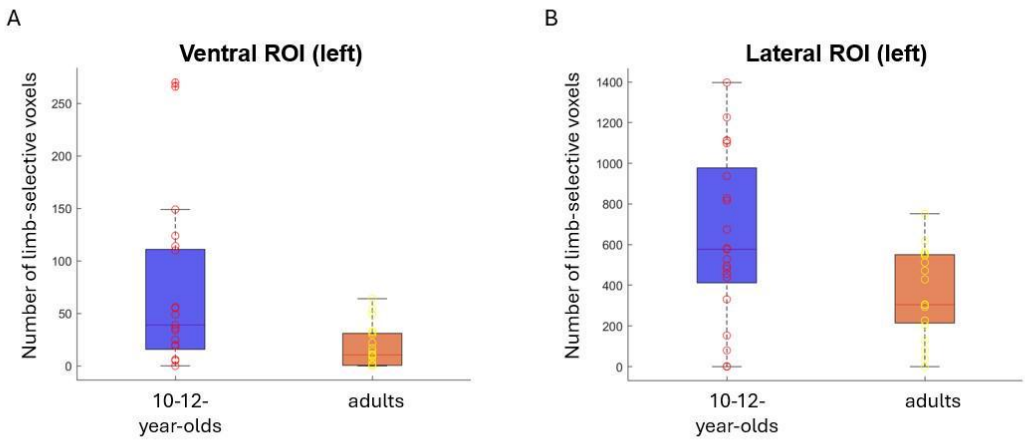
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Fellowship of the Daimler and Benz Foundation (awarded to Dr. Marisa Nordt)

Title: Development of limb-selective regions in the ventral and lateral temporal lobe from school age to adulthood

Authors: *S. R. COHNEN[1], L. KAHLER[1], M. NORDT[2,1];
[1]Univ. Hosp. RWTH Aachen, Aachen, Germany; [2]Mol. Neurosci. and Neuroimaging, JARA-Brain Inst. II, Res. Ctr. Juelich, Juelich, Germany

Abstract: The temporal lobe contains functional regions that are involved in visual processing of categories like faces, hands or words. These category-selective regions are of great importance for social perception and cognitive skills, like reading. Some category-selective regions, such as regions selective for faces and hands, exist both on the ventral and on the lateral side of the temporal lobe. It has been shown that ventral category-selective regions undergo development

during childhood. For instance, while ventral word-selective regions expand during the school years, ventral limb-selective regions shrink from childhood to adulthood. However, it is currently unknown if hand-selective regions on the lateral surface of the temporal lobe shrink as well. To address this gap in knowledge, we collected functional magnetic resonance imaging (fMRI) data of 14 children aged four to six years (M = 5.45 years; SD = 0.74), 18 children aged ten to twelve years (M = 11.3 years; SD = 0,9) and 17 adults (M = 36.93 years; SD = 11,42). During fMRI, participants viewed blocks of images of 10 visual categories (adult and child faces, words, pseudowords, bodies, hands, shovels, pens, corridors, and houses) while performing an oddball task. We next examined if mean selectivity to hands differed across the age groups in anatomically defined regions-of-interest (ROIs) both on the lateral and on the ventral surface of the temporal lobe. Our results first replicate the decrease in hand-selectivity in ventral ROIs from childhood to adulthood shown previously. In addition, we show that mean selectivity to hands also decreases significantly in lateral temporal ROIs in the left, but not right hemisphere. These findings advance our understanding of the development of category-selective regions and of the development of visual streams more broadly.



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Natural Science Foundation of China Grant NSFC32271081

Title: Joint Coding Principles of Motion-sensitive Neurons in IT Cortex Revealed by Dynamic Visual Stimuli

Authors: *W. JIN, B. GONG, J. LUO, P. BAO;
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Abstract: Motion processing is traditionally associated with dorsal stream regions such as MT and MST, whereas ventral stream areas, including inferotemporal (IT) cortex, are typically linked to static object recognition. Using fMRI in macaques viewing both static and dynamic stimuli across eight visual categories (faces, bodies, objects, and scrambled noise), we identified three motion-sensitive regions: MT/MST, FST, and a third area located in anterior IT cortex within area TEa. We refer to this region as the anterior fundus motion area (AFM). To characterize its response properties, we performed electrophysiological recordings from AFM, obtaining responses from ~400 units during 2-second dynamic and static video presentations. A majority of units (79%) exhibited a significant preference for dynamic stimuli (motion selectivity index > 0.33). For comparison, recordings from category-selective regions including face patch AL and two body-selective areas (MSB, ASB) revealed markedly lower motion selectivity, with MSB peaking at only 12%. These findings indicate a functional dissociation between AFM and classical category-selective patches. Furthermore, dynamic stimuli enhanced category selectivity for faces and bodies while reducing selectivity for scenes. A substantial proportion of neurons shifted their category preference toward bodies under dynamic conditions, revealing a population-level bias for animate motion. Together, our results demonstrate that motion-selective responses extend into high-level ventral visual cortex and suggest that anterior IT integrates dynamic information to support recognition of moving agents and socially relevant stimuli.

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Nanosymposium

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Title: Spatial (Allocentric) Coding for Memory-Guided Actions in Naturalistic Environments: An fMRI Study

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Abstract: When performing an action toward a remembered target object, we must combine visual perception and spatial coding processes to correctly guide our limbs toward a successful outcome. In this study, we investigated the cortical mechanisms for memory-guided actions toward naturalistic objects using functional MRI (fMRI). Participants (n=29) performed an Experimental task and a Control task. In both tasks, participants viewed a kitchen scene with six fruits randomly displayed along a countertop (Encoding; 2s), the positions of which had to be remembered. During a subsequent delay phase (Delay 1; 1s jittered), the fruits disappeared, leaving an empty kitchen scene. Afterwards, only five of the original fruits re-appeared, with a possible manipulation - they appeared in their original (Encoding) locations (No Shift) or one, three, or all five fruits were shifted (Shift) imperceptibly leftward or rightward (Test; 1.3 s). In a second delay phase (Delay 2; 1s jittered), only the empty kitchen scene was presented. Finally, during the Response phase (3s), participants performed an action: in the Experimental trials, they reached toward the missing object position (Test) or, in the Control trials, they indicated via button press whether the Encoding phase had a duplicated fruit. We found differential engagement of early-to-late occipitotemporal cortex in the Encoding phase for the Experimental vs. Control tasks. We then found recruitment of bilateral intraparietal sulcus (spatial updating / visuomotor transformation), as well as early (likely V1) and later (lateral occipitotemporal gyrus) visual regions during the Test phase, with visual region activation persisting into Delay 2. During the Response phase, we found sensitivity to Shift (i.e., indicative of updating) in intraparietal sulcus bilaterally and into higher order scene / allocentric occipitotemporal (parahippocampal place area, PPA) cortex. These findings extend and corroborate previous findings that showed specific early-to-higher occipital regions for allocentric coding of simplistic visual landmarks. Overall, these preliminary findings provide novel insights into the progression of visual scene perception into spatial coding and movement planning for memory-guided actions for naturalistic scenes and objects.

Disclosures: B.R. Baltaretu: None. **J. Crawford:** None. **K. Fiehler:** None.

Nanosymposium

NANO004: Higher Visual Areas: Processing in the Ventral Visual Stream

Location: SDCC Rm 11

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO004.09

Topic: E.06. Vision

Support: NIH Grant R01EY035157
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Title: Fmri evidence of perceptual completion of occluded objects at early stages of the human ventral visual pathway

Authors: *F. TONG[1], D. D. COGGAN[2];
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Abstract: In everyday vision, an object of interest is often partially obscured by other foreground objects, thereby leading to an incomplete representation on the retina. Nevertheless, we typically perceive occluded objects as complete entities due to amodal completion. Despite extensive research, the relative contributions of low- and high-level visual areas to amodal completion has remained unclear, as few investigations have spanned the visual hierarchy. Moreover, the role of top-down attention in object completion is not well understood. Here, we used fMRI pattern analysis to quantify the degree of object completion that takes place across successive levels of the human visual pathway and to determine whether such completion was dependent on attention to the objects. We performed fMRI scanning at 7T while participants viewed images of natural objects and those same objects occluded by two complementary sets of non-overlapping horizontal bars (which if combined would cover the entire image). Although the two different occluded views shared no image-level information, cortical response patterns were highly similar in both early visual areas (V1-V4) and high-level object-selective areas, suggesting that perceptual completion emerges at a very early stage of cortical processing. In Experiment 2, the same pattern of results emerged while the observer attended to a task at central fixation, indicating automatic mechanisms for perceptual completion. Taken together, these results support the notion that amodal completion is intrinsic to the neural computations performed by the early visual system.

Disclosures: F. Tong: None. **D.D. Coggan:** None.

Nanosymposium

NANO004: Higher Visual Areas: Processing in the Ventral Visual Stream

Location: SDCC Rm 11

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

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Topic: E.06. Vision

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Title: Long-term stability of visual representations in the primate ventral stream

Authors: *G. RAMOS-TRASLOSHEROS[1], C. R. PONCE[2];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Neurobio., Harvard Univ., Boston, MA

Abstract: Primates rely on the ventral stream to extract visual information from their environment. Ventral representations are thought to progress from simple features in V1 to more complex ones in anterior inferotemporal cortex (aIT). While IT neurons show response

normalization to multiple objects, the precise representations and their sensitivity to feature and spatial context remains unknown beyond hand-curated image sets. Moreover, while the structure of these representations remains unclear, even less is understood about their long-term stability; for instance, although rodent visual representations are thought to be unstable at the single-neuron level, the stability of primate ventral stream representations over time remains largely uncharacterized.
To address these gaps, we optimized preferred images of neurons in the macaque ventral stream using closed-loop image generation and perturbation approaches, and tracked visual response properties using floating microelectrode arrays in V1, V4, and posterior IT (PIT) (16/16/32 and 32/32/32 channels per area in two macaques) over 300-400 days.
We found that the critical image regions driving strong neuronal responses were typically high-contrast localized features rather than whole objects. Embedding these local features into arbitrary natural images increased neuronal responses. Most neurons preferred local features on uniform gray backgrounds over black or white, with notable exceptions. These findings suggest that ventral stream representations are local and selective to feature context, which may be implemented by sharply tuned excitatory and broadly tuned inhibitory neurons.
In long-term recordings (over one year), most multiunit sites showed stable selectivity and dynamics over months, with occasional transitions likely reflecting electrode drift. This stability held across V1, V4, and PIT at multiunit and population levels, and extends previous findings from IT face patches. Overall, our results provide a detailed characterization of ventral stream representations, emphasizing their contextual sensitivity and robust long-term stability. This persistent stability supports the promise of vision restoration therapies targeting single neurons or microcircuits.

Disclosures: G. Ramos-Traslosheros: None. **C.R. Ponce:** None.

Nanosymposium

NANO004: Higher Visual Areas: Processing in the Ventral Visual Stream

Location: SDCC Rm 11

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO004.11

Topic: E.06. Vision

Title: Visualizing the unseen: perceptographer, an AI engine for reconstructing brain-stimulation-evoked perceptual events

Authors: *D. NGUYEN[1,2], E. SHAHBAZI[1], S. LEDET[1], R. BHUIYAN[3], A. CZAJKA[3], A. AFRAZ[1];
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Abstract: We recently developed a novel paradigm, “perceptography,”[1] to visualize complex perceptual distortions evoked by local stimulation of the inferotemporal (IT) cortex. Perceptography uses machine learning to create and optimize specific complex image distortions

which the animal struggled to distinguish from the state of being cortically stimulated. This paradigm opens the door to scientific measurement of subjective perceptual events but comes with a serious image generation challenge.
Without a theory linking neural activity to visual perception, perceived visual distortions caused by brain stimulation may be of any nature. Thus, to avoid bias, our image generation engine, aimed at mimicking stimulation-evoked visual distortions, should be able to span the gamut of image space.
State-of-the-art AI provides two fundamentally different approaches for image generation (e.g., face generation): Generative adversarial networks (GAN), which are suitable for natural faces but struggle with off-manifold distortions to the face, and diffusion models (DM), which can make any image given a text prompt but have difficulty fine-tuning image/face identities in a continuous manner[2].
We introduce Perceptographer, a novel structure designed to solve this problem. It combines a GAN (StyleGANEX), an autoencoder, and a DM (pix2pix-instructor&LLM) to create a novel, flexible engine to navigate this dense multidimensional space. We invert each GAN-DM output image into a perturbable latent space, enabling Perceptographer to generate off-manifold distortions, apply varied distortion levels, and reconstruct any random point in a continuous feature space.
Perceptographer presents a novel framework for visualizing brain-stimulation-evoked perceptual events in different visual areas. This framework overcomes the limitations of current image generation models in handling complex, off-manifold image distortions, providing new opportunities for visualizing and understanding brain-stimulation-evoked perceptual phenomena across multiple cortical regions.
References:
1- Shahbazi, E., Ma, T., Pernuš, M., Scheirer, W. and Afraz, A., 2024. Perceptography unveils the causal contribution of inferior temporal cortex to visual perception. Nature Communications, 15(1), p.3347.
2- Nestor, A., Lee, A.C., Plaut, D.C. and Behrmann, M., 2020. The face of image reconstruction: progress, pitfalls, prospects. Trends in cognitive sciences, 24(9), pp.747-759.

Disclosures: D. Nguyen: None. **E. Shahbazi:** None. **S. Ledet:** None. **R. Bhuiyan:** None. **A. Czajka:** None. **A. Afraz:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

Location: SDCC Rm 25A

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Presentation Number: NANO005.01

Topic: G.07. Biological Rhythms and Sleep

Support: The National Natural Science Foundation of China grants (32071010 and 32100799)

Title: An amino acid metabolite from wake-active monoaminergic neurons constitutes a sleep homeostasis molecule

Authors: *H. CAO[1], K. WANG[1], J. ZHAO[1], P. YUAN[2], Z. ZHANG[1];
[1]Ctr. for Excellence in Brain Sci. and Intelligence Technol., Shanghai City, China; [2]Fudan Univ., Shanghai.

Abstract: Wakefulness produces sleep-promoting substrates for sleep homeostasis. Disrupted homeostasis is associated with sleep disorders, yet no sleep medicine currently targets sleep homeostasis as a therapeutic strategy. Studies over the past century reported that the cerebrospinal fluid (CSF) contains substances reflecting homeostatic sleep pressure. However, the identity of the functional sleep homeostasis molecule, and neural substrate for producing and sensing it, remains mysterious. Here, we discovered that an amino acid metabolite in the CSF reflects physiological sleep homeostasis in nocturnal and diurnal animals, using targeted mass spectroscopy and liquid chromatography. We further combined single-nuclei transcriptome, novel biosensors, structural biology, *in vivo* calcium imaging, optogenetics and pharmacology to dissect its neuronal source and effector. We found that wake-active monoaminergic neurons produce it in an activity-dependent manner, as a necessary intermediate metabolite for producing neuromodulators. And notably, we identified a subpopulation of sleep-promoting hypothalamic neurons as the effector, which selectively expresses the metabolite-specific receptor (a G protein-coupled receptor), and thereby the neuronal excitability encodes sleep pressure and mediates the sleep homeostatic process. Our study demonstrates a novel ligand-receptor pair as a long-sought signaling for controlling sleep homeostasis in the brain.

Disclosures: H. Cao: None. **K. Wang:** None. **J. Zhao:** None. **P. Yuan:** None. **Z. Zhang:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

Location: SDCC Rm 25A

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

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Topic: G.07. Biological Rhythms and Sleep

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Title: $Gaba_A$ receptor knock-down of thalamocortical relay neurons disrupts sleep spindles morphology and fear memory extinction

Authors: F. KATSUKI[1], M. BAUER[2], M. VAUGHN[3], R. E. BROWN[5], J. S. HAAS[4], R. BASHEER[6], ***D. S. UYGUN**[7];
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Abstract: The thalamus is critical in regulating electroencephalographic (EEG) waves of wakefulness and sleep. Yet, the molecular and circuit mechanisms orchestrating these waves are not entirely understood. *in vivo* CRISPR-Cas9 gene editing enables high precision knock-down (KD) of molecules within circuits & cell-types. We used this technology to study synaptic GABA inhibition onto the thalamocortical (TC) relay neurons in sleep oscillations.
We bred vesicular glutamate transporter subtype 2 mice expressing Cre recombinase (vGlut2-cre mice) with lox-stop-lox-Cas9 mice to generate vGlut2-Cas9 offspring. Offspring express Cas9 in vGlut2+ cells, including the majority of TC relay neurons. We generated single-guide RNAs to target alpha1 subunits of $GABA_A$ receptors, the synaptic $GABA_A$ receptor isoform of mouse TC relay neurons, and delivered them locally by AAV. We recorded EEG to study sleep spindles. Compared with baseline records (repeated-measures), KD of alpha1 in TC relay neurons reduced 10-15 Hz (σ; the frequency band of spindles) power in spindle enriched NREM sleep and altered the morphology of the spindles (N=14) including the amplitude during the waxing phase vs the total amplitude (ratio) [t (13) 2.28, p = 0.04]. In separate mice (between-subjects), the waxing amplitude/total amplitude ratio difference in sleep spindle morphology was repeatable [t (12) = 2.1536, p = 0.0262]. Here, the mice also showed persistent behavioral hallmarks (freezing behavior) of fearful memories on a contextual fear conditioning protocol, when compared to a control group (ANVOCA, F (1, 66) = 7.58, p = 0.0076) - control mice had sham-sgRNAs targeting a non-expressed synaptic $GABA_A$ receptor isoform in TC relay neurons.
Our work suggests properly tuned spindles require synaptic $GABA_A$ receptors on TC relay neurons, where thalamic reticular nucleus outputs are received. Disruption of the underlying circuits may be common to sleep spindle disruption and recovery from a traumatic event. Our results may have relevance for developing novel objective screens for post-traumatic stress disorder or other stress related mental illnesses.
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Disclosures: F. Katsuki: None. **M. Bauer:** None. **M. Vaughn:** None. **R.E. Brown:** None. **J.S. Haas:** None. **R. Basheer:** None. **D.S. Uygun:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

Location: SDCC Rm 25A

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO005.03

Topic: G.07. Biological Rhythms and Sleep

Title: Mitochondrial origins of the pressure to sleep

Authors: ***R. SARNATARO**[1], C. VELASCO DOMINGUEZ[1], N. MONACO[1], A. KEMPF[2,1], G. MIESENBÖCK[1];
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Abstract: To obtain a comprehensive, unbiased view of molecular changes in the brain that may underlie the need for sleep, we have characterized the transcriptomes of single cells isolated from rested and sleep-deprived flies. Transcripts upregulated after sleep deprivation, in sleep-control neurons projecting to the dorsal fan-shaped body (dFBNs) but not ubiquitously in the brain, encode almost exclusively proteins with roles in mitochondrial respiration and ATP synthesis. These gene expression changes are accompanied by mitochondrial fragmentation, enhanced mitophagy, and an increase in the number of contacts between mitochondria and the endoplasmic reticulum, creating conduits for the replenishment of peroxidized lipids. The morphological changes are reversible after recovery sleep and blunted by the installation of an electron overflow in the respiratory chain. Inducing or preventing mitochondrial fission or fusion in dFBNs alters sleep and the electrical properties of sleep-control cells in opposite directions: hyperfused mitochondria increase, whereas fragmented mitochondria decrease, neuronal excitability and sleep. ATP levels in dFBNs rise after enforced waking because of diminished ATP consumption during the arousal-mediated inhibition of these neurons, which augments their mitochondrial electron leak. Consistent with this view, uncoupling electron flux from ATP synthesis relieves the pressure to sleep, while exacerbating mismatches between electron supply and ATP demand (by powering ATP synthesis with a light-driven proton pump) precipitates sleep. Sleep, like ageing, may be an inescapable consequence of aerobic metabolism.

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Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

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Presentation Number: NANO005.04

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant MH085927

Title: Melatonin Promotes Sleep by Activating Slo1 Channels via MT_1 Receptors in the Suprachiasmatic Nucleus

Authors: K. VEDANTHAM[1], A. AHMAD[2], L. NIU[3], Y. SHUI[3], F. LEMTIRI-CHLIEH[4], D. KABACK[5], X.-M. MA[3], X. HU[6], S.-P. YEE[5], ***Z.-W. WANG**[3];
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CT; [3]UConn Sch. of Med., Farmington, CT; [4]Neurosci., Univ. of Connecticut Hlth. Ctr., Farmington, CT; [5]UConn Hlth., Farmington, CT; [6]Neurosci., Uconn Hlth., Farmington, CT

Abstract: Melatonin, a hormone secreted by the pineal gland, is well known for its sleep-promoting effects. However, the specific melatonin receptor and downstream effector mediating these effects remain unidentified. Previous studies have shown that both the MT_1 melatonin receptor and the large-conductance, Ca^{2+}- and voltage-activated K^+ channel Slo1 (BK channel) are expressed in the suprachiasmatic nucleus (SCN), the brain's master circadian clock. It is unclear whether MT_1 and Slo1 interact functionally and whether such interactions influence SCN neuronal electrical properties and sleep behavior. To address these questions, we generated de novo *MT1* and *Slo1* knockout mice in the CBA/CaJ genetic background and examined the physiological roles of MT_1 and Slo1 using EEG/EMG recordings and whole-cell patch-clamp analysis of SCN neurons. CBA/CAJ mice were chosen because, unlike C57BL/6 and other common laboratory strains, they can synthesize and secrete melatonin. We found that knockout of either *MT1* or *Slo1* substantially reduced REM and NREM sleep (~60% and ~10%, respectively, in $MT_1^{-/-}$ mice; ~70% and ~25%, respectively, in $Slo1^{-/-}$ mice) and significantly increased wake duration (~15% in $MT_1^{-/-}$ and ~30% in $Slo1^{-/-}$ mice) compared to littermate controls. Both knockouts exhibited prolonged action potential durations (~30% longer in $MT_1^{-/-}$ and roughly twofold longer in $Slo1^{-/-}$ neurons) and a complete loss of afterhyperpolarization in SCN neurons, with non-additive effects observed in *MT1*/*Slo1* double-knockout mice. Notably, these electrophysiological and behavioral changes were largely restricted to the daytime, when mice are typically less active. Consistently, we found that Slo1 expression in the SCN was approximately threefold higher during the daytime than at night, contrasting with earlier studies where researchers reported higher nighttime and lower daytime expression. Co-immunoprecipitation assays using various MT_1 and Slo1 fragments demonstrated that these two proteins physically interact via specific domains. Together, our findings support a model in which melatonin promotes sleep by activating Slo1 channels through MT_1 receptors in the SCN.

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Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

Location: SDCC Rm 25A

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

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Topic: G.07. Biological Rhythms and Sleep

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Title: Sleep and Circadian Rhythm Disruption by NPTX2 Loss of Function via Orexin Pathway

Authors: *S.-E. ROH[1], M. XIAO[2], K. CHEONG[3], C. KWAK[4], A. D. DELGADO[5], A. V. SAVONENKO[8], A. BAKKER[6], H.-B. KWON[7], P. F. WORLEY[9];
[1]Neurol., Johns Hopkins Sch. of Med., Baltimore, MD; [2]JHMI, Lutherville Timonium, MD; [3]Neurosci., Johns Hopkins Med. Inst., Baltimore, MD; [4]JHU, Baltimore, MD; [6]Psychiatry and Behavioral Sci., [7]Neurosci., [5]Johns Hopkins Univ., Baltimore, MD; [8]Pathology, NIH Ctr. for Scientific Review, Bethesda, MD; [9]Dept Neurosci/Neurol, Johns Hopkins Sch. Med., Baltimore, MD

Abstract: Sleep and circadian rhythm disruption (SCRD) is an early, modifiable risk factor in aging and neurodegenerative diseases, yet its molecular underpinnings remain unclear. Orexin A, a hypothalamic wake-promoting neuropeptide, is down-regulated in narcolepsy, and the synaptic immediate-early gene Neuronal Pentraxin 2 (NPTX2) that is co-expressed in orexin neurons, is similarly reduced in narcolepsy and the levels in cerebrospinal fluid (CSF) predicts the transition from normal aging to mild cognitive impairment (MCI). We report a strong correlation of CSF NPTX2 and orexin A in two independent cohorts of cognitively normal older adults, but this link is dampened in Alzheimer's disease (AD). NPTX2 KO mice, despite normal orexin and orexin receptor (OXR) expression, exhibit broad sleep and circadian disruption including unstable circadian onset, rest-phase hyperactivity, excessive daytime sleepiness, elevated microarousals, reduced sleep-spindle density, and broad EEG spectral shifts. Mechanistically, NPTX2 binds both orexin receptors (OXR1/2) at the cell surface. NPTX2 KO mice also show hypersensitivity to TAK-925, a clinical OXR2 agonist, implying receptor hypersensitization when NPTX2 scaffolding is lost. While dual orexin receptor antagonists (DORAs) reduce amyloid and tau pathology, targeted modulation of NPTX2-OXR interactions may offer circuit- and cell-type-specific therapies for sleep disturbances in aging, narcolepsy, and early neurodegeneration.

Disclosures: S. Roh: None. **M. Xiao:** None. **K. Cheong:** None. **C. Kwak:** None. **A.D. Delgado:** None. **A.V. Savonenko:** None. **A. Bakker:** None. **H. Kwon:** None. **P.F. Worley:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

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Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO005.06

Topic: G.07. Biological Rhythms and Sleep

Support: HHMI

Title: Breaking the clock: Structure-guided mutagenesis of clock proteins results in 48-hour period

Authors: *N. PETERSEN[1], M. ROSBASH[2];
[1]HHMI/Brandeis, Somerville, MA; [2]HHMI/Brandeis Univ., Waltham, MA

Abstract: Circadian timekeeping depends on protein-protein interactions as well as post-translational modifications, yet the structural details that underlie these events remain largely uncharacterized. We used AlphaFold 2.3 to predict the interaction region between the Drosophila PERIOD (PER) protein and the kinase Doubletime (DBT). The structure identified two conserved helical domains of PER, SYQ and LT, as critical for the interaction. We made mutations within these domains and used mutant period rescue constructs to assess their effects on circadian period and temperature compensation in vivo. A charge-reversal mutation (E to K) in the SYQ domain caused severe period lengthening and temperature sensitivity, which was rescued by a compensatory charge-reversal mutation in a predicted interacting LT domain residue (K to E); this validated an AlphaFold predicted interaction between two charged residues in SYQ and LT. Three additional mutations in the SYQ domain showed varying effects on period length and temperature compensation, with one uniquely displaying period shortening at higher temperatures. Most strikingly, two mutations in the LT domain (L770A, L775A) resulted in the longest recorded periods for single point mutations in period, with free-running periods > 44 hours at 25C. These also showed extreme temperature sensitivity with a magnitude of change >20 hrs. over the physiological temperatures tested. RNA-sequencing analysis of the L775A mutant shows that these behavioral changes are paralleled by altered molecular cycling. Our findings underscore the power of AlphaFold in predicting a crucial protein-protein interaction even with a largely disordered protein partner and reveal that the PER-DBT binding interface plays a crucial role in maintaining proper timekeeping and temperature-compensated circadian rhythms.

Disclosures: N. Petersen: None. **M. Rosbash:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

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Presentation Number: NANO005.07

Topic: G.07. Biological Rhythms and Sleep

Support: The EGL Charitable Foundation

Title: From molecule to oblivion: How general anesthetics permit pain-free surgery

Authors: *M. BARON;
Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: GABAergic general anesthetics are presumed to act by suppressing the activity of $GABA_A$-receptor expressing neurons distributed throughout the brain, particularly in the cerebral cortex. I will present an alternative model based on the discovery that exposing neurons in a small brainstem nucleus, the mesopontine tegmental anesthesia area (MPTA), to GABAergics

induces loss-of-consciousness while lesioning it renders animals relatively insensitive to anesthetics delivered systemically. Specifically, I have identified a population of "effector-neurons" which, when excited chemo-genetically, induce anesthesia, apparently via dedicated ascending and descending axonal projections. The excitation appears to reflect disinhibition by inhibitory interneurons that express an extrasynaptic $GABA_A$-receptor isoform including the δ-subunit. Agents that drive this isoform selectively, including THIP and GABA at nanomolar concentrations, the GABAergic anesthetics pentobarbital and Propofol at concentrations present in the brain during clinical anesthesia, and some neurosteroids, are pro-anesthetic. These observations imply that anesthetics cause unconsciousness by pharmacologically co-opting circuitry that evolved to effect sleep, fainting and other natural events that involve bistable brain-state transitions.

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Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

Location: SDCC Rm 25A

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO005.08

Topic: G.07. Biological Rhythms and Sleep

Support: NINDS 5T32NS115723-04

Title: Multiscale Dynamics of Traveling Waves in Human Cortex During Propofol Anesthesia

Authors: *V. ZARR[1], T. DAVIS[2], B. GREGER[4], E. H. SMITH[3];
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Abstract: Despite substantial work, the spatiotemporal neural dynamics underlying medically induced loss of consciousness (mLOC) remain unclear. Recent studies in non-human primates (NHPs) show that traveling waves change direction upon anesthetic induction. We hypothesized that burst suppression in human multiunit action potential times (MUA), firing rates, and local field potential (LFP) activity evokes neural traveling waves, and that the spatiotemporal properties of these waves differ between awake and mLOC brain states. We analyzed direct cortical recordings from Utah-style microelectrode arrays in two adult epilepsy patients undergoing monitoring during propofol-induced LOC. Following identification of burst suppression, we regressed the timing of LFP and neuronal firing against the spatial coordinates of the array's microelectrodes. Traveling waves were defined as statistically significant regression slopes using F-tests against permutation distributions (500 shuffles). Both L1 and L2 regularization were used, with multiple hypotheses controlled for using permutation testing. We found that both single-unit activity and LFP activity propagated as traveling waves during burst suppression. We then sought to characterize the spatiotemporal features of the detected waves. LFP and firing rate wave directions shifted significantly during mLOC. LFP wave speed

remained stable across brain states, while firing rate wave speed decreased significantly during mLOC. Additionally, the speed of MUA traveling waves decreased over the time course of anesthesia and slower MUA waves during burst suppression bursts were associated with increased neuronal firing. These results provide the first evidence of spatiotemporal traveling wave organization in the human cortex during anesthesia and suggest that traveling waves may contribute to modulating neuronal excitability in unconscious states.

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Nanosymposium

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Title: Sleep/wake regulation through KCTD2,5

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Abstract: Sleep is an essential and conserved behavior from vertebrates to invertebrates, yet the intracellular molecular signaling pathways that determine daily sleep amounts and the neuronal substrates for homeostatic sleep need are largely unknown. Previously, it was reported that loss-of-function *insomniac* mutant flies showed reduced total sleep time (Stavropoulos and Young, Neuron 2011). This *insomniac* gene is conserved as *Kctd2,5,17* from fly to mammals. KCTD family is known to work as adaptors for the E3 ubiquitin ligase Cullin3 and recognize substrates for ubiquitination by this complex. Here, we examined sleep/wakefulness of mice deficient for *Kctd2* and *Kctd5* in *synapsin1*-positive neurons since the late infancy resulted in significantly increased NREM sleep total time with elevated EEG delta power during NREM sleep. Next, we examined sleep/wakefulness of mice deficient for *Kctd2* and *Kctd5* in *Vglut2*-positive excitatory neurons, which also showed significantly increased NREM sleep total time, with elevated EEG delta power during NREM sleep. We demonstrated that conditional knockout of KCTD2/5 in neuron cells cause hypersomnia phenotypes and these genes regulate sleep/wake cycle in excitatory neurons. Interestingly, these KCTD2/5 conditional KO mice showed longer NREM

sleep time which is completely opposite from *insomniac* deficient fly. This gene acts as a factor that suppresses arousal in flies, but in mammals, due to different neural circuits and compensatory regulatory mechanisms, its loss may have resulted in hypersomnia. Our results suggest that the evolutionally conserved KCTD2/5 mediates, together with ubiquitination of substrates, the intracellular signaling crucial for the regulation of daily sleep amount and sleep need at the organismal level. This study was conducted with the approval of the Ethics Committee for Animal Experiments of the University of Tsukuba.

Disclosures: A. Koyama: None. **T. Fujiyama:** None. **S.J. Kim:** None. **S. Nakata:** None. **K. Iwasaki:** None. **J. Ma:** None. **S. Kanno:** None. **M. Kakizaki:** None. **S. Mizuno:** None. **S. Takahashi:** None. **H. Funato:** None. **M. Yanagisawa:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

Location: SDCC Rm 25A

Time: Saturday, November 15, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO005.10

Topic: G.07. Biological Rhythms and Sleep

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Title: Anatomical and transcriptional divergence in lateral hypothalamus vesicular GABA transporter neurons

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Abstract: In the central nervous system (CNS) the projectome is a critical component as it provides the structural framework through which the CNS mediates critical behaviors and physiological processes. In this view, neurons organize into subpopulations that differentially target postsynaptic regions and support different neurobiological functions, leveraged by expression of key transcripts. Although changes in gene expression is the driving force behind cell morphology, connectivity, and function, a comprehensive understanding of the interplay between the transcriptome and projectome remains elusive. Here, we merged single nucleus RNA sequencing (snRNAseq) of vesicular GABA transporter neurons (VGAT) in the lateral hypothalamus (LH) with retrograde tracing to test if changes in gene expression correlates with the projectome. We targeted LH VGAT neurons projecting to the ventrolateral preoptic area (VLPO), ventral tegmental area (VTA) and central nucleus of the amygdala (CeA) with the retrograde tracer (AAV2retro-CAG-FLEX-rc [Jaws-KGC-GFP-ER2]) in VGAT-IRES-cre mice. Our first experiment targeted the CeA; LH tissue was extracted and submitted for snRNAseq (n=8 mice per region). We found 5,089 VGAT nuclei (*Slc32a1+)* with *Gfp* co-expression in 259 nuclei, hence representing the subpopulation of CeA-projecting LH VGAT neurons. *Slc32a1*

nuclei organized into 18 clusters using unsupervised clustering with principal component analysis and uniform manifold approximation and projection (UMAP) for dimensionality reduction. *Gfp*+ nuclei were intermingled throughout all 18 clusters. Differential gene expression analysis (Log2 fold change, >0.25; minimum percent, 25%) revealed a total of 1,461 genes significantly enriched in *Gfp*+ nuclei (Wilcoxon Rank Sum test with False Discovery Rate correction; adjusted p-value <0.05). This included neurotransmitter receptors and subunits, G-protein coupled receptors, voltage gated ion channels and synaptic adhesion proteins, suggesting that CeA-projecting LH VGAT neurons represent a transcriptional variant from a common cell type. To assess inter-subpopulation heterogeneity, we integrated datasets from all three experiments (VLPO, VTA, and CeA). We used the UMAP centroid positions of each subpopulation to calculate mean squared Euclidian distances, a distance-based measure of transcriptional heterogeneity, and found moderate values between data sets, indicating that VTA, VLPO and CeA-projecting LH VGAT neurons are transcriptionally divergent. Overall, this suggests that changes in gene expression correlate with the projectome, and specifically efferent projection pathways.

Disclosures: S.K. Pintwala: None. **E. Arrigoni:** None. **P. Fuller:** None.

Nanosymposium

NANO005: Sleep Regulation: From Molecular to External Factors

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Topic: G.07. Biological Rhythms and Sleep

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Title: Identification of photoperiod as a regulator of dopamine-mediated behavior in female mice

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Abstract: Photoperiod is the primary environmental signal that drives seasonal changes in behavior. Our previous work demonstrated that photoperiod exerts sex-specific effects on synaptic dopamine dynamics within the nucleus accumbens (NAc). Emerging evidence points to the dopamine transporter (DAT) as a potential site mediating these sex-specific photoperiodic influences on NAc dopamine signaling. The NAc plays a central role in the brain's reward circuitry, linking motivation to action, and alterations in synaptic dopamine dynamics here can significantly impact behavior. Cocaine, a psychostimulant that targets monoamine transporters such as DAT, produces strong behavioral effects, making it a useful tool for assessing DAT function and dopamine physiology. In this study, we investigated how seasonally relevant

photoperiods affect DAT function in the NAc and dopamine-dependent behaviors in both male and female mice. Our findings reveal that females exposed to a Short, winter-like photoperiod display reduced cocaine-induced hyperlocomotion. In contrast, females raised under a Long, summer-like photoperiod exhibit enhanced dopamine release and increased cocaine-induced DAT inhibition, yet show reduced sensitivity to cocaine-associated learning. Together, these results provide evidence that photoperiod exerts female-specific effects on DAT function in the NAc and on behaviors mediated by DAT.

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Nanosymposium

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Title: Hypothalamic neural circuit underlying circadian regulation of torpor in mice

Authors: *D. ONO[1], S. MIYAZAKI[2], A. YAMANAKA[3], M. MIEDA[4], T. J. NAKAMURA[5], H. YAMAGUCHI[6];
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Abstract: In mammals, daily rhythms in physiology and behavior are governed by the central circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. This internal clock synchronizes bodily functions with external cues such as the light-dark cycle and ambient temperature, which follow a roughly 24-hour rhythm. During winter, animals face significant environmental challenges, including cold temperatures and restricted food availability. To survive, several species enter energy-conserving states such as torpor or hibernation, characterized by dramatic drops in metabolic activity. Although the circadian system is implicated in regulating the timing of torpor, the precise neural mechanisms remain elusive. To investigate how the circadian clock influences torpor, we examined core body temperature (Tb) dynamics in wild-type (WT) and Cry1/Cry2 double knockout (CryDKO) mice subjected to torpor-inducing conditions. WT mice exhibited a tightly controlled drop in Tb limited to the late subjective night through early subjective day, whereas CryDKO mice showed a continuous

reduction in Tb over the full 24-hour cycle. These results indicate that the circadian system imposes temporal constraints on the onset of torpor. We then explored the neural pathways by which the SCN exerts this control. Optogenetic stimulation of SCN GABAergic neurons projecting to the preoptic area (POA) effectively suppressed torpor. Tracing experiments revealed that these SCN neurons form direct connections with two distinct neuronal populations in the POA: excitatory glutamatergic neurons that promote torpor, and inhibitory GABAergic neurons that suppress these excitatory cells. We will present evidence that highlights how this pathway enables the circadian system to gate the timing of torpor in response to environmental pressures.

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Nanosymposium

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Title: Time-of-day dependent modulation of nociceptive response in Drosophila

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Abstract: Animals rely on endogenous circadian clocks to fine-tune sensory responses throughout the day, enabling them to adapt to temporal changes in their external environment. A representative example is the daily variation in nociceptive responsiveness observed in mammals. However, the underlying mechanisms remain unclear, largely due to the complexity of mammalian neural circuits. In this study, we used fruit flies (*Drosophila melanogaster*) as a simple model to uncover the molecular basis of circadian-regulated nociception. We first demonstrated that adult wild-type flies exhibit significant variation in heat-induced jump behavior throughout the day, mirroring the daily fluctuations observed in mammals. Consistent with this notion, intracellular calcium levels in the peripheral nociceptive neurons fluctuated with

the daily variation in heat-induced jumping. Conversely, circadian clock mutants failed to show these daily variations in thermo-nociceptive behavior and calcium responses, suggesting that *Drosophila* nociceptive system is likely under the control of circadian clock as well. To gain deeper insight at the circuit level, we identified previously uncharacterized subsets of nociceptive regulatory neurons that transmit noxious information from the periphery to the central brain. We discuss a potential mechanism involving these newly identified neurons, addressing the previously unanswered question as for what neurons/circuits indeed regulate the time-of-day variation in nociceptive responsiveness in a clock-dependent manner.

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH intramural

Title: Multi-modal Calcium Dynamics Underlie Circadian Timekeeping in SCN NMS neurons

Authors: *Q. TANG[1], S. HATTAR[2];
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Abstract: Introduction:Over the past several decades, genetically-encoded fluorescent indicators (GEFIs) have revolutionized neuroscience by enabling cell-type-specific optical recording of neural activity. While most applications have focused on brain regions where stimulus-evoked activity correlates with behavior on the scale of seconds to minutes, many fundamental behavioral and physiological processes such as feeding, thermoregulation, and circadian timekeeping occur over hours to weeks. However, adapting optical recording techniques to these longer timescales presents unique challenges, particularly in accurately measuring and interpreting neural activity across extended recording durations. As a result, even studies using similar data have reached divergent conclusions, largely due to differences in data analysis and interpretation. This lack of standardization risks misinterpretation, miscommunication, and reduced reproducibility.
Methods:Using long-term fiber photometry imaging of GCaMP7s. We recorded spontaneous calcium activity from neuromedin-S (NMS) neurons from suprachiasmatic nucleus (SCN). With our newly developed analytical framework, we acquired multi-modal calcium dynamics in the SCN.Results:The calcium dynamics in the SCN NMS neurons consists of multiple layers of circadian rhythm: Sinusoidal slow fluctuation of baseline intracellular calcium; Binary action potential driven calcium transients; and time-of-day dependent light response. Conclusions:1. Calcium is a key player in multiple complex circadian timekeeping mechanisms in the SCN. 2. Long-term fiber photometry calcium imaging is a powerful tool to study the biological rhythm.

3. The fundamental principles of our analytical framework extend beyond circadian research and apply broadly to long-term optical recording studies of brain circuits that govern behavior and physiology over days to weeks.

Disclosures: Q. Tang: None. **S. Hattar:** None.

Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

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Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO006.01

Topic: I.03. Decision Making

Title: Synaptic mechanisms of inhibitory plasticity improve decision-making under input-specific noise

Authors: *B. SHEN[1], S. C. SONG[2], K. LOUIE[3], P. W. GLIMCHER[4], R. C. FROEMKE[5]; [1]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [2]Pathology, [3]Ctr. for Neural Sci., [4]New York Univ., New York, NY; [5]Otolaryngology, NYU Med., New York, NY

Abstract: Synaptic plasticity in inhibitory interneurons plays critical roles in shaping neural computations. In decision-making, such plasticity is believed to be essential for improving neural coding and decision-making deliberation by filtering noise. However, given the diversity of interneuron types and different potential sources of noise, how interneurons interact with noise remains poorly understood. Given the stereotyped targeting of interneuron subtypes to different pyramidal neuron dendritic regions, we hypothesize that different interneuron classes exert distinct control over noise from different dendritic origins. Specifically, somatostatin-positive (SST+) interneurons, which target distal dendrites of pyramidal neurons, are expected to suppress noise that shares the same dendritic pathway as the input but have limited influence on noise from other compartments. In contrast, parvalbumin-positive (PV+) interneurons, which target perisomatic regions, may exert broader inhibitory control across noise sources from multiple dendritic pathways. To test this, we combined in vitro recordings from genetically identified interneurons in mouse auditory cortex with computational modeling of decision circuits. Using spike-timing-dependent plasticity protocols, we found that SST+ interneurons exhibited the strongest inhibitory potentiation, followed by PV+ and NDNF neurons, while VIP+ interneurons showed no significant plasticity on pyramidal neurons. Computational simulations revealed that SST+ plasticity selectively enhances signal-to-noise ratio of neural coding by suppressing input-specific noise, while PV+ plasticity provides broader suppression to noise originating from different pathways. Despite their differences, both types of inhibitory plasticity improved decision accuracy, primarily by prolonging the deliberation time of evidence accumulation through slowing ramping activity dynamics. These results demonstrate that different interneuronal plasticity enhances decision-making by suppressing noise in a pathway-specific manner. It highlights a synaptic mechanism of different interneuron subtypes that shapes circuit-level computations under decision-making uncertainty.

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Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

Location: SDCC Rm 23A

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO006.02

Topic: I.03. Decision Making

Title: Optimal Utility: Maximizing reward in a noisy brain by picking a curved utility function

Authors: *S. SINHA[1], A. TYMULA[2], P. W. GLIMCHER[3];
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Abstract: Pascal introduced the first framework for human decision-making, arguing that humans maximize long-term average returns. Bernoulli later proposed that humans maximize a transformation of value now called utility. By the 1970s, it was clear human choices were not consistently pursuing even the most byzantine utility functions. In response, Kahneman and Tversky laid the foundation for predictive theories that abandoned consistency and fixed utility functions in favor of non-stationary multi-parameter descriptive models.

Why would biological choosers evolve to be inconsistent and non-stationary? In this project, we employ a new approach to explain the why. We use a mixture of proof-based, analytic, and Montecarlo approaches to ask: 1) What is the average yield of a given utility function to a chooser with a specific internal noise in a well-defined environment? 2) What utility function maximizes average earnings conditional on her internal noise and environment?

We show that for a noisy chooser, expected earnings maximization cannot be achieved using a perfectly linear utility function as Pascal had proposed. When representational precision is constrained, sigmoidal, convex, and concave utility functions can all be optimal, depending on the distributional structure of the environment.

We also quantify the expected gains in earnings that would result from relaxing the resource constraint in the brain and compare these gains with the biological costs of noise reduction. This allows us to calculate the optimal level of noise in the representation of value, under different cost functions-thus endogenizing both the utility function and the choosers' noise term. We illustrate how the choice environment affects the optimal allocation of additional cognitive resources to valuation.

Under these representational assumptions, many puzzling phenomena become easily explainable. The K&T sigmoidal value function, Bernoulli's concave, and Pascal's linear utility function all emerge as rational strategies required of choosers who face different environments with varying degrees of precision. Our approach has important implications for revealed preference theory and what we can infer from observed choices. We propose that, rather than "revealed preference theory," a more accurate description of a rational theory of human decision making may be "revealed decision-environment theory." If our theory is correct, then one could predict the

utility (and hence choice structure) of any chooser for any given environment fully understood by the chooser (a significant constraint). This opens avenues for understanding the mechanisms driving human choice behavior.

Disclosures: S. Sinha: None. **A. Tymula:** None. **P.W. Glimcher:** None.

Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

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Topic: I.03. Decision Making

Support: NIMH P50MH094258
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Title: Is the whole the sum of its parts? Neural computation of consumer bundle valuation in humans

Authors: L. CROSS[1], ***R. WEBB**[2], J. P. O'DOHERTY[3];
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Abstract: Humans are often tasked with making decisions about bundles of multiple items and very little is known about how the human brain aggregates, computes and represents value in such cases. We investigated how the human brain evaluates consumer items, both individually and in bundles, and how this activity relates to choice behavior. Participants underwent a deep-fMRI scanning protocol while we elicited behavioral valuations for single and bundled items. Behaviorally, we find that bundle values are sub-additively discounted compared to the sum of individual item values. Neurally, we find that the same distributed network in pre-frontal cortex computes the value of a bundle and its constituent individual items, but the value representation undergoes a normalization that actively re-scales across bundle and single item contexts. These findings suggest that generalized value regions contextually adapt within a valuation hierarchy, as opposed to utilizing an absolute value code.

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Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

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Presentation Number: NANO006.04

Topic: I.03. Decision Making

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Title: Iterative Bayesian inference explains the dynamics of perceptual organization of natural scenes

Authors: T. BISWAS[1], J. VACHER[3], S. MOLHOLM[2], P. MAMASSIAN[4], ***R. COEN-CAGLI**[1];
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Abstract: Perceptual decision making is classically conceptualized as evidence integration theory - the notion that sensory inputs are perceived by sequentially accumulating noisy samples from the environment and averaging out the noise. Modeling with evidence integration has captured perceptual and neural dynamics elicited by parametric stimuli in simple tasks, but studies of natural vision reveal richer dynamics that remain poorly understood. In this study, we propose that samples in time are not accumulated from a noisy external environment, but from internal representations formed through Bayesian inference where the statistics of sensory inputs are refined iteratively. Thus, we aim to test if iterative Bayesian inference determines perceptual dynamics when processing natural stimuli. To test this, we focus on natural image segmentation. We measured human perceptual segmentation using a recently published experimental design: participants judged whether pairs of regions in an image were in the same segment (‘same’) or not (‘different’). Subjective segmentation maps were reconstructed for each participant with optimization on ‘same’/‘different’ responses per pair. By examining single-trial responses where perceived segments were inconsistent with the segments established by the subjective map, we observed that participants presented a bias toward responding ‘same’ when the two regions were close and ‘different’ when far. Furthermore, decision times increased with distance for ‘same’ responses, but decreased with distance for ‘different’ responses, and this effect was larger for participants with stronger bias. For further inquiry, we developed image-computable segmentation models of the classical evidence integration theory and iterative Bayesian inference theory. Although both model types fit aggregate decision-time distributions similarly well, we found that the spatiotemporal dynamics observed in the data were captured only by iterative inference incorporating a Bayesian spatial proximity prior. This work highlights the importance of considering iterative Bayesian computations to understand human perceptual dynamics when exact inference is intractable, as in most real-life situations.

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Topic: I.03. Decision Making

Support: Alfred P. Sloan foundation and the Nomis foundation G-2020-14019

Title: Neurocompuational Underpinning of Valuation Uncertainty in Healthy Humans

Authors: *H.-K. CHUNG[1], H.-H. LI[2], P. N. TOBLER[1];
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Abstract: Adaptive decision-making requires comparing and selecting options based on their value. Values are thought to be represented in a noisy fashion and not known with certainty. From a Bayesian perspective, the width of prior beliefs and noise in (likelihood) value encoding jointly influence valuation and choice. However, empirical evidence that people track such uncertainty during valuation is limited, partly because traditional methods fail to capture it at the single-trial level. Moreover, while dopamine (DA), norepinephrine (NE), and acetylcholine (ACh) are thought to reduce neural noise in general, causal links to valuation uncertainty remain unclear. To address these gaps, we conducted a randomized, placebo-controlled, double-blind study using psychoactive drugs. We tested how reboxetine (NE enhancer), methylphenidate (DA enhancer), and nicotine (ACh enhancer) affect valuation uncertainty. Participants indicated the minimum and maximum subjective value of lotteries, with the range in the reported willingness-to-pay (WTP) indexing perceived uncertainty in each trial. A Bayesian inference model was used to dissociate sources of valuation noise (prior beliefs vs. encoding noise) while accounting for reporting criteria. Experiment 1 (n = 25 human participants) validated our uncertainty measure: confidence ratings decreased with WTP range (Bayes Factor (BF) = 42), and WTP variability was associated with wider range (BF = 60). Moreover, range increased with the mean and variance of the lottery outcome magnitudes (both BF > 100). In Experiment 2 (n = 160 human participants; 80 women), participants received 4 mg reboxetine, 20 mg methylphenidate, 2 mg nicotine, or placebo, with 40 participants per group. Both methylphenidate (BF = 58) and reboxetine (BF = 22) reduced WTP range, indicating lower perceived uncertainty. Modeling revealed that methylphenidate reduced decision conservatism (BF = 20) and decreased encoding noise that scaled with outcome magnitude (BF = 40), whereas reboxetine reduced the width of the prior (BF = 28), baseline (likelihood) encoding noise (BF = 27), and magnitude-dependent encoding noise (BF = 48). Together, our results demonstrate that decision-makers can represent and track valuation uncertainty on a trial-by-trial basis, and our computational model provides a mechanistic account of this process. Both DA and NE decreased perceived valuation uncertainty and attenuated the extent to which noise scaled with outcome magnitude. Additionally, DA lowered the conservative decision criterion, becoming more certain overall across noise conditions, while NE reduced both prior noise and encoding noise in value representation.

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NANO006: Computational Modeling of Decision and Behavioral Processes

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Presentation Number: NANO006.06

Topic: I.03. Decision Making

Title: Correlated variability drives relative value coding in deep neural networks

Authors: *K. LOUIE[1], S. SINHA[1], D. LEVY[2];
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Abstract: Value is a fundamental variable in decision-making, integrating potential cost and benefit information into a unitary quantity to guide the selection process. While rational choice theory assumes that options are assigned absolute values, independent of other available alternatives, biological decision processes often rely on comparative evaluation and decision-related brain circuits employ a relative rather than absolute value code. While relative value coding is widely observed, it is unknown why such coding arises and what function it serves. Here, we examine what environmental and internal factors drive relative reward coding in deep neural networks trained in economic decision tasks. We find that relative value coding: (1) arises naturally in deep networks, (2) decreases when coding capacity (layer size, network depth) is expanded, and (3) increases when inputs exhibit more statistical structure (correlated variability). Furthermore, the degree of relative value coding is modulated by both internal and external noise. Together, these findings suggest that relative valuation reflects an efficient coding process, optimizing network performance under information processing constraints.

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Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

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Presentation Number: NANO006.07

Topic: I.03. Decision Making

Title: Mathematically modelling excessive demand in a lab asset market reveals that timing the price bubble by playing a dividend hot-potato game boosts earnings: Toward a neuroeconomic biomarker of excessive demand

Authors: *J. L. HARACZ;
Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Objective: In research seeking a neural marker of “excessive demand” (ExD), this term is defined as demand that promotes disequilibria in asset or goods markets and drives prices

above fundamental values (e.g., an asset-price bubble). Neuroimaging studies are elucidating the neuroeconomics of asset-price bubbles. In a lab asset-market study, a market-level ExD measure, calculated from summed subject-level desired share holdings, outperformed other demand proxies in explaining asset-price changes. This result yields the question of what motivates subjects' desired share holdings. The present lab asset-market study answers this question. Methods: In a classic lab asset-market design, 9 experiments each included 9 subjects. Experiments included 15 2.5-minute periods of trading an asset with a dividend-based fundamental value that declined across periods. To capture desired holdings, the end of each Period 1-14 was followed by a survey that elicited each subject's number of asset shares that they want to hold at the end of the next period. Results: Potential motivating factors for holding shares were analyzed with a regression equation of the form $DS_{i,t+1} - S_{i,t}$ (i.e., subject i's desired change in share holdings, calculated as the desired number of shares in upcoming Period t+1 minus the number of shares held in the current Period t) = $b_0 + b_1$(Variable 1) + b_2(Variable 2) ⋯ + b_7(Variable 7), where $b_{x>0}$ is the coefficient for a potential explanatory variable x: Variable 1 (price trend [i.e., momentum]) = (aveP_{t-1} - aveP_{t-2})/aveP_{t-2}; Variable 2 (asset price deviation from fundamental value) = (aveP_{t-1} - FV_t)/FV_t; Variable 3 (number of shares held) = $S_{i,t}$; Variable 4 (prior period's excess bids) = $B_{i,t-1} - O_{i,t-1}$; Variable 5 (forecasted price trend) = (Fave$P_{i,t}$ - aveP_{t-1})/aveP_{t-1}; Variable 6 (change in holdings) = $S_{i,t} - S_{i,t-1}$; Variable 7 (purchasing power given last period's average price) = $C_{i,t}$/aveP_{t-1}; where FV_t is each share's dividend-based fundamental value in Period t, Fave$P_{i,t}$ is subject i's survey-elicited forecast of the average price for Period t, and $C_{i,t}$ is subject i's amount of cash in Period t. This model explained 27.4% of the variance in subjects' desired change in share holdings. A model with $S_{i,t}$ as the only variable explained 21.0% of this variance. Conclusions: Subjects' number of shares held was best able to explain the desired change in holdings for the next period. This explanatory variable's negative sign means that subjects tended to hold dividend-yielding shares for a limited time before selling them. Subjects ending up without shares earned more than those left with shares ($p = 0.0035$), suggesting a winning strategy of playing dividend hot potato.

Disclosures: J.L. Haracz: None.

Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

Location: SDCC Rm 23A

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO006.08

Topic: I.03. Decision Making

Support: UZH University Research Priority Program "Brain Network Mechanisms in Learning and Development" (URPP AdaBD)

Title: Risk attitudes are causally shaped by noisy inference in parietal magnitude coding

Authors: *C. C. RUFF;
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Abstract: Humans and many animals systematically pick smaller but certain rewards over larger, uncertain ones - a tendency termed *risk aversion*. Classical accounts attribute it to motivational preferences or fear of uncertainty. Recent computational theories instead argue that risk aversion can arise from *perceptual* biases: noisy neural coding coupled with Bayesian inference makes people *underestimate* large, risky pay-offs, producing seemingly risk-averse choices even for risk-neutral decision makers. Behavioural studies support this idea, but its neural basis has remained unclear. Here I report two experiments in which we provide causal evidence that risk aversion arises from noisy perceptual inference about payoff magnitudes in parietal cortex. All experiments used the numerical population receptive field (nPRF) model to characterise which brain regions are (non-linearly) tuned to numerical magnitudes. This identified an "approximate number system" (ANS) in parietal cortex that showed reliable tuning to payoff magnitudes. Critically, we could use the numerical tuning curves of individual subjects to assess the noisiness of these neural representations and link this to risk-taking behavior. In Experiment 1 (n=30), we measured neural noise in this system during economic choices, tracking trialwise fluctuations. Participants behaved more variable and less risk-neutral on trials where neural patterns in the ANS were less well-aligned with specific numerical magnitudes. This indicates that fluctuations in risky choice behavior can be driven by noise in perceptual magnitude representations. In Experiment 2 (n=40), we perturbed numerically-tuned regions in the right intraparietal area using transcranial magnetic stimulation (TMS). Supporting the causal role of the ANS for economic decision-making, the nPRF model fits showed reduced responses and increased noise after stimulation, which related to more variable and less risk-neutral risky choices. Together, our experiments show that risky choices are causally influenced by perceptual biases originating from noise in parietal magnitude representations. They also demonstrate how advanced neuroimaging methods from visual neuroscience allow empirical tests of theoretical choice models with neural data.

Disclosures: C.C. Ruff: None.

Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

Location: SDCC Rm 23A

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO006.09

Topic: I.03. Decision Making

Support: NIMH R01MH127375
Simons Foundation
Pew Foundation

Title: Decision-making requires active thalamic engagement, not just cortical integration

Authors: *B. SIEVERITZ, R. KIANI;
New York Univ., New York, NY

Abstract: Perceptual decision making has been extensively linked to frontoparietal cortical circuits. In tasks requiring the integration of dynamic sensory evidence to inform saccadic eye movements, neural activity in the lateral intraparietal cortex (LIP) and dorsolateral prefrontal cortex (dlPFC) reflects the accumulation of evidence over time. These findings have contributed to the belief that cortico-cortical interactions play a dominant role in decision formation. However, frontoparietal areas are reciprocally connected with the mediodorsal (MD) and ventrolateral (VL) thalamic nuclei. A prevailing view suggests that these thalamic nuclei act as relays of cortical information. If true, both decision-related and stimulus-related signals should be similarly represented across thalamus and cortex, and behavioral deficits following thalamic disruption should be transient as cortical circuits that are recurrently-connected to each other would compensate. To test these predictions, we trained two macaque monkeys to perform a reaction time version of the direction discrimination task with random dots. In the first experiment, we recorded spiking activity from the MD and VL thalamus, as well as the interconnected prearcuate gyrus in the frontal cortex. Decoding analyses revealed that both cortical and thalamic sites encoded the upcoming choice more strongly than stimulus strength, and stimulus decoding was similar across areas. However, choice decoding was significantly more accurate and rose earlier in the prearcuate gyrus compared to either thalamic nucleus. In a second experiment, we reversibly inactivated MD or VL thalamus during task performance. Inactivation of either nucleus induced a strong ipsilateral choice bias and a reduction in sensitivity to motion information. These behavioral effects persisted within and across sessions, with no sign of cortical compensation. Our results challenge the notion that the MD and VL thalamus serve merely as relays for cortical computation. Instead, they suggest that these thalamic nuclei are integral components; and potential bottlenecks; of the decision-making circuit, essential for translating accumulated evidence into action.

Disclosures: B. Sieveritz: None. **R. Kiani:** None.

Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

Location: SDCC Rm 23A

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Presentation Number: NANO006.10

Topic: I.03. Decision Making

Support: NIH intramural ZIA-MH002988
NIH K99 EY032102

Title: Computational modeling of decision and metacognitive noise reveals trait-like features of stochastic information processing

Authors: *S. LOPEZ-GUZMAN;
Div. of Intramural Research, Unit on Computat. Decision Neurosci., Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Decision-making behavior is stochastic. Current computational models of decision-making mimic this variability by injecting noise at different putative stages of information processing. These models are useful for predicting choice behavior, interrogating candidate mechanisms of decision-making, and tying behavior to neural activity. However, decision-making noise is often considered a nuisance variable with the focus of attention being given to other signatures of decision-making considered relevant individual differences. Here, we explore whether noise in decision and confidence behavior is a shared feature that reflects individual differences in information processing. Across Studies 1 and 2 (total N = 503) human participants performed a delay discounting task and a risk preference task. In study 3 (N=149) online participants performed the same tasks with a few differences: a) participants rated their confidence after every choice, and b) they performed a perceptual decision-making task where they simultaneously reported the orientation of a visual grading and their confidence. We fit utility models with different specifications for decision noise to choice behavior from studies 1 and 2. For study 3, we developed a novel computational approach to modeling both choice and confidence behavior by combining utility models and the CASANDRE model of confidence, deriving measures of decision noise and metacognitive noise (meta-uncertainty). We find that across all decision models and studies, decision noise (i.e., choice stochasticity or cognitive imprecision parameters) correlated across value tasks (r = 0.47-0.75). In study 3, confidence behavior both across perceptual and value-based decision tasks, was well fit by a computational model that uses decision uncertainty to inform confidence. Moreover, second-order metacognitive noise (meta-uncertainty) was also strongly correlated across tasks even extending beyond the value domain (r = 0.17-0.58). We conclude that decision noise 1) can be modeled as noise at different stages of the decision, 2) is conserved across different economic choice tasks, and 3) is useful information for higher-order cognitive processes like metacognition. Similar to decision noise, noise at the metacognitive stage is conserved across different types of decision-making tasks. These results point to common computational mechanisms behind variability in choice and confidence behavior as “trait-like”. Future endeavors relate these types of noise to other individual differences or psychopathology, and attempt to employ these computational models to tie neural variability to behavioral variability.

Disclosures: S. Lopez-Guzman: None.

Nanosymposium

NANO006: Computational Modeling of Decision and Behavioral Processes

Location: SDCC Rm 23A

Time: Saturday, November 15, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO006.11

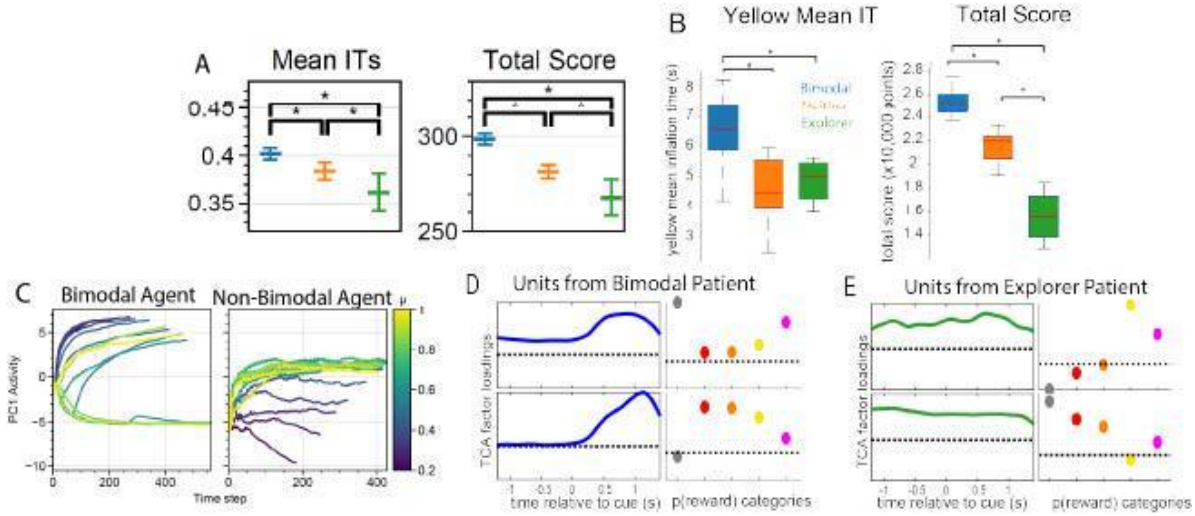
Topic: I.03. Decision Making

Support: NIMH R01MH128187

Title: Analogous neural representations underlying risky decision-making in deep reinforcement learning agents and humans

Authors: *A. PRICE[1], A. LIU[2], R. COWAN[1], T. DAVIS[1], N. SHAHDOUST[3], S. RAHIMPOUR[1], B. SHOFTY[1], J. D. ROLSTON[4], E. H. SMITH[1], A. BORISYUK[2];
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Abstract: The Balloon Analog Risk Task (BART) is a wide-spread decision-making task that models risk taking and impulsive behavior. We trained deep reinforcement learning agents on BART to gain insight into the diversity of neural and behavioral representations that underlie BART behavior. We leveraged these insights to better understand how human BART participants may be understanding the task and how internal population representations underlie performance of both artificial and human agents. BART participants were instructed to maximize the points earned throughout the task session. Points were rewarded based on the size of the balloon when inflation was terminated and no points were received, nor taken away, if a balloon popped. Actor-Critic agents were trained on a variation of BART using a standard proximal policy optimization procedure. The activity of the recurrent layer nodes was clustered using k-means (k=6), and then the agents were clustered (k=3) based on their proportion of node-types. The three agent classes resulted in having unique behavioral strategies and were classified as Bimodal, Neither or Explorer. Single neuron activity was recorded from patients undergoing neuromonitoring for epilepsy. Tensor component analysis was used to gain insights into the representations of the pseudopopulation of human units. The classifications identified from the agents fit the human behavioral data well. Both Bimodal agents and humans scored significantly better on their respective versions of BART and had significantly longer inflation times (Fig 1A (agent) and 1B (human)). Units from patients classified as Bimodal also exhibited better reward uncertainty (risk) representations than their Explorer counterparts, similar to the curvilinear representations of the bimodal artificial agents (Fig 1C, 1D and 1E) which led to better peformance. Here, we gained insight into the neural underpinnings of risky decisions using actor-critic networks, finding that in both humans and agents, more nonlinear encoding of reward probabilities resulted in improved decision making under risk.



Disclosures: A. Price: None. **A. Liu:** A. Employment/Salary (full or part-time):; Meta. **R. Cowan:** None. **T. Davis:** None. **N. Shahdoust:** None. **S. Rahimpour:** None. **B. Shofty:** None. **J.D. Rolston:** None. **E.H. Smith:** None. **A. Borisyuk:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH U24MH130968
NIH R01MH133181
NIH R01MH113005
CIHR PJT-180565
Schmidt Science Fellows

Title: Decoding developmental time in the brain: transcriptomic signatures across diverse contexts

Authors: *S. VENKATESAN[1], J. WERNER[2], Y. LI[3], J. GILLIS[1];
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Abstract: Single-cell transcriptomics has revolutionized our understanding of cell type identities in the developing brain. However, predicting a cell type's developmental state from its transcriptome remains a challenge. Our meta-analysis of human brain datasets comprising over 2.8 million cells reveals both tissue-level and cell-autonomous predictors of developmental age. We show that global tissue cell type composition predicts age within individual studies, but fails to generalize across studies, whereas specific cell type proportions reliably track developmental time across datasets. Furthermore, we use regularized regression models to identify transcriptomic signatures of cell-autonomous maturation in the developing brain. A cell type-agnostic model using 462 genes achieves the highest accuracy (error = 2.6 weeks), robustly capturing developmental dynamics across diverse cell types and datasets. This model generalizes to human neural organoids, accurately predicting normal developmental trajectories (R = 0.91) and disease-induced shifts in vitro. Furthermore, it extends to the developing mouse brain, revealing an accelerated developmental tempo relative to humans. Our work provides a unified framework for comparing neurodevelopment across contexts, model systems, and species. Future work will evaluate the generalization of transcriptomic signatures predictive of developmental time across diverse species. These results will provide insights into the evolutionary conservation and reprogramming of gene regulatory programs determining neurodevelopmental tempo.

Disclosures: S. Venkatesan: None. **J. Werner:** None. **Y. Li:** None. **J. Gillis:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

Location: SDCC Rm 11

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Presentation Number: NANO007.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: SickKids Restracomp Program
Ontario Graduate Scholarship
NSERC Canadian Graduate Scholarship - Doctoral
David Stephen Cant Graduate Scholarship in Stem Cell Research - University of Toronto

Title: Comparative genomics of primate pluripotent stem cell-derived neural cultures: insights into human brain expansion

Authors: *W. CHOI[1,3], H. HOU[1], M. AHMED[1], C. CHAN[1], A. AGGARWAL[1,3], A. TIAN[1], N. DHALIWAL[1], J. A. MUFFAT[2,3], M. WILSON[1,3], Y. LI[1,3];
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Abstract: The massive expansion and complex folding pattern of the human brain, particularly the cerebral cortex, are thought to be the foundation of our unique intellectual abilities. Compared to other primates such as macaque, the human cortex contains approximately 10-fold more neurons. This is due to a larger pool of neural stem and progenitor cells (collectively known as neural precursors or NPs) in the developing human cortex, where these NPs differentiate into the neurons and glia that make up the postnatal brain. However, the cellular and molecular processes that govern human NP expansion remain largely undefined. Here, we compared the differentiation and proliferation of human and macaque pluripotent stem cell-derived NPs in high growth factor (GF) maintenance medium and low GF differentiation medium to investigate mechanisms underlying human cortical expansion. We determined the relative proportions of NPs and neurons by immunostaining for SOX2 and DCX, respectively, and assessed NP proliferation with EdU assay. In low GF conditions, human NPs maintain their proliferative capacity longer, whereas macaque NPs more readily differentiate into neurons and reduce their proliferation. Bulk RNA-Seq analysis revealed that early transcriptomic responses to GF reduction involve upregulation of neuronal genes in both species, with a stronger response observed in macaque. We also found that E2F targets were enriched among genes upregulated under low GF conditions in human but not in macaque, pointing to a potential mechanism underlying species-specific differences in NP proliferative capacity. Pharmacological inhibition of E2F activity increased neuronal differentiation and reduced proliferation in human NPs. Altogether, our work suggests that under low GF conditions, human NPs have enhanced proliferative potential due to increased E2F signaling, which may contribute to human cortical expansion.

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Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

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Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS118580
TSC Alliance
Team Mia Research Fund

Title: Exploring aberrant fate decisions during forebrain development in tuberous sclerosis complex

Authors: *N. A. ELSAYED[1], E. HARMON[2], J. M. IRISH[2], R. A. IHRIE[2], K. C. ESS[2]; [1]Vanderbilt Univ., Nashville, TN; [2]Pediatrics - Neurol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Tuberous Sclerosis Complex (TSC) is a genetic neurodevelopmental disorder caused by mutations in the *TSC1/TSC2* genes, with upregulation of mammalian target of rapamycin (mTOR) signaling causing unregulated cell growth and abnormal differentiation. Within the brain, dysplastic cortical lesions called tubers emerge during fetal development. Although tuber growth appears to stop postnatally, repercussions do not. Patients suffer debilitating neurological sequelae, including epilepsy, autism, and neuropsychiatric disorders. The molecular mechanisms underlying tuber development remain unclear, and existing human cell models propose competing hypotheses for tuber cells of origin. These hypotheses are based primarily on transcriptomic data, highlighting a need for further mapping of these models and comparison to patient tissue at the translational and post-translational level. In this study, we sought to understand changes in cellular identity during dorsal- or ventral-directed neural development of *TSC2* mutant cells and their association with cellular phenotypes found in tubers. Three independent isogenic sets of patient-derived induced pluripotent stem cells (iPSCs) were generated. Each isogenic set was composed of three lines with heterozygous, homozygous, and corrected mutations in the *TSC2* gene. iPSCs were differentiated into forebrain organoids and collected at defined intervals for Cytometry by Time-of-Flight (CyTOF) analysis. Our custom built 40-antibody CyTOF panel encompassed both markers of neural lineage and phosphoproteins downstream of the mTOR, EGFR, and MAPK signaling pathways. Across isogenic lines, $TSC2^{-/-}$ neural progenitor cells presented a distinct and persistent protein phenotype as early as ten days following neural induction, including elevated SOX2 and YAP1 expression coinciding with p-S6 S240/244 and p-4E-BP1 phosphorylation events. CyTOF analysis of patient tubers showed similar abnormal cell signatures. Furthermore, immunostaining of early dorsal $TSC2^{-/-}$ organoids revealed unexpected expression of markers typically associated with ventral forebrain specification. These results indicate a broader level of identity dysregulation than previously reported. Functional neuronal data using multi-electrode arrays revealed elevated firing rates in both $TSC2^{+/-}$ and $TSC2^{-/-}$ mutant neurons. Collectively, these data suggest that cell fate and function are altered at very early neurodevelopmental stages in TSC, with consequences likely persisting into postnatal life.

Disclosures: N.A. Elsayed: None. **E. Harmon:** None. **J.M. Irish:** None. **R.A. Ihrie:** None. **K.C. Ess:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

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Presentation Number: NANO007.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R24 MH114788

Title: A single-cell transcriptomic atlas for the diversity of immune cells in the developing human brain

Authors: *S. MALAIYA[1], B. HERB[2], J. WERNER[3], S. VENKATESAN[4], P. NANO[6], A. BHADURI[7], C. COLANTUONI[8], J. GILLIS[5], S. A. AMENT[9];
[1]Sch. of Med., [2]IGS, Univ. of Maryland Baltimore, Baltimore, MD; [3]Physiol. Dept., [4]Dept. of Physiol., [5]Univ. of Toronto, Toronto, ON, Canada; [6]UCLA, Los Angeles, CA; [7]Univ. of California Los Angeles, Los Angeles, CA; [8]Johns Hopkins Med. Institutions, Baltimore, MD; [9]Dept. of Psychiatry and Inst. for Genome Sci., Univ. of Maryland Baltimore Sch. of Med., Baltimore, MD

Abstract: Immune cells play essential yet poorly understood roles in brain development, with the earliest microglia appearing in human neural tissue by 5.5 weeks of gestation. Single-cell RNA sequencing is a powerful tool to characterize the cellular diversity of the developing human brain, yet prior studies were underpowered to characterize rare immune populations. To overcome this barrier, we uniformly processed and integrated eight scRNA-seq datasets from the human neocortex, spanning all three trimesters of its prenatal development, as well as postnatal ages from infancy to adulthood. Cell annotation with canonical cell type markers identified over 60,000 immune cells. Clustering of these cells revealed the diversity of microglia and peripheral immune cells present at each stage of development, including rare cell states present only transiently during development. Pseudotermporal trajectories predicted dynamics related both to the maturation and reactive states of these immune cells. Collectively, these analyses reveal the diversity of immune cells in the developing human brain in unprecedented detail.

Disclosures: S. Malaiya: None. **B. Herb:** None. **J. Werner:** None. **S. Venkatesan:** None. **P. Nano:** None. **A. Bhaduri:** None. **C. Colantuoni:** None. **J. Gillis:** None. **S.A. Ament:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

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Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH U01MH130962

Title: Clonally resolved atlas of the newborn mouse forebrain

Authors: *G. YUAN[1], M. KUNST[4], M. STEYERT[2], M. KEEFE[2], R. MATHIEU[4], C. VAN VELTHOVEN[4], A. ALVAREZ-BUYLLA[3], H. ZENG[4], T. J. NOWAKOWSKI[1];
[1]Neurolog. Surgery, [3]Eli and Edythe Broad Ctr. for Regeneration Med. and Stem Cell Res., [2]Univ. of California San Francisco, San Francisco, CA; [4]Allen Inst. for Brain Sci., Seattle, WA

Abstract: How hundreds of cell types of the mammalian brain emerge from a limited set of progenitor cells during development remains largely unknown. Using lentiviral barcoding, we uncover a comprehensive map of developmental cell lineage relationships underlying the cell diversity of the newborn mammalian brain, focusing on cortex, thalamus, hippocampus, striatum, and olfactory bulb. Our study reveals a new principle of mammalian development: glutamatergic neurons and astrocytes are “born together and stay together”, as both cell classes are closely lineage-related and show limited dispersion across brain regions; by contrast, GABAergic neurons and OPCs represent cell classes “born together and rear apart”, with majority of clonally related cells found across multiple brain regions. Finally, by extending our unbiased cell lineage analysis to the mouse model of Autism Spectrum Disorders, we reveal altered differentiation trajectories in the striatum of 16p11.2 microdeletion. Together, our findings provide a comprehensive atlas of clonal cell lineage relationships across a newborn mammalian brain in both normal and neurodevelopmental disorder contexts, offering a foundational resource for understanding mammalian brain development.

Disclosures: G. Yuan: None. **M. Kunst:** None. **M. Steyert:** None. **M. Keefe:** None. **R. Mathieu:** None. **C. van Velthoven:** None. **A. Alvarez-Buylla:** None. **H. Zeng:** None. **T.J. Nowakowski:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

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Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.06

Topic: A.01. Neurogenesis and Gliogenesis

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Alfred P. Sloan Foundation
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Klingenstein-Simons Fellowship from the Esther A. & Joseph

Klingenstein Fund
Simons Foundation
NIH BRAIN Initiative Cell Atlas Network (UM1MH130991)

Title: Maps to Mechanisms: Interrogating the Patterning of Human Cortical Areas

Authors: *P. R. NANO[1], D. JAKLIC[2], J. SOTO[2], J. MIL[2], A. BHADURI[1];
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Abstract: The cerebral cortex derives its power from specialized areas, and area-specific phenotypes characterize virtually all neurodevelopmental disorders. These include molecular shifts in the prefrontal cortex (PFC) and the posteriorly located visual cortex (V1). Importantly, the transcriptomic distinctions between the PFC, V1 and other areas are dampened in neurodevelopmental disorders, suggesting a link between the patterning of cortical areas and disease etiology. Translating these findings into therapeutic interventions requires addressing precisely when and how areal patterning typically occurs. Through four pooled, CRISPR activation screens in human primary cortical tissues (HPCT), we have evaluated the ability of PFC-enriched transcription factors (TFs) to pattern the PFC. Our screens identify novel roles for the TFs SOX4 and YBX1 in the activation of PFC fate. These results are recapitulated in parallel organoid screens, enabling the mechanistic interrogation of the necessity of SOX4 and YBX1 to PFC patterning and their ability to compete with established V1 determinants such as NR2F1. By revealing novel mechanisms of PFC fate specification, our work demonstrates the ability of our functional genomics platform to interrogate the ability of intrinsic regulators to dictate areal fates in development and disease.

Disclosures: P.R. Nano: None. **D. Jaklic:** None. **J. Soto:** None. **J. Mil:** None. **A. Bhaduri:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

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Presentation Number: NANO007.07

Topic: A.01. Neurogenesis and Gliogenesis

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NIH grant R01HD082131 to C.D.
NIH grant R01NS112399 to C.D.
NOMIS Foundation Award to C.D.

Title: A coming-of-age story: neuronal control of behavior in early life

Authors: *H. KAPLAN[1], B. L. LOGEMAN[1], T. YAWITZ[4], C. SANTIAGO[2], M. TALAY[3], C. SEO[1], D. D. GINTY[5], B. REN[6], C. G. DULAC[1];
[1]Harvard Univ., Cambridge, MA; [2]Harvard Univ., Boston, MA; [3]MCB, Harvard Univ., Cambridge, MA; [4]Columbia Univ., New York, NY; [5]Dept Neurosci, Harvard Med. Sch., Boston, MA; [6]UC San Diego, La Jolla, CA

Abstract: How does animal behavior emerge developmentally? This question, and particularly the underlying contributions of genetic versus environmental information, has been fiercely debated by generations of scientists, leading Nikolaas Tinbergen to codify developmental issues as one of his Four Questions in the study of animal behavior. Despite this historical spotlight, modern studies of neuronal circuit development have largely focused on sensory and cortical systems, or prenatal stages. To gain insight into the development of behavioral control, we focused on the preoptic area (POA) of the hypothalamus, a brain region that governs both social behaviors, such as mating or parenting, and homeostatic control functions, such as sleep or thermoregulation. Recent work has identified molecularly defined neuronal types in the POA that appear dedicated to specific social or homeostatic functions. However, this work has been carried out exclusively in adults; how these cell types emerge developmentally remains unknown. We therefore molecularly profiled POA cell types in mice using single-nucleus RNA-sequencing and paired ATAC-sequencing at eight ages from late embryo to adult, encompassing key developmental transitions such as birth, weaning, and puberty (Kaplan et al., Nature 2025). We identified key stages of POA development, including the perinatal emergence of sex differences, postnatal maturation and refinement of signaling networks, and nonlinear transcriptional changes accelerating at the time of weaning and puberty. We found that a cell type's functional role partly predicted its maturational timing: cell types involved in social behavior tended to mature later than those involved in homeostatic control. To examine the functional implications of these molecular trajectories, we are now performing calcium imaging recordings in mouse pups as young as 10-days-old, in response to social cues, and during changes in sleep state. Having charted the development of POA cell types in wild-type animals, we next asked how POA development is affected by environmental inputs. To do so, we performed snRNA-seq in six mouse lines, each impaired in a distinct sensory modality crucial for POA function. This uncovered a major role for pheromone input in the timing of POA cell type maturation, while other sensory inputs have little to no effect. Altogether, our work paints a picture of POA development as surprisingly sensitive to extrinsic factors, such as pheromone input or sex hormones, and lays the foundation for future work addressing the origin of instinctive behaviors and their control at various life stages.

Disclosures: H. Kaplan: None. **B.L. Logeman:** None. **T. Yawitz:** None. **C. Santiago:** None. **M. Talay:** None. **C. Seo:** None. **D.D. Ginty:** None. **B. Ren:** None. **C.G. Dulac:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

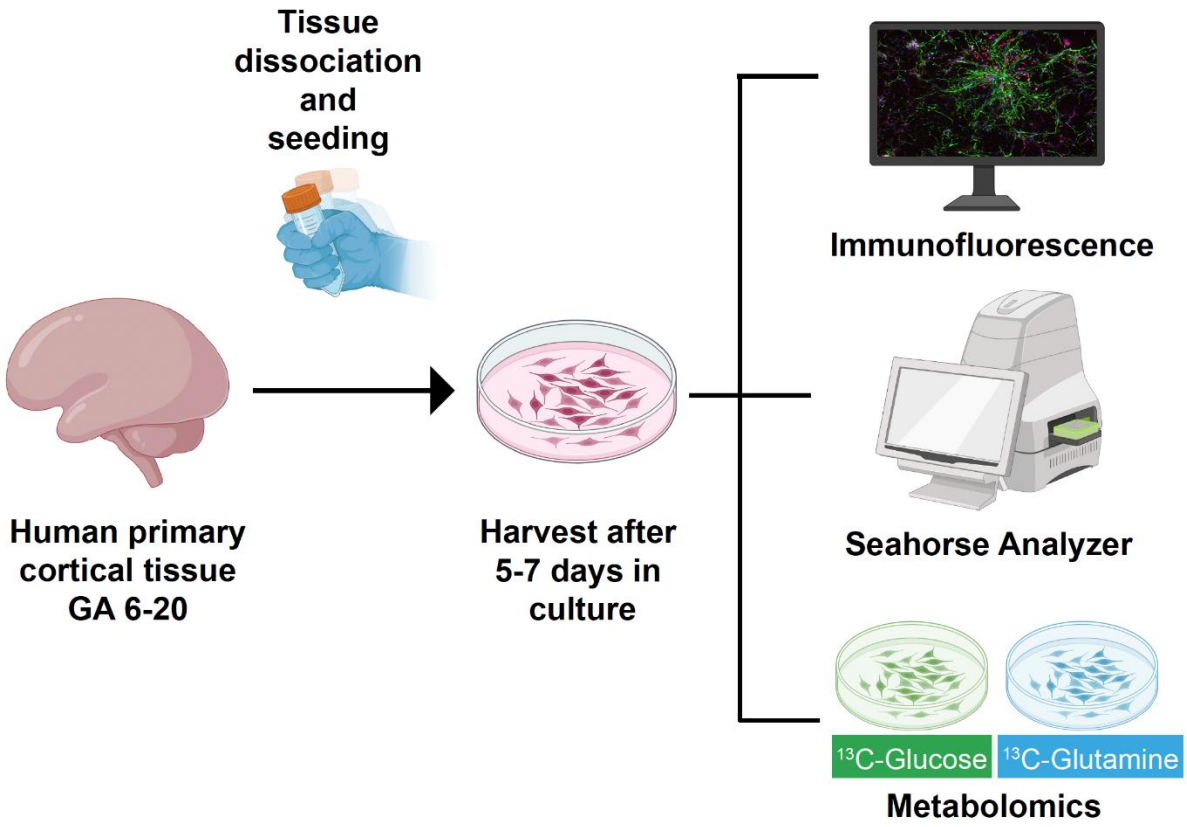
Presentation Number: NANO007.08

Topic: A.01. Neurogenesis and Gliogenesis

Title: Metabolic Atlas of Early Human Cortical Development Identifies Cell Fate Regulators

Authors: *J. A. SOTO, J. MIL, P. R. NANO, R. KAN, C. NGUYEN, H. R. CHRISTOFK, A. BHADURI;
Biol. Chem., UCLA, Los Angeles, CA

Abstract: The brain undergoes rapid and dynamic changes during early cortical development, a period marked by intense cellular proliferation, differentiation, and synaptic plasticity. Recent research has begun to highlight the crucial role of cellular metabolism in regulating these processes, underscoring the importance of metabolic signaling for proper cortical formation. Yet, we lack a fundamental understanding of the metabolic activity enabling such development processes. To fill this gap, we generated a comprehensive metabolic atlas analyzing the bulk metabolic transitions that occur across multiple time points of cortical development across human and cortical organoid samples. Seahorse assays highlighted dynamic changes in metabolic properties across 5 different timepoints in our data set prompting further investigation. Metabolite levels and pathway activity were quantified by performing LC-MS/MS with U-C^{13} labelled glucose and glutamine. A notable trend from our atlas shows that glycolytic and pentose phosphate pathway (PPP) abundance increased in both systems. To further probe the role of glycolysis and the PPP in radial glia specification, we manipulated glucose availability and PPP activity in cortical organoids using both nutrient restriction and pharmacological inhibitors. These interventions resulted in reproducible changes in cell fate output, including a relative increase in astrocytes, inhibitory neurons, and outer radial glia. To confirm that these effects were due to altered radial glia fate specification rather than secondary effects, we performed genetic knockdown of PPP enzymes in a radial glia-enriched population and observed consistent phenotypic outcomes. These findings support a model in which metabolic pathways such as glycolysis and the PPP play a regulatory role in neural stem cell fate decisions. Understanding the link between metabolism and early cortical development could offer new insights into long-term neurodevelopmental health, where disrupted metabolic pathways are known to contribute to developmental disorders.



Disclosures: J.A. Soto: None. **J. Mil:** None. **P.R. Nano:** None. **R. Kan:** None. **C. Nguyen:** None. **H.R. Christofk:** None. **A. Bhaduri:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.09

Topic: A.01. Neurogenesis and Gliogenesis

Support: RO1NS131223

Title: Neurodevelopmental, physiological, and genomic defects in models of Nprl2-associated focal cortical dysplasia and the potential for rapamycin pharmacotherapy

Authors: *A. BISWAS[1], S. BRUCKMEIER[2], E. PATTIE[2], A. AUBER[6], C. COLANTUONI[7], B. N. MATHUR[3], S. A. AMENT[4], P. H. IFFLAND[5];
[2]Anat. and Neurobio., [3]Pharmacol., [4]Dept. of Psychiatry and Inst. for Genome Sci., [5]Neurol., [1]Univ. of Maryland SOM, Baltimore, MD; [6]St. Mary's Col. of Maryland, Westminster, MD; [7]Johns Hopkins Med. Institutions, Baltimore, MD

Abstract: Somatic mutations in *NPRL2* cause focal cortical dysplasia type 2 (FCD2), a neurodevelopmental disorder marked by cortical dyslamination and epilepsy. We modeled FCD2 in mice via CRISPR/Cas9 knockout (KO) of *Nprl2* specifically in the progenitors of superficial (Layer 2/3) cortical excitatory neurons. *Nprl2* KO induced hallmarks of FCD2, including mTOR hyperactivation, cortical dyslamination, and reduced seizure threshold. Electrophysiological recordings revealed increased capacitance and reduced excitability in KO neurons. Surprisingly, restoring mTOR activity with rapamycin rescued only a subset of functional defects. To investigate molecular mechanisms, we applied Curio Seeker (Slide-seq) spatial transcriptomics to produce cortical atlases of neonatal mice in three groups: *Nprl2* KO, *Nprl2* KO with rapamycin, and controls. *Nprl2* KO was associated with 201 cortical layer-specific gene expression changes (FDR < 0.05), including layer-specific dysregulation of neurodevelopmental transcription factors and synaptic components, as well as broader activation of neuroinflammatory signatures. Laminar identity in layer 2/3 was preserved across groups, but layer 5 markers were ectopically expressed in superficial layers in *Nprl2* KO animals. Comparisons to expression profiles of mature cortical neurons revealed developmental delays in establishing mature layer-specific expression profiles. Although many transcriptional changes were attenuated by rapamycin, transcriptional differences persisted in edited Layer 2/3 neurons. Moreover, rapamycin induced aberrant gene expression patterns throughout the cortex. These findings identify *Nprl2* as a regulator of cortical circuit assembly and transcriptional identity, and demonstrate that mTOR inhibition only partially restores function, highlighting targets for novel therapeutic intervention in mTOR-related cortical malformations.

Disclosures: A. Biswas: None. **S. Bruckmeier:** None. **E. Pattie:** None. **A. Auber:** None. **C. Colantuoni:** None. **B.N. Mathur:** None. **S.A. Ament:** None. **P.H. Iffland:** None.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grants 1R01 NS118067-01A1
JPB foundation
BlueRock Therapeutics
Marie-Josée Kravis Women in Science Endeavor

Title: Mapping hPSC-derived dopaminergic models to the human brain across development and aging: A multi-model single-cell atlas

Authors: *V. D. BOCCHI[1], K. TO[2], P. STORM[3], J. PETT[2], P. ZUMBO[4], C. XU[2], T. KIM[5], S. KOO[6], L. WEBER[1], D. YANG[1], J. PIAO[7], R. A. BARKER[9], V. TABAR[8], D. BETEL[10], M. PARMAR[11], S. A. TEICHMANN[12], L. STUDER[13];
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Kingdom; [3]Wallenberg Neurosci Ctr., Lund Univ., Lund, Sweden; [4]Weill Med. College, Cornell Univ., New York, NY; [5]DGIST, Daegu, Korea, Republic of; [6]Yonsei Univ., Seoul, Korea, Republic of; [8]Neurosurg., [7]Mem. Sloan Kettering Cancer Ctr., New York, NY; [9]Cambridge Ctr. Brain Repair, Cambridge, United Kingdom; [10]Inst. for Computat. Biomedicine,Weill Cornell Med. Col., New York, NY; [11]Developmental and Regenerative Neurobio., Wallenberg Neurosci Ctr., Lund University, Sweden; [12]Cambridge Stem Cell Inst., Cambridge, United Kingdom; [13]Ctr. for Stem Cell Biol., Sloan Kettering, New York, NY

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder marked by the selective loss of midbrain dopamine (mDA) neurons in the substantia nigra. Human pluripotent stem cells (hPSCs) offer a promising platform for modeling PD and developing regenerative therapies. While numerous protocols have been established to generate mDA neurons, systematic benchmarking remains difficult due to inconsistent nomenclature, variable cell composition, and the lack of a unified reference map for unbiased classification.

To address this challenge, we generated the first single-cell atlas of the human brainstem across the first and second trimesters, enabling accurate identification of mDA neurons and off-targets produced in vitro. We also constructed a consensus map of adult and aging mDA subtypes to assess subtypes and maturation fidelity. This high-resolution reference harmonizes annotations across studies and defines cell-type-specific gene signatures, regulatory networks, and signalling pathways.

Using this in vivo reference, we trained machine learning models to classify single-cell data from over 20 mDA differentiation protocols—including monolayer cultures, tricultures with astrocytes and microglia, organoids, assembloids, and grafts to create a multi-model atlas of hPSC-derived mDA neurons. This analysis revealed substantial variation in cell-type composition and mDA authenticity across differentiation protocols. We observed the presence of off-target populations originating from the dorsal and ventral midbrain, diencephalon, and hindbrain together with non-neuronal cells like astrocytes and fibroblasts. Notably, we identified hybrid mDA-like states co-expressing markers of multiple lineages, including pSNpr neurons and noradrenergic neurons, suggesting incomplete in vitro specification. These hybrid states were largely absent in grafted cells, indicating a shutdown of aberrant programs post-transplantation. Finally, we identified stress- and maturation-linked gene modules, showing that cellular stress impairs mDA maturation and is mitigated in tricultures, assembloids, and grafts that show better specification and maturation. Altogether, this integrated atlas provides a foundational resource for benchmarking mDA differentiation and guiding the development of authentic stem cell-derived models for PD research.

Disclosures: V.D. Bocchi: None. **K. to:** None. **P. Storm:** None. **J. Pett:** None. **P. Zumbo:** None. **C. Xu:** None. **T. Kim:** None. **S. Koo:** None. **L. Weber:** None. **D. Yang:** None. **J. Piao:** None. **R.A. Barker:** None. **V. Tabar:** Other; Co-founder, scientific advisor, and have received research support from BlueRock Therapeutics. **D. Betel:** None. **M. Parmar:** Other; Owner of Parmar Cells AB and coinventor of the following patents: WO2016162747A2, WO2018206798A1, and WO2019016113A1. M.P. performs paid consultancy and commissioned research for Novo Nordisk AS Cell. **S.A. Teichmann:** Other; scientific advisory board member of ForeSite Labs, OMass Therapeutics, Qiagen, Xaira Therapeutics, a co-founder and equity holder of TransitionBio and Ensocell Therapeutics, Non-executive director of 10x Genomics, and a part-time employee of GlaxoSmithKline. **L. Studer:** Other; Co-founder, scientific advisor,

and have received research support from BlueRock Therapeutics, Co-founder of DaCapo Brain Science.

Nanosymposium

NANO007: From Neural Stem Cells to Cell Fate

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO007.11

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01MH134799-02

Title: Multiomic profiling of the developing mouse spinal cord

Authors: *C. M. RIVERA[1], D. T. HOPKINS[1], H. POLDSAM[2], J. E. JOHNSON[3], A.-H. POOL[4], H. C. LAI[5];
[1]Neurosci., [2]UT Southwestern, Dallas, TX; [3]Neurosci., UT Southwestern Med. Ctr., Dallas, TX; [4]Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [5]UT Southwestern Med., Dallas, TX

Abstract: During embryogenesis, unique transcription factors define 11 discrete progenitor domains that control the differentiation of defined cell types in the dorsal spinal cord. However, the relationship between developing progenitor domains and molecular features in the adult spinal cord is not clearly defined. Our goal is to construct molecular and spatial atlases to help us understand the process through which embryonic progenitor domains give way to the vast heterogeneity of cell types found in the adult spinal cord. We have selected three representative developmental points to generate these atlases: E11.5 (embryonic), P4 (early postnatal) and P56 (adult). Using the 10x Multiome platform for single nuclei RNA-sequencing (snRNA seq) and snATAC-seq, we have generated gene expression and open chromatin region maps of the spinal cord along the rostral - caudal axis. Our current data reveals that most excitatory neurons in the dorsal horn come from the dI5 lineage. We have started mapping these cell types to their developmental spinal cord lineage using spatial transcriptomics at all three developmental timepoints. By using spinal cord-Cre mouse lines, we will unambiguously trace the lineage of these progenitor domains to generate a molecular-lineage map of the spinal cord. This multi-atlas effort will allow us to gain a better understanding of the developmental and structural organization of the spinal cord.

Disclosures: C.M. Rivera: None. **D.T. Hopkins:** None. **H. Poldsam:** None. **J.E. Johnson:** None. **A. Pool:** None. **H.C. Lai:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.01

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

Support: NIH Grant K01-AG083732

Title: Machine Learning Detects Behavioral Biomarkers of Disease and Prevention in Alzheimer's Models

Authors: *S. R. MILLER[1], J. J. PALOP[2];
[1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]Gladstone Inst. & UCSF, South San Francisco, CA

Abstract: Computer-vision and machine-learning (ML) approaches are being developed to provide scalable, unbiased, and sensitive methods to assess mouse behavior. Here, we used the ML-based variational animal motion embedding (VAME) segmentation platform to assess spontaneous behavior in humanized App knock-in and transgenic APP models of Alzheimer's disease (AD) and to test the role of AD-related neuroinflammation in these behavioral manifestations. We found marked alterations in spontaneous behavior in $App^{NL\text{-}G\text{-}F}$ and 5xFAD mice, including age-dependent changes in motif utilization, disorganized behavioral sequences, increased transitions, and randomness. Notably, blocking fibrinogen-microglia interactions in 5xFAD-$Fgg^{\gamma 390\text{-}396A}$ mice largely prevented spontaneous behavioral alterations, indicating a key role for neuroinflammation. Thus, AD-related spontaneous behavioral alterations are prominent in knock-in and transgenic models and sensitive to therapeutic interventions. VAME outcomes had higher specificity and sensitivity than conventional behavioral outcomes. We conclude that spontaneous behavior effectively captures age- and sex-dependent disease manifestations and treatment efficacy in AD models.

Disclosures: S.R. Miller: None. **J.J. Palop:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.02

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

Title: Eeg-based predictive modeling of cognitive impairment and dementia pathology using advanced signal processing and machine learning

Authors: *I. MICHAELEVSKI[1], P. BUZAEVA[1], M. BAIRACHNAYA[3], Y. ZUNTZ[2], D. ABOOKASIS[1], A. PINHASOV[1];
[2]Electrical and electronics engineerinh, [1]Ariel Univ., Ariel, Israel; [3]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Early detection of cognitive impairment and neurodegenerative diseases like Alzheimer's (AD) is critical for timely intervention and monitoring. This study introduces a novel EEG-based framework for identifying early cognitive dysfunction using two complementary murine models: socially submissive (Sub) Sabra mice, which exhibit early synaptic and cognitive deficits, and transgenic 5xFAD mice, which display amyloid-driven pathology and progressive decline. We performed longitudinal EEG recordings in freely moving Sub, 5xFAD, and respective control mice (Dominant Sabra and wild-type C57BL/6, respectively) at 3, 6, and 9 months. Electrodes targeted the prefrontal (PFC), posterior parietal (PPC), visual cortex (VC), and hippocampus. **Results:** In Sub mice, we observed: (A) absent theta-driven connectivity in VC and increased Granger causality from right VC to PPC during tasks; (B) reduced theta-gamma phase-amplitude coupling (PAC) between VC and PFC; (C) lower beta/gamma power in PFC and VC. In 5xFAD mice, we found: (A) reduced hippocampal signal energy; (B) consistent phase shifts across 1-100 Hz bands; (C) disrupted PAC in hippocampus and PFC; (D) increased alpha/delta amplitude variability; (E) low-frequency dominance; and (F) decreased cortical excitability and connectivity. **Analysis:** We extracted 200+ EEG features using methods including spectral density, Hilbert spectra, PCA/ICA, Granger causality, coherence, bispectrum, PAC, singular spectrum analysis, and discrete wavelet transforms (Mayer kernel). **Classification:** Machine learning models showed strong performance: Sub vs. Dom mice were classified with 89% accuracy using Katz/Higuchi fractal metrics and k-NN; 5xFAD vs. WT mice reached 96% accuracy using Ensemble Boosted Trees. C**onclusion:** EEG-derived features combined with machine learning enable early detection of electrophysiological markers of cognitive decline. This approach may support preclinical diagnosis and tracking of AD and related disorders.

Disclosures: I. Michaelevski: None. **P. Buzaeva:** None. **M. Bairachnaya:** None. **Y. Zuntz:** None. **D. Abookasis:** None. **A. Pinhasov:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.03

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

Support: NIH RF1 AG051504-5

Title: Blood-based gene expression levels are associated with Alzheimer's disease related phenotypes and preserved in brain transcriptome

Authors: *X. CHEN[1], X. WANG[2], Z. QUICKSALL[3], T. NGUYEN[8], D. REYES[4], V. LOWE[4], R. PETERSEN[5], K. KANTARCI[4], M. ALLEN[9], M. CARRASQUILLO[6], K. NHO[10], A. J. SAYKIN[11], J. S. REDDY[3], N. ERTEKIN-TANER[7];
[2]Dept. of Quantitative Hlth. Sci., [3]Quantitative Hlth. Sci., [1]Mayo Clin., Jacksonville, FL; [4]Dept. of Radiology, [5]Dept. of Neurol., Mayo Clin., Rochester, MN; [6]Dept. of Neurosci., Mayo Clin., St

Johns, FL; [7]Neurosci. and Neurol., Mayo Clin., Jacksonville, FL; [8]Neurosci., Mayo Clin. Florida, Jacksonville, FL; [9]mayo clinic, Neptune Beach, FL; [10]Radiology and Imaging Sci., Indiana Univ. Sch. of Med., Carmel, IN; [11]Radiology and Imaging Sci., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Background: Most current biomarkers of Alzheimer's disease (AD) inform on core AD neuropathologies, namely amyloid, tau and neurodegeneration (ATN). However, molecular disease mechanisms underlying AD are complex and heterogeneous, highlighting the need for cost-effective blood-based biomarkers that reflect brain molecular changes beyond ATN. **Methods:** Using blood transcriptome from the Mayo Clinic Study of Aging (MCSA) and the Alzheimer's Disease Neuroimaging Initiative, we identified genes and consensus co-expression network modules associated with AD or mild cognitive impairment (MCI) diagnosis and cognitive scores using differential gene expression and weighted-gene co-expression network analyses (WGCNA). We confirmed preservation of blood modules in brain transcriptome across seven cohorts. Blood transcriptome of independent antemortem AD/MCI (ANMerge and Emory University) and Parkinson's disease (PD) cohorts (PDBP and PPMI) were used for replication. Available RNAseq-based blood transcriptomes were further deconvoluted to obtain cell-type specific expression. 5XFAD amyloidosis mouse transcriptome was used for in vivo validation. **Results:** Blood transcriptome modules M5 and M8 are upregulated in AD/MCI and associate with worse cognitive scores whereas M1, M10, M13, M16, and M21 are downregulated in AD/MCI and associate with better cognition. Risk-associated M5 and M8 are enriched in myeloid cells (basophils, monocytes, and/or neutrophils) and preserved in brain microglial modules, whereas protective M13 and M21 are enriched in lymphocytes (natural killer or B cells). These four modules are also enriched in immune-related biological pathways. Associations between myeloid cell-enriched modules (MCEMs) with neurodegenerative disease were replicated in the independent AD/MCI and PD cohorts. The majority of deconvoluted transcripts in MCEMs are upregulated in AD/MCI and in the 5XFAD amyloidosis model. In blood modules preserved in brain transcriptome, we prioritized differentially expressed transcripts (including known AD risk genes such as *SPI1*) as centrally-linked peripheral signatures of AD and related disorders (ADRD). **Conclusions:** We identified blood co-expression network modules associated with AD/MCI phenotypes, enriched in peripheral immune cells and preserved in brain transcriptome. We replicated and validated our findings of MCEMs. Peripheral expression signatures identified reveal potential disease mechanism in AD/ADRD. These modules and individual transcripts may serve as potential therapeutic targets and theragnostic biomarkers for AD/ADRD and cognitive decline.

Disclosures: X. Chen: None. **X. Wang:** None. **Z. Quicksall:** None. **T. Nguyen:** None. **K. Kantarci:** None. **M. allen:** None. **K. Nho:** None. **A.J. Saykin:** None. **J.S. Reddy:** None. **N. Ertekin-Taner:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.04

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

Support: HR21C1003 Korea Health Industry Development Institute (KHIDI)
HI22C0724 Korea Health Industry Development Institute (KHIDI)
HR22C1734 Korea Health Industry Development Institute (KHIDI)
RS-2024-00406876 Korea Health Industry Development Institute (KHIDI)
2024-ER0505-01 Korea National Institute of Health (K-NIH)

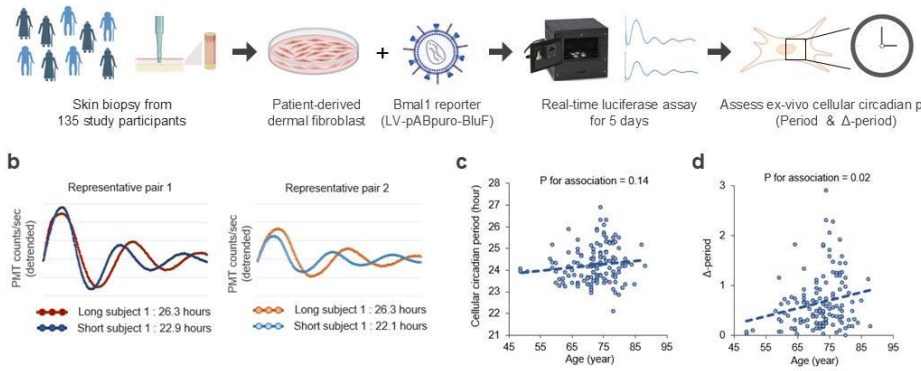
Title: Cellular circadian period and its deviation associate with Alzheimer’s disease pathophysiology and aging-related neurodegeneration in older adults with cognitive impairment

Authors: *H. ROH[1], S. SON[2], E. KIM[3];
[2]Dept. of Psychiatry, [1]Ajou Univ. Sch. of Med., Suwon, Korea, Republic of; [3]Dept. of Brain Sci., Ajou Univ. Sch. of Med., Suwon, Kyunggi-Do, Korea, Republic of

Abstract: Alterations in circadian rhythms are recognized in Alzheimer's disease (AD) and other neurodegenerative disorders, but the significance of ex-vivo cellular circadian periods and Δ-period (deviation from 24 hours) in patient-derived cells remains largely unexplored. Here, we examined ex vivo cellular circadian periods in patient-derived fibroblasts from 135 older adults with cognitive complaints and investigated their associations with biomarkers of AD, neurodegeneration, and cognitive function. A longer cellular circadian period was associated with more advanced tau pathology, greater neural injury and degeneration, and increased inflammation. In contrast, greater Δ-period was not linked to tau pathology but showed even stronger associations with neural injury and degeneration. Grey matter density analysis revealed that a longer circadian cellular period was primarily associated with AD-related regions, while greater Δ-period exhibited broader associations, including areas implicated in aging-related neurodegeneration. Furthermore, the greater Δ-period was associated with multiple cognitive domains, reflecting a stronger link to cognitive impairment. Survival analysis demonstrated that a longer circadian cellular period was associated with a higher risk of clinical progression. These findings suggest that cellular circadian period lengthening reflects AD-related neurodegeneration and disease progression, whereas greater Δ-period is more closely linked to aging-related neurodegenerative processes. The distinct but overlapping associations of these ex-vivo cellular circadian measures highlight their potential as biomarkers for AD and aging-related neurodegeneration and clinical progression.

Graphical abstract for 2025-S-5531-SfN (Hyun Woong Roh, Ajou University School of Medicine, Korea)

Figure 1. Experimental scheme and distribution of cellular circadian period and Δ-period by age



A. Experimental scheme for this study, B. Two representative pair cases for long and short cellular circadian period, C. Association between cellular circadian period and age, D. Association between Δ-period and age.

Figure 2. Associations of cellular circadian period and Δ-period with ATN(IV) criteria AD biomarkers and clinical characteristics

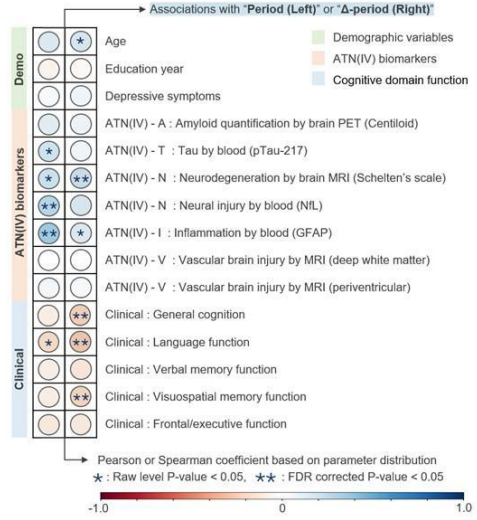
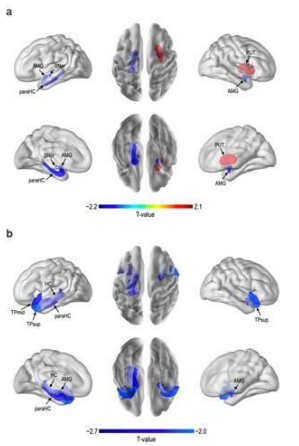


Figure 3. Cellular circadian period and Δ-period negatively associated with AD associated grey matter density after adjusting covariates



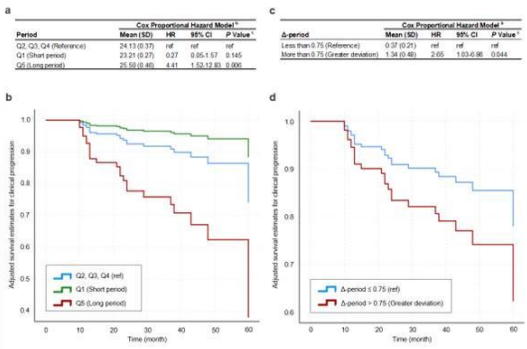
Brain regions associated with Period [b]	P-Value
Amygdala, left	0.027
Amygdala, right	0.040
Putamen, right	0.037
Parahippocampal gyrus, left	0.042
Substantia nigra, pars reticulata, left	0.047

Brain regions associated with Δ-period [b]	P-Value
Amygdala, left	0.009
Amygdala, right	0.020
Parahippocampal gyrus, left	0.014
Superior temporal pole, left	0.012
Superior temporal pole, right	0.033
Hippocampus, left	0.023
Dorsal raphe nucleus	0.032
Middle temporal pole, left	0.050

Table 1. Generalized linear model for association of cellular circadian period and Δ-period with T (tau proteinopathy), N (neurodegeneration), and I (inflammation) biomarkers

	Independent variable : Period (hr)			
	Non-adjusted model		Adjusted model	
Dependent variables [a]	β (SE)	*P* Value	β (SE)	*P* Value [b]
T (tau pathology)				
Plasma pTau-217	0.27 (0.10)	0.008	0.23 (0.09)	0.012
N (neurodegeneration)				
Plasma NfL	0.24 (0.05)	< 0.001	0.22 (0.05)	< 0.001
MRI Schelten's scale	0.10 (0.05)	0.042	0.10 (0.05)	0.033
I (inflammation)				
Plasma GFAP	0.19 (0.05)	< 0.001	0.14 (0.04)	< 0.001
	Independent variable : Δ-period (hr)			
	Non-adjusted model		Adjusted model	
Dependent variables [a]	β (SE)	*P* Value	β (SE)	*P* Value [b]
T (tau pathology)				
Plasma pTau-217	0.27 (0.16)	0.082	0.22 (0.14)	0.125
N (neurodegeneration)				
Plasma NfL	0.34 (0.09)	< 0.001	0.32 (0.09)	< 0.001
MRI Schelten's scale	0.21 (0.08)	0.005	0.21 (0.07)	0.004
I (inflammation)				
Plasma GFAP	0.18 (0.07)	0.014	0.15 (0.06)	0.018

Figure 4. Longitudinal associations of cellular circadian period and Δ-period with clinical progression.



Disclosures: H. Roh: None. **S. Son:** None. **E. Kim:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

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Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.05

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

Support: NIH Grant U01MH110274

Title: Altered Brain Synergistic Core in Patients with Mild Cognitive Impairment and Alzheimer's Disease

Authors: *W. YIN[1], C.-H. CHEN[2,1], W. JIANG[3,1], T. LI[1], W. LIN[1];
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Abstract: Background: Traditional functional connectivity analyses measure statistical dependencies between brain regions but fail to capture hidden features within BOLD signals. Recent advances in information theory decomposition enable separation of neural interactions into synergistic (supporting complex cognition) and redundant (sensorimotor processing) components. We hypothesized that synergetic and redundant communications among brain regions in Alzheimer's disease (AD) and its prodromal stage, mild cognitive impairment (MCI) are altered and disease stage dependent. **Method:** We analyzed a subset of resting-state fMRI data from the ADNI database, acquired using an identical protocol across three groups: MCI (n=142, 55.9-96 years), normal controls (NC, n=152, 57.4-95.4 years), and AD (n=49, 55.3-91.3 years). Preprocessing followed the conventional pipeline, with BOLD signals extracted from 232 parcellated regions. Using integrated information decomposition—a novel framework quantifying information sharing—we decomposed pairwise interactions into synergistic and redundant signals. Whole-brain and network-level metrics were computed as mean synergy/redundant values across all regions or within canonical functional networks. Linear regression was used to compare group-level differences, adjusting for age. **Results:** Quantitative analysis revealed a significant stepwise degradation of whole brain synergy across the continuum from NC, MCI to AD (mean value, NC: 0.7579, MCI:0.7388, AD:0.7117; NC vs MCI: $p=0.033$, MCI vs AD: $p=0.028$, NC vs AD: $p<0.001$). Similar progressive patterns were also observed at the network level in the dorsal attention, executive control, default mode networks (all $p<0.05$). The visual, ventral attention and subcortical networks showed significant decline only at the MCI-AD transition ($p<0.05$). Contrastingly, with the exception of the ventral attention network ($p=0.019$) demonstrating significant redundancy decline during NC-MCI transition, no significant differences were observed at whole-brain and network-level ($p>0.05$). **Conclusion:** Our study demonstrated progressive alteration of brain synergistic interactions across the Alzheimer's disease continuum, offering potential biomarkers for disease staging and new targets for network-based interventions.

Disclosures: W. Yin: None. **C. Chen:** None. **W. Jiang:** None. **T. Li:** None. **W. Lin:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.06

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

Support: T-Neuro Inc. research support

Title: Age-related blood CD8 T cell binding to a non-self peptide/HLA increases Alzheimer's and related MCI biomarker sensitivity and expands assay sensitivity to HLA-A2-negative patients

Authors: *C. J. WHEELER[1], D. VAN DAM[2,5], Y. VERMEIREN[3,4], P. DE DEYN[5,3];
[1]T-Neuro DX, StemVax Therapeutics, Soc for Brain Mapping & Therapeut., Aptos, CA;
[2]Biomed. Sci., [3]Biomed. Sciences, Inst. Born-Bunge, [4]7Faculty of Med. and Hlth. Sciences, Vaccine and Infectious Dis. Inst., Univ. of Antwerp, Antwerp, Belgium; [5]Neurol. and Alzheimer Res. Ctr., Univ. of Groningen, Groningen, Netherlands

Abstract: Background: We recently developed a CD8 T cell-induced mouse model that recapitulates definitive hallmarks of Alzheimer's disease (AD) and allowed prediction of previously unknown properties of human sporadic AD (PMID: 38995966). Identification of a peptide antigen to which the inducing T cells respond in this model (a non-Aβ epitope on Amyloid Precursor Protein [APP]) allowed us to quantify analogous T cells in humans, using an $APP_{471\text{-}479}$/Human Leukocyte Antigen(HLA)-A2 multimer (pHLA) and flow cytometry on blood. Levels of these T cells distinguished AD and related MCI from normal aging controls with high accuracy (AUC: 0.883-0.892), but the APP-based assay is suitable only for HLA-A2-positive (about half of all) patients. **Methods:** Lower level CD8 T cell binding to a non-self ($_{ALIAPVHAV}$/HLA-A2) multimer correlated with APP multimer binding in HLA-A2-positive patients (0.42-0.58x relative to APP/HLA-A2; r = 0.81; $P < 0.001$). Surprisingly, this multimer also bound at similar levels to HLA-A2-negative patients' T cells. We quantified $KLRG1^+$ CD8 T cells in HLA-A2-positive (n = 79) and -negative (n = 44) subjects binding to the non-self multimer by flow cytometry, using Alzheimer's disease (AD), mild cognitively impaired with or without CSF biomarkers consistent with AD (MCI-AD and MCI-other, respectively), and normal aging cohorts. We then assessed correspondence of multimer binding to AD and MCI status. **Results:** As with APP pHLA in HLA-A2-positive patients, levels of T cells binding to the non-self pHLA multimer were significantly diminished in AD and MCI-AD in all patients ($P < 0.015$ by 2-sided T-Test). In receiver-operating characteristic (ROC) analysis of the HLA-negative cohort, area under the curve (AUC) was 0.878 for AD ($P < 0.00001$; 79% sensitivity, 99% specificity), 0.816 for MCI-AD ($P < 0.00001$; 78% sensitivity, 100% specificity), and 0.663 for MCI-other ($P < 0.0001$; 84% sensitivity, 47% specificity). These values are close to or better than the APP-specific CD8 T cells in HLA-A2-positive individuals, with similar discrimination of AD-related from other MCI. Moreover, AD and especially MCI-AD tracking was improved in the HLA-A2-positive cohort relative to APP/HLA-binding (AUC 0.912 for AD; 0.921 for MCI-AD). **Conclusions**: Subtype-independent pHLA multimer binding by $KLRG1^+$ CD8 T cells strongly corresponds to AD and MCI-AD in individuals regardless of HLA-A2 status, providing a working basis for expanding our blood biomarker assay to all patients. Ongoing work will

focus on increasing the detection sensitivity of HLA-independent multimer binding to further increase assay utility.

Disclosures: C.J. Wheeler: A. Employment/Salary (full or part-time):; T-Neuro Inc., NovAccess Global. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); T-Neuro Inc., patents PCT/US2016/049598, WO2017/040594, and PCT/US2019/017879, NovAccess Global. **D. Van Dam:** None. **Y. Vermeiren:** None. **P. De Deyn:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.07

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

Title: Csf immune activation markers in mci and alzheimer's disease

Authors: *A. ALNOBANI[1], A.-C. RAULIN[1], R. PETERSEN[2], M. HECKMAN[1], T. KANEKIYO[1];
[2]Neurol., [1]Mayo Clin., Jacksonville, FL

Abstract: Title: CSF immune activation markers in MCI and Alzheimer's DiseaseAuthors:Alla Alnobani1, Launia White2, Ana-Caroline Raulin1, Chengjie Xiong2, Yan Asmann2, Yingxue Ren2, Ronald C. Petersen3, Michael Heckman2, Takahisa Kanekiyo11 Department of Neuroscience, Mayo Clinic, Jacksonville, FL2 Department of Quantitative Health Sciences, Mayo Clinic, Jacksonville, FL3 Department of Neurology, Mayo Clinic Rochester, MNAlzheimer’s disease (AD) progression is driven by a complex interplay of genetic, demographic, and immune-related factors. We investigated how APOE status, sex, and age affect immune activation markers in CSF from 200 participants with mild cognitive impairment (MCI, N=131) and AD (N=69) from the Mayo Clinic Study of Aging and ADRC. CSF levels of Aβ42, Aβ40, p-tau181, NfL, and GFAP were measured using Simoa Quanterix and 384 inflammatory proteins were quantified through Olink inflammation panel. Linear and Cox regression models assessed associations between APOE, age, sex and other clinical variables such as hypertension, diabetes, smoking, obesity, and dyslipidemia, and CSF biomarker levels. APOE ε4 carriers had significantly lower Aβ42 ($\beta = -0.45$, $p = 5.96e-05$) and higher p-tau181 ($\beta = 0.41$, $p = 0.007$). Age was positively associated with GFAP, NfL, and multiple cytokines including CXCL9, TNFRSF11B, and SCGB1A1 (all $p < 1e-12$). We also found that APOE ε4 associates with higher IL2RB and SIT1 increased ($\beta = 0.33$-0.44, $p < 0.005$) and lower CD22 ($\beta = -0.36$, $p = 0.0014$) in CSF, suggesting APOE ε4-linked immune activation and reduced inhibitory signaling. We also found that higher CSF GFAP level is associated with the risk of CDR conversion in MCI cases (HR = 2.77, $p = 0.0011$). In addition, higher CSF levels of GALNT3, PCDH1, CD48, EPO, and IKBKG were associated with the decreased risk of the CDR conversion (HR = 0.39-0.50, all $p < 0.005$). These results indicate the contributions of immune activations to the

progression of AD and potential CSF immune activation markers to predict the conversion of MCI to AD.

Disclosures: A. Alnobani: None. **A. Raulin:** None. **R. Petersen:** None. **M. Heckman:** None. **T. Kanekiyo:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.08

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

Support: R01-AG082052
R01-666 AG054059
P50 AG033514
R01 AG027161
R01 AG021155
Horizon S10 OD025245

Title: Development of language composites for enhanced sensitivity to multiple plasma biomarkers

Authors: *D. HE[1], R. LANGHOUGH[3,2], C. VAN HULLE[3,2], K. BASCHE[5], E. JONAITIS[4], B. HERMANN[2], H. ZETTERBERG[2], S. C. JOHNSON[6], K. D. MUELLER[7];
[1]Communication Sci. and Disorders, [2]Univ. of Wisconsin Madison, Madison, WI; [3]; [4]Madison, WI, ; [5]Geriatrics, Univ. of Wisconsin-Madison Sch. of Med. and Publ. Hlth., Madison, WI; [6]Med., Univ. Wisconsin, Madison, WI; [7]Communication Sci. & Disorders, Univ. of Wisconsin - Madison, Madison, WI

Abstract: Language deficits are among the earliest detectable signs of Alzheimer`s disease (AD), often preceding cognitive symptoms. Yet, language-specific composites, particularly integrating connected speech measures critical for capturing real-world communication breakdowns, remain unexplored. Bridging this gap is pressing to enhance early AD detection, identify individuals at risk for language-dominant variants, and enable timely personalized interventions. We developed language composites for their sensitivity to multiple AD plasma biomarkers (phosphorylated-tau217 [p-tau217], neurofilament light chain [NfL], glial fibrillary acidic protein [GFAP]). We analyzed longitudinal cohort data from 824 adults (mean age at baseline = 61.94) in the Wisconsin Registry for Alzheimer's Prevention, including cognitively unimpaired-stable (n=661), cognitively unimpaired-declining (n=152), and MCI (n=11) individuals. Individual language indicators were selected from connected speech (Cookie Theft picture description and Logical Memory story recall) and single-word tasks (animal and letter fluency). We constructed three language composites: a *theoretical composite* based on expert knowledge, a *connected speech-specific composite* derived as a subset of the theoretical

composite, and an *empirical composite* optimized for predicting early AD cognitive progression. Each composite was computed as the sum of equally weighted z-scores of the selected measures. Sensitivity was assessed by the simple age slope for increased plasma biomarkers using linear mixed-effects models adjusted for sex and literacy. The empirical language composite exhibited superior and consistent sensitivity (i.e., faster longitudinal decline) with all three biomarkers (p-tau217: β = -0.0336; NfL: β = -0.0251; GFAP: β = -0.0222; all $p < 0.01$) than individual measures. Proper name delayed and immediate recall declined fastest uniquely associated with p-tau217 (delayed: β = -0.0414; immediate: β =-0.0351), even surpassing the composites, while animal fluency selectively declined with increased NfL (β = -0.0251) and GFAP (β = -0.0227) levels. Our findings suggest that an empirical language composite comprising words per minute, filled pauses, animal fluency, and proper name immediate and delayed recall captures holistic cognitive-language decline associated with multiple biomarkers, while individual measures may imply a biomarker-specific profile of language decline. Together, our results support the clinical utility of plasma biomarkers and language-specific composites jointly informing individuals at high risk of future cognitive-communicative decline.

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Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.09

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

Support: NIA R01-AS DC-018561-02S1

Title: Whispers of dementia from the auditory brain in a preclinical model of a novel early biomarker of Alzheimer’s Disease risk

Authors: *A. GUNGOR AYDIN[1], E. B. TORRES[2], M. D. TAMBINI[3], E. YOUSSEF[4], K. M. BIESZCZAD[5];
[1]Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ; [2]Psychology Dept, Rutgers Univ. Dept. of Psychology, Piscataway, NJ; [3]Rutgers New Jersey Med. Sch., Newark, NJ; [4]Psychology, [5]Psychology, Behavioral and Systems Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: Hearing loss in mid-life has been identified as the largest modifiable risk factor for Alzheimer’s Disease (AD) (Livingston et al., 2024), yet the mechanistic link between auditory function and dementia remains unclear. If a common biological mechanism of neurodegeneration underlies both, then a very early biomarker of AD risk may be tractable in an auditory neural signal. Sound-evoked auditory brain signals may thus serve as a window into early biobehavioral changes that precede AD progression. Thus, we determined if an auditory neural signature of genetic AD risk could be detected in animal models of AD as a candidate prodromal diagnostic

for early intervention strategies using the non-invasive, rapidly acquired auditory brainstem response (ABR). ABR morphology has long been proposed as a marker for AD (Tarawneh et al., 2021), its clinical utility has been limited by insufficient sensitivity and specificity. We applied a novel multidimensional parametric feature extraction method, developed for neurodevelopmental disorders (Torres et al., 2013, 2023), to 100s of single-trial ABRs in genetically-modified rats to show we can accurately predict AD genetic risk, stratify AD by root cause, and predict outcomes of novel interventions. AD models used were CRISPR/Cas9 genetically modified knock-in rats (N=15; male & female) that harbor familial early-onset AD risk mutations to amyloid precursor protein ($App^{Swedish}$) and/or Psen1 ($Psen1^{LF}$) vs. wildtype humanized genes (Tambini et al., 2019; Tambini & D'Adamio, 2020). This powerful knock-in AD model makes no a priori assumption about the pathogenic mechanisms nor the behavioral consequences thereof; only the unbiased genetic one, and at the APP locus, whose proteolysis has a central role in *both* familial and sporadic AD pathogenesis. We found that normal-hearing App^{Swe} (Swedish familial AD risk variant) rats separate clearly from controls and from $Psen1^{LF}$ rats in multidimensional parameter space, with increasing separation in older age and is sex-dependent. Notably, auditory training normalizes the ABR of App^{Swe} rats into overlapping parameter space as trained controls, which supports a central vs. peripheral (e.g., cochleopathic) source of dysfunction. These preclinical data are the first ever to show how the auditory system provides a biomarker for early-life detection of AD and lays the groundwork to test the synergy of auditory and cognitive functions in human dementia.

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Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

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Presentation Number: NANO008.10

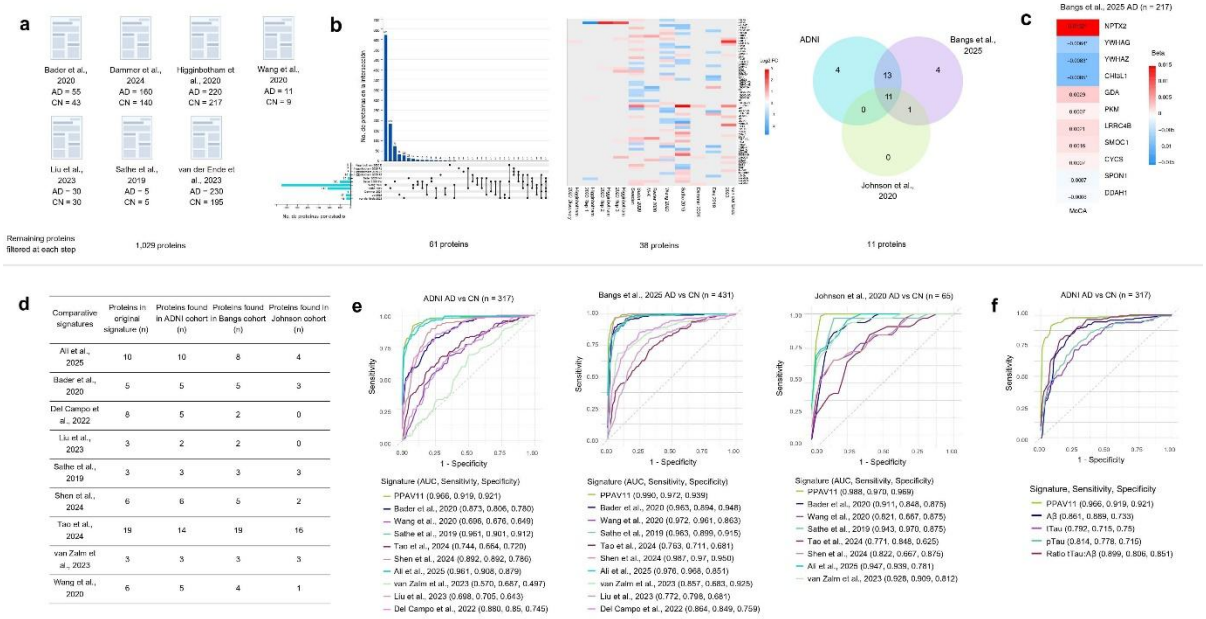
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Comparative study of proteomic biomarker signatures for Alzheimer's Disease diagnosis

Authors: *M. ZAMBRANO-ASTORGA, A. MORENO ULLOA;
Ctr. de Investigación Científica y de Educación Superior de Ensenada, Baja California, Ensenada, Mexico

Abstract: Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder traditionally defined by beta-amyloid plaques (Aβ) and phosphorylated Tau (pTau) tangles. However, biomarker strategies centered on these core proteins offer limited diagnostic performance and fail to capture the full molecular complexity of AD. Recent proteomic studies have proposed novel cerebrospinal fluid (CSF) signatures, yet many lack validation across diverse cohorts. Here, we conducted a comparative evaluation of our previously identified CSF proteomic panel—PPAV11—against nine published proteomics-based biomarker sets (Figure 1d) and canonical

AD markers (Aβ, pTau, and total Tau). The PPAV11 panel was derived through data mining and co-occurrence filtering from seven proteomic studies (n = 1,350), retaining proteins that were consistently dysregulated across datasets (Figure 1a-b). The evaluation of diagnostic performance was assessed via logistic regression-based receiver operating characteristic (ROC) analysis across three independent cohorts (n = 813). Associations between PPAV11 proteins and cognitive scores (Montreal Cognitive Assessment (MoCA)) were analyzed using the Limma framework. PPAV11 consistently outperformed all proteomics panels (Figure 1e) and AD core biomarkers (Figure 1f), achieving AUC-ROC values of 0.96-0.99 with sensitivity and specificity exceeding 0.919 across cohorts. Regarding biological significance, PPAV11 includes proteins associated with neurogenesis, metabolism, and cell death. Moreover, four of the 11 proteins showed significant correlations with cognitive performance (FDR < 0.05). These results highlight PPAV11 as a reproducible and biologically informative biomarker panel with superior diagnostic accuracy compared to existing biomarkers. The findings support its potential for clinical application and underscore the value of integrative data mining approaches in advancing biomarker validation for complex diseases.



Comparative signatures	Proteins in original signature (n)	Proteins found in ADNI cohort (n)	Proteins found in Bangs cohort (n)	Proteins found in Johnson cohort (n)
Ali et al., 2025	10	10	8	4
Bader et al., 2020	5	5	5	3
Del Campo et al., 2022	8	5	2	0
Liu et al., 2023	3	2	2	0
Sathe et al., 2019	3	3	3	3
Shen et al., 2024	6	6	5	2
Tao et al., 2024	19	14	19	16
van Zalm et al., 2023	3	3	3	3
Wang et al., 2020	6	5	4	1

Figure 1. Discovery of PPAV11 and comparative evaluation of the panel with new and core biomarker panels. (a) Overview of the seven proteomic studies used for the initial discovery of PPAV11. (b) Schematic of the PPAV11 discovery pipeline, including co-occurrence filtering and directionality of dysregulation. Proteins present in at least two studies with consistent direction of change were retained in the final panel. (c) Correlation of PPAV11 with Montreal Cognitive Assessment (MoCA); statistical significance expressed with asterisk. (d) Summary of nine previously published biomarker panels and the number of overlapping proteins tested in each validation cohort. (e) Comparative diagnostic performance of PPAV11 versus other proteomic signatures, and (f) versus core AD CSF biomarkers, assessed via logistic regression–based ROC curve analysis across three independent cohorts.

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Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.11

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

Title: Highly Sensitive and Precise Detection of Blood-Based Neurodegeneration Markers NFL, GFAP, pTau217, and Aβ1-42 Using a Microfluidics-Based Next-Generation Immunoassay Platform

Authors: J. GROVER, B. TASADDUQ, M. CONNOR, M. RINK, M. PUTNAM, ***Y. NOAM**; Bio-Techne, Wallingford, CT

Abstract: Quantitation of biomarkers in biological fluids is becoming increasingly critical for the diagnosis, prognosis, and monitoring of neurodegenerative diseases. Different biomarkers reflect distinct pathological processes: for example, neurofilament light chain (NFL) serves as a general marker of neurodegeneration, phosphorylated tau 217 (pTau217) is indicative of amyloid-related pathology, and glial fibrillary acidic protein (GFAP) reflects neuroinflammation. While cerebrospinal fluid (CSF) analysis has traditionally provided valuable insights, there is a growing demand for blood-based biomarker detection due to its accessibility and minimally invasive nature. However, the lower concentrations of these biomarkers in blood necessitate highly sensitive and robust analytical platforms. Here, we describe enhancements to the Simple Plex^{TM} platform using the Ella instrument, an automated benchtop bioanalyzer, for the reliable and sensitive quantitation of low-abundance neurodegenerative biomarkers in blood. We developed novel assay panels targeting pTau-217, GFAP, NFL, and Amyloid beta 1-42, enabling 100% detectability at femtogram levels across healthy plasma samples, while maintaining high analytical performance and precision. The microfluidics-integrated workflow included 15 minutes of sample preparation with time-to-result of 2.5 hours. High-sensitivity assay panels were evaluated for precision, detectability, parallelism, and accuracy. Limits of Detection (LOD) were 0.53 pg/mL (NFL), 0.13 pg/mL (GFAP), 0.10 pg/mL (pTau217), and 0.26 pg/mL (Aβ1-42), with functional Lower Limits of Quantitation (fLLOQ) at 2.50, 0.63, 0.16, and 1.31 pg/mL, respectively. High sample precision was evident by single-digit coefficients of variation at endogenous analyte levels (mean CVs = 4.3%, 7.0%**,** 8.3%, 4.1%). Parallelism experiments confirmed consistency of performance in serum and plasma matrices by yielding linear (80-120%) sample behavior upon serial dilution, across assays (101%, 104%, 113%, 103%). Importantly, the assays employ antibody pairs consistent with those used in established commercial assays with known diagnostic utility, showing strong correlation ($R^2 > 0.97$) with existing methods. In conclusion, this work validates a sensitive, analytically robust and automated benchtop solution for the quantitation of neurodegenerative biomarkers in blood, offering a promising tool for advancing biomarker-driven research and clinical applications in neurodegeneration.

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Nanosymposium

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Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.12

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

Title: Analytical and clinical assessment of plasma p-Tau217, BD-Tau, p-Tau181, p-Tau212, and p-Tau205 assays in Alzheimer's disease subjects

Authors: A. CHENNA[1], B. C. YEE[1], J. HERRERA[2], K. MALYAVANTHAM[2], C. J. PETROPOULOS[1], *J. W. WINSLOW[3];
[1]LabCorp-Monogram Biosci., South San Francisco, CA; [2]Quanterix Corp, Billerica, MA; [3]Labcorp, South San Francisco, CA

Abstract: As of 2025, approximately 7.2 million Americans aged 65 and older are living with Alzheimer's disease (AD), a number expected to rise sharply with the aging population. Elevated levels of phosphorylated tau (p-Tau) and brain-derived tau (BD-Tau) proteins in plasma have emerged as reliable biomarkers of AD, reflecting the presence of amyloid plaques and neurofibrillary tangles, key pathological features of the disease. Recent advances in ultra-sensitive immunoassay technologies now allow for the detection of multiple p-Tau species in blood, providing a promising avenue for early diagnosis, disease staging, and risk stratification. In this study, we assessed the analytical and clinical performance of six plasma biomarkers: p-Tau217, BD-Tau, p-Tau181, p-Tau212, p-Tau205 (Quanterix-ALZpath), and p-Tau217 (Quanterix, Fujirebio). AD samples (n = 100) were pre-selected based on a validated Fujirebio p-Tau217 assay cutoff (>0.18 pg/mL) indicative of amyloid pathology and compared to cognitively healthy controls (n = 75). Samples were analyzed using Quanterix Simoa® HD-X assays and the Fujirebio Lumipulse® G1200 system. The median levels of all five Quanterix-based markers and Fujirebio p-Tau217 were significantly elevated in the AD group compared to age-matched controls ($p < 0.0001$). Diagnostic performance, as measured by area under the receiver operating characteristic curve (AUC), was high across all assays: p-Tau217 (ALZpath, AUC = 0.973), Fujirebio p-Tau217 (AUC = 0.953), p-Tau181 (AUC = 0.878), p-Tau212 (AUC = 0.893), p-Tau205 (AUC = 0.808), and BD-Tau (AUC = 0.815). Strong correlations were observed between p-Tau217 (ALZpath) and Fujirebio p-Tau217 ($r = 0.919$), as well as with BD-Tau ($r = 0.733$), p-Tau181 ($r = 0.888$), p-Tau212 ($r = 0.757$), and p-Tau205 ($r = 0.532$). These results demonstrate that plasma p-Tau217, BD-Tau, p-Tau181, p-Tau212, and p-Tau205 levels all robustly differentiate AD from healthy controls. High-sensitivity immunoassays for these markers offer a practical, minimally invasive approach for clinical and research applications in AD diagnostics and therapeutic monitoring.

Disclosures: A. Chenna: A. Employment/Salary (full or part-time):; Labcorp-Monogram Biosciences. **B.C. Yee:** A. Employment/Salary (full or part-time):; Labcorp-Monogram Biosciences. **J. Herrera:** A. Employment/Salary (full or part-time):; Quanterix Corp. **K. Malyavantham:** A. Employment/Salary (full or part-time):; Quanterix Corp. **C.J. Petropoulos:** A. Employment/Salary (full or part-time):; Labcorp-Monogram Biosciences. **J.W. Winslow:** A. Employment/Salary (full or part-time):; Labcorp-Monogram Biosciences.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.13

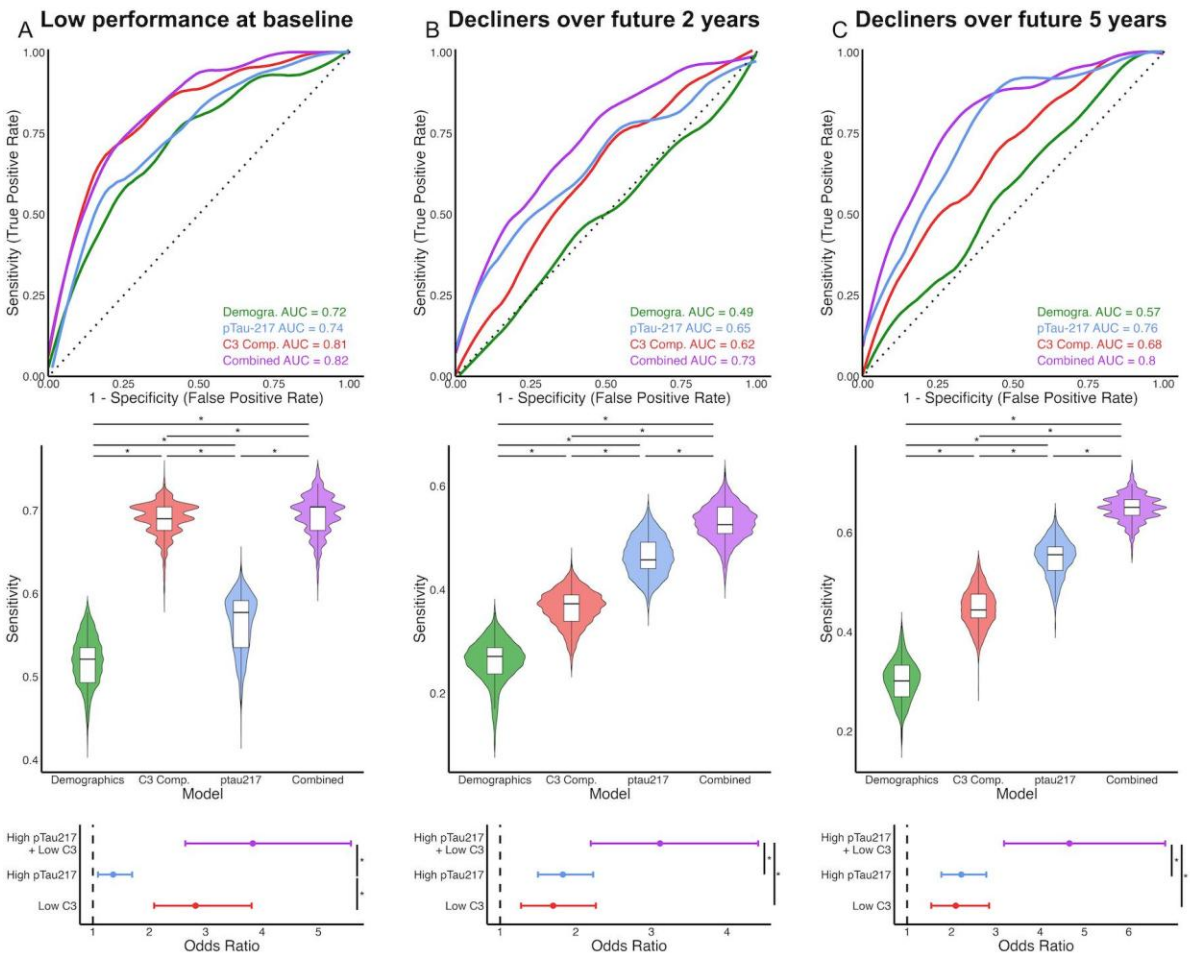
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG066683
P30 AG066519

Title: Integrating digital memory tasks and plasma pTau217 for early detection in Alzheimer's disease

Authors: *C. R. VANDERLIP[1], C. E. STARK[2];
[1]UC Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Plasma pTau-217 has emerged as a sensitive and specific biomarker for early detection of Alzheimer’s disease, yet the timeline linking pathology to cognitive decline remains poorly defined. In parallel, digital cognitive assessments offer a scalable approach for capturing subtle changes in cognition, but their standalone predictive utility is still being established. In this study, we examined whether combining these two low-burden, tools improve identification of cognitively unimpaired individuals at elevated risk for future cognitive decline. We analyzed data from 954 amyloid-positive, cognitively unimpaired individuals who completed a brief digital memory assessment and underwent a blood draw for plasma pTau-217. We assessed their ability to predict future decline on the Preclinical Alzheimer’s Cognitive Composite and Mini-Mental State Exam over a five-year period. Models that combined low memory performance with elevated pTau-217 outperformed models using either measure alone, even after adjusting for age, sex, education, and baseline cognitive performance. Importantly, while predictive performance was consistent across sexes, the combined model was significantly more accurate in APOE4 non-carriers than carriers, suggesting that digital memory assessments may capture vulnerability to decline more effectively in the absence of genetic risk. Together, these findings support the integration of digital cognitive assessments and plasma biomarkers as a practical, scalable, and highly sensitive approach for identifying individuals in the earliest stages of Alzheimer’s disease.



Disclosures: C.R. Vanderlip: None. **C.E. Stark:** None.

Nanosymposium

NANO008: Diagnostics and Biomarkers for Alzheimer's Disease and Related Dementias

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO008.14

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

Title: Native Methylation Sequencing for Detection and Monitoring of Alzheimer’s Disease, Parkinson’s and Other Neurodegenerative Conditions

Authors: *C. POLLARD;
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Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disorder, yet current diagnostics, such as CSF protein analysis and PET imaging, are invasive, expensive, and

not recommended for presymptomatic screening. As a result, they are typically applied late in disease progression, when treatment options are limited. While protein biomarkers like amyloid and tau have diagnostic value, they correlate poorly with disease progression and are only useful to understand drug efficacy when therapies target the proteins themselves. Their disease-specific nature also limits applicability across the broader neurodegenerative spectrum.Circulating cell-free DNA (cfDNA) released from dying neurons offers a scalable, noninvasive alternative. cfDNA retains methylation patterns reflective of the cell type of origin, enabling detection of ongoing neurodegeneration and identification of disease-relevant neuronal subtypes. This framework is broadly applicable across neurodegenerative diseases characterized by selective neuronal loss, providing both cell-specific resolution and disease-relevant insight.We found that by identifying neuron-derived cfDNA in the blood, we could not only predict neurodegeneration with high accuracy, but could also differentiate between distinct disease types. To accomplish this, a model was first created by sequencing purified primary neuron cells from various subtypes using the ONT PromethION platform. Differentially methylated regions (DMRs) were identified under stringent criteria (≥10 CpGs per window, ≥10× coverage per CpG, ≥20% methylation difference, adj. $p<0.05$). Cell type-specific methylation signatures from primary cortical, dopaminergic, and spinal motor neurons were established to enable cell of origin identification of neuron-derived cfDNA in plasma of patients. In a cohort of 300 patients, we found that neuron-specific cfDNA signatures enabled accurate identification of neurodegenerative disease. Cortical neuron-derived cfDNA achieved perfect separation between Alzheimer's disease (AD) cases and age-matched controls ($p<.0001$), while dopaminergic neuron-derived cfDNA similarly achieved a perfect separation between Parkinson's disease (PD) cases and their respective controls. When distinguishing between other diseases neuron-derived cfDNA profiles enabled 96.8% accuracy in predicting disease type. These results demonstrate the power of cfDNA methylation profiling as a novel method for noninvasive liquid biopsy for early detection, longitudinal monitoring, therapeutic validation and the ability to identify and differentiate between neurodegenerative diseases.

Disclosures: C. Pollard: A. Employment/Salary (full or part-time):; Resonant, LLC.

Nanosymposium

NANO009: Traumatic Brain Injury: Mechanisms, Models, and Therapeutic Interventions

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Time: Sunday, November 16, 2025, 8:00 AM - 10:30 AM

Presentation Number: NANO009.01

Topic: D.04. Brain Injury and Trauma

Support: MURI, 2023

Title: Establishing an In Vivo Human Organoid Model for Traumatic Brain Injury with Depth-Titrated Cortical Impact

Authors: *C. SMITH[1], R. DONAHUE[2], J. LEE[2], J. KIM[2], E. NOEL[2], V. E. JOHNSON[2], H.-C. I. CHEN[3], D. JGAMADZE[3];

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Abstract: Human-specific cellular responses to traumatic brain injury (TBI) cannot be studied using conventional rodent models, which lack the unique molecular, cellular, and structural characteristics of the human brain. To overcome this translational barrier, we developed a chimeric *in vivo* model of TBI by engrafting human induced pluripotent stem cell (iPSC)-derived cortical organoids into the cortex of immunosuppressed rats, followed by depth-titrated controlled cortical impact (CCI). Cortical organoids were differentiated for 50 days using standard embryoid body protocols, then transplanted into aspirated cortical cavities and allowed to integrate for 60 days. CCI was performed at injury depths of 1 mm, 2 mm, or 3 mm (velocity: 2.5 m/s), after which rats were sacrificed at 30 days post-injury (dpi) to evaluate depth-dependent histopathological outcomes. Hematoxylin and eosin staining demonstrated progressive injury severity with increasing depth, including cavity enlargement, delamination, and disorganization of cortical structure. Human-specific SC121 immunostaining confirmed organoid survival and identity across all injury conditions. Quantification of human cell density within the injury cavity revealed a depth-dependent biomechanical gradient of tissue vulnerability: mean densities were 7.41 ± 0.90 cells/100 μm^2 for 1 mm, 6.62 ± 2.60 cells/100 μm^2 for 2 mm, and 4.96 cells/100 μm^2 for 3 mm injury. Despite structural compromise, $SC121^+$ cell percentages remained >99.8%, indicating persistent human cell identity. Based on pilot survival and pathology data, 2 mm was selected as the optimal injury depth for experimental studies—producing moderate, consistent damage while preserving some degree of organoid integrity to enable histological analysis. To assess dynamic tissue responses, we compared acute (3 dpi) and subacute (30 dpi) phases at 2 mm. Ongoing studies examine injury responses in engrafted human cerebral organoids and surrounding rat cortex at 3 and 30 dpi. Key measures include lesion size, $SC121^+$ cell density, glial and immune markers (GFAP, Iba1), vascular remodeling (CD31), neuronal integrity (PAX6, NeuN), axonal injury (APP), and apoptosis (Caspase-3). These data aim to clarify how injury impacts graft survival and integration over time. This platform raises the possibility of studying human-relevant TBI pathology under controlled biomechanical conditions in a chimeric animal model.

Disclosures: C. Smith: None. **R. Donahue:** None. **J. Lee:** None. **J. Kim:** None. **E. Noel:** None. **V.E. Johnson:** None. **H.I. Chen:** None. **D. Jgamadze:** None.

Nanosymposium

NANO009: Traumatic Brain Injury: Mechanisms, Models, and Therapeutic Interventions

Location: SDCC Rm 24A

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Presentation Number: NANO009.02

Topic: D.04. Brain Injury and Trauma

Support: NIH R01AG055559
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AARF-19-617868

Title: A common molecular mechanism in Traumatic Brain Injury, Vascular Dementia and Alzheimer's Disease

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Abstract: Traumatic brain injury (TBI) and vascular dementia (VaD) are two major risk factors for late-life cognitive decline and often co-occur with Alzheimer's disease (AD) pathology. Despite their diverse etiologies, a shared molecular mechanism has not been fully elucidated. We previously identified *cis* phosphorylated tau (cis P-tau) as an early pathogenic form of tau induced after TBI, which drives neurodegeneration independent of tau aggregation. Here, we extend this work to VaD and demonstrate that cerebral hypoperfusion also induces robust accumulation of cis P-tau in both human patients and the bilateral common carotid artery stenosis (BCAS) mouse model. Using a monoclonal antibody that selectively targets cis P-tau, we show that antibody treatment prevents gliosis, demyelination, and cognitive deficits in BCAS mice. Single-cell transcriptomic analysis reveals that cis P-tau drives widespread gene expression changes across multiple brain cell types, especially downregulation of pathways related to axonal transport, synaptic structure, and myelination in excitatory neurons. Remarkably, cis P-tau antibody rescues over 90% of these altered transcripts. These findings suggest that cis P-tau is a convergent molecular driver linking TBI and VaD pathologies, and support its therapeutic targeting as a strategy to prevent dementia in patients with brain injuries or vascular risk.

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Nanosymposium

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Topic: D.04. Brain Injury and Trauma

Support: NIH/NINDS Grant 4RF1NS125578
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Title: Targeting Gasdermin-D improves secondary injury mechanisms and pro-inflammatory signaling post-traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is a major public health concern that affects millions of individuals globally and causes nearly 70,000 deaths annually in the United States. TBI induces a post-injury cascade, composed of increased pro-inflammatory activity, cell death mechanisms, and imbalances in cellular homeostasis that contribute to chronic quality of life disruptions after injury. An important pathway of the secondary injury cascade is the inflammasome, a multi-protein complex that is mediated by caspase-1 signaling to lead to the release of pro-inflammatory cytokines interleukin (IL)-1β and IL-18 and to pyroptotic cell death through the cleavage of Gasdermin D (GSDMD). Additionally, extracellular vesicles (EVs)—cargo-messenger particles that mediate intercellular communication—are released after TBI and can lead to the perpetuation of inflammatory and pathological mechanisms systemically and within the central nervous system. Importantly, novel therapies to mediate post-injury secondary mechanisms are needed to improve outcomes for patients. Thus, in this project we investigated whether targeting pyroptosis can improve pro-inflammatory outcomes and pathological markers post-TBI in a mouse model. Four-month-old GSDMD knockout (KO) mice and C57BL/6 controls (WT) underwent sham or moderate controlled cortical impact (CCI) and were sacrificed after 3 days. Cortical lysates were collected for electro-chemiluminescent immunoassay (ECLIA) detection of pathological and inflammatory mediators, and EVs for flow cytometry analysis. Behavioral testing was also completed to assess changes in cognitive outcomes after TBI. We determined that KO mice had decreased levels of the pro-inflammatory cytokines IL-6 ($p < 0.01$), IL-1β ($p < 0.01$), and tumor necrosis factor-α (TNF-α; $p < 0.05$) in comparison to WT mice after TBI. Furthermore, there was also a reduction in neuronal-derived and IL-1β-expressing EVs ($p < 0.01$) in KO mice 3-days post-TBI when compared to WT mice. Overall, our findings demonstrate that the inhibition of GSDMD can improve pro-inflammatory cytokine signaling in the cerebral cortex and reduce EV-mediated pathology-associated communication. Therefore, targeting the inflammasome pathway by GSDMD inhibition presents a promising therapeutic avenue for improving post-injury outcomes after TBI.

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Nanosymposium

NANO009: Traumatic Brain Injury: Mechanisms, Models, and Therapeutic Interventions

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Topic: D.04. Brain Injury and Trauma

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Title: Therapeutic effect of neural induced-stem cell-derived exosomes via regulating ERK/p38/NF-κB in a traumatic brain injury

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Abstract: Background: Traumatic brain injury (TBI), results from sudden external forces, such as falls or accidents, and it can cause immediate and long-term damage to the central nervous system. In this study, the therapeutic effects of neural-induced human adipose-derived stem cell-derived exosomes (NI-Exo) on TBI were investigated. Methods: Exosomes were isolated and characterized through nanoparticle tracking analysis, cryo-transmission electron microscopy, and western blotting analysis. The therapeutic effects of NI-Exo were assessed in LPS-stimulated human microglial cells and traumatic brain-injured mice via behavioral tests (rotarod, elevated body swing, and cylinder tests), qPCR, western blotting analysis, and immunostaining. Results: In the in vitro study, NI-Exo significantly downregulated pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) and upregulated anti-inflammatory cytokines (IL-4 and IL-10). In the in vivo study, NI-Exo (1×10^4 or 1×10^5 particles/mL) was administered intracerebroventricularly 1-hour post-surgery to verify the effect on the in vivo model. In the TBI mouse model, NI-Exo improved asymmetric behaviors and reduced tissue disruption and cell loss. In addition, the protein levels of pro-apoptosis (p53, ROCK1, and Bax) decreased and those of anti-apoptosis (Mcl-1) increased NI-Exo-treated group compared with that of TBI group. Mechanistic investigations revealed that NI-Exo inhibited ERK and p38 phosphorylation, highlighting its role in mitigating neuroinflammation via the ERK/p38/NF-κB signaling pathway. Conclusion: Therefore, NI-Exo promoted anti-inflammation in human microglia and TBI mouse models; it also improved anti-apoptosis in TBI models, thereby offering a promising therapeutic potential for TBI treatment through the ERK/p38/NF-κB pathway.

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Nanosymposium

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Topic: D.04. Brain Injury and Trauma

Support: AHA 23TPA1069224
NIH 5R01NS126273-03

Title: Fatty acid synthetase-mediated signaling attenuates myelin pathology following traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is caused by trauma to the brain, either through open-head (with skull fractures) or closed-head (without skull fractures) injuries. The majority of TBIs are closed-head injuries (CHI), often resulting from falls in the elderly, motor vehicle accidents, and sports-related trauma. TBI is a significant global health concern, leading to long-term disability and mortality. In the U.S., there are approximately 2.87 million TBI-related emergency visits, hospitalizations, and deaths annually. Beyond the physical damage, TBI results in long-term cognitive, emotional, and functional impairments, largely due to demyelination of white matter tracts from loss of oligodendrocytes, and disrupted lipid metabolism. Myelination is a key therapeutic target in TBI treatment. Synthesis of omega-3 fatty acids (omega-3-FA) through acyl-CoA synthetases (ACSLs) is essential for cell function and survival. GW3965 (ACSL6 activator), have demonstrated neuroprotective effects in neurological conditions such as in stroke and transient ischemic attack (TIA) models by regulating lipid metabolism, reducing demyelination, and enhancing recovery. Given that 80% of TBIs are mild, closed-head injuries, and GW3965 has demonstrated neuroprotective effects in other neurological models, it holds promise as a therapeutic agent for TBI. This study seeks to expand upon existing evidence neurological benefits and evaluate the therapeutic potential of GW3965 in mild closed-brain TBI. By investigating its effects on myelin integrity and functional recovery, we aim to contribute critical insights into the role of lipid metabolic pathways in TBI pathophysiology and identify novel therapeutic targets to improve clinical outcomes.

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Nanosymposium

NANO009: Traumatic Brain Injury: Mechanisms, Models, and Therapeutic Interventions

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Presentation Number: NANO009.06

Topic: D.04. Brain Injury and Trauma

Support: Legacy Foundation Award

Title: Cerebrovascular damage and loss of the neurogenic response after mild traumatic brain injury

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Abstract: Various factors including exercise, antidepressants and brain injury normally trigger the hippocampus to increase its generation of new neurons (neurogenesis). This *neurogenic response* is important for the beneficial effects of exercise on cognitive function, the efficacy of antidepressants, and for recovery from brain injury. However, we recently reported that a single concussive-like injury causes the loss of the neurogenic response in adult mice. Specifically, using a closed head mild traumatic brain injury (mTBI) induced by an electromagnetic impactor (4.7m/s, 2.2mm depth), we showed that after a first mTBI, a subsequent mTBI does not trigger the normally expected increases in neurogenesis. This loss was observed as early as 3 weeks but also up to 2 months after the first injury, suggesting that it is not a transient deficit. We further showed that while the neural stem cell (NSC) pool remains intact, the proliferative response of NSCs to the subsequent injury is absent. New preliminary data from our lab show that unlike non-injured mice, running fails to induce proliferation within the hippocampus of mice with a prior mTBI, suggesting that this impairment may be generalized to the various stimuli that normally induce increases in neurogenesis. Assessment of mechanisms underlying the failure of NSCs to respond to stimuli that normally cause their division suggests that thickening of the skull, which could potentially dampen the severity of second mTBI, unlikely explains the lack of response. Markers of increased quiescence nor senescence did not support such intrinsic changes within the NSCs. Preliminary data however have led us to begin interrogating whether neurovascular injury after a mTBI contributes to the loss of the neurogenic response. These include observed cerebrovascular increases in Mfge8 and amyloid beta, hallmarks of cerebral amyloid angiopathy (CAA); persistent cerebral increases in the angiotensin II type 1A receptor; and data demonstrating that a slow pressor dose of angiotensin II (7 days) in non-injured mice mimics the loss of the neurogenic response when challenged with a single mTBI. NSCs abundantly wrap their processes around blood vessels to receive nutrients, oxygen and proliferating cues when blood vessels normally dilate. Thus, we hypothesize that impairments in cerebrovascular reactivity after mTBI contribute to the loss of the neurogenic response. Identifying why the NSCs fail to respond to stimuli that should trigger increases in their proliferation can lead to strategies to help reengage the neurogenic response to improve cognitive recovery and the trajectory of healthy cognitive aging.

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Nanosymposium

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Topic: D.04. Brain Injury and Trauma

Support: UCSF Program for Breakthrough Medical Research Award
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Title: Sema3a-dependent homeostatic plasticity promotes adaptive sleep microarchitecture following brain injury

Authors: *D. NECULA[1], P. H. CHIPMAN[3], H. LI[4], S. POLURI[5], G. W. DAVIS[3], J. T. PAZ[2]; [2]Neurol., [1]Gladstone Inst. and UCSF, San Francisco, CA; [3]Biochem. & Biophysics, [4]UCSF, San Francisco, CA; [5]UC Berkeley, Berkeley, CA

Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability, affecting 69 million people worldwide. Sleep disruption is among the most prevalent and debilitating consequences. However, while sleep disturbances are common, they are not universal even in well-controlled rodent studies. This would suggest the presence of endogenous mechanisms that attempt to buffer sleep from perturbation. We investigated whether presynaptic homeostatic plasticity (PHP) supports sleep recovery following controlled cortical impact to the primary somatosensory cortex of C57BL/6J mice. We focused on the reticular nucleus of the thalamus (nRT), a GABAergic structure whose cortical inputs degenerate after TBI, rendering it a likely setting for PHP. Furthermore, the nRT plays a central role in generating and modulating many sleep-related rhythms. Whole-cell patch clamp performed over the first seven days following injury revealed altered spontaneous excitatory postsynaptic current amplitude and frequency in the nRT. This compensatory response was not present in a Semaphorin 3A loss-of-function mutant, recently shown to be necessary for PHP in adult mouse hippocampus. Multiunit recordings in thalamic brain slices showed increased spiking in nRT neurons in response to stimulation of the internal capsule. In turn, we observed reduced spiking in the ventrobasal thalamocortical nucleus, which is consistent with the increased activity in its nRT inputs. By contrast, the Sema3a mutant exhibited a hyperexcitable ventrobasal nucleus and reduced burst activity. At the chronic timepoint, in vivo ECoG recordings of brain-wide activity revealed a wildtype-specific modulation of sleep-related bandpower and oscillatory activity, namely in slow oscillations/delta waves and spindles. An increase in the ratio of putative “pro-memory” slow oscillations to putative “anti-memory” delta waves, coupled with enhanced spindle activity and stable bandpower, points to a potential electrophysiological recovery state in the wildtype not seen in the Sema3a mutant. Lastly, lack of Sema3a signaling also exacerbated lesion volume at 1.5 months post-injury as measured by laser profilometry. Taken together, our data suggest that homeostatic compensations activated in the acute post-TBI period may drive resilience of sleep microarchitecture to cortical injury and ameliorate structural damage.

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Nanosymposium

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Topic: D.04. Brain Injury and Trauma

Title: Long-term rem sleep and behavioral dysregulation after penetrating brain injury in rats

Authors: *Z. AURFAN[1], S. J. THAMS[2], M. G. RISLING[3], M. GÜNTHER[1];
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Abstract: Introduction: Penetrating traumatic brain injury (pTBI) can lead to persistent neurological and functional impairments. However, its chronic effects on sleep, cognitive function, and affective behavior remain incompletely understood. This study investigated long-term alterations in sleep architecture, cortical oscillatory activity, and behavior in a rat model of pTBI. **Methods:** Two cohorts of male Sprague Dawley rats (mean weight 391 gram) were randomized to either pTBI or sham procedures. pTBI was induced using a 2 mm wide rod that penetrated the left cerebral hemisphere (2.5 mm lateral and posterior to bregma), at a depth of 5 mm and a velocity of 100 m/s. In cohort 1 (sham $n = 3$, pTBI $n = 3$), telemetric EEG and EMG implants were used to record sleep for 24-hour periods, once per week, over eight weeks. Time in wakefulness (W), rapid eye movement (REM) sleep, non-rapid eye movement (NREM) sleep, and cortical oscillations were quantified. In cohort 2 (sham $n = 14$, pTBI $n = 15$), cognitive and affective behaviors were assessed using the open field test (OPF), elevated plus maze (EPM), Y-maze (YM), novel object recognition (NOR), and novel location (NOL) tests at 1, 4, and 8 weeks post-injury (WPI). **Results:** Compared to sham animals, pTBI rats had significantly reduced REM sleep from 1 to 8 WPI ($p = 0.021$), while total NREM and W were unaffected. Power spectral analysis revealed significantly decreased theta power during REM ($p < 0.0001$) and W ($p < 0.0001$). Alpha power was reduced during REM ($p < 0.0001$) and W ($p = 0.0323$), whereas gamma power increased during REM ($p = 0.0006$). These oscillatory changes were present at all times. In behavioral tests, pTBI rats spent less time in the center of the OPF arena at all times ($p < 0.0001$), and less time in the open arms of the EPM ($p < 0.0001$), indicating increased anxiety-like behavior. In the Y-maze, no differences were observed until 8 WPI, when pTBI rats made significantly more incorrect spontaneous alternations ($p = 0.0101$), suggesting impaired spatial working memory. No differences were found in NOR or NOL performance. **Conclusion:** Despite partial recovery of REM sleep duration, neurophysiological features of REM remain persistently disrupted, indicating lasting network dysfunction. This may drive both sustained anxiety-like behavior and delayed spatial memory deficits. Future studies aim to uncover the molecular and structural mechanisms underlying these long-term consequences of penetrating brain injury.

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Topic: D.04. Brain Injury and Trauma

Support: NIH R01DC01379801A1

Title: Post-tbi sensitivity of the auditory and vestibular systems to intense noise exposure

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Abstract: Traumatic brain injury (TBI) is a significant public health concern, with long-lasting effects for individuals and communities. While the effects of TBI on cognitive and motor functions are well documented, sensory dysfunctions are often overlooked due to the complexity of TBI presentations in patients. Among these less-explored but clinically significant outcomes are hearing and balance impairments. In mild TBI (mTBI), these dysfunctions primarily manifest as sensorineural, caused by transient or permanent damage to hair cells and associated neural pathways. CDC reports that individuals at higher risk for mTBI, such as military personnel and athletes, are also frequently exposed to high-intensity noise, which alone can cause irreversible cochlear and vestibular damage. The limited understanding of the temporal dynamics and pathophysiology of audiovestibular symptoms in mTBI increases the risk of dual insults in these populations, where noise exposure potentially exacerbates mTBI-related sensory deficits, complicating rehabilitation efforts. We investigated short- and long-term changes in audiovestibular function in ecologically valid models of mTBI and combined mTBI+Noise (n=4/group male Brown Norway rats, 14-16 weeks). mTBI was induced using a 450 g weight dropped from 1 meter. To reflect clinical scenarios, the dual-insult cohort was exposed to broadband noise (4-16 kHz, 110 dB SPL, 1 hr) four days after injury, a period associated with early cognitive recovery. ABR measurements revealed that mTBI alone induced significant shifts in hearing thresholds, with delayed recovery at low frequencies (D7) compared to higher frequencies (D3). cVEMPs evoked by 1 kHz stimuli showed transient changes in threshold and P1-N1 amplitude, while temporary amplitude shifts were also observed at 8 kHz. All measures recovered by D3. No effect of mTBI on cVEMP latency was noted. In the dual-injury cohort, one day post-noise, ABR thresholds showed additive effects at high frequencies: significant shifts were observed at 4, 8, 16, 24, and 32 kHz in both noise-only and noise+mTBI groups, with no further elevation in the dual group. No threshold differences were seen at lower frequencies or for click stimuli. cVEMP thresholds and P1-N1 amplitudes at 1 and 8 kHz were significantly affected by noise, but the presence of prior mTBI did not exacerbate these changes. No alterations in cVEMP latency were detected across groups. These findings suggest that mTBI does not acutely amplify the impact of subsequent noise trauma; however, whether a prior head injury influences the trajectory of long-term sensory recovery remains under investigation.

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Nanosymposium

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Topic: D.04. Brain Injury and Trauma

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Title: Stress, Traumatic Brain Injury, and Psychedelics

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Abstract: Traumatic brain injury (TBI) caused by closed skull impact can result in temporary or permanent damage to brain tissue and function. This injury has been associated with various comorbidities, including cognitive dysfunction, anxiety, depression, and posttraumatic stress disorder (PTSD). To determine whether stress could worsen these outcomes prior to TBI, C57BL/6J mice were assigned to 3 groups: naïve, non-stressed with TBI, or stressed with TBI. Naïve mice were confined to their home-cages and experienced neither stress nor TBI. Non-stressed mice were exposed daily for 1 hr to a shuttle box, while stressed mice received learned helplessness training over the same time. At the end of 3 weeks, non-stressed and stressed mice were exposed to TBI (mild closed head injury). These same mice were given vehicle or dimethyltryptamine (DMT) 1 hr after TBI and they received the same treatment 24 hr later. Recovery preceded for 3 days, after which mice were assessed for anxiety-, depressive-, and PTSD-like behaviors, as well as for working and episodic memory. Mice were euthanized 21 days post-TBI for brain morphological and immunohistochemical analyses. In comparison to naïve controls, only mild deficits in working and episodic memory were detected in non-stressed TBI mice regardless of drug treatment. Significantly, vehicle-treated stressed TBI mice exhibited anxiety-, depressive-, and PTSD-like behaviors, as well as. severe impairments in working and episodic memory. DMT-administration to the stressed TBI mice prevented presentation of these emotional abnormalities and their cognitive deficits were similar to levels of non-stressed TBI animals. Analyses of brains from vehicle-treated stressed TBI mice found increased microglial activation and enhanced hippocampal damage relative to DMT-treated stressed TBI mice. Together, DMT normalizes stress-induced emotional responses, preserves cognitive performance to levels of the non-stressed TBI mice, decreases microglial activation, and reduces neural insult due to secondary injury related to neuroinflammation.

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Nanosymposium

NANO010: Vestibular and Visual Neuroprosthetics

Location: SDCC Rm 23A

Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.01

Topic: F.05. Brain-Machine Interface

Support: Stichting de Weijerhorst
Heinsius Houbolt Foundation
ZonMW/Health Holland
MedEL (providers of device)

Title: The VertiGO! Trial: current standings and next steps in evaluating the safety and efficacy of prolonged daily stimulation in a multi-canal vestibulo-cochlear implant (VCI) prototype

Authors: *M. DE KOCK[1], M. TEN HOOR[1], B. VERMORKEN[1], S. VAN BOXEL[1], E. DEVOCHT[1], A. PEREZ FORNOS[2], N. GUINAND[2], R. VAN DE BERG[1];
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Abstract: The vestibular system is one of three integral sensory systems that relay information to the brain. Once integrated, this input drives motor outputs associated with gaze stability, postural adjustments, and maintenance of static and dynamic balance. When vestibular function is significantly reduced or lost in both ears, this is classified as bilateral vestibulopathy (BVP) - a disorder with profound reduction in patient quality of life. Patients typically experience a multitude of symptoms, management of which is often insufficient or limited to vestibular rehabilitation. Currently, there is no effective treatment available. As such, the Geneva-Maastricht group is investigating a combined multi-canal vestibulo-cochlear implant (VCI). This VCI aims to partially restore vestibular function in patients suffering from BVP. This ongoing trial is evaluating the safety and efficacy of prolonged multi-canal VCI stimulation.
Ten cases with BVP and ipsilateral severe sensorineural hearing loss were implanted with the newest VCI prototype. Following standard cochlear implant (CI) rehabilitation, the vestibular electrodes were fitted and activated. The effect of VCI stimulation was assessed using several outcomes, including those related to VOR response, self-motion perception, posture, balance, and quality of life. For an extensive protocol, please refer to doi.org/10.1371/journal.pone.0301032.
Current surgical protocol resulted in precise electrode positioning. In nine out of ten subjects, eye movements were elicited through electrical vestibular stimulation. Acclimatization to the stimulation was rapid and free of adverse effects in all subjects. Furthermore, VCI fitting and initial activation elicited eye movements and perceptions that were well-aligned. Dynamic ranges of vestibular implant electrodes were stable over time. Auditory performance using the VCI is comparable to regular CI use. Finally, all subjective data are currently being processed.
Based on the above results, VCI implantation and activation is considered safe, with no serious adverse events recorded. Furthermore, VCI modulation demonstrated promising outcomes in numerous measures. These results confirm the artificial vestibular system's safety and efficacy in a hospital setting, supporting its readiness for daily use. The next step is to initiate the home-use

part of the trial, scheduled to begin in October 2025. Consistent long-term stimulation is needed to demonstrate the adaptation effects of VCI modulation on gaze stabilization, movement perception, and daily-life experiences.

Disclosures: M. de Kock: None. **M. ten Hoor:** None. **B. Vermorken:** None. **S. van Boxel:** None. **E. Devocht:** None. **A. Perez Fornos:** None. **N. Guinand:** None. **R. van de Berg:** None.

Nanosymposium

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Location: SDCC Rm 23A

Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.02

Topic: F.05. Brain-Machine Interface

Support: NIH Grant DC018061
NIH Grant DC000023
NIH Grant DC021106

Title: Prosthetic semicircular canal stimulation restores gaze and postural stability in nonhuman primate locomotion

Authors: *O. R. STANLEY, R. WEI, S. THOMAS, K. E. CULLEN;
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Abstract: Vestibular prostheses provide artificial sensory input to the peripheral vestibular system and have been shown to activate the vestibulo-ocular and vestibulo-spinal reflexes (VOR and VSR), eliciting robust eye and head movements in both humans and animal models. Despite the importance of these reflexes for postural and visual stability, the effects of vestibular prosthetic stimulation on behavior in naturalistic contexts remain poorly understood. We previously characterized behavioral differences between vestibular-intact rhesus macaques versus those with bilateral vestibular loss (BVL) during locomotion, including increased head motion, limb variability, and gaze instability - patterns that closely resemble those seen in human patients.To directly assess whether multichannel vestibular prosthesis (MVP) stimulation can restore motor function during active behavior, we designed a study in which we recorded behavior from three BVL monkeys (one male, two female) walking on a treadmill and traversing a linear platform. A head-mounted camera, 6D inertial measurement unit (IMU), and reflective marker system recorded eye and head movement, while limb movements were obtained via markerless pose estimation (DeepLabCut) from synchronized videos. During each session, the animal was first acclimated to a baseline MVP stimulation rate (150 Hz) after which head-motion-coupled rate modulation was activated. Stimulation was modulated based on IMU measurements aligned to the plane of either the posterior or anterior canal, selected based on which canal-specific stimulation evoked the strongest head movement response in each animal. The stimulation was designed to mimic the dynamic properties - notably phase lead and high-pass gain - of irregular vestibular afferents, which preferentially drive VSR pathways.After

adaptation to modulated stimulation, animals exhibited significant reductions in head movement amplitude and variability, as well as increased consistency in step timing and amplitude, compared to both baseline and non-stimulated conditions. Although minimal improvements in VOR gain were observed, the resulting stabilization of head motion led to improved gaze stability.Taken together, these findings demonstrate that vestibular input delivered via MVP can be rapidly incorporated into motor control strategies to improve postural and gaze stability. However, they also underscore the need for further study and development of stimulation approaches to support broader behavioral recovery across the full repertoire of vestibular-dependent functions.

Disclosures: O.R. Stanley: None. **R. Wei:** None. **S. Thomas:** None. **K.E. Cullen:** None.

Nanosymposium

NANO010: Vestibular and Visual Neuroprosthetics

Location: SDCC Rm 23A

Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.03

Topic: F.05. Brain-Machine Interface

Support: NIH NRSA Fellowship F31-DC020390

Title: Biomimetic stimulation mappings improve balance corrections in nonhuman primates with a vestibular implant

Authors: *O. M. LEAVITT BROWN[1], K. E. CULLEN[2];
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Abstract: The vestibular system is essential for generating appropriate postural responses in a dynamic world. Patients with bilateral vestibular loss continue to experience significant disability despite rehabilitation. The vestibular implant is an innovative solution that restores vestibular reflexes by bypassing damaged neuroepithelium and directly stimulating vestibular afferents. While these devices have shown promise in clinical trials, biomimetic stimulation paradigms may further enhance efficacy. Here, we evaluated whether stimulation patterns mimicking vestibular afferent firing properties improve postural control in a rhesus monkey model.
Rhesus monkeys were trained to maintain a natural perching posture on a force plate within a chamber mounted to a motion platform delivering roll-tilt perturbations (40°/s peak velocity, 500°/s² acceleration, 10° displacement). Head motion was recorded using a wireless IMU and optical tracking; joint kinematics were estimated using DeepLabCut and Anipose. Postural responses from three intact animals (20 trials each) served as controls.
We developed a behavioral model to quantify postural deficits following bilateral vestibular loss using dynamic perturbations that exceeded the kinematic range of prior studies. Head motion became hypermetric and reversed, and response magnitude scaled with angular acceleration, which had not been dissociated from velocity previously. Strikingly, these deficits emerged

within the short-latency response window, challenging the long-held view that this phase is primarily proprioception-driven and highlighting an early vestibular contribution to posture. Next, we tested whether prosthetic stimulation of the anterior semicircular canal could restore function. Gyroscope signals were transformed into canal-plane velocities and mapped to stimulation pulse rates using four encoding strategies: a “static” clinical mapping; biomimetic mappings based on regular or irregular afferents; and a novel “super-irregular” mapping with enhanced high-frequency dynamics. Of these, only the irregular afferent-inspired mapping robustly restored postural response amplitude and direction.
These findings establish a rhesus monkey model for vestibular prosthesis-driven postural restoration and show for the first time that mimicking irregular afferent dynamics yields the most effective recovery. Future experiments will include neural recordings to advance understanding of vestibular contributions to postural control.

Disclosures: O.M. Leavitt Brown: None. **K.E. Cullen:** None.

Nanosymposium

NANO010: Vestibular and Visual Neuroprosthetics

Location: SDCC Rm 23A

Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.04

Topic: F.05. Brain-Machine Interface

Title: Neuropixels probes can capture single-unit activity from the chinchilla vestibular nerve during vestibular-implant stimulation

Authors: *A. CHHABRA[1], D. ROBERTS[4], K. WIBOONSAKSAKUL[5], C. FERNANDEZ BRILLET[1], A. CHENG[5], K. E. CULLEN[2], T. HARRIS[6], C. C. DELLA SANTINA[3]; [1]Biomed. Engin., [2]Dept. of Biomed. Engin., [3]Otolaryngology- Head and Neck Surgery, The Johns Hopkins Univ., Baltimore, MD; [4]Neurol., Johns Hopkins University, SOM, Baltimore, MD; [5]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [6]HHMI JRC, Ashburn, VA

Abstract: Multichannel vestibular prostheses (MVPs) are under active development to restore head-motion sensation, yet optimizing electrode placement and stimulus waveforms remains a challenge because conventional microelectrodes rarely yield well-isolated signals from afferents and are overwhelmed by stimulation artifacts. High-density probes like the Neuropixels could, in principle, provide a high-throughput, neuron-level assay of both on- and off-target activation, but they have never been applied to cranial nerves as they were primarily developed for recording from cortex or brainstem nuclei.We performed an acute, terminal feasibility study in one adult chinchilla (n=1). A refurbished 1 cm rodent Neuropixels 1.0 array (384 recording sites) was advanced into the internal auditory canal under microscopic guidance. Charge-balanced biphasic short current pulses (200 µA, 25 µs/phase) were delivered with a platinum-iridium electrode positioned in the horizontal-canal ampulla and returned through the common crus. Raw artifacts produced were approximately five- to ten-fold larger than spontaneous neural waveforms. Stimulus triggers were digitally synchronized to the Neuropixels sampling clock, allowing

construction of a trial-averaged artifact template; subtracting this template reduced residual artifact to baseline and unmasked clear spikes. Simple spike-sorting of a 15 minute recording segment revealed five putative neurons spread along the probe shank whose baseline firing rates were consistent with eighth-nerve afferents. Due to the acute and terminal nature of the experiment, controlled motion or sound stimuli were not delivered, video oculography was not recorded, and no phase-locked modulation was observed.Taken together, these results demonstrate, for the first time, that Neuropixels technology can be used to resolve single-unit activity from a cranial nerve despite the large artifacts generated by clinically relevant implant currents. The ability to record multiple afferents simultaneously in the presence of stimulation paves the way for a rapid, repeatable assay of off-target activation - precisely the tool needed to refine electrode geometry and stimulus design for next-generation MVPs. Future work will extend the approach to chronic implants, incorporate controlled vestibular and auditory stimuli, and scale the method to the rhesus monkey model.

Disclosures: A. Chhabra: None. **D. Roberts:** None. **K. Wiboonsaksakul:** None. **C. Fernandez Brillet:** None. **A. Cheng:** None. **K.E. Cullen:** None. **T. Harris:** None. **C.C. Della Santina:** None.

Nanosymposium

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Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.05

Topic: F.05. Brain-Machine Interface

Support: NIH R01 DC2390

Title: Beneficial effects of alternative stimulation pulse shapes for sensory prostheses: insights from vestibular prosthesis-evoked reflexes and population neural activity

Authors: *K. WIBOONSAKSAKUL[1], A. CHHABRA[1], K. E. CULLEN[2], C. C. DELLA SANTINA[1];
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Abstract: Sensory prosthesis research has advanced at a rapid pace, yet standard stimulation pulse shapes have remained largely unchanged. Most prostheses (e.g., cochlear, vestibular, and tactile prostheses) typically utilize symmetric pulses to deliver electrical stimulation to peripheral nerves. Recent neurophysiological studies, however, have shown that these symmetric pulses do not always reliably evoke firings in stimulated afferents, thus impeding optimal restoration of sensory inputs to the brain. One promising strategy from cochlear implant literature is the use of alternative pulse waveforms—specifically, asymmetric pulses, characterized by a brief, high-amplitude cathodic phase followed by a long, low-amplitude anodic phase. Testing alternative waveforms for sensory prostheses can be challenging due to lack of simple, objective measures in animal models and the limited number of implanted patients. Here, we leveraged the unique

properties of the vestibular system—the readily quantifiable reflexes that stabilize gaze and posture by generating eye and head movements, as well as their well-defined neural pathways—to investigate whether alternative pulse shapes are beneficial to vestibular prosthesis performance. In monkeys with bilateral vestibular loss implanted with a vestibular prosthesis targeting the nerve bundles of each semicircular canal ampulla, we applied electrical stimulation using symmetric and asymmetric waveforms and quantified the evoked reflexes by measuring eye and head movements. Simultaneously, we recorded both evoked potentials and single-unit responses from afferent-target neurons in the vestibular nuclei using high-density silicon probes. In comparison to symmetric pulses, asymmetric pulses evoke markedly stronger reflexive eye and head movements. Correspondingly, asymmetric pulses resulted in larger evoked potentials compared to standard pulses. Overall, single-unit recordings revealed a strong correlation between neural recruitment and enhanced behavioral performance. A simple population model suggests asymmetric pulses are more effective in recruiting afferent fibers that are further away from stimulating electrodes. Taken together, our findings indicate that asymmetric pulses can increase vestibular prosthesis performance. We speculate that these beneficial effects will prove translatable to other sensory prostheses and, eventually, clinical practice. Critically, our single-unit recordings and population model results will be useful for validating and improving existing biophysical models of peripheral nerve stimulation and also for identifying challenges to overcome.

Disclosures: K. Wiboonsaksakul: None. **A. Chhabra:** None. **K.E. Cullen:** None. **C.C. Della Santina:** None.

Nanosymposium

NANO010: Vestibular and Visual Neuroprosthetics

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Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.06

Topic: F.05. Brain-Machine Interface

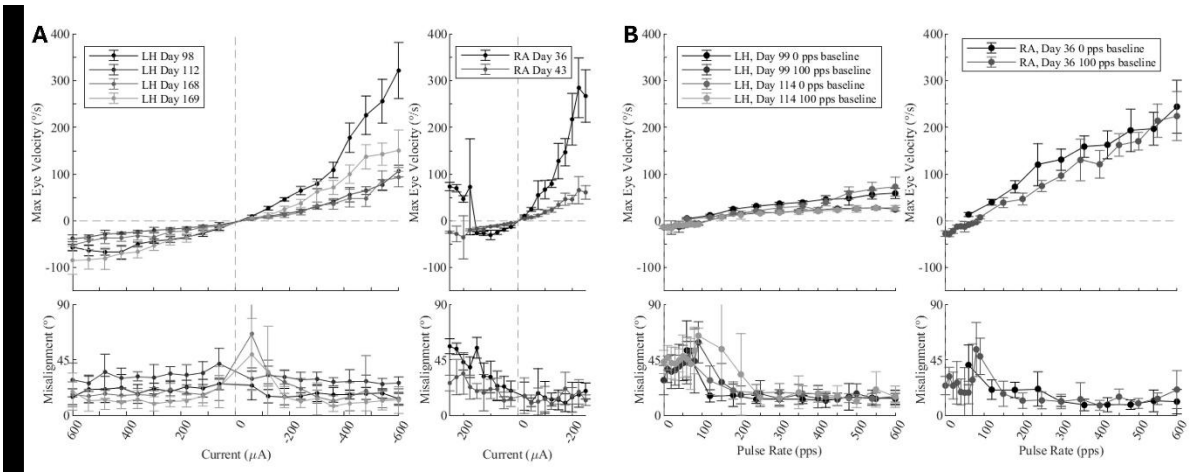
Support: NIH R01DC018300
NIH R01DC002390

Title: Direct Current and Pulsatile Stimulation of the Vestibular System using Chronically Implanted Ionic Conduits in a Nonhuman Primate

Authors: *K. MUELLER[1], E. O. VESPER[2], C. FERNANDEZ BRILLET[2], W. M. THOMAS[1], B. MORRIS[2], C. C. DELLA SANTINA[1], G. Y. FRIDMAN[1];
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Abstract: Modern neural implants use pulsatile, charge-balanced, biphasic stimulation to deliver information to nervous tissue. These electric pulses have a phase-locked excitatory effect on neurons that can be modulated by altering amplitude, frequency, phase duration, and interphase

gap. While this approach avoids the buildup of toxic byproducts at the electrode-tissue interface, pulsatile stimulation generally cannot inhibit neural activity—imposing limitations on its therapeutic effects. Ionic direct current stimulation (iDC) is a technique that can directly excite, inhibit, and sensitize neural tissue. This type of stimulation is delivered via ionic fluid conduits. Prior rodent studies using iDC have noted its ability to suppress nociceptive fibers in peripheral nerves and broaden the dynamic range of evoked eye reflexes in the vestibular system. However, long-term studies in larger animal models have not yet been conducted. In this work, we present the first chronic implantations of iDC conduits in the vestibular system of a nonhuman primate. As is seen with metal electrodes, pulsatile stimulation evoked excitatory responses through iDC conduits, and adapting the system to an excitatory baseline allowed for relative inhibition. Further, we demonstrated that iDC stimulation evokes both excitatory and inhibitory responses without the need to adapt to an excitatory baseline. For the left ear, responses were recorded from the horizontal canal for 5.5 months after implantation. The conduits in the right ear continue to function after 3 months. While responses varied between days, both pulsatile and iDC stimulation delivered through the ionic conduits generated robust eye movements in both the left and right ear implants.



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Nanosymposium

NANO010: Vestibular and Visual Neuroprosthetics

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Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.07

Topic: F.05. Brain-Machine Interface

Support: NIH Grant # 4UH3NS095557 – 03

Title: Combining Intracortical Visual Prosthesis (ICVP) stimulation with auditory image-recognition information improves person-finding performance

Authors: ***M. P. BARRY**[1,3], G. SORCI[4], K. STIPP[2], V. L. TOWLE[5], P. GRANT[4,6], M. ROYSTER[4], B. BAK[7], F. J. LANE[2,4], S. SANI[8], R. W. BYRNE[9], M. J. BAK[7], J. P. SZLYK[4], S. COGAN[10], G. DAGNELIE[3], P. R. TROYK[1];
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Abstract: "What is that?" is a question often asked by individuals using visual prostheses. These individuals may be able to detect high-contrast objects but not identify what they are. We investigated the benefits and limitations of combining a visual prosthesis with the OrCam MyEye to find specific people. The first human participant (P1) of the Intracortical Visual Prosthesis study, who had only bare light perception, received 25 wireless floating microelectrode arrays (WFMAs) in the right dorsolateral visual cortex in February 2022. Array stimulation has elicited up to 19 independent phosphenes in the lower left visual field that, when driven by a camera, produce some functional vision. P1 wore glasses equipped with: (1) a thermal sensor used to drive ICVP stimulation when a person was in view; and (2) a MyEye preprogrammed to verbally identify people in view. When multiple people were visible, the MyEye would randomly identify one or more of those people. For each of 126 trials, 2-3 people stood 14-180° apart 3.0-3.6 m from P1. On each trial, P1 was told how many people were present and their 5-6 possible locations. P1 guessed who was at each occupied position and attempted to give a specified person a high five (touch hands). Permutation tests with 10^5 repetitions each were used to determine statistical significance. Performance in guessing who was where was at chance with the thermal-sensing ICVP alone (10/34 correct, mean error (±SD): 32±46°). Use of the MyEye, both with and without ICVP, reduced guessing error (60/92 correct, mean error 4.9±7.8°; $p < 10^{-5}$ vs. ICVP alone). Error in identifying people with the MyEye was inversely related to average separations between people, dropping from 8.4±8.6° error (for separations of 14-17°, 16 trials) to 0±0° error (for separations of 146-180°, 20 trials) ($p < 2x10^{-5}$). Use of the MyEye alone, with its wide field of view and lack of specificity, left P1 standing at an empty space when giving a high five more frequently than when using the ICVP (MyEye only: 10/46 trials; ICVP both with and without MyEye: 2/80 trials; $p < 10^{-3}$). Use of both systems together allowed P1 to both choose the correct person and approach them with the least error (average distance to target with ICVP only: 170±160 cm; MyEye only: 72±48 cm; both together: 47±27 cm; $p < 0.003$ for all pairwise differences). These results suggest that combining location information from visual prostheses with contextual information from image recognition algorithms can improve functional performance more than either technology alone. Focusing an algorithm's processing on the same stimulus as that detected by the prosthesis will be important for optimizing performance and reducing confusion.

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Bak: A. Employment/Salary (full or part-time):; MicroProbes for Life Science, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MicroProbes for Life Science, Inc.. **J.P. Szlyk:** None. **S. Cogan:** F. Consulting Fees (e.g., advisory boards); Qualia Oto.. **G. Dagnelie:** None. **P.R. Troyk:** A. Employment/Salary (full or part-time):; Sigenics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sigenics, Inc..

Nanosymposium

NANO010: Vestibular and Visual Neuroprosthetics

Location: SDCC Rm 23A

Time: Sunday, November 16, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO010.08

Topic: F.05. Brain-Machine Interface

Support: R01EY023336

Title: Endogenous neural activity impacts perception of phosphenes in a human sEEG patient

Authors: *D. OSWALT[1], M. BEAUCHAMP[2], D. YOSHOR[3];
[2]Neurosurg., [3]Dept. of Neurosurg., [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Visual cortical prostheses (VCPs) are devices that attempt to use direct electrical stimulation of early visual cortex to drive useful visual percepts. Previous and current VCPs have used electrical stimulation delivered in a manner independent of ongoing cortical activity. Because salient features of neural activity are known to impact perception of visual or sensory stimuli, we expect a similar interaction for electrically evoked stimuli. For example, low frequency oscillations at 8-12 Hz, are a prominent feature of activity in early visual cortex with multiple threads of evidence suggesting their importance in cortical processing. Improvements in both detection and discriminability of visual and tactile have been associated with reductions in alpha power and with presentation of stimuli at particular phases of the alpha rhythm. We expect same may be true for delivery of electrical stimulation.
Electrical stimulation was applied to intracranial electrodes implanted in a patient undergoing clinical monitoring for medically refractory epilepsy. Electrophysiological recordings were collected during electrical stimulation delivered to a stereo-EEG electrode implanted in early visual cortex. Brief (20-50 ms) 200 Hz pulse trains were applied at 0.7mA, a current near the threshold to evoke perception of a phosphene on that electrode. When comparing the evoked responses, the trials where the subject reported a phosphene were paired with a larger evoked response in comparison to the trials where the subject did not report perception of a phosphene. In evaluating the 1 s window prior to delivery of electrical stimulation, we found a change in alpha power was indeed related to phosphene perception. The average power in the 8-12Hz range in the 1 s prior to stimulation was significantly higher in trials where the subject did not perceive a phosphene compared to the trials where the subject did perceive a phosphene. Continued study is needed to understand the interplay between endogenous neural activity and

electrical stimulation induced sensory experiences. Better understanding of the neural features that mediate perception will help develop better stimulation strategies for VCPs that minimize the current required to drive perception of phosphene pattens and lead to better safety and device longevity.

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Nanosymposium

NANO011: Neuronal Dynamics Underlying Memory

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO011.01

Topic: I.07. Long-Term Memory

Support: NIH R01EY020851
Simons Foundation, Simons Collaboration on the Global Brain award 543033

Title: Field potentials sensitively and efficiently capture visual memory representations in inferotemporal cortex

Authors: C. M. HACKER[1], S. BOHN[1], B. L. FOSTER[2], N. C. RUST[1];
[1]Psychology, [2]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: When studying the neural correlates of cognitive functions, a general assumption is that recording groups of isolated neurons is superior to more aggregate measures like the local field potential (LFP) for capturing neural representations. Contrary to that belief, we find that conclusions about the neural representations of visual memory are the same whether they are made using spikes or LFPs, but that, strikingly, LFPs required at least 3-fold less data to achieve comparable levels of decoding. Specifically, we compared spike and LFP data simultaneously recorded from inferotemporal cortex (ITC) of four macaque monkeys engaged in a single-exposure visual memory task in which they viewed a sequence of images and indicated whether each was novel or repeated. We examined the neural representations of four stimulus attributes known to influence visual memory: novelty, delay, memorability, and contrast. We found that the format of the neural representations in population spiking and high-gamma activity (50-150 Hz) of the LFP were strikingly aligned in terms of direction, magnitude, temporal dynamics, and subtle differences across animals. By training linear decoders to distinguish novel from repeated images on the basis of differences in activity levels, we show that an animal's memory task performance can be predicted as well from high-gamma activity as spikes, using the same decoding scheme, but that at least 3-fold more data is required to reach the same decoding level with spikes than with LFPs. Importantly, we also demonstrate that this close alignment is specific to the high-gamma range of the LFP and examine what analysis decisions are necessary to facilitate this alignment.
Overall, we demonstrate that high-gamma activity in the LFP is sensitive enough to capture

neural representations of visual memory as well as spikes, but that the lower data requirements make high-gamma the more efficient option. Insofar as a major goal of basic research performed in animals is that insights about the healthy brain may one day inform treatments for neurological and neuropsychiatric disorders in humans, understanding the relationship between the signals most often recorded from humans (field potentials) and animals (spikes) is essential. By considering population coding schemes in identifying neural representations for comparison, our results suggest that magnitude codes are well-positioned for translation across model species. More generally, our results show that high-gamma activity is a robust, sensitive, efficient, and common neural measurement that allows for both reliable translation across scales of measurement and species, supporting translational work.

Disclosures: C.M. Hacker: None. **S. Bohn:** None. **B.L. Foster:** None. **N.C. Rust:** None.

Nanosymposium

NANO011: Neuronal Dynamics Underlying Memory

Location: SDCC Rm 25A

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Presentation Number: NANO011.02

Topic: I.07. Long-Term Memory

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European Research Council (ERC) under the European Union's Horizon 2020 (Grant agreement No. 101001121)

Title: Cortical signatures of hippocampal ripples in human sleep

Authors: *P.-C. CHEN[1], J. STRITZELBERGER[2], B. STARESINA[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. Hosp. Erlangen, Erlangen, Germany

Abstract: Background:
Hippocampal sharp-wave ripples are critical for memory consolidation and are typically detected via intracranial EEG in humans. While prior studies have shown ripple-associated hippocampal-cortical coordination, it remains unclear whether ripples also enhance cortical-cortical interactions and whether such ripple-related signatures are observable non-invasively via scalp EEG.**Methods:**
We analysed overnight sleep recordings from 16 epilepsy patients implanted with Behnke-Fried electrodes (33 hippocampal contacts total). Each patient additionally had 21 scalp EEG electrodes. Sleep was manually staged using AASM criteria. Ripples during non-rapid eye movement sleep were defined as 80-120 Hz events with envelope amplitudes >2.5 SD above the mean, excluding periods with pathological interictal discharges. Ripple-free surrogate events were drawn from surrounding 10-minute windows. EEG signals were re-referenced (scalp: common-average; iEEG: white-matter). Phase-locking values (PLVs) were computed in 0.5 s windows with 0.1 s steps for ripple vs. surrogate comparisons at the participant level.**Results:**

We observed significant increases in hippocampal-cortical PLVs in the spindle band (12-16 Hz) following ripples, indicating enhanced synchrony between hippocampal and cortical sites. Importantly, ripple-triggered increases in cortico-cortical PLVs were also observed in the spindle band, particularly across long-range scalp channel pairs. These PLVs were statistically greater than matched surrogate events.**Conclusions:**
Our findings reveal that hippocampal ripples not only facilitate hippocampal-cortical communication but also enhance widespread cortical synchrony, especially in the spindle band. This supports the idea that ripples orchestrate large-scale cortical dynamics during sleep. These findings also highlight that detecting ripple-related spindle synchrony at the scalp level may advance non-invasive EEG biomarkers for ripples in the human brain.

Disclosures: P. Chen: None. **J. Stritzelberger:** None. **B. Staresina:** None.

Nanosymposium

NANO011: Neuronal Dynamics Underlying Memory

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Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO011.03

Topic: I.07. Long-Term Memory

Support: NIH R01 NS1007806
1K23NS104252
FACES
NYU Department of Neurology

Title: Spatiotemporal Patterns Differentiate Hippocampal Sharp-Wave Ripples from Interictal Epileptiform Discharges in Mice and Humans

Authors: *J. SHIN[1], A. MASLAROVA[2], A. NAVAS-OLIVE[4], M. VOROSLAKOS[1], S. HENIN[3], G. BUZSAKI[2], A. A. LIU[1];
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Abstract: Hippocampal sharp-wave ripples (SPW-Rs) are high-frequency oscillations critical for memory consolidation in mammals. Despite extensive characterization in rodents, their detection in humans is limited by coarse spatial sampling, interictal epileptiform discharges (IEDs), and a lack of consensus on human SPW-R localization and morphology. Here, we demonstrate that mouse and human hippocampal ripples share spatial, spectral and temporal features, which are clearly distinct from IEDs. In 1024-channel hippocampal recordings from APP/PS1 mice, SPW-Rs were distinguishable from IEDs by their localization to the CA1 pyramidal layer, narrowband frequency peaks, and multiple ripple cycles on the unfiltered local field potential. In sleep data recorded from surgical epilepsy patients, hippocampal ripples showed similar narrowband frequency peaks and multiple ripple cycles in CA1 and the subiculum but were absent in the dentate gyrus. Conversely, IEDs showed a broad spatial extent and wide-band frequency power.

By selecting channels based on 1/f-corrected peri-event power spectral density (PSD) to identify narrowband high frequency peaks we were able to reject ~40% of the automatically detected events. In addition, we introduce a semi-automated, human ripple detection toolbox (*ripmap*) separating event waveforms by low-dimensional embedding to further reduce false-positive rate in selected ripple channels. Our approach improves ripple detection in humans, by utilizing rodent SPW-R spatial, spectral, and morphological features to standardize the initial selection of candidate ripple in a semi-automated manner, thus providing a firm foundation for future human memory research.

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Nanosymposium

NANO011: Neuronal Dynamics Underlying Memory

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Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO011.04

Topic: I.07. Long-Term Memory

Support: NSF GRFP DGE-2236662

Title: Arousal state modulation of human hippocampal ripples across wake and sleep

Authors: *E. M. SIEFERT[1,2], Y. Y. CHEN[1,4], K. A. DAVIS[3], H. CHEN[1], A. C. SCHAPIRO[2], B. L. FOSTER[1];
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Abstract: Hippocampal replay is the reactivation of spiking activity patterns from previous experiences and is thought to be a key mechanism for memory consolidation. These replay events can be captured as high-frequency oscillatory bursts - termed ‘ripples’ - in the hippocampal field potential across species. Broadly, replay/ripple events are thought to occur during offline states like sleep and awake rest, as these times allow the brain to replay memories with limited interference from new sensory experiences. However, ripple rates vary dramatically across sleep stages, challenging the idea that they are solely driven by offline periods. Moreover, recent human studies observed ripples during active cognitive tasks, indicating that ripples can occur outside restful, offline states. What mechanism explains the predominance of ripples in offline states but accounts for their variability across sleep stages and cognitive tasks? We propose that dynamic dips in arousal may promote ripple genesis across states, with larger arousal reductions during certain sleep stages driving high offline ripple rates, and smaller transient fluctuations accounting for their occurrence during awake behaviors. To test this, direct recordings were made from the human hippocampus during both wake and overnight sleep (n = 15; 82 hippocampal contacts). Arousal was tracked with staging during sleep and pupillometry during wake. Identified ripples were analyzed for modulation by arousal state. Consistent with

prior work, ripple rate varied profoundly across sleep stages, being maximal during NREM sleep, greatly reduced during REM, and minimal during wake. Interestingly, this sleep-stage modulation was more pronounced in the anterior than posterior hippocampus, while the wake ripple rate in both regions was matched. Similarly, during separate wake recordings with pupillometry, ripple rate tracked arousal state, being highest during the smallest pupil states. As in sleep, this arousal-related modulation was strongest in the anterior hippocampus. Altogether, hippocampal ripples were found to track arousal fluctuations across both sleep and wake, increasing in rate when arousal dipped. These results refine our understanding of offline states and suggest that dips in arousal during wake may mirror that of sleep, providing opportunistic moments for consolidation interspersed with awake behaviors. Mechanistically, these results bridge arousal and ripple physiology, aligning with growing evidence implicating drops in acetylcholine - an arousal-related neurotransmitter that is indexed by pupil size and fluctuates across sleep stages - in ripple genesis and memory consolidation.

Disclosures: E.M. Siefert: None. **Y.Y. Chen:** None. **K.A. Davis:** None. **H. Chen:** None. **A.C. Schapiro:** None. **B.L. Foster:** None.

Nanosymposium

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Presentation Number: NANO011.05

Topic: I.07. Long-Term Memory

Support: HHMI
NIMH F32 MH123003

Title: Hippocampal neurofeedback promotes generation of spatial representations that jump directly to distant locations

Authors: *M. E. COULTER[1], A. GILLESPIE[2], J. CHU[3], E. L. DENOVELLIS[5], D. F. LIU[6], T. NGUYEN[8], K. WADHWANI[7], B. SHARMA[9], X. DENG[10], U. EDEN[11], C. KEMERE[4], L. M. FRANK[12];
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Abstract: Humans can remember places we have been and experiences we have had without overt behavioral signs that these memories have been retrieved. Humans can retrieve specific memories based on internal goals, and remembering does not require external retrieval cues. In

addition, remembering an experience distant in time or place does not seem to require mentally traversing all intermediate times or places. Instead, the brain can mentally teleport or "jump" directly to the memory. Thus, memory retrieval is a process that can be expressed in the brain separately from (1) the decision to act based on the content of the memory, (2) specific external cues that trigger the memory, and (3) intervening experiences that separate the current state from the past event. However, current animal models of memory typically present sensory cues to trigger retrieval and assess retrieval based on action. As a result, it is difficult to determine whether measured patterns of neural activity relate to the cue(s), the retrieved memory, or the behavior. We therefore asked whether we could develop a paradigm to isolate retrieval-related neural activity in animals without retrieval cues or the requirement of a behavioral report. To do this, we focused on hippocampal "place cells." These cells primarily represent the animal's current location (local representations), but they can also represent previously visited locations distant from the animal's current location (remote representations). It is not known whether animals can deliberately engage specific remote representations, so we developed a closed-loop neurofeedback system to reward rats for generating remote spatial representations. Although no specific retrieval cues were presented, rats learned to activate hippocampal remote representations corresponding to experimenter-chosen spatial locations, thus demonstrating deliberate memory retrieval. During the task, the remote target location was not visible from the animal's position, and the rat took advantage of this feature by generating representations that "jumped" to the target area (as in human memory), rather than continuous representations that included all intermediate locations between the animal's actual location and the target. Notably, our paradigm differs from previous neurofeedback approaches because the animal did not receive continuous sensory feedback. Our work establishes a model for studying how the brain can deliberately reinstate representations related to previous experience and this paradigm can be used for direct study of memory retrieval in the typical brain and in learning and memory disorders.

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Nanosymposium

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Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

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Topic: I.07. Long-Term Memory

Support: NIH/NINDS ZIA NS003144

Title: A Representation Of Temporal Structure In Human Anterior Temporal Lobe

Authors: *I. M. BRIGHT[1], A. VAZ[3], S. INATI[2], M. W. HOWARD[4], K. A. ZAGHLOUL[5];
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Abstract: Episodic memories are closely linked to the spatial and temporal contexts in which they occur. Although the neural encoding of spatial context has been extensively studied, the mechanisms by which the brain represents temporal context are less established. Previous studies have demonstrated that temporal context produces a recency effect in neural activity patterns, with decreasing similarity as temporal distance increases. However, the time scale of this change varies widely across reports, ranging from seconds to tens of minutes. To investigate how temporal context is encoded in the human brain, we recorded single-unit activity from the anterior temporal lobe of epilepsy patients as they performed a paired associates memory task. We observed that neural population activity exhibited recency effects over multiple timescales simultaneously, gradually changing over trials, lists, and entire sessions. Critically, these changes were consistent across repeated experiences, allowing for the decoding of task structure. Taken together, these results inform how the temporal component of episodic memory is represented at the population level in human cortex, shedding light on the neural basis of episodic memory organization.

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Nanosymposium

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Presentation Number: NANO011.07

Topic: I.07. Long-Term Memory

Support: UKRI Future Leaders Fellowship
UK Medical Research Council grant

Title: Investigating the role of neural reactivation in credit assignment

Authors: *L. GLITZ[1], S. NALLURU[2], A. HESS[3], W. CLARKE[3], V. MANCINI[3], S. ABBASI-RAD[5], J. CAMPBELL[3], J. O'REILLY[4], H. BARRON[3];
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Abstract: A number of computational frameworks - most prominently reinforcement learning - have conceptualised reward as the driving force behind human and animal learning. However, animals and humans not only show reward-seeking behaviour in response to directly rewarded stimuli, but also in response to stimuli that have not been directly reinforced. These behaviours appear to draw on alternative neural mechanisms that allow reward, or credit, to be assigned to

stimuli that have not been paired with reward directly. A candidate mechanism for assigning credit to stimuli that have not been directly paired with reward involves 'reverse replay'. Reverse replay can be defined as the reactivation of temporally sequenced memories, in the reverse order to previous behavioural experience. Reverse replay can be observed in the spiking activity of neurons in the hippocampus, during periods of rest/sleep. However, it remains unclear whether representing reward information prior to stimuli/events that lead to reward can allow for value information to be propagated backwards and encoded in representations along the incoming trajectories. Here we investigated the relationship between reverse replay and credit-assignment in humans, taking advantage of near whole brain imaging. Participants learned trajectories from a starting location to a target location. In the second stage of the task, some of the target locations were rewarded. Crucially, however, there were also 'foil' locations, which were only statistically correlated with the starting cues. Some of these foil locations also contained reward, allowing us to look at backpropagation of value to the starting cues for both causal and statistically correlated trajectories. Using ultra-high field fMRI in combination with decoding of neural activity during rest, we show evidence for value propagation during memory reactivation in periods of rest. We reveal the neuroanatomical circuits that contribute to value propagation and demonstrate how reward information can be misattributed to starting locations. Our findings reveal a mechanism where memory replay can assign credit to distal cues, yet inherent biases in this mechanism have propensity to construct false beliefs which may be relevant to neuropsychiatric conditions such as psychosis.

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Nanosymposium

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Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO011.08

Topic: I.07. Long-Term Memory

Support: Simons Junior Fellowship

Title: Experience-dependent alterations of hippocampal-neocortical dialogue during sleep

Authors: *C. LAFFERTY[1], G. BUZSAKI[2];
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Abstract: The hippocampus is conventionally viewed as the cortex's instructor, triggering the reinstatement of waking cortical activity patterns during sleep. However, recent evidence indicates that the cortex plays an active role in these interactions, suggesting that the consolidation process is more like a dialogue than a rehearsal. Consequently, the mechanistic details of how the hippocampus and cortex jointly coordinate systems-wide reactivations during

sleep are not yet known. In particular, it is not clear how this dialogue might support the integration of new information into existing schemas. Here, I address these questions using a combination of in vivo electrophysiology and widefield imaging of cortical activity to study experience-dependent changes in hippocampal-cortical interactions during sleep. Thy1-GCaMP6f transgenic mice were prepared for widefield cortical imaging of one brain hemisphere by thinning the overlying skull and index-matching the surface to the underlying tissue. Mice were then implanted with a 128-channel silicon probe targeted to the dorsal CA1 region of the hippocampus. To examine interactions between hippocampus and cortex during content-specific reactivation of hippocampal ensembles, mice were trained to obtain water on an 8-maze. During early training, mice were given access to only one arm of the maze and during late training the second arm was introduced to provide mice with a novel experience on a background of pre-existing knowledge. Place cell activity recorded in CA1 during behavior was used to identify reactivation templates. During post-behavior sleep, we measured the fraction of spikes occurring during sharp-wave ripples (SPW-Rs) that exhibited prior place activity in the maze to select SPW-Rs that exhibited content-specific reactivation. When we compared patterns of cortical activity time-locked to SPW-Rs in the top (content) and bottom (control) sextiles of reactivation scores, we found that content-specific SPW-Rs alone are associated with a distinct cortical dynamic characterized by activation of the default mode network. Content and control SPW-Rs did not differ significantly in terms of their amplitude, or duration and the observed difference in cortical activity remained pronounced even when content and control SPW-Rs were randomly subsampled to have matching SPW-R features. These data suggest that SPW-Rs having otherwise similar properties may differentially engage cortex on the basis of the content they encode. These results provide a striking account of experience-dependent hippocampal-cortical dialogue that forms the bedrock of most systems consolidation theories.

Disclosures: C. Lafferty: None. **G. Buzsaki:** None.

Nanosymposium

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Topic: I.07. Long-Term Memory

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Title: The neural basis of image selection during free viewing behavior in a model of Alzheimer’s Disease

Authors: *D. E. SHEETS[1], D. A. RUFF[1], K. ALLEN[1], J. MORRISON[3], M. R. COHEN[2]; [2]Neurobio., [1]Univ. of Chicago, Chicago, IL; [3]UC Davis, Woodland, CA

Abstract: Approximately 38 million people worldwide are living with a diagnosis of Alzheimer's disease (AD), and it is estimated that an additional 50 million people are unknowingly in early stages of the disease. Most patients are diagnosed with AD many years after disease onset, which is too late for most lifestyle changes and pharmaceutical treatments to be effective. This highlights an urgent need to develop robust and reliable methods for early detection of AD and to identify neuronal changes that might be targets for new treatment. This requires comparing human behavior to an animal model with known disease onset time and behavioral similarity to humans. We are therefore studying a macaque model of AD (Beckman et al., 2021) to test the hypothesis that image selection will be a useful platform to identify how disease progression affects complex behavior and neural processing.
We made daily recordings from chronically implanted electrode arrays in visual area V4 while monkeys performed tasks that probed visual processing and image recognition memory. As in humans with AD, we observed early changes in the monkeys' preference to look at novel images over familiar. This behavioral change was accompanied by worsening reliability of V4 population encoding of novel but not familiar images. Additionally, for each monkey and human participant, we identified the low- and high-level visual features that predicted which images they chose to look at. We found that these preferences changed during disease progression in our monkeys, and are potentially a good platform for identifying people who are at risk of developing AD. We also identified a potential neural explanation for the observation that human AD patients mix up information. While the tuning of V4 neurons for visual features stayed largely stable during disease progression, the representations of different features became increasingly entangled. These findings illustrate that subtle yet robust changes in the visual system can be harnessed for early detection of AD and to understand the neural reasons for behavioral changes during disease progression.

Disclosures: D.E. Sheets: None. **D.A. Ruff:** None. **K. Allen:** None. **J. Morrison:** None. **M.R. Cohen:** None.

Nanosymposium

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Presentation Number: NANO011.10

Topic: I.07. Long-Term Memory

Support: NIH U01 NS117838
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R61 MH135109

Title: This is the way: Neural dynamics during real-world wayfinding in humans

Authors: *C. S. INMAN[1], T. S. DAVIS[2], U. TOPALOVIC[3], L. GARCIA[5,2], M. STANGL[6], A. KAZEMI[2], M. HOLLEARN[1], J. CAMPBELL[2], L. AUGUSTIN[1], D. ELIASHIV[7], V. R. RAO[9], I. FRIED[10], N. HASULAK[11], S. HILLER[8], N. A. SUTHANA[4];
[1]Psychology, [2]Univ. of Utah, Salt Lake City, UT; [4]Neurosurg. / Biomed. Engin., [3]Duke Univ., Durham, NC; [5]; [6]Biomed. Engin., Boston Univ., Boston, MA; [7]UCLA, Los angeles, CA; [8]UCLA, Los Angeles, CA; [9]Neurol., Univ. of California, San Francisco, San Francisco, CA; [10]DEPT NEUROSURGERY, UCLA Sch. Med., Los Angeles, CA; [11]Phoenix Res. Consulting, Phoenix, AZ

Abstract: The ultimate goal of neuroscience is to understand and explain real-world behavior in terms of brain activity and to use these insights to develop therapeutic approaches for neural disorders. Traditional neuroimaging methods, such as fMRI, require participants to remain stationary, which limits the complexity and realism of research studies. By using mobile recording devices synchronized with intracranial EEG recordings in epilepsy patients with an implanted deep brain recording system, we can study the neural basis of everyday human activities such as navigation, wayfinding, and memory encoding in a more natural way that captures the complexity, scale, and functional characteristics of real-world experiences. We asked five RNS participants to learn a 0.75-mile route around campus, providing only instructions to remember the route well enough to navigate back to the starting point. Subjects walked the route 7-8 times across two days, with the 1st walk guided (encoding) and 6-7 of the walks navigated by the participant themselves (navigation retrieval). Local field potential data between 1-85 Hz was continuously collected throughout each participant's walk, synchronized with a suite of 1st-person experience sensors at millisecond precision (eye-tracking, psychophysiology, movement speed, audio, head- and chest-mounted video, etc.). Findings across all participants suggest that theta band power (5-8 Hz) throughout the temporal lobe significantly increases when participants are navigating outdoors relative to indoor navigation. Interestingly, changes in velocity do not fully explain changes in hippocampal theta power around events or outdoors. Building on these findings, we find that aperiodic broadband spectral slope decreases around turns of the body and head, while the prevalence of true theta oscillations primarily decreases after navigation errors, like making an incorrect turn or the moment the participant realizes they are lost. Taken together, these findings suggest that hippocampal theta oscillations are not correlates of the structure of the environment or a person’s movement through it, but a response-based opportunity to update our mental representation of the world as we find our way. This novel, naturalistic cognitive neuroscience paradigm opens new pathways to studying the neural dynamics of navigation, wayfinding, and memory processes at the temporal and spatial scales of real-world experiences in freely moving humans.

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Nanosymposium

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Topic: I.07. Long-Term Memory

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NIH R01-NS125250
NIH R01-NS107357

Title: Cholinergic modulation of human intracranial brain oscillations during memory

Authors: *T. GEDANKIEN[1], B. C. LEGA[3], J. JACOBS[2];
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Abstract: Cholinergic dysfunction is a hallmark of Alzheimer's disease and other memory disorders. Yet, the neurophysiological mechanisms linking cholinergic signaling to memory remain poorly understood. In two studies, we administered scopolamine, a muscarinic cholinergic antagonist, to neurosurgical patients with intracranial electrodes as they performed episodic memory tasks. When scopolamine was present at encoding, we observed impaired memory performance which was coupled to disruptions to both the amplitude and phase alignment of hippocampal theta oscillations during encoding. However, when scopolamine was present during retrieval alone, we observed disruptions to slow theta during retrieval without impaired memory performance. Our results indicate that cholinergic signaling primarily supports memory encoding by coordinating the temporal dynamics of hippocampal theta oscillations. However, our findings challenge the notion that memory retrieval relies on a similar mechanism, and instead suggest that hippocampal theta universally reflects an encoding-related neural state. These findings motivate updates to current models of acetylcholine's role in memory and may inform future therapies targeting rhythmic biomarkers of memory dysfunction.

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Nanosymposium

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NEI (P30 EY008126)
NIH OD (1S10D021771)

Title: Experience reorganizes content-specific memory traces in macaques

Authors: *S. ABBASPOOR[1], A. ALJISHI[2], K. L. HOFFMAN[3];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Psychology, [3]Psychological Sci., Vanderbilt Univ., Nashville, TN

Abstract: The ability to flexibly learn from past experiences is crucial for animals to adapt to changing environments. The hippocampus and related neocortical networks play a key role in this process. Rodent studies show durable memories rely on joint firing patterns among neuron groups, or "cell assemblies" that reactivate during sleep. How sleep reactivation occurs for distinct memoranda over time is unclear, and the existence of such assemblies in the macaque hippocampus remains largely unknown. To investigate cell assembly reactivation for specific experiences in macaques, we developed a neurobehavioral method for studying memory formation under freely-moving and sleep conditions. In one of two corners of a test enclosure, monkeys learned to select a 4-item sequence of objects presented across 4 touchscreens. One corner's object sequence had been learned 2-5 weeks earlier ("Old"), and the opposite corner's sequence used new objects ("New") or were reissued the next day ("Recent"). Monkeys successfully learned 14 and 22 unique 4-item sets, respectively, and performed better on Old than New sets, indicating long-term memory savings. During task and overnight sleep, we recorded neuronal ensemble activity in the hippocampus and connected cortical regions using high-density linear arrays (31-273 units/session). An SVM decoder trained on ensemble activity reliably classified trial identity (Old vs. New/Recent), establishing the selectivity of the neural population activity during experience. We identified cell assemblies by decomposing population vectors via activation covariance and classified them as Old, New/Recent, or Nonselective. We found that sleep reactivation is shaped by prior experience, with older and recently formed memories leading to stronger and more stable assembly reactivation than new sequences. New-biased assemblies underwent flexible reorganization and gradually formed higher-order links with old-biased assemblies, giving rise to 'metassemblies'—hierarchically coordinated networks that may support the integration of new and old experiences. To understand the cellular basis of these dynamics, we examined CA1 laminar organization and found that superficial pyramidal neurons contributed more to new-biased assemblies, whereas deep pyramidal neurons were more consistently involved over time. As memories aged, coactivity between these layers increased, suggesting subpopulations become progressively intertwined to support stable memories. These results reveal flexible, experience-driven memory ensemble organization and key network traits supporting long-term memory in primates.

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Nanosymposium

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Topic: I.07. Long-Term Memory

Support: Neurtex Brain Research Institute

Title: Neuronal excitability and sparse coding of remembered episodic memories in the human hippocampus

Authors: *C. W. TALLMAN[1], P. N. STEINMETZ[2], J. T. WIXTED[1];
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Abstract: Sparse coding is the leading theory of episodic memory representation in the human hippocampus. This theory holds that individual neurons represent few memories, and individual memories are represented by few neurons. Prior analyses of intracranial single-unit recordings from epilepsy patients identified a sparse coding signal during a continuous recognition memory task (Urgolites et al., 2022; Wixted et al., 2018). We detected the same signal in an independent dataset with a delayed study-test design (Faraut et al., 2018; Chandravadia et al., 2022), and we additionally found support for the neuronal allocation hypothesis. Specifically, the sparse coding signal was evident only in the hippocampus and only for remembered items associated with neural excitability at encoding (Tallman et al., under revision). Here, we tested whether the same excitability/sparse-coding patterns were also present in the continuous recognition memory experiment analyzed by Urgolites et al. (2022). Activity was recorded from 396 neurons in the hippocampus and from additional neurons in the amygdala, ACC, and PFC while patients (N=34) judged whether 120 words were novel or repeated. Words were repeated after 0, 1, 3, 7, 15, or 31 intervening items and grouped into short (0-3) lags presumed to reflect working memory and long (7-31) lags, presumed to reflect long-term memory (Rubin et al., 1999). Sparse coding and neural excitably effects would be expected only for long-lag items. Spike counts were normalized per trial and neuron. Excitability at encoding was defined as an increase from low pre-stimulus firing to high during-stimulus firing. Spike count distributions between repeated (second presentation) and novel (first presentation) items were compared using quantile-quantile (QQ) plots, and differences in skewness were statistically tested. Evidence of sparse coding was defined as a significant increase in skewness for repeated vs. novel items. Sparse coding effects were found only in the hippocampus. Critically, the effects were also observed (1) only for remembered items (hits) with long lags ($\Delta_{skew} = 0.71$, $p = .044$), (2) only for items with excitability at encoding ($\Delta_{skew} = 1.67$, $p = .008$), and, more specifically, (3) only for remembered items (hits) with excitability at encoding ($\Delta_{skew} = 2.07$, $p = .005$). These remarkably selective effects were evident in tests of long-term memory in two independent datasets collected by two separate labs, with differences in experimental design (e.g., memoranda, spike sorting). Thus, the observed selectivity of the sparse coding signal would appear to reflect general mechanisms of episodic memory and not procedure-specific details.

Disclosures: C.W. Tallman: None. **P.N. Steinmetz:** None. **J.T. Wixted:** None.

Nanosymposium

NANO011: Neuronal Dynamics Underlying Memory

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Time: Sunday, November 16, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO011.14

Topic: I.07. Long-Term Memory

Support: ERC 101001121

Title: Ripples facilitate human memory consolidation through reactivation of learning-related neurons

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Abstract: Sleep is fundamental for stabilizing newly formed memory traces. In rodents, hippocampal ripples drive consolidation of spatial information through coordinated reactivation of navigation-specific cells (e.g., place cells). However, the processes that support human memory consolidation are less well understood, particularly at the cellular level. Are learning-related neurons, i.e., those tuned to specific learned stimuli, preferentially recruited in service of memory consolidation? To tackle this question, we recorded activity of more than 1,400 neurons in the human medial temporal lobe (MTL) during an associative learning task and tracked their activity throughout subsequent overnight sleep. We found that MTL neurons exhibit robust reactivation during ripples, with significantly stronger activation during sleep ripples than during awake ripples. Crucially, ripple-mediated reactivation was stronger in neurons whose preferred stimuli were successfully recalled compared to those neurons whose preferred stimuli were not successfully recalled, directly linking ripple-triggered reactivation to human memory performance. Finally, we extended our analysis to the whole-brain level using macroscale intracranial recordings. We show that during epochs when learning-related neurons were active, large-scale activity patterns resembled those observed during initial stimulus presentation. This suggests that MTL reactivation supports cortical communication and integration of newly acquired information. Together, these findings uncover a mechanism by which ripples recruit learning-related neurons to support memory consolidation during human sleep.

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Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

Location: SDCC Rm 33

Time: Sunday, November 16, 2025, 8:00 AM - 10:30 AM

Presentation Number: NANO012.01

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant RF1MH133777

Title: A two-phase framework for similarity search across single-neuron data modalities

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Abstract: Efforts to classify neurons across transcriptomic, morphological, and electrophysiological domains lack an integrative framework. The bulk of single-cell studies captures only one data modality, while Patch-seq and related methodologies for multimodal data collection are constrained by scalability. This siloing of data collection has led to neuron classifications that rely predominantly on modality-specific attributes. Furthermore, as the space of data collection technologies diversifies and new studies add to the existing pool of data, technical variation compounds, hindering effective data integration. While existing batch correction methods for single-cell RNA-seq data are effective with limited reference samples, their reliance on batch identity as a model conditioner limits scalability in frameworks where new data are continually added. Additionally, there is a lack of consensus method for correcting technical bias across studies in other data modalities such as morphology and electrophysiology recordings. The relative sparsity of concurrent multi-modal measurements and their underrepresentation of some neuronal classes render them insufficient on their own for building a robust, unified framework for classification.
Here, we present a two-phase framework for learning joint neuron representations from -omics, morphology, and electrophysiology data using contrastive learning. In the first phase, unimodal encoders trained on large volumes of data with modality-specific augmentations produce embeddings that are resilient to technical variation across studies and chemistries. In the second phase, transcoders trained on Patch-seq and other multimodal datasets, which offer complementary measurements of the same cell, learn to project these unimodal embeddings into a shared representation space. From this framework, we construct a model across multiple data modalities, enabling the retrieval of cells with high similarity to a given reference cell. We benchmark these embedding models and demonstrate comparable performance to existing batch correction methods, even when trained without access to labels or batch identity. Finally, we provide API tools and tutorials to facilitate querying across a broad multi-tissue, multi-study atlas of mouse neurons. Such an approach to single-cell data integration that prioritizes scalability, leverages the vast reserves of archived single-cell data, and models noise through data augmentation in a way that mirrors the effects of technical variation will effectively facilitate the generating of a comprehensive neuron atlas to support neuroscience discovery.

Disclosures: B. Wang: None. **E. Schoenbeck:** None. **M. Babadi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hepta Bio.. **B. Huo:** None.

Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Title: A Brain Cell Type Resource Created by Large Language Models and a Multi-Agent AI System for Collaborative Community Annotation

Authors: R. LI[1], W. CHEN[1], Z. LI[1], R. CASTAÑEDA[2], J. LI[1], N. MAURYA[1], A. SOLANKI[1], H. HE[3], Z. WU[5], H. XU[4], M. J. HAWRYLYCZ[6], ***W. ZHENG**[1];
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Abstract: Single-cell RNA sequencing (scRNA-seq) has transformed brain cell type identification, yet annotating novel or rare types remains challenging due to incomplete reference markers. While Large Language Models (LLMs) trained on biomedical literature show promise, their use is limited by factual errors and weak biological reasoningTo address these limitations, we developed a multi-agent AI system—Brain Cell type Annotation and Integration using Distributed AI (BRAINCELL-AID)—for annotating brain cell types. BRAINCELL-AID includes a query agent and an annotation agent to leverage fine-tuned large language models (LLMs) trained on curated gene sets to learn principles of gene co-functionality. A literature agent and a retrieval-augmented generation (RAG) agent further enhance reliability by grounding annotations in peer-reviewed biomedical literature. Trained and evaluated on over 7,000 gene sets from MSigDB, BRAINCELL-AID achieved high concordance with biological ground truth: 77% of mouse and 74% of human gene sets contained annotations highly relevant to the known biology, when assisted by the RAG component. BRAINCELL-AID currently provides annotations for over 20,000 brain cell type-specific marker gene sets derived from single-cell RNA-seq data across 5,300+ brain cell type clusters spanning the entire mouse brain, integrating multiple biological signatures and contextual information. BRAINCELL-AID enables novel insights into brain cell function by identifying region-specific gene co-expression patterns and inferring functional roles of gene ensembles, associating gene sets with novel regulatory factor. BRAINCELL-AID also predicts new combinatorial roles, such as dual-transmitter signaling through the spatially restricted co-expression of Slc6a3, Gtf2a1l, and Aldh1a1 across SN, VTA, and RAmb modulating dopaminergic signaling with potential GABAergic co-transmission—the detection of similar molecular signatures in RAmb represents a novel observation. This highlights BRAINCELL-AID's ability to generate testable hypotheses about gene function and circuit mechanisms in understudied regions, supporting scalable, interpretable discovery in neuroscience.To ensure transparency and expert validation, the accurate, literature-backed BRAINCELL-AID annotations are provided as a shared resource for neuroscience community to collaboratively evaluate, refine, analyze and annotate brain cell types (https://biodataai.uth.edu/GSIS), enabling human-AI collaboration. This hybrid framework lays the groundwork for high quality annotation of a comprehensive, expert-informed brain cell atlas across species.

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Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Presentation Number: NANO012.03

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant UM1MH130981

Title: Single cell multi-omics integration with augmented variational autoencoders

Authors: ***N. WANG**[1,2], D. TURNER[1], V. NIETO CABALLERO[1], C. CARDENAS[1], H. FEINBERG[1], N. SCOTT[1], M. DEBERARDINE[1], S. DAN[1], D. YUAN[2], J. SCHEMBRI[1], L. CACERES[1], J. W. PILLOW[1,3,4], C. LEE[2], F. M. KRIENEN[1,5];
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Abstract: Advances in single-cell technologies and next-generation sequencing enable the routine acquisition of datasets containing millions of cells or nuclei. These datasets are often combined from many donors and different species, which poses new challenges for downstream analyses. Dataset integration - a starting point of most large-scale analyses - is impacted by unwanted sources of variation, such as batch effects. Existing approaches, such as scVI, integrate data using conditional variational autoencoders (VAEs) but may struggle with more complex data. We revisit scRNAseq integration by extending VAEs with a generalized linear model that enables easily adjustable batch correction strength and faster runtime performance. We examine how model architecture design impacts integration performance on commonly used benchmarking datasets. To demonstrate applications of our approach, we explore gene feature selection on a multi-species atlas of mammalian basal ganglia, perform cross-platform integration of human and mouse whole brain atlases, and integrate mouse and marmoset data across brain regions and developmental time points. Finally, we compare approaches for integrating single-cell multi-omics into spatial transcriptomics data. Our contributions include 1) a comprehensive overview and recommendations for single-cell integration and applications and 2) a flexible and scalable method for large-scale cross-species atlas integration.

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Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Presentation Number: NANO012.04

Topic: J.06. Computation, Modeling, and Simulation

Support: Division of Intramural Research, National Library of Medicine

Title: Exploring human neocortical cell types in the NLM Cell Knowledge Network

Authors: *Y. ZHANG[1], B. PENG[2], K. ARVIND[1], A. DESLATTES MAYS[1], M. DILLER[1], C. EASTWOOD[3], R. LECLAIR[1], Z. LU[1], A. V. PANKAJAM[1], N. ROTENBERG[1], R. SCHEUERMANN[1], W. SPEAR[1], B. R. XU[1];
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Abstract: The human neocortex consists of highly heterogenous cell types involved in complex brain functions, such as sensory perception, cognition, language, etc. Using single nucleus RNA-seq, Jorstad et al. (2023) [PMID: 37824655] reported a dataset with 1.1 million nuclei and identified 24 cell subclasses and 153 transcriptomically distinct cell types across 8 human neocortical areas by grouping similar transcriptional profiles into clusters, forming a cross-areal consensus taxonomy. However, this dataset has not been fully connected with prior knowledge, such as cell ontology, disease, and drug information, which limits its usability for the broader community.

Here, we present the National Library of Medicine Cell Knowledge Network (NLM-CKN), an open resource developed to capture and represent knowledge relating to human neocortical and other cell types. The NLM-CKN integrates single cell experimental data-derived cell characteristics with prior knowledge using an ontological framework. A conceptual data model was developed to represent this cell type knowledge in a collection of standardized, semantically structured (SSS) assertions (subject-predicate-object). The assertions are represented in a semantic knowledge graph, where biomedical entities, including cells, tissues, biomarkers, pathways, drugs, and diseases, are represented as nodes, and their relations as edges. Validated computational analysis pipelines were applied to the single cell genomics data, including a random forest machine learning-based algorithm, NS-Forest, to identify the minimal marker gene combinations, to molecularly characterize these cell types. To account for the hierarchical organization of the cell subclasses and cell types (i.e., clusters), a strategy of combining subclass and cluster markers via combinatorial evaluation in decision trees was adopted. We identified 460 unique marker genes that optimally classify the 153 cell types.

The NLM-CKN is intended to serve as a trusted source of knowledge about cellular phenotypes derived from validated computational pipelines applied to single cell experimental data and from the application of large language AI models for natural language processing of the scientific literature. The NLM-CKN is synergistic with other community resources by using the biomedical entities derived from high-quality reference ontologies from the OBO Foundry (e.g., Cell Ontology, UBERON, MONDO) and the SSS assertions from definitive sources of trusted information (e.g., Open Targets), allowing discovery of novel linkages through knowledge network traversal. A prototype of the NLM-CKN is accessible at http://cell-kn-mvp.org.

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Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Topic: J.06. Computation, Modeling, and Simulation

Support: NIMH Grant U24MH130919
NIMH Grant U24MH130918
NIMH Grant U24MH130988

Title: Evaluating AI-readiness of datasets shared through the BICAN Consortium Data Ecosystem

Authors: *P. M. BAKER[1], S. S. GHOSH[2], D. JARECKA[2], M. E. MARTONE[3], P. L. RAY[1], H. XU[4], C. L. THOMPSON[1];
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Abstract: The BRAIN Initiative Cell Atlas Network (BICAN, http://www.brain-bican.org) is a cutting edge, multi-institute consortium for multi-modal brain cell-type classification and atlasing across multiple species and developmental stages. The BICAN Data Ecosystem includes specimen portals, data repositories, metadata catalogs, software tools, workflows, and standards that work together to streamline and standardize access to diverse digital assets, supporting efficient use and reuse across the consortium and the broader scientific community. AI/ML tools are gaining considerable traction for supporting data analysis and experimental design within research communities. High-quality, well-documented, and expertly-curated datasets such as those generated by the BICAN consortium are critical for building and validating such models. To facilitate the use of BICAN datasets for these use cases, the consortium is developing best practices for establishing and maintaining 'AI-readiness' of consortium datasets, allowing us to maximize the impact of BICAN data for facilitating scientific discovery and generating novel insights. Multiple community groups have drafted and shared their guidelines for 'data-readiness for AI' (DRAI), including the NIH's Bridge2AI program, which outlines seven core criteria (CC) for biomedical data: 1) FAIRness, 2) Provenance, 3) In-depth Characterization, 4) Pre-Model Explainability, 5) Ethics, 6) Sustainability, and 7) Computability. (doi: https://doi.org/10.1101/2024.10.23.619844). The BICAN Data Ecosystem includes a comprehensive range of public-facing infrastructure that supports the core AI-readiness criteria identified by the proposed NIH Bridge2AI framework: BICAN Endorsed Standards and FAIR checklists (1); BICAN Metadata Schema Repository (CC 3, 4); BICAN-BY-NR licensing and

Informed Consent schema (CC 5); R24 Data APIs (CC 3, 6, 7); NIMP Specimen Portal (CC 3, 4, 5); BICAN-KB (CC 2, 4); BICAN Data Catalog (CC 3, 6). We will show how these readiness elements have and are being integrated in the BICAN data ecosystem when generating BICAN datasets. We are also developing and testing processes for DRAI evaluations of BICAN datasets, and we will discuss future work towards addressing any gaps and improvements. In addition to this pre-modeling work, BICAN has an AI Interest Group for supporting consortium AI modeling efforts, where issues around model validation and transparency are discussed. As DRAI criteria evolve with AI models and their requirements, we are working within BICAN to iteratively develop and share BICAN DRAI guidelines and workflows to support the development of cutting-edge AI use cases.

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Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Presentation Number: NANO012.06

Topic: J.06. Computation, Modeling, and Simulation

Support: Brain Aneurysm Foundation Award

Title: Validation and enhancement of automated EEG-based prediction of delayed cerebral ischemia following subarachnoid hemorrhage: a multi-center study

Authors: *Z. JIN[1], E. GILMORE[2], B. WESTOVER[3], J. KIM[4];
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Abstract: Background: Delayed cerebral ischemia (DCI) is a major cause of morbidity and mortality following aneurysmal subarachnoid hemorrhage (aSAH). Early detection and intervention are critical for improving outcomes. We previously developed an automated EEG-based prediction model using a "max carry-forward" approach, which dynamically selects EEG features with the largest intergroup differences within a 6-hour prediction window. We performed this study to validate the model's generalizability across medical centers using an independent patient cohort. **Methods:** We analyzed continuous EEG data from 79 aSAH patients from Yale New Haven Hospital (YNHH) as an external validation cohort, compared against our original 113-patient Massachusetts General Hospital (MGH) training dataset. Nine core EEG features were extracted hourly from days 2-10 post-aSAH: (1) epileptiform discharge (ED) burden, (2) Shannon entropy, (3) spectral power in delta, theta, alpha, beta, and total bands, (4) alpha/delta ratio, and (5) percent alpha variability. Additional spatial features included vascular territory-specific measures (anterior/middle/posterior cerebral artery distributions) and

hemispheric asymmetry indices. Model performance was evaluated using stratified 5-fold cross-validation under three scenarios: (1) external validation applying the original MGH-trained model to YNHH data, (2) retraining and testing exclusively on the Yale cohort, and (3) training and testing on combined MGH-YNHH data (n=192 patients). **Results:** Cross-institutional validation yielded distinct performance profiles across modeling strategies. Direct application of the MGH-trained model to YNHH data achieved moderate cross-site transferability (AUC = 0.67, day 7 post-aSAH), while institution-specific retraining substantially improved discrimination (AUC = 0.71, day 6 post-aSAH). The federated approach using combined MGH-YNHH data demonstrated intermediate predictive accuracy (AUC = 0.68, day 6), suggesting that institutional heterogeneity significantly impacts model performance beyond dataset size effects. **Conclusions:** This multi-center validation demonstrates the feasibility and potential clinical utility of EEG-based DCI prediction models, particularly when incorporating institution-specific training data. Ongoing work focuses on implementing LSTM architectures to better capture temporal dependencies, expanding multi-institutional training datasets, and integrating clinical variables to enhance prediction accuracy and clinical applicability.

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Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Presentation Number: NANO012.07

Topic: J.06. Computation, Modeling, and Simulation

Support: RF1MH133777

Title: A comprehensive framework for integration of multimodal single-cell omics data with probabilistic contrastive learning

Authors: *Y. XU[1,2], E. SCHOENBECK[2], M. BABADI[2,3], B.-X. HUO[2];
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Abstract: Data integration has been a long-standing challenge presented in single-cell omics data analysis, from batch effect correction within a single modality data to modality alignment across different data modalities. The rapidly increasing volume and diversity of single-cell data with modern technologies has only been exacerbating the situation. Current batch-removal methods have demonstrated great success in single-cell gene expression (scRNA-seq) data. However, the use of these tools in other modalities, for example single-cell chromatin accessibility (scATAC-seq) data, haven't been extensively evaluated. With more single-cell epigenomics technologies becoming available and accessible to research groups, millions of single cells have been profiled at different epigenetic levels, calling for an urgent need for reliable tools for batch correction across these modalities. Meanwhile, removing batch effects and integrating a single modality of data only give a limited view of cellular biology. Cross-

modality integration that combines multiple data modalities, from gene expression to chromatin accessibility, and even to chromatin structure, could potentially unlock a systematic understanding of cellular and molecular mechanisms. Current computational tools for modality alignment primarily focused on addressing modality differences, leaving within-modality batch correction under-addressed. To reach an effective and reliable integration for multi-omics single-cell data, we need a versatile computational tool that can handle data integration at multiple levels.Here we present a comprehensive integration framework through probabilistic contrastive learning. This framework addresses both within-modality batch correction and cross-modality alignment at the same time. We first show how our approach alleviates an unsolved problem of artificial alignment in single-cell data integration, compared to other existing methods. Then, we benchmark our method with major integration tools and demonstrate a superior performance of our framework in addressing both within-modality batch correction and cross-modality alignment at the same time. Finally, we show a comprehensive use case of integrating 9 modalities of mouse brain datasets, covering more than 1 million single cells collected through the BRAIN Initiative studies. We show that cross-study and -modality integration of mouse brain datasets with our method constructs a highly robust reference for multiple downstream analyses, including cross-study and -modality cell mapping and cell-type annotation.

Disclosures: Y. Xu: None. **E. Schoenbeck:** None. **M. Babadi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hepta Bio.. **B. Huo:** None.

Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

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Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant U24MH130918 (BICAN Knowledgebase)
NIH Grant R24MH117295

Title: Brainkb: a scalable infrastructure facilitating neuroscientific discovery via knowledge graphs and multi-agent systems

Authors: *T. CHHETRI[1,2], Y. CHEN[2], P. TRIVEDI[2], D. JARECKA[2], P. RAY[3], L. NG[4], S. S. GHOSH[2];
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Abstract: Scientific knowledge—expressed as assertions with corresponding evidence in scientific publications—is expanding rapidly. Assertions are the explicit claims or conclusions made in the publication, supported by experimental methods, and data. However, this knowledge

remains scattered across preprints, publications, data repositories, knowledge-bases, and presentations, each offering varying levels of recency and completeness. This fragmentation and rapid pace of scientific discovery have led to information overload. Addressing this challenge requires the precise capture of scientific knowledge—namely, assertions—along with essential provenance information, such as publications, methodologies, experiments, and data, which serve as evidence connecting assertions to their supporting context. These issues are magnified in neuroscience, where complex relations are often across subfields such as genomics, imaging, behavior, and disease. To tackle these challenges, we introduce BrainKB, an open-source web-based knowledge-base platform of the BRAIN Initiative Cell Atlas Network (BICAN) project. BrainKB uses knowledge graphs (KGs)—a graph data model—to represent and connect disparate neuroscientific knowledge in a FAIR (Findable, Accessible, Interoperable, and Reusable)-compliant manner where nodes represent entities of interest such as assertions along with associated evidence and provenance information, while edges capture the relationships among them, thus supporting new discovery. For example, the Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD) identified early vulnerability of somatostatin-positive inhibitory interneurons in Alzheimer's disease—an assertion supported by transcriptomic and spatial transcriptomic analysis and linking to Brain Initiative Cell Census Network (BICCN) reference atlas. By organizing complex and distributed scientific knowledge into an interconnected graph, BrainKB reduces information overload: it allows researchers to explore scientific assertions in context and efficiently trace claims across publications, datasets, and experiments. To achieve this, BrainKB uses agents powered by large language models to extract information and integrate it with curated resources such as ontologies and neuroscience schemas.

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Nanosymposium

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Presentation Number: NANO012.09

Topic: J.06. Computation, Modeling, and Simulation

Title: Bloom: Brain cell representations across species and modalities

Authors: *R. GALA[1], R. LIU[1], Z. YAO[1], S. OTTO[1], T. S. MOLLENKOPF[1], S. MUFTI[1], S. SENGUPTA[2], H. ZENG[1], U. SÜMBÜL[1];
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Abstract: Atlas-scale multimodal data—such as paired scRNA-seq and scATAC-seq—for brain cells are now becoming available across multiple species. There is a growing need for scalable models that can handle feature heterogeneity across modalities, while also accounting for differences and similarities across species. Such models can be used to study cell types in different contexts, and design experimental tools to manipulate them. We propose *Bloom*, a

large-scale transformer model pre-trained on multi-modal single-cell measurements, to obtain cell-level representations towards this goal. We use DNA sequences to inform the model about the individual genes and chromatin regions. We employ DNA language models, such as DNABERT2 and EVO2, to obtain fixed-length embeddings for DNA sequences of interest. These sequence-informed representations of genes and chromatin regions are combined with corresponding expression and accessibility values form the per-cell input to our model. *Bloom* is trained to predict gene expression or chromatin accessibility for query genes or regions, given a context set of genes and regions for individual cells. Through this objective, it learns cell representations that are useful for various tasks, including cell type annotation. *Bloom* can also impute missing data within- and across-modalities, and can also generate hypotheses to narrow down interactions between genes and regulatory regions.

Disclosures: R. Gala: None. **Z. Yao:** None. **S. Otto:** None. **T.S. Mollenkopf:** None. **S. Mufti:** None. **S. Sengupta:** None. **H. Zeng:** None. **U. Sümbül:** None.

Nanosymposium

NANO012: AI-Driven Integration and Analysis across Neuroscience Datasets

Location: SDCC Rm 33

Time: Sunday, November 16, 2025, 8:00 AM - 10:30 AM

Presentation Number: NANO012.10

Topic: J.06. Computation, Modeling, and Simulation

Support: U24MH136793

Title: Ai-Powered network-based drug repurposing for multiple neurological and psychiatric disorders via integration of single-cell and bulk multi-omics data

Authors: *Y. XIA;
Yale Univ., New Haven, CT

Abstract: Background: Neurological and psychiatric disorders are widespread and disabling, yet effective treatments remain a challenge. Genetic research is crucial for understanding these disorders and developing personalized therapies, as two-thirds of FDA-approved drugs are backed by human genetic evidence. Linking genetic variation to drug development requires understanding the intricacies of various brain cell types, which single-cell omic data now enables. These data revolutionize our understanding of how genetic variants impact brain function, leading to more targeted therapy regimens.**Methods:** We applied summary-data-based Mendelian randomization (SMR) to GWAS summary statistics for 21 brain-related traits (including schizophrenia, bipolar disorder, Alzheimer's disease, and Parkinson's disease). Seven QTL modalities—chromatin accessibility (caQTL), DNA methylation (mQTL), bulk expression (eQTL), PsychENCODE single-cell expression (sc-eQTL), splicing (sQTL), and protein abundance (pQTL)—were integrated to prioritize causal genes. We then performed single-cell analysis on the PsychENCODE BrainScope atlas (>2.8 million prefrontal cortex nuclei across 28 cell types) to map each SMR-prioritized gene to its relevant cell-type(s). Next, we constructed

multimodal, cell-type-specific networks integrating single-cell level protein-protein interactions (PPI), gene regulatory networks (GRN), and gene-coexpression networks. Finally, we applied AI-powered network-based drug repurposing algorithms to this graph to prioritize potential therapeutic candidates.**Results:** We identified 2,808 candidate causal genes. Cross-disorder analysis revealed 674 genes shared by two or more conditions . Among these, 54 were transcription factors and 82 encoded ligands or receptors, highlighting druggable targets. Single-cell mapping showed that SMR-prioritized genes had significantly higher cell-type-specific expression and lower inter-individual variability compared to background genes. The multimodal graph revealed cell-type-specific subnetworks, and network-based drug repurposing pinpointed compounds targeting hub genes in disease-relevant cell populations.**Conclusions:** By integrating SMR, single-cell mapping, and multimodal graph construction, we uncover cell-type-driven regulatory mechanisms across multiple brain disorders. Applying AI powered network-based drug repurposing to these cell-type-specific graphs yields prioritized therapeutic candidates. This pipeline links genetic risk to precise cellular contexts and accelerates the identification of repurposable drugs in neuropsychiatric disease.

Disclosures: Y. Xia: None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.01

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

Support: National Institutes of Health grant U01MH116487
Chan Zuckerberg Biohub - San Francisco
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The SYNGAP1 Research Fund
The Sorensen Foundation Fellowship in Child & Adolescent Psychiatry
DSAPN F99/K00: 1F99NS139536-01

Title: Convergence of Autism Proteins at the Cilium

Authors: *E. KOSTYANOVSKAYA[1], M. LASSER[1], B. WANG[1], J. SCHMIDT[1], E. BADER[1], K. MCCLUSKEY[1], C. BUTEO[1], A. R. SINDLEDECKER[1], J. ARBELAEZ[1], O. CASTILLO[1], J. DEA[1], K. HELDE[3], D. B. KASTNER[1], A. T. EHRLICH[1], M. STATE[1], A. WILLSEY[1], H. WILLSEY[2];
[2]Pscyhiatry and Behavioral Sci., [1]Univ. of California San Francisco, San Francisco, CA;
[3]SynGAP research Fund, Mill Valley, CA

Abstract: Hundreds of high confidence autism genes have been identified, yet the relevant molecular underpinnings are unclear. Our recent functional genetics work has suggested that these genes may share a common function at the cilium, a membrane-bound organelle critical for

neurogenesis, brain patterning, and neuronal activity-all processes strongly implicated in autism. Autism commonly co-occurs with conditions that are known to have strong ciliary underpinnings, including congenital heart disease, hydrocephalus, and blindness, but the role of autism genes at the cilium has not been systematically investigated. Here we demonstrate that autism proteins converge in expression, localization, and function at cilia, and that patients with pathogenic variants in these genes have cilia-related co-occurring conditions and biomarkers of disrupted ciliary function. This degree of convergence among genes spanning disparate functional annotations strongly suggests that cilia are relevant to autism biology and could be explored for therapeutic potential in treating impairing co-occurring conditions.

Disclosures: E. Kostyanovskaya: None. **M. Lasser:** None. **B. Wang:** None. **J. Schmidt:** None. **E. Bader:** None. **K. McCluskey:** None. **C. Buteo:** None. **A.R. Sindledecker:** None. **J. Arbelaez:** None. **O. Castillo:** None. **J. Dea:** None. **K. Helde:** None. **D.B. Kastner:** None. **A.T. Ehrlich:** None. **M. State:** None. **A. Willsey:** None. **H. Willsey:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.02

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

Support: R01MH12012
K01MH123757

Title: Distinct, parallel pathways of trafficking striatal GPCRs to primary cilia

Authors: P. DIRESU[1], H. CAHILL[1], C. BUTEO[2], B. L. KIEFFER[3], M. VON ZASTROW[4], ***A. T. EHRLICH**[5];
[1]Univ. of California, San Fransisco, San Fransisco, CA; [2]Univ. of California, San Fransisco, San Francisco, CA; [3]Strasbourg Translational Neurosci. and Psychiatry, INSERM UMR-S 1329, Univ. de Strasbourg (UNISTRA), Strasbourg, France; [4]Dept Psychiatry, UCSF, San Francisco, CA; [5]Univ. of California San Francisco, San Francisco, CA

Abstract: The primary cilium is a specialized, membrane-bound organelle characterized by a microtubule-based core and a selective diffusion barrier that restricts protein entry to those bearing a ciliary localization sequence (CLS). While CLSs vary between proteins and lack a conserved motif, ciliary trafficking of membrane proteins is largely thought to be mediated by TULP3. In this study, we investigate whether multiple transport pathways operate concurrently to deliver G protein-coupled receptors (GPCRs) to primary cilia within the same cellular environment. Focusing on two striatal-enriched GPCRs, dopamine receptor D1 and GPR88—both localized to the primary cilia of medium spiny neurons—we identify distinct, receptor-specific trafficking mechanisms. D1 requires its carboxyl terminus for ciliary targeting, while GPR88 relies on its third intracellular loop. Disruption of TULP3 via CRISPR results in partial

loss of D1 but complete loss of GPR88 ciliary localization, implicating TULP3-dependent and -independent mechanisms. Overexpression of the small GTPase RAB23 rescues D1 ciliary localization in TULP3 knockout cells, supporting a parallel transport model. Furthermore, using the RUSH system, we observe differential trafficking itineraries: D1 accesses cilia via the plasma membrane (lateral entry), whereas GPR88 traffics directly to cilia. Our findings reveal that neuromodulatory GPCRs utilize distinct, parallel trafficking pathways to reach primary cilia, even within the same cell type.

Disclosures: P. Diresu: None. **H. Cahill:** None. **C. Buteo:** None. **B.L. Kieffer:** None. **M. Von Zastrow:** None. **A.T. Ehrlich:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.03

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

Support: R01DK114008
F31DK142351
Indiana University Indianapolis Graduate Fellowship

Title: Ligand induced ciliary G-protein coupled receptor localization changes in primary neurons

Authors: *J. BOONE[1], K. BREWER[1], C. VAISSE[2], N. BERBARI[1];
[1]Biol., Indiana Univ. Indianapolis, Indianapolis, IN; [2]Univ. of California San Francisco, San Francisco, CA

Abstract: Solitary cellular appendages, called primary cilia, function as signaling centers on many cell types, including cells throughout the developing and adult central nervous system. Defects in cilia or their associated signaling are associated with altered behaviors and learning deficits in humans and animal models. Often these microtubule-based organelles coordinate specific G-protein coupled receptor (GPCR) signaling at the ciliary membrane, as observed in photoreceptors in vision and olfactory sensory neurons in olfaction. Unlike the cilia on these sensory neurons, which express a lone ciliary GPCR per cell, here we report that neurons from other brain regions decorate their cilia with more than one GPCR. Specifically, we have found that cilia from the mouse hypothalamus simultaneously localize Melanocortin receptor 4 (MC4R) and melanin concentrating hormone receptor 1 (MCHR1) in vivo. We investigate the implications of dual GPCR localization in primary neuronal cultures in vitro under different metabolic states mediated through ligands that act on MC4R; agouti-related peptide (AgRP) and alpha-melanocyte stimulating hormone (α-MSH). How cilia regulate the signaling of GPCRs remains unclear, but dynamic ligand dependent receptor localization seems to be an emerging theme for ciliary mediated signaling. Results from these studies will provide a better

understanding of mammalian cilia function and may also reveal mechanisms associated with their roles in behaviors.

Disclosures: J. Boone: None. **K. Brewer:** None. **C. Vaisse:** None. **N. Berbari:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.04

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

Title: Neuronal primary cilia modulate synaptic formation and plasticity

Authors: *J. GUO;
Univ. of Calgary, Calgary, AB, Canada

Abstract: All neurons possess primary cilia, hair-like signaling antennae protruding from the cell soma. Long considered as evolutionary remnants of little significance, primary cilia in the past decade have sparked enormous interest, fueled by the discoveries that mutations in 150+ genes that encode cilia-specific proteins can lead to 30+ human autosomal recessive disorders termed “ciliopathies”. The brain is particularly vulnerable to ciliary defects. Patients of ciliopathies show neurological and cognitive deficits such as ataxia, developmental dyslexia, and are often diagnosed with intellectual disability (ID), autism spectrum disorders (ASD), schizophrenia, bipolar disorder, depression, and anxiety. Using ciliopathy genetic mouse models, we found that neuronal primary cilia are centralized signaling nodes to influence spine morphogenesis, synaptic transmission, and plasticity. Mice with acutely induced ciliary defects in the cortex at the adolescence stage show social and learning defects. These data establish the direct causal link between primary ciliary signaling and synaptic development/function and reveal a previously underappreciated signaling mechanism central for neuronal development and neurodevelopmental disorders.

Disclosures: J. Guo: None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.05

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

Support: MOST-110-2917-I-564-010
Program for Breakthrough Biomedical Research, UCSF
NORC, UCSF
NIH R01

Title: Excavation of neuronal ciliome reveals the molecular signature of cilia-mediated synaptic functions

Authors: *C.-H. CHANG[1], V. TRINH[3], N. LOKESH[1], L. DINH[4], I. LO[4], J. SIMMS[4], M. POWNALL[1], M. KALOCSAY[5], M. NACHURY[2];
[2]Ophthalmology, [1]UCSF, San Francisco, CA; [3]Texas A&M Univ., College Station, TX; [4]Gladstone Institute, UCSF, San Francisco, CA; [5]Exptl. Radiation Oncology, MD Anderson Cancer Ctr., Houston, TX

Abstract: The neuronal cilium, a microtubule-based organelle, acts as a signaling center in the central nervous system. Individuals with defects in neuronal cilia exhibit anatomical abnormalities in brain structures and psychiatric symptoms. Recent research has shown frequent interactions between neuronal cilia and synapses, suggesting that cilia might monitor synaptic activities. However, the mechanisms by which neuronal cilia transduce signals and communicate with the neighboring synapses remain largely unknown. Despite significant progress in identifying ciliary components in kidney cells over the past decade, studying proteins comprising the neuronal cilia is still in the early stage. To overcome the difficulty in biochemically purifying cilia, we utilized TurboID, an engineered proximity labeling enzyme, and genetically modified the *Arl13b* gene in mice to produce the *Arl13b-GFP-TurboID* allele. Although ARL13B levels vary across different brain cell types, strong and specific biotin signals were observed in all cilia, indicating the biotin signals leveraging from the *Arl13b-GFP-TurboID* mouse as a universal cilia marker in the mouse brain. Quantitative proteomic analysis identified 232 proteins in neuronal cilia, including synaptic proteins, transporters, adhesion molecules, and neurotransmitter receptors. We validated several proteins from each functional category, and expansion microscopy revealed a close association between GPCRs, synaptic receptors, and neighboring synapses *in vivo*. The significant differences between neuronal and kidney cilia proteomes underscore the diversity and specialization of mammalian ciliary proteomes. The presence of adhesion molecules and neurotransmitter receptors on neuronal cilia membranes suggests their involvement in peri-synaptic transmission. To explore this function, we removed primary cilia from pyramidal neurons, resulting in notable behavioral changes with deteriorated neuronal activity. Overall, our findings provide detailed molecular insights into the expanding roles of neuronal cilia and their implications in neuropsychiatric disorders.

Disclosures: C. Chang: None. **V. Trinh:** None. **N. Lokesh:** None. **L. Dinh:** None. **I. Lo:** None. **J. Simms:** None. **M. Pownall:** None. **M. Kalocsay:** None. **M. Nachury:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.06

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

Support: NIH CA235749
NIH CA274595
NIH GM143276
NSF IOS-2143711

Title: Spatial proteomic landscape of brain cilia reveals new regulators of cortical development

Authors: X. LIU[1], O. TORRES GUTIERREZ[2], Y. AL ISSA[2], E. CAI[2], ***X. GE**[3];
[1]Mol. and Cell Biol., [2]Univ. of California, Merced, Merced, CA; [3]Univ. of California Riverside, Riverside, CA

Abstract: The primary cilium is a microtubule-based organelle that functions as a crucial signaling hub for the cell. Disruption of ciliary function leads to ciliopathies, a wide spectrum of disorders. A common feature of most ciliopathies is brain structural abnormalities, including malformations in cortical organization and axonal tract. This underscores the critical role of primary cilia in brain development.
The key step toward understanding brain ciliary function is identifying its *bona fide* protein components, a challenging task due to the cilium's small size and *in vivo* complex dynamics. To address this, we employed proximity labeling combined with quantitative mass spectrometry to define ciliary proteins in neural progenitors in the developing brain. First, our dataset identified region-specific proteins that localize to the primary cilium during key stages of brain development. Second, among the new ciliary proteins, we validated the ciliary localization of translation machinery components, suggesting an unexpected link between local protein synthesis and ciliary signaling in neural progenitors. Finally, we investigated the mechanistic roles of two new ciliary candidates associated with human diseases: MARCKS, a protein previously implicated in birth defects, and CKAP2L, a protein associated with Filippi syndrome. Functional analyses revealed that MARCKS is essential for the formation and maintenance of cilia, while CKAP2L is required for the transport of specific ciliary signaling components. CKAP2L knockout in mice results in defects in maintaining the neural progenitor pool, which subsequently leads to abnormalities in cortical architecture.
Collectively, our findings uncover a new layer of molecular complexity in the primary cilia of neural stem cells and offer insight into region-specific ciliary composition. This dataset provides a unique resource for understanding ciliary functions in brain development and the molecular etiology of developmental disorders

Disclosures: X. Liu: None. **O. Torres Gutierrez:** None. **Y. Al Issa:** None. **E. Cai:** None. **X. Ge:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.07

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

Support: NIH Grant R01DK060540
NIH Grant R01DK106404
Canadian Institutes of Health Research, Doctoral Foreign Study Award

Title: Assessing the in vivo PKA activity status at primary cilia of neurons

Authors: *P. K. PANDHER, A. BLAKE, A. BEDNARSKA, X. YUE, S. ZHANG, C. VAISSE, C. M. ALEXANDRE;
Diabetes Ctr., Univ. of California, San Francisco, San Francisco, CA

Abstract: Neuronal excitability is influenced by neuromodulators. Many neuromodulator receptors such as the dopamine receptor D1 (DRD1), somatostatin receptor 3 (SSTR3), serotonin receptor 6 (5-HT6R), and melanocortin-4 receptor (MC4R), localize to neuronal primary cilia. The primary cilium is an antennae-like organelle used as a signalling hub by cells, including most neurons. Primary cilia allow for segregation of signaling pathways. For example, most neuronal cilia express adenylate cyclase 3 (ADCY3) allowing for generation of cyclic AMP (cAMP) by stimulatory Gs coupled ciliary GPCRs. An increase in the ciliary pool of cAMP activates a local pool of Protein Kinase A (PKA) that is distinct from the cytoplasmic pool (PMID: 33932338). The cumulative effects of neuromodulators acting on GPCRs at primary cilia can be assessed by measuring the effects of a downstream effector of cAMP, such as PKA. Currently, there are no tools available to measure the activation status of PKA in cilia *in vivo.* To this end, we have developed an adeno-associated virus (AAV) delivered, Cre recombinase dependent ciliary PKA activity reporter. This reporter consists of a chimeric protein that includes a peptide phosphorylated by PKA -derived from vasodilator-stimulated phosphoprotein ($VASP_{148-164}$)-, a red fluorescent protein (RFP), and a ciliary localization signal. Phosphorylation of $VASP_{148-164}$ can be detected by a phospho-specific antibody, serving as a proxy for PKA activity. This Cre-dependent ciliary PKA activity reporter is administered in the lateral ventricle, enabling widespread brain diffusion of the virus, in neonate mice expressing a neuron specific Cre recombinase. Our preliminary data suggests that there is ciliary PKA activity in region specific subpopulations of *vGlut2* and *Gad2* expressing neurons. Furthermore, our data demonstrates that ciliary PKA activity is correlated with physiological Gs-coupled receptor activity in MC4R expressing neurons and can be inhibited through downregulation of *Adcy3* expression. Future studies will assess which GPCRs are expressed at the primary cilia of these specific neuronal subregions. In summary, our novel *in vivo* ciliary PKA activity reporter allows for reading the integrated output of ciliary GPCR signaling through ADCY, providing broader insight into how neuropeptides modulate and influence neuronal activity.

Disclosures: P.K. Pandher: None. **A. Blake:** None. **A. Bednarska:** None. **X. Yue:** None. **S. Zhang:** None. **C. Vaisse:** None. **C.M. Alexandre:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.08

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

Title: Rab23 mediates ciliogenesis and the leptin signaling response in the hypothalamus

Authors: *C.-H. HOR;
Hong Kong Baptist Univ., Kowloon Tong, Hong Kong

Abstract: RAB23 is a small GTPase that mediates ciliary trafficking and the Sonic Hedgehog signaling pathway. In humans, biallelic mutations in *RAB23* cause Carpenter syndrome, which is commonly associated with childhood obesity in affected patients. The underlying mechanisms by which the loss of *Rab23* leads to the development of obesity remain largely elusive. Here, we demonstrate that conditional knockout (CKO) of *Rab23* in the neural progenitor cells using Nestin-cre driver line, and the adeno-associated virus (AAV)-driven deletion of *Rab23* in the adult hypothalamus result in increased body weight and food intake, as well as a compromised response to leptin-induced satiety signals. Intriguingly, we observed a reduced number of ACIII-positive ciliated neurons and a dysregulated leptin-melanocortin signaling pathway in the hypothalamus neurons, indicating that dysregulated ciliary signaling in these nuclei may play a role in causing hyperphagic obesity in the *Rab23* mutant mice. Collectively, our findings reveal a novel role of *Rab23* in the hypothalamus regulation of satiety response.

Disclosures: C. Hor: A. Employment/Salary (full or part-time):; Hong Kong Baptist University.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.09

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

Support: MRC Doctoral Training Programme
School of Clinical Medicine Cambridge Trust Scholarship
Sackler Trust
New York Stem Cell Foundation (NYSCF) - Robertson Investigator
Chan Zuckerberg Initiative (CZI) - Ben Barres Early Career Investigator
Wellcome Trust

Title: Proximity proteomics of primary cilia in human hypothalamic neurons

Authors: *V. MACARELLI[1], T. SROKA[2], J. HANSEN[1], U. AXELSSON[3], E. LUNDBERG[1], S.-H. SHEU[4], F. MERKLE[5];
[1]Stanford Univ., Stanford, CA; [2]Saarland Univ., Homburg, Germany; [3]KTH - Royal Inst. of

Technol., Solna, Sweden; [4]Chan Zuckerberg Imaging Inst., Redwood City, CA, ; [5]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Background: Primary cilia are “sensory antennae” that project from the plasma membrane of most cell types, including mammalian neurons. In appetite-regulatory hypothalamic neurons primary cilia help sense metabolic factors to regulate food intake. However, the mechanisms by which primary cilia exert this function across different hypothalamic nuclei remain poorly understood. **Aims**: The sensory function of primary cilia is closely linked to the receptors and proteins that are present within these organelles. Therefore we hypothesized that characterizing the proteins present in the primary cilia of hypothalamic neurons would shed mechanistic insights into their sensory role and identify new therapeutic targets for obesity and related metabolic diseases. **Methods**: We targeted the ascorbate peroxidase APEX2 to primary cilia in human induced pluripotent stem cells (hiPSC)-derived hypothalamic neurons predominantly of the hypothalamic arcuate nucleus (ARC) to biotinylate and identify ciliary proteins. **Results**: Among the cilia-enriched proteins, we identified synaptic proteins, neurotransmitter receptors, and cell-cell adhesion and axon guidance proteins, extending recent findings that primary cilia interact with neuronal synapses. We also found genes associated with increased body weight and metabolic phenotypes that could represent new therapeutic targets including the lysophosphatidic receptor 1 (LPAR1), which we validated is cilia-localized and we confirmed that its ligand (LPA) mediates ciliary shortening. These findings provide insights into the molecular mechanisms by which primary cilia functionally impact appetite-regulatory neurons.

Disclosures: V. Macarelli: None. **T. Sroka:** None. **J. Hansen:** None. **U. Axelsson:** None. **E. Lundberg:** None. **S. Sheu:** None. **F. Merkle:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.10

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

Support: BRF Scientific Innovation Award
1F32NS136265-01
NIH T32NS007292

Title: Modulation of excitatory synaptic strength by SSTR3-mediated ciliary signaling in vivo

Authors: *N. F. WONG[1], L. TERESHKO[3], P. SENGUPTA[1], G. G. TURRIGIANO[2];
[1]Biol., [2]Dept of Biol., Brandeis Univ., Waltham, MA; [3]Biogen, Cambridge, MA

Abstract: Primary cilia of pyramidal neurons (PNs) in the visual cortex (V1) localize high levels of the somatostatin receptor 3 (SSTR3) neuropeptide receptor. We previously showed that SSTR3-mediated ciliary signaling modulates excitatory synaptic strength and number onto

cultured neocortical PNs, thereby shifting the balance of excitation/inhibition (Tereshko et al. 2021). To determine whether SST signaling also regulates excitatory synapses *in vivo* during postnatal development, we used an AAV-mediated short hairpin to knockdown SSTR3 in rat primary V1. The AAV was stereotactically injected into V1 of adolescent P14-P16 rats. 3-10 days after injection, whole-cell voltage clamp of GFP-expressing V1 PNs was performed to measure postsynaptic strength. We also performed posthoc image analysis on these neurons to quantify spine density and morphology. We found that SSTR3 knockdown increased the number and strength of excitatory synapses onto layer 2/3 PNs, without impacting inhibitory synaptic strength. This increase in synaptic strength was transient, while growth in synapse number was more persistent. Moreover, SSTR3 knockdown in adult animals (~P40) similarly affected excitatory synapses of layer 2/3 PNs, indicating that SSTR3 signaling regulates excitatory postsynaptic strength from adolescence through adulthood. In contrast, although layer 5 PNs also express ciliary SSTR3, knockdown of SSTR3 in these cell types did not affect synaptic strength, suggesting a layer-specific role for ciliary SSTR3 signaling in modulating excitatory synapses. SSTR3 KD can result in an overall increase in cFOS-labeled cells, reflecting an increase in overall neuronal activity. This modulation of neuronal activity by ciliary neuropeptidergic signaling suggests a disruption of visual processing, but not learning, during a vision-dependent prey capture learning paradigm. We propose this as a novel mechanism by which neuronal circuit can maintain their homeostatic neuronal set-point through SSTR3-mediated ciliary signaling.

Disclosures: N.F. Wong: None. **L. Tereshko:** None. **P. Sengupta:** None. **G.G. Turrigiano:** None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.11

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

Support: T32DK007418
A.P. Giannini Postdoctoral Fellowship
R01HD089918
R01AR054396

Title: Primary cilia as signaling centers for neuronal appetite control.

Authors: *M. J. KONJIKUSIC;
Biochem. and Biophysics, UCSF, San Francisco, CA

Abstract: Regulation of central energy balance is critical for long term health. Energy balance is dictated by feeding behaviors, satiety and hunger. A central regulator of this process is the Leptin/Melanocortin pathway in hypothalamic neurons. Work from our lab and others has found

that primary cilia, small antenna-like projections of the cell, help orchestrate portions of the leptin/melanocortin pathway in the hypothalamus. In support of the role of primary cilia in energy homeostasis is that almost all diseases associated with cilia-dysfunction, present with obesity. Interestingly, recent work has linked mutations in *GPR75*, an orphan G-Protein Coupled Receptor, in humans to leanness, and to resistance to obesity on high fat diets in mice. Recent reports have found that GPR75 similarly localizes to primary cilia in cultured hypothalamic neurons. However, GPR75 is more widely expressed in the brain than just in the hypothalamus. There has been debate about whether GPR75 remains as an orphan receptor or whether previous reports have properly identified a ligand. Moreover, what cell types in the brain depend on GPR75 for feeding behaviors, what pathways are affected by GPR75 activity, and whether cilia-enrichment is necessary for GPR75 activity remains unexplored. In this work, we describe that GPR75 is a basally active GPCR using cell culture models. GPR75 basal activity affects both its localization to the primary cilium and activity of PKA at the primary cilium. Basal trafficking of GPR75 functions through a GRK2/B-Arrestin dependent pathway. Moreover, basal activity of GPR75 is inhibited by blocking activity of the arachidonic acid pathway. Interestingly, we have localized endogenously tagged GPR75-mCherry to cortical neuronal cilia in mouse brain sections, not in the hypothalamus. Altogether this work shows GPR75 is basally active, localizes to cilia in the brain outside of just the hypothalamus, and affected through the arachidonic pathway. Ongoing work is using conditional mouse genetics to discover what cell types have GPR75 activity, and how cilia affect this activity *in vivo*.

Disclosures: M.J. Konjikusic: None.

Nanosymposium

NANO013: Primary Cilia Signaling and Function

Location: SDCC Rm 25A

Time: Sunday, November 16, 2025, 1:00 PM - 4:00 PM

Presentation Number: NANO013.12

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

Support: GRK2381
Excellence Strategy

Title: Disease-linked mutations reveal ciliary dysfunction as a converging mechanism in parkinson’s disease

Authors: N. KARMALI, ***N. MOHD RAFIQ**;
Univ. of Tübingen, Tübingen, Germany

Abstract: Synaptojanin-1 (SJ1) is a neuron-enriched phosphoinositide phosphatase essential for the uncoating of endocytic proteins during synaptic vesicle recycling. A Parkinsonism-linked R258Q mutation that selectively impairs its 4-phosphatase activity causes severe neurological phenotypes in humans and SJ1^RQKI mice. Using iPSC-derived dopaminergic (DA) neurons from SJ1 knockout and SJ1^RQKI lines, we observed not only enhanced clustering of endocytic

factors at synaptic terminals, but also significant elongation of primary cilia. These cilia exhibited abnormal accumulation of Cav1.3 calcium channels and ubiquitin chains, pointing to a defect in protein turnover at the ciliary base—where SJ1 was found to localize—indicating a novel role in maintaining ciliary homeostasis and signaling in DA neurons (Rafiq et al 2024, Volos et al 2025).To explore whether ciliary dysfunction represents a broader pathogenic mechanism in Parkinson's disease (PD), we analyzed iPSC-derived oligodendrocyte lineage cells harboring the LRRK2 p.G2019S mutation. Single-cell transcriptomics revealed expansion of ciliated populations and downregulation of cilium-related pathways, including defects in cilium movement and semaphorin-plexin signaling. Pseudotime trajectory analysis also uncovered altered sonic hedgehog (SHH) signaling dynamics (Dehestani et al 2025, preprint).Together, these findings suggest that disruption of ciliary structure and signaling is a shared consequence of distinct PD-associated mutations. Our work positions SJ1 as a critical regulator of cilia in DA neurons and highlights cilia-related pathways as convergent points of vulnerability in Parkinson's disease.

Disclosures: N. Karmali: None. **N. Mohd Rafiq:** None.

Nanosymposium

NANO014: Oxidative Stress and Cellular Mechanisms in Alzheimer's Disease and Brain Injury

Location: SDCC Rm 33

Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO014.01

Topic: C.01. Brain Wellness and Aging

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Gun och Bertil Stohnes Stiftelse
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Lindhés Advokatbyrå

Title: Oxysterols as mediators of neurodegeneration: A mechanistic view

Authors: *R. LOERA-VALENCIA;
Karolinska Institute/Tecnologico de Monterrey, Chihuahua, Mexico

Abstract: Oxysterols, oxidized derivatives of cholesterol, have emerged as key metabolic mediators linking systemic dyslipidemia and brain dysfunction. Among these, 27-hydroxycholesterol (27-OHC)—a peripheral oxysterol that crosses the blood-brain barrier—has gained attention for its multifaceted neurotoxic effects. Our research has systematically demonstrated that 27-OHC accumulation disrupts neuronal homeostasis through mitochondrial

dysfunction, altered cholesterol trafficking, and synaptic impairment. In hippocampal neurons, chronic exposure to 27-OHC leads to dysregulation of the PI3K/Akt pathway, promoting tau hyperphosphorylation and memory deficits. These synaptic impairments are exacerbated by 27-OHC-induced disruptions in NMDA receptor signaling and cholesterol metabolism, creating a self-reinforcing loop of neuronal vulnerability.
Beyond neurons, we reveal that oxysterols exert distinct yet convergent neurodegenerative mechanisms in glial cells. In astrocytes, 27-OHC impairs cholesterol efflux and increases oxidative stress, shifting their phenotype toward a pro-inflammatory and metabolically compromised state. This astrocytic dysfunction compromises neuronal support and amplifies neuroinflammation. In oligodendrocytes, our data suggest that oxysterol-mediated redox imbalance disrupts myelin integrity and mitochondrial bioenergetics, contributing to white matter degeneration observed in aging and Alzheimer's disease (AD).
Using multi-omics approaches and behavioral assays in murine models, we provide a comprehensive framework in which oxysterols act as upstream regulators of cell-type-specific dysfunction. Importantly, metabolomic profiling of oxysterol species in AD brains reveals a consistent elevation of 27-OHC alongside perturbations in other oxysterols such as 24S- and 7-ketocholesterol, underscoring a broader dysregulation of sterol homeostasis in the diseased brain. These findings position oxysterols not only as biomarkers but also as mechanistic drivers of neurodegeneration through integrated disruptions in neuronal, astrocytic, and oligodendrocytic function. Targeting oxysterol synthesis, transport, or downstream signaling offers a promising avenue for therapeutic intervention across a spectrum of neurodegenerative conditions.

Disclosures: R. Loera-valencia: None.

Nanosymposium

NANO014: Oxidative Stress and Cellular Mechanisms in Alzheimer's Disease and Brain Injury

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Topic: C.01. Brain Wellness and Aging

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Title: Differentiation status of neural progenitor cell-derived neurons affects protein expression differentially under conditions of oxidative stress

Authors: *R. S. DUNCAN[1], S. M. RIORDAN[1], P. KOULEN[2];
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Abstract: Neural progenitor cells (NPCs) are commonly used to generate differentiated neurons as *in vitro* model systems of neuronal differentiation, neurodegeneration, and neuroprotection. Since many neurodegenerative diseases exhibit pathophysiological oxidative stress, we

determined whether oxidative stress affected the proteomes of undifferentiated and differentiated NPCs differently. In order to determine such a potential effect of differentiation status on the cellular response to oxidative stress, we exposed both undifferentiated and differentiated NPCs to 10μM tert-butyl hydroperoxide (tBHP) for 24 hours. We used a proteomics approach to identify differentially expressed proteins resulting from either differentiation status, oxidative stress, or both. We identified enriched biological process pathways including ones regulating synapse maturation (1.3-fold, $p < 0.01$), metal ion transport (0.92-fold, $p < 0.05$), and synaptic signaling (0.92-fold, $p < 0.05$), and negatively regulating programmed necrotic death (1.3-fold, $p < 0.05$). Our results consisted of 7,538 identified proteins from which we identified at least 40 differentially expressed proteins known to be involved in neuronal differentiation as well as several novel proteins that have not been previously identified associated with the differentiation process. Furthermore, we determined the effect of differentiation status on the cells' response to oxidative stress. Oxidative stress altered the expression levels of over 80 proteins involved in the cell cycle and differentiation process in undifferentiated NPCs, while in differentiated cells, it altered the expression of over 100 proteins involved in synaptic and cytoskeletal function.

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Nanosymposium

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Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01AG071512

Title: The protective roles of the biliverdin reductase/bilirubin axis in the brain

Authors: *B. D. PAUL[1], C. VASAVDA[4], R. KOTHARI[5], S. CHAKRABORTY[2], S. J. TRIPATHI[3], A. A. PIEPER[6], B. THOMAS[7], S. H. SNYDER[5];
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Abstract: Bilirubin is one of the most measured metabolites in the blood, yet its exact functions are still being elucidated. Biliverdin reductase A (BVRA) is the main biosynthetic enzyme for bilirubin and a component of heme catabolism responsible for converting biliverdin to bilirubin. While the antioxidant and anti-inflammatory roles of bilirubin in vitro and in peripheral tissues have been well studied, the functions of bilirubin in the brain have been less explored. We show that bilirubin, being lipophilic, protects the lipid rich compartments of cells and prevents lipid

peroxidation and plays a complementary role to the protective effects of the major water-soluble antioxidant, glutathione (GSH), which protects the hydrophilic compartments. The brain is lipid-rich and metabolically highly active and is especially susceptible to lipid peroxidation. We have shown that mice lacking BVRA display elevated lipid peroxidation, mitochondrial dysfunction, and compromised ability to neutralize free radicals and are highly susceptible to neuronal damage. Furthermore, we have shown that bilirubin directly scavenges superoxide radicals (O_2•−) generated during mitochondrial respiration and mediates neuroprotection. Additionally, we show that BVRA, mediates synaptic signaling through the focal adhesion kinase (FAK). Thus, studying the actions of BVRA and bilirubin will yield deeper insights into a novel and hitherto underappreciated neuroprotective pathway in the brain, which can be harnessed to develop therapeutics for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and stroke.

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Nanosymposium

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Presentation Number: NANO014.04

Topic: C.01. Brain Wellness and Aging

Support: R01AG071512 BDP
R21AG073684 BDP

Title: Neuroprotective roles of the cystathionine gamma-lyase/hydrogen sulfide axis in the brain

Authors: *S. CHAKRABORTY[1], S. J. TRIPATHI[1], E. VAZQUEZ-ROSA[2], S. BARKER[3], K. CHAUBEY[4], H. FUJIOKA[5], E. MILLER[7], M. FILIPOVIC[8], S. H. SNYDER[9], A. A. PIEPER[6], B. D. PAUL[1];
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Abstract: The reverse transsulfuration pathway includes several enzymes that help regulate the levels of glutathione, homocysteine, and the gasotransmitter hydrogen sulfide (H_2S). In the brain, maintaining optimal levels of these substances is essential for regulating cerebral blood flow, ensuring the integrity of the blood-brain barrier, promoting neuroplasticity, and maintaining redox status. A key component of this pathway is cystathionine gamma-lyase (*Cth*), the neuronal

enzyme responsible for generating the semi-essential amino acid cysteine and H_2S. Notably, H_2S regulates protein activity through sulfhydration/persulfidation, modifying reactive cysteine residues. This process is key in neurodegenerative disorders like Alzheimer's disease and traumatic brain injury. However, the specific mechanisms are not fully understood. We have addressed this question by systematically characterizing *Cth*$^{-/-}$ mice by analyzing neuropathology and persulfidation status. The *Cth* $^{-/-}$ mice display early neurodegeneration that progresses with age, as well as elevated oxidative stress, neuroinflammation, and associated changes. These pathological changes are accompanied by behavioral abnormalities and aberrant stress responses. Taken together, our results suggest that *Cth* in the brain critically regulates redox balance, and persulfidation status, which together protect the brain from neurodegeneration and related behavioral abnormalities.

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Topic: C.01. Brain Wellness and Aging

Support: ERC CoG 864921

Title: Protein thiol alterations drive pathologic liquid-liquid phase separation in the aging brain

Authors: *M. FILIPOVIC;
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Abstract: Cellular homeostasis relies on the precise regulation of chemical processes, including protein posttranslational modifications (PTMs) and biomolecular condensation. Aging disrupts the equilibrium of these processes, increasing susceptibility to disease and mortality. Using advanced chemoproteomic techniques, we investigated cysteine PTMs in the mouse brain. Our findings reveal that age-related increases in thiol oxidation promote the formation of biomolecular condensates, leading to protein aggregation. In contrast, protein persulfidation, regulated by endogenous sulfide production, inhibits biomolecular condensation, thereby preserving protein function. These cysteine PTMs significantly alter the phase separation properties of synapsin-1, a key regulator of synaptic activity, contributing to impaired neurotransmitter release associated with aging and neurodegenerative diseases. Furthermore, increased oxidation of cysteine in GAPDH, an essential glycolytic enzyme, induces condensation and aggregation, while persulfidation promotes the dissolution of these condensates. Mice deficient in cystathionine γ-lyase (CSE), the enzyme responsible for endogenous sulfide

production, exhibit reduced lifespans and spontaneously develop neurofibrillary tangles with age. Our results highlight the therapeutic potential of protein persulfidation in reversing aberrant liquid-liquid phase separation (LLPS), offering new avenues for using sulfide donors to mitigate age-related diseases.

Disclosures: M. Filipovic: None.

Nanosymposium

NANO014: Oxidative Stress and Cellular Mechanisms in Alzheimer's Disease and Brain Injury

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Topic: C.01. Brain Wellness and Aging

Support: NIH 2P20GM121307-06
Alzheimer's Association

Title: Reactive sulfide species in Vascular cognitive impairment and dementia in Aging and Alzheimers disease

Authors: K. MURNANE[1], ***G. K. KOLLURU**[2];
[2]Pathology, [1]LSU Health-Shreveport, Shreveport, LA

Abstract: Vascular cognitive impairment and dementia (VCID) is a well-recognized phenomenon contributing to neurodegeneration and long-term cognitive dysfunction in numerous disease states, including Alzheimer's disease. Importantly, VCID is associated with impaired cerebral blood flow (CBF) and changes in blood-brain barrier (BBB), which can be significantly affected by aging; however, the underlying mechanisms of VCID are poorly defined. Reactive sulfur species (RSS), including hydrogen sulfide (H_2S), persulfide (RSSH), and polysulfide (RSS(n)), are emerging as critical signaling molecules in human health and disease. Cystathionine gamma lyase (CSE) is the most prominent H_2S/RSS-producing enzyme in the endovascular system and an essential component in regulating key vascular functions. Our group has shown that CSE-dependent H_2S/RSS production is critically important for the regulation of vascular function and remodeling, and in patients with clinical vascular disease. Our data indicate that CSE/RSS levels decrease with age in mice and humans alike. We observed that CSE/RSS participates in regulating cerebral vascular tone and dilation, controlling blood flow. A deficiency of CSE/RSS leads to a redox imbalance and increased reactive oxygen species (ROS)/superoxide levels, as well as endoplasmic reticulum (ER) stress, resulting in impaired revascularization and blood flow, which can contribute to cognitive impairment. We observed significant improvement that can be rectified by overexpression of CSE or exogenous RSS-donor therapy in young and aged WT and CSEKO mice. Exogenous CSE/RSS can decrease ROS, improve blood flow, BBB, and rectify cognitive impairment. Additionally, our observations indicate decreased CSE expression and subsequent RSS levels in *$APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}}$*

^{F}/AD mice on cognitive function. These results will provide novel insights into the therapeutic roles of sulfide on neurovascular blood flow and cognitive function.

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Nanosymposium

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Topic: C.01. Brain Wellness and Aging

Support: DFG RTG2550/1736 project ID 411422114

Title: Thiol-based regulation of synaptic clustering of gephyrin

Authors: *E. H. W. BRUCKISCH[1], T. GEHLING[2,3], F. LIEBSCH[4], F. R. EGGERSMANN[5], L. JACOBS[2,3], B. DEJANOVIC[6], J. RIEMER[2,3], P. KLOPPENBURG[2], G. SCHWARZ[4]; [1]Dept. of Chem. and Biochem., Univ. of Cologne, Inst. for Biochem., Cologne, Germany; [3]Inst. for Biochem., [2]Univ. of Cologne, Cologne, Germany; [4]Univ. of Cologne, Koeln, Germany; [5]Univ. of Cologne, Cologne, ; [6]Inst. of Biochem., Cologne, Germany

Abstract: Gephyrin is the principal scaffolding protein at inhibitory post-synapses where it clusters glycine and GABA type A receptors ($GABA_ARs$). Gephyrin undergoes multiple post-translational modifications and protein interactions that collectively regulate inhibitory synapse formation. Recently, it was demonstrated that reactive oxygen species (ROS) play a central role in enhancing inhibitory signal transmission thus extending their role beyond oxidative stress in disease and aging. However, the underlying molecular mechanisms mediating these functions have remained elusive. Gephyrin harbors multiple surface-exposed cysteines that are spread over its three functional domains (G, C, E). Cysteine residues have been found to undergo reversible S-palmitoylation and S-nitrosylation. While earlier studies reported an increase in gephyrin synapses in a palmitoylation dependent manner of Cys212 and Cys284, we now also found that the same two residues mediate nNOS-mediated S-nitrosylation of gephyrin leading to more, but smaller gephyrin clusters. Furthermore, we found that dynein light chain (DLC), a ligand binding to gephyrin, and nNOS collectively modulate inhibitory synapses through a reciprocal regulation of gephyrin S-nitrosylation and S-palmitoylation. In addition, we show that H_2O_2-induced oxidation of gephyrin cysteines triggered reversible, synaptic multimerization through disulfide bridge formation providing more receptor binding sites, leading to proteolytic protection and enhanced liquid-liquid phase separation. We identify mitochondria-derived ROS as a physiological source of ROS and observed oxidized gephyrin multimers *in vivo* supporting the functional relevance of gephyrin redox regulation. Finally, recent structural work identified the molecular basis of gephyrin clustering by E-domain-mediated phase separation and filament formation which is impacted by one of the surface-expressed and oxidation sensitive cysteines.

Collectively, our findings suggest that cysteines in gephyrin control synaptic localization and clustering as regulatory redox-switches thereby establishing a link between neuronal and mitochondrial activity.

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Nanosymposium

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R01 AG056259
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Title: Redox regulation of neuroinflammatory pathways contributes to damage in alzheimer’s disease Brain

Authors: L. N. CARNEVALE, *S. A. LIPTON;
The Scripps Res. Inst., La Jolla, CA

Abstract: In addition to the presence of abnormal protein aggregates, Alzheimer’s disease (AD) is marked by chronic neuroinflammation, a process that exacerbates neuronal synaptic dysfunction and progression of the disease. While cGAS-STING (cyclic GMP-AMP synthase - stimulator of interferon genes) activation has emerged as a key component of neuroinflammation, it has also posed a challenge in therapeutic targeting, particularly in the context of neurodegeneration. One issue is that STING plays a critical role in immune defense, but its activation by endogenous factors in AD contributes to damaging neuroinflammation. However, the mechanisms driving this aberrant activation have remained unclear, limiting therapeutic progress. In this study, we identify protein S-nitrosylation of STING (forming SNO-STING) as a novel redox modification that mediates STING oligomerization/activation and promotes aberrant immune signaling in AD. Using redox chemical biology, mass spectrometry, and in vivo models, we demonstrate that pathologically-relevant levels of SNO-STING are present in postmortem human sporadic AD brains, hiPSC-derived microglia exposed to amyloid-β (Aβ) and α-synuclein (αSyn) aggregates, and 5xFAD transgenic mice. S-nitrosylation

facilitates disulfide-bond formation and thus contributes to STING oligomerization, leading to type I interferon signaling and early neuroinflammation. This activation is a key event in the progression of synaptic loss and neuronal damage in the disease. Importantly, STING activation remains a critical barrier for therapeutic targeting of STING, as inhibiting key activation steps has proven challenging for Big Pharma. Our work identifies the cysteine residue underlying S-nitrosylation of STING as a druggable target, offering a potential strategy for modulating STING activation and mitigating neuroinflammation. By investigating redox-driven immune activation in the context of neurodegeneration, we not only expand our understanding of STING regulation, but also propose a translational approach to targeting this pathway to promote brain resilience and halt disease progression.

Disclosures: L.N. Carnevale: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The Scripps Research Institute. **S.A. Lipton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The Scripps Research Institute.

Nanosymposium

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Topic: C.01. Brain Wellness and Aging

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Title: Mitochondria-derived double-stranded RNA may contribute to neuroinflammation in brain aging and Alzheimer’s disease

Authors: *R. L. DOSER, T. J. LAROCCA;
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Abstract: Mitochondrial dysfunction and inflammation are highly interconnected and both central to brain aging and neurodegeneration. However, it remains unclear what initiates these adverse biological processes early on in aging, which limits our ability to explain or prevent age-related cognitive dysfunction and neurodegenerative diseases, such as Alzheimer’s disease (AD). One potent activator of inflammatory signaling is cytoplasmic double-stranded RNA (dsRNA), and the mitochondrial transcriptome may be a major source of endogenous dsRNA via bidirectional transcription of the circular mitochondrial chromosome resulting in long, complementary RNAs. Mitochondria-derived dsRNA (mt-dsRNA) released into the cytoplasm has been shown to contribute to cellular senescence, certain cancers and autoimmune disorders, but it is not clear whether mt-dsRNA cause neuroinflammation with brain aging or AD.

Therefore, we are investigating whether mt-dsRNA release may be an unrecognized and targetable driver of neuroinflammation in aging and AD. To initially test this hypothesis, we performed multiple bioinformatics analyses of large RNA-seq datasets on human brain aging and AD, and on related in vitro cell/neuron RNA-seq datasets. We found evidence of mt-dsRNA formation and cytoplasmic accumulation in older and AD brains, and that the abundance of mitochondrial RNA correlates with inflammatory signaling and dsRNA binding protein gene expression. To follow up on these findings in vitro, we have optimized the tagging and subcellular visualization of mitochondrial transcripts in fibroblast-derived transdifferentiated neurons from young, old and AD donors. Using these advanced cell culture and subcellular imaging techniques in combination with transcriptomics and treatments that enhance mitochondrial health or block avenues of dsRNA release, we have gained a more mechanistic insight into the cause and effect of mt-dsRNA release in aging and AD.

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

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Title: Neuron-specific intracellular shift of extracellular matrix proteases as an earlier event preceding neuroinflammation in an Alzheimer's disease-like mouse model

Authors: *M. MOROTTI[1], C. CODAZZI[2], F. D'ALELIO[1], C. D'AMELIO[2], C. FEROLETO[1], C. CALIGIURI[2], I. PAOLETTI[1], D. D. LI PUMA[2], G. DE CHIARA[3], L. LEONE[2,1], C. GRASSI[2,1], M. V. PODDA[2,1];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia globally, yet its earliest molecular events remain inadequately characterized. Neuropathological changes commence long before clinical symptoms manifest, suggesting a potential window for preventive intervention, contingent upon the identification of reliable, mechanism-linked early markers. Recent evidence has highlighted the extracellular matrix (ECM) as a crucial regulator of neuronal homeostasis, synaptic integrity, and the neuroimmune interface. Among ECM-modifying enzymes, matrix metalloproteinases (MMPs) are of particular interest due to their involvement in neurovascular remodeling and inflammation during advanced

stages of AD, although their role in the prodromal phase remains unclear. In this study, we examined the dynamics of ECM-associated proteases in a mouse model of sporadic AD induced by labial HSV-1 infection followed by repeated viral reactivation via thermal stress (TS). This model replicates distinct pathological stages: 2×TS mice, subjected to two monthly reactivation cycles, exhibit mild cognitive impairment-like features, while 7×TS mice develop functional and molecular AD-like phenotypes. Samples were collected at defined time points and analyzed using histological, immunofluorescent, and molecular methods. A shift in the localization of a specific ECM-regulating protease, accompanied by localized matrix disruption, was particularly evident in neurons of 2×TS HSV-1 mice compared to 2×TS mock and 7×TS mice. Notably, nuclear localization of MMPs occurred in the absence of cytokine induction or glial activation, supporting its identification as an early, inflammation-independent event. Colocalization of MMP with apoptotic markers suggests a potential link to early neuronal stress. This phenomenon was independently validated in a triple transgenic AD mouse model, supporting its generalizability across AD models. Our results identify a novel mechanism involving neuron-specific intracellular translocation of ECM enzyme localization that may contribute to the onset of neuronal vulnerability in AD and might be relevant as a potential early biomarker or therapeutic target.

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Nanosymposium

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Topic: C.01. Brain Wellness and Aging

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Rebecca E. Barchas, MD, Professor in Translational Psychiatry of Case Western Reserve University

Title: The role of early biomarkers in depression linked to Alzheimer's disease in both clinically relevant human brains and the TgF344AD rat model for the purpose of elucidation and therapeutic development.

Authors: *K. CHAUBEY[1,2,3,4], E. F. VAZQUEZ-ROSA[1,2,3,4], C. CINTRON-PEREZ[1,2,3,4], K. FRANKE[1,2,3,4], J. R. VOORHEES[5], X. ZHU[1], A. A. PIEPER[1,2,3,4];
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Hosp. Cleveland Med. Ctr., Cleveland, OH; [4]Sch. of Med., Inst. for Transformative Mol. Med., Cleveland, OH; [5]Inotiv, St. Louis, MO

Abstract: Alzheimer's disease (AD), a progressive neurodegenerative disorder characterized by impaired neuropsychiatric function, is associated with reduced brain NAD^+/NADH ratios. Depression, a hallmark symptom frequently manifesting prior to cognitive decline, underscores the need to identify early disease mechanisms. Using the TgF344AD rat model, which also exhibits depression-like behavior that precedes cognitive decline, we identified early-stage proteomic signatures and validated their clinical relevance through cross-species comparison with human AD databases. Nine-month-old rats, an age when TgF344AD rats display depression-like behavior without cognitive impairment, were administered either vehicle or (-)-P7C3-S243, a neuroprotective compound that preserves and restores NAD^+/NADH homeostasis in diseased states, for six months. Strikingly, (-)-P7C3-S243 treatment fully prevented depression-like phenotypes. Brain proteomic profiling via UPLC-MS/MS on a Q-Exactive Orbitrap HF-X system revealed 345 significantly dysregulated proteins in vehicle-treated TgF344AD rats versus vehicle-treated wild-type littermates. Notably, directional changes in many of these proteins aligned with human AD brain proteomic datasets. Critically, six months of daily (-)-P7C3-S243-treatment normalized a subset of AD-associated proteins in TgF344AD rats. Protein levels were verified in human and rat AD brain. Interestingly, relatively few protein level changes were seen in wild-type mice treated with (-)-P7C3-S243, indicating a homeostatic stabilizing effect of this agent. This study establishes conserved molecular signatures of depression in AD across the TgF344AD rat model and human patients, demonstrating the model's translational relevance. Furthermore, we pinpoint shared pathological protein networks reversed by preserving NAD^+/NADH homeostasis in the AD brain, offering mechanistic insights into AD-associated depression and identifying therapeutic targets.

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Presentation Number: NANO014.12

Topic: C.01. Brain Wellness and Aging

Support: Department of Veterans Affairs Merit Award I01BX005976
The Rebecca E. Barchas, MD, Professor in Translational Psychiatry of Case Western Reserve University and the Morley-Mather Chair in Neuropsychiatry of University Hospitals of Cleveland Medical Center
American Heart Association and Paul Allen Foundation Initiative in Brain

Health and Cognitive Impairment (19PABH134580006)
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NIH/NIA 1 U01 AG073323

Title: Pharmacological restoration of brain NAD^+/NADH prevents and reverses neurodegeneration and neuropsychiatric impairment after traumatic brain injury.

Authors: *E. F. VAZQUEZ-ROSA[1], S. BARKER[2], L. DOU[5], K. CHAUBEY[3], C. CINTRON-PEREZ[1], K. FRANKE[1], F. CHENG[5], A. A. PIEPER[4];
[2]Pathology, [3]Dept. of Psychiatry, [1]Case Western Reserve Univ., Cleveland, OH; [4]Psychiatry, Case Western Reserve Univ., Shaker Hts, OH; [5]Cleveland Clin., Cleveland, OH

Abstract: Traumatic brain injury (TBI) elevates long-term risks of chronic neurodegeneration and age-related diseases such as Alzheimer's and Parkinson's disease, driven in large part by pathological reductions in brain NAD^+/NADH, a critical component of energy metabolism in cellular homeostasis. Post-TBI depletion of brain NAD^+/NADH, exacerbated by oxidative stress, neuroinflammation, mitochondrial dysfunction, and DNA damage, creates a maladaptive cascade linking acute injury to progressive neurological decline. The neuroprotective compound P7C3-A20, an aminopropyl carbazole that restores NAD^+/NADH homeostasis in disease states, has demonstrated therapeutic potential across various preclinical models of neurodegeneration, including age-related cognitive decline, stress-induced depression, stroke, Parkinson's disease, and Alzheimer's disease. To evaluate the efficacy of stabilizing NAD^+/NADH in TBI, we employed a multimodal TBI (mmTBI) mouse model utilizing an overpressure chamber to create a precisely calibrated and reproducible combination of global concussion, acceleration/deceleration, and early blast wave exposure. Mice subjected to this injury develop progressive and chronic cognitive deficits associated with prolonged axonal degeneration leading to neuronal cell loss, blood-brain barrier deterioration, DNA damage, oxidative stress, and neuroinflammation, as well as peripheral metabolic changes in the blood resembling those reported in human TBI. We found that preservation of NAD^+/NADH by P7C3-A20 rescued NAD^+-dependent Sirtuin 1 (Sirt1) deacetylase activity, which is normally suppressed post-TBI, leading to mitigated neurotoxic tau acetylation, neurodegeneration, and cognitive impairment. Remarkably, delayed P7C3-A20 treatment one year post-TBI in mice, the equivalent of decades in people, restored blood-brain barrier integrity, arrested neurodegeneration, and reversed cognitive deficits. We have also established that P7C3-A20 treatment following TBI effectively prevents the acceleration of Alzheimer's disease (AD)-like deficits, including learning impairment and amyloid plaque accumulation, in 5xFAD mice, a preclinical model of AD. Collectively, our results demonstrate that P7C3-A20-mediated maintenance of brain NAD^+/NADH homeostasis effectively prevents and reverses neurodegeneration and neuropsychiatric impairment after TBI, including protection from accelerated AD.

Disclosures: E.F. Vazquez-Rosa: None. **S. Barker:** None. **L. dou:** None. **K. Chaubey:** None. **C. Cintron-Perez:** None. **K. Franke:** None. **F. Cheng:** None. **A.A. Pieper:** None.

Nanosymposium

NANO014: Oxidative Stress and Cellular Mechanisms in Alzheimer's Disease and Brain Injury

Location: SDCC Rm 33

Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO014.13

Topic: C.01. Brain Wellness and Aging

Title: Epa-pparα Signaling drives maladaptive fatty acid reprogramming and neurovascular degeneration following repetitive brain injury

Authors: *E. KARAKAYA;
Med. Univ. of South Carolina, Charleston, SC

Abstract: Repetitive mild traumatic brain injury (rmTBI) is a key antecedent to chronic traumatic encephalopathy (CTE), yet the metabolic determinants of cerebrovascular vulnerability remain poorly defined. Here, we identify eicosapentaenoic acid (EPA)-induced activation of peroxisome proliferator-activated receptor alpha (PPARα) as a critical driver of maladaptive neurovascular remodeling in the injured brain. While omega-3 polyunsaturated fatty acids (PUFAs) are classically considered neuroprotective, our findings reveal a context-dependent liability of EPA following rmTBI. Using a cyclic fish oil-enriched high-fat diet in male C57BL/6J mice, we observed sustained cortical accumulation of EPA under homeostatic conditions, but selective EPA depletion and PPARα overactivation following repetitive less-than-mild TBI (rlmTBI). This depletion coincided with impaired vascular perfusion, endothelial damage, and transcriptional signatures of beta-oxidation, extracellular matrix remodeling, and angiogenic dysfunction. Mechanistic in vitro experiments in brain microvascular endothelial cells showed that EPA altered metabolic substrate utilization, induced PPARα expression, and disrupted angiogenic capacity under permissive fatty acid oxidation conditions (AICAR + L-carnitine). Notably, these effects were not observed with docosahexaenoic acid (DHA), indicating isoform-specific toxicity. In silico regulatory network analysis confirmed PPARα as a master regulator of EPA-induced endothelial transcriptomic shifts. Finally, spatial lipidomics and proteomics of postmortem human CTE cortex demonstrated long-chain PUFA accumulation and perivascular extracellular matrix expansion, mirroring murine findings and confirming translational relevance. These data establish PPARα as a critical metabolic switch downstream of EPA in the injured brain and challenge prevailing assumptions regarding universal omega-3 benefit. This work reveals a vascular-centric mechanism of maladaptive fatty acid metabolism and introduces PPARα as a candidate target for stratified dietary interventions in TBI recovery and CTE prevention.

Disclosures: E. Karakaya: None.

Nanosymposium

NANO014: Oxidative Stress and Cellular Mechanisms in Alzheimer's Disease and Brain Injury

Location: SDCC Rm 33

Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO014.14

Topic: C.01. Brain Wellness and Aging

Title: Divergent Language Aging Trajectories: A Multimodal Brain-Behaviour Analysis

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Abstract: Age-related decline underlies cognitive functions such as sensorimotor control, executive functioning, memory, and language production (LP), whereas language comprehension (LC) tends to remain intact or improve across healthy adult lifespan. Preservation of LC can have structural and functional origins identifiable from key brain regions of the language network (LAN). To investigate this hypothesis, we analyzed the relationships among resting-state brain functional connectivity (rsFC) derived from functional magnetic resonance imaging (fMRI) signals, structural connectivity (SC) derived from diffusion MRI metrices, and behaviour (LC and LP) using a cross-sectional cohort of healthy adults (N = 652; aged 18-88). Six cognitive tasks assessing LC and LP were employed, with neuroimaging measures focused on region-specific connections within the LAN. Using generalized additive mixed models (GAMMs), complex brain-behaviour interactions were identified. Behavioral analyses revealed established age-related dichotomy, LC abilities in vocabulary and proverb comprehension improved and in syntactic and semantic comprehension remained stable, whereas LP tasks, e.g., verbal fluency, picture priming, and tip of tongue exhibited significant decline across the lifespan. SC exhibited decline in both intra- and inter-hemispheric fronto-temporal and frontal lobe connections, contrasted by preserved or enhanced temporal lobe connectivity, supporting a pattern of frontal vulnerability concomitant with temporal resilience. Age-related FC patterns demonstrated overall preservation, reflecting compensatory mechanisms to sustain functional integrity despite structural degradation. GAMM analyses revealed complex relationships between brain connectivity and language performance across age. Thus, integrating knowledge of brain structure, function, and language abilities, we identified the brain network mechanisms associated with dichotomous language behavior along lifespan.

Disclosures: P. Jain: None. **A. Akhter:** None. **A. Banerjee:** None.

Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.01

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

Support: Dementia Australia Research Foundation Project Grant
Dementia Australia Research Foundation MCR Fellowship
NHMRC GNT2020624
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NHMRC GNT2029740
NHMRC GNT2000660
NHMRC GNT1136241

Title: Development of a novel cyclotide to target neuronal excitotoxicity

Authors: D. ARIAWAN[1], J. VAN DER HOVEN[1], O. TIETZ[1], ***J. VAN EERSEL**[2], L. M. ITTNER[1];
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Abstract: Excessive neuronal activity can lead to cellular toxicity and thereby neuronal death. This excitotoxic event is a shared feature of a broad range of neurological disorders including dementia, stroke, and epilepsy. In the event of neuronal excitotoxicity, there is increased interaction between glutamatergic NMDA receptors (NMDARs) containing the subunit 2B (NR2B) and postsynaptic density protein 95 (PSD-95), which facilitates toxic downstream signalling. This signalling cascade can be blocked using a therapeutic peptide known as Nerinetide or NR2b9c. The peptide binds to PSD-95 and thereby blocks interactions between PSD-95 and NMDAR. However, the peptide is unstable in human plasma which led to ineffective outcomes in clinical trials. Cyclotides are naturally occurring cyclic peptides with three disulfide bonds, offering remarkable stability. In this study, we modified a cyclotide backbone to enhance neuronal uptake and grafted in the therapeutic NR2b9c sequence. This was then evaluated tested in primary neurons and in mouse models of epilepsy to determine clinical efficacy. Primary neurons treated with the NR2B9c cyclotide (c5R-NR2B9c) showed significant resistance to cell death induced by exposure to high concentrations of NMDA or Amyloid-beta, demonstrating protection from excitotoxicity. Further, administration of c5R-NR2B9c in a mouse model of epilepsy resulted in increased survival and reduced seizure severity after seizure induction. Overall, we show that modifying cyclotides can enhance delivery of therapeutic peptides into neuronal cells, which can be utilized to protect neurons from excitotoxicity.

Disclosures: D. Ariawan: None. **J. van der Hoven:** None. **O. Tietz:** None. **J. van Eersel:** None. **L.M. Ittner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Celosia Therapeutics.

Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.02

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

Support: Cure Epilepsy

Title: Optimization of a tau-targeting gene therapy for the treatment of epilepsy

Authors: *I. WEE[1], J. VAN EERSEL[3], L. M. ITTNER[2];
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Abstract: Hyperphosphorylated and aggregated Tau is a central pathological feature of Alzheimer's disease (AD), driving synaptic dysfunction and neurodegeneration. Increasing evidence implicates tau-induced excitotoxicity—a process wherein pathological Tau enhances glutamatergic signaling and calcium dysregulation—as a shared mechanism contributing to both AD progression and the network hyperexcitability characteristic of epilepsy. Past research has shown that phosphorylation of tau by the neuronal p38γ MAP kinase at T205 promotes its removal from the PSD-95: NMDA receptor complex and thereby prevents toxic downstream signaling associated with excitotoxicity. In support of this, we demonstrated that delivering p38γ MAP kinase gene through AAV significantly reduces epileptiform discharges, behavioral deficits, and seizure-associated mortality in various epilepsy mouse models. To advance this therapeutic strategy clinically, we focused on improving a key technical aspect of AAV-based gene delivery: genome packaging efficiency. A persistent challenge in AAV production is that the virus packaging process often generates a high proportion of empty capsids and carries the risk of reverse packaging of vector backbone sequences (i.e. non-therapeutic sequence), which can compromise both safety and therapeutic efficacy. To enhance genome packaging efficiency, we designed vector backbones that exceed the AAV packaging limit by inserting stuffer sequences (sequences 1, 2, and 3) in eight different combinations. Our results demonstrate that increasing the size of the vector backbone (>4.7kb) can significantly reduce reverse packaging. Specifically, fewer backbone sequences were detected when using a vector containing 1-stuffer sequence compared to no stuffer vectors, with further reductions observed with the addition of 2- or 3-stuffer sequences through qPCR. Further, in the primary neuron culture pre-treated with p38γ-rAAVs, we observed better protection against NMDA-induced excitotoxicity in cultures treated with 2- or 3-stuffer vectors compared to those treated with no-stuffer or 1-stuffer vectors. Consistently, a similar pattern of protection was observed *in vivo*, where the childhood epilepsy mice treated with p38γ-rAAVs from the 3-stuffer vector showed greater resistance to seizure-associated death compared to those receiving the vector with no stuffer. These findings highlight the impact of backbone size in optimizing genome packaging efficiency and improving the quality of rAAV-based gene therapies, as well as demonstrating the clinical efficacy of targeting tau for the treatment of excitotoxicity associated with epilepsy.

Disclosures: I. Wee: A. Employment/Salary (full or part-time):; Macquarie University. Other; CURE Epilepsy. **J. van Eersel:** A. Employment/Salary (full or part-time):; Macquarie University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CURE Epilepsy. **L.M. Ittner:** A. Employment/Salary (full or part-time):; Macquarie University. E. Ownership Interest

(stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Celosia Therapeutics.

Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.03

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

Support: R01AG072758
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U54NS100717
F32AG085960
Freedom Together Foundation
CIRM

Title: Investigating the role of progranulin microglial replacement therapy in Frontotemporal Dementia and Parkinson's Disease

Authors: *S. NAGUIB[1], L. FAN[2], H. DAVTYAN[4], J. CHADAREVIAN[4], S. KIM[5], Y. VOSKOBIYNYK[7], J. T. PAZ[8], T. M. DAWSON[6], M. BLURTON-JONES[9], S. GONG[1], L. GAN[3];
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Abstract: Microglia, the brain’s resident immune cells, play a dual role in maintaining homeostasis and responding to cellular stress. Their activation is a hallmark of neurodegenerative diseases, including Frontotemporal Dementia (FTD) and Parkinson's disease (PD), yet it remains unclear whether their effects are primarily protective or detrimental to disease progression. Recent advancements, such as CSF1R inhibitor-based microglia replacement in mice, have paved the way for therapeutic interventions aimed at modifying microglial function. In this study, we investigated the potential of progranulin (PGRN)-overexpressing microglia as a novel therapeutic approach for both FTD and PD.PGRN is a secreted protein implicated in both FTD and PD. To evaluate its therapeutic potential, we genetically engineered microglia to overexpress PGRN and transplanted them into neonatal *GRN-/-/hCSF1* mice, as hCSF1 will facilitate engraftment. Behavioral assays, *ex vivo* electrophysiology, and snRNAseq revealed that transplantation of wild-type PGRN-expressing microglia (MG-WT) increased brain PGRN levels and induced transcriptomic changes in mouse astrocytes, including reduced expression of GFAP and C4b, which are markers of astrogliosis and complement pathway activation. Additionally, MG-WT transplantation rescued thalamic

hyperexcitability observed in *GRN-/-/hCSF1* mice, highlighting the protective effects of PGRN even at wild-type levels.Building on this platform, we extended the approach to a PD mouse model. Immunodeficient mice were bilaterally injected with mouse preformed fibrils (mPFFs) that induce a-synuclein inclusions by 4 months. PGRN-overexpressing microglia were injected 1 month following mPFFs, and by 4 months, mice receiving PGRN-overexpressing microglia displayed improved motor performance on the rotarod and reduced dopaminergic neuron loss compared to mice that received wildtype microglia. These findings underscore the therapeutic potential of PGRN-microglial replacement strategies to modulate disease progression in multiple contexts.

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Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

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Presentation Number: NANO015.04

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

Support: NIH/NIA R01 grants AG018440
NIH/NIA R01 grants AG073979

Title: Anti-sense Oligonucleotide as a therapeutic for synucleinopathies: pharmacokinetic, safety and efficacy evaluation

Authors: *R. AHAMMAD[1], B. J. SPENCER[2], R. RISSMAN[2];
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Abstract: Effective blood-brain barrier (BBB) penetration is a significant challenge for antisense oligonucleotide (ASO) therapies targeting neurodegenerative diseases. We utilized a peptide ($ApoB^{11}$) mediated transport delivery of an ASO to the CNS following systemic delivery to reduce expression of targeted transcripts for neurodegenerative diseases. This study evaluates the pharmacokinetics, CNS penetration, and therapeutic efficacy of $ApoB^{11}$:2'-OMe ASO-α-Syn, an ASO for α-synuclein (α-Syn) suppression in synucleinopathies. After a single intraperitoneal (IP) injection (2 mg/kg) in C57BL/6 mice, $ApoB^{11}$:ASO-α-Syn showed robust brain penetration, reaching peak concentrations (C_{max} = 0.14 nMol/mg) at 1.5 hours and an extended brain half-life ($t_{1/2}$ = 646.2 hours), indicating prolonged CNS retention. Immunofluorescence confirmed widespread uptake in neurons and endothelial cells. The ASO also accumulated in the liver (C_{max} = 419.5 nMol/mg, $t_{1/2}$ = 104.9 hours), consistent with receptor-mediated uptake. Acute and subacute toxicity studies revealed no systemic toxicity at the highest non-lethal dose (32 mg/kg). In a mouse model of dementia with lewy body (DLB) mice overexpressing human α-Syn,

ApoB[11]:ASO-α-Syn reduced α-Syn mRNA and protein levels in the hippocampus and cortex by ~50% at 16 mg/kg. Notably, treatment also improved both motor and cognitive performance along with neurodegeneration in these mice. These results demonstrate that ApoB[11] is an effective ASO carrier for CNS delivery, supporting its potential as a therapeutic strategy for synucleinopathies.

Disclosures: R. Ahammad: None. **B.J. Spencer:** None. **R. Rissman:** None.

Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.05

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

Support: Moseley Foundation Grant 2019-2021
Moseley Foundation Grant 2022-2023
NIH Director's New Innovator Award 1DP2EB028110-01

Title: Direct Cell Reprogramming-based Therapies Targeting the Cerebrovascular Niche Reduce Disease Burden in the Triple Transgenic Mouse Model of Alzheimer’s Disease

Authors: *D. F. ALZATE-CORREA[1], J. STRANAN[1], M. ANGELICA[1], S. JARAMILLO-GARRIDO[1], N. AREIZA-MAZO[1], J. FITZGERALD[2], C. C. ASKWITH[5], N. HIGUITA-CASTRO[3], O. KOKIKO-COCHRAN[2], D. GALLEGO-PEREZ[4];
[2]Neurosci., [3]BME, [4]Biomed. Engineering/Surgery, [1]The Ohio State Univ., Columbus, OH;
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Abstract: Alzheimer’s Disease (AD) is a neurodegenerative condition characterized by cognitive decline, and neuropathological changes such as amyloid-beta plaques and neurofibrillary tangles. In AD patients, Blood-Brain Barrier (BBB) damage and cerebral blood flow (CBF) reduction are evident long before the main neuropathological lesions appear, indicating that deficits in cerebrovascular function play a key role in AD onset and development. Consequently, our research program uses cell reprogramming -based therapies to target neurovascular deficiencies in AD and evaluate their effect on disease burden. To implement safe and effective vascular cells therapies, fibroblasts pre-labeled with 5-bromo-2’-deoxyuridine (BrdU) were electroporated with three transcription factors (*Etv2, Foxc2, Fli1*, abbreviated as *EFF*) that induce direct reprogramming of fibroblast into induced endothelial cells (iECs). These iECs were then intracranially injected into the lateral ventricles (LV) of triple transgenic mouse model of AD mice (3xTg-AD) and wild-type controls to evaluate their therapeutic efficacy via behavioral assessments, immunostaining, transcriptomics, and biochemical assays. Our findings indicate that *EFF*-primed cells exhibited an angiogenic transcriptional profile consistent with endothelial cell characteristics as early as 24h post-electroporation. Furthermore, a single injection of *EFF*-primed cells in the brains of 3xTg-AD mice induced an increase in global CBF,

reenforcing its potential role to modify the cerebral vascular compartment. In addition, when multiple doses of *EFF*-primed cells were injected in 3xTg-AD, a significant reduction in spatial memory deficits was observed. Of note, the injected cells survive in the brain for several weeks and migrate to different brain regions such as the sensorimotor cortex and hippocampus, where they increase the vascular area. Moreover, *EFF*-primed cells correlated with reduced cortical and hippocampal amyloid-beta loads in cortical layers IV and V. Finally, transcriptomic analysis identified particular gene expression changes related to glucose metabolism and fatty acid oxidation. Among these genes, PPAR-α, draws the attention given that its activation has been previously linked to reduction in amyloidogenic processes, tau phosphorylation, and neuronal inflammation. These findings suggest that reprogramming fibroblasts into iECs can effectively target the neurovascular compartment and reduce cognitive deficits and pathological processes associated with AD.

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Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.06

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

Support: NIA Grant P01AG062817

Title: Small molecule Shc inhibitors dose-dependently reduce microglial inflammation and rescue memory loss in two mouse models of Alzheimer's disease

Authors: *M. LO TUFO[1], C. MONTGOMERY[2], C. GARCIA[4], A. TOMILOV[5], J. DI LUCENTE[3], I. MAEZAWA[6], L.-W. JIN[7], G. CORTOPASSI[2];
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Abstract: Genetic reduction of Shc protein has been shown to ameliorate neurobehavioral deficits in the PS1/APP mouse model of Alzheimer's disease (AD). We have used Artificial Intelligence to identify ~100 novel small molecule Shc Inhibitors (ShcIs). We used computational chemistry methods (Docking and Molecular Dynamics) to show the likely site of binding of ShcIs to Shc protein. We have shown that ShcIs inhibit A-beta amyloid-driven human microglial inflammation, and suppress the downstream pathways considered to drive that inflammation. Our *in vitro* studies of ShcI-301 and ShcI-401 in BV2 microglial cells demonstrate ShcIs are dose-dependently anti-inflammatory and induce homeostatic microglial morphology by

increasing ramification. Lead ShcIs were administered to PS1/APP and ApoE4 mouse models of Alzheimer's disease, these produced a significant rescue of inflammation and Long-Term Potentiation (LTP) related outcomes in both models.
Thus, we have identified a novel target for therapeutic intervention into AD progression, Shc, and have evaluated ~100 novel molecules proven to engage that target. Lead molecules 301 and 401 bind the same site on Shc protein by computational chemistry methods, significantly and dose-dependently inhibit inflammation in microglia, and Shc 401 rescues neuroinflammation in both PS1/APP and ApoE4 mouse models of Alzheimer's.

Disclosures: **M. Lo Tufo:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Beiwe Bio. **C. Montgomery:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Beiwe Bio. **C. Garcia:** None. **A. Tomilov:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Beiwe Bio. **J. Di Lucente:** None. **I. Maezawa:** None. **L. Jin:** None. **G. Cortopassi:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Beiwe Bio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Beiwe Bio.

Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

Location: SDCC Rm 11

Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.07

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

Support: AMED JP25ym0126817
Fujita Mind-BRIDGe of J-PEAKS
JSPS KAKENHI 21K07257

Title: PKA/Rap1 signaling cascade in stratal medium spiny neurons: Implication for motor control and Parkinson’s disease therapy

Authors: ***H. SANO**[1,2], D. TSUBOI[3,2], S. CHIKEN[4], A. NAMBU[5], K. KOBAYASHI[6], K. KAIBUCHI[3,2];
[1]Intl. Ctr. for Brain Sci. (ICBS), [2]Fujita Mind-Brain Res. & Innovation Ctr. for Drug Generation (Fujita Mind-BRIDGe), [3]Div. of Cell Biology, Intl. Ctr. for Brain Sci. (ICBS), Fujita Hlth. Univ., Toyoake, Japan; [4]Section of Multilayer Physiol., [5]Div. of Behavioral Develop., [6]Section of Viral Vector Develop., Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Medium spiny neurons (MSNs) in the striatum play a central role in motor regulation. Dopamine D1 receptor (D1R)-expressing MSNs, also known as the *direct* pathway MSNs, play a critical role in the initiation and promotion of voluntary movements. While our previous studies showed that the D1R-protein kinase A (PKA)-Rap1-mitogen-activated protein kinase (MAPK)

signaling pathway modulates reward-related behaviors in the ventral striatum (Nagai et al., Neuron, 2016; Tsuboi et al., Cell Rep, 2022), its role in motor control within the dorsal striatum remained to be elucidated. We investigated how the PKA/Rap1 cascade regulates voluntary movements in the *direct* pathway, especially under Parkinson's disease (PD) state. We generated PD model mice by injecting 6-hydroxydopamine. Subsequently, the constitutive active form (CA) of PKA or Rap1 was expressed in the striatal D1R-MSNs using adeno-associated virus vectors. The motor deficits were evaluated through the analysis of forelimb use asymmetry and spontaneous rotational behavior. Furthermore, we recorded neuronal activities in the basal ganglia (BG) of awake mice. To further evaluate the therapeutic potential of PKA CA, it was expressed in both D1R- and D2R-MSNs in PD model mice and macaque monkeys. The expression of PKA CA or Rap1 CA in D1R-MSNs significantly improved motor function, reducing forelimb use asymmetry and rotational behavior in PD mice. These behavioral improvements suggest that activation of the PKA/Rap1 cascade in the *direct* pathway alleviates PD-related motor deficits. In wild-type mice, electrical stimulation of the motor cortex evokes a triphasic response (early excitation, inhibition, and late excitation) in the substantia nigra pars reticulata (SNr), the output nucleus of the BG. This triphasic response pattern was disrupted in PD mice, showing only early and late excitations. Notably, expression of PKA CA or Rap1CA led to the restoration of the triphasic response pattern in PD mice, suggesting functional recovery of the cortico-striato-SNr *direct* pathway. In addition, the expression of PKA CA in both the D1R- and D2R-MSNs ameliorated motor deficits in PD models of mice and macaque monkeys. These findings demonstrate that the PKA/Rap1 signaling cascade is crucial for restoring *direct* pathway function and voluntary movements in PD, and may serve as a promising therapeutic target for drug development for PD.

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Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

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Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO015.08

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

Support: NIH Grant GM146257
NIH Director's Transformative Research Award for Accelerating Leading-edge Science in ALS (ALS2) Initiative
South Carolina SmartState Endowment for Economic Growth

Title: Small molecules targeting Rab10-mediated intracellular trafficking machinery

Authors: S. MALASALA, Y.-H. CHEN, *Q. LU;
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Abstract: AbstractBackground: Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by the accumulation of amyloid-beta (Aβ) plaques and tau aggregates. Recent findings have implicated Rab10, a key regulator of intracellular trafficking, as a candidate resilience factor in AD. Rab10 expression and phosphorylation is increased in AD brains. Genetic variants associated with compromised Rab10 function confer protection against AD progression. As Rab10 plays an important role in Aβ production, autophagy deregulation, and neuroinflammation, it is a potential and novel therapeutic target. This study aims to develop small-molecule modulators targeting Rab10. **Methods:** We utilized a structure-based approach to screen 80 million small molecules to identify Rab10 modulators. Computer-aided drug design (CADD) and cell-based assays were employed to analyze the drug likeness and validate the efficacy of the top-ranked chemical compound hits. The modulator efficacy is determined by using immunofluorescent light microscopy and biochemical assays. **Results:** For the five top-ranked candidates (R1-R5), molecular dynamics simulations demonstrated their stable interactions with Rab10. R1 showed the most favorable interactions, including the key hydrogen bonding to Thr23 that is essential for GTP binding and interactions with Mg^{2+}. ADME/Tox analysis confirmed favorable pharmacokinetic profile, including blood-brain barrier permeability, good gastrointestinal absorption, and low toxicity. In cultured HEK293 cells, immunofluorescent imaging revealed that R4 induced endosomal swelling, whereas R1-R3 and R5 recovered normal endosomal expression pattern after lipopolysaccharides (LPS) induced endosomal aggregates, demonstrating their various impacts on Rab10. Western blot analysis confirmed these findings for R1-R5 as they altered the expression of endosomal markers EEA1 and LAMP1 as well as Rab10 phosphorylation downstream of LRRK2. **Conclusion:** Rab10 small molecules present therapeutic and biomarker potential, and they provided the foundation for optimization to advance Rab10-targeting AD intervention.

Disclosures: S. Malasala: None. **Y. Chen:** None. **Q. Lu:** None.

Nanosymposium

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Presentation Number: NANO015.09

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

Support: NIH Director’s Transformative Research Award ALS2 Initiative R01GM146257/ R01OD031672

Title: Cdc42 inhibitor preclinical effects in the G93A mutant hSOD1 mouse model of ALS

Authors: *M. E. SPAETH HERDA[1], S. MALASALA[3], M. A. MCGARR[2], A. G. WEAVER[2], A. E. KOLY[2], C. PENA[2], V. D. MYHRE[2], Y.-H. CHEN[4], Q. LU[5], D. A. LINSEMAN[2]; [2]Biol. Sci., [1]Univ. of Denver, Denver, CO; [3]Dept. of Chem. and Biochem., [4]Univ. of South Carolina, Columbia, SC; [5]Dept of Chem. and Biochem., The Univ. of South Carolina, Columbia, SC

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder for which therapeutics targeted to the underlying pathogenetic mechanisms are lacking. Members of the Rho GTPase family (Rho, Rac, and Cdc42) play diverse roles in the nervous system and alterations in both Rac and Rho function have been implicated in ALS. Yet, little is known about the role of Cdc42 in this disease. Here, we investigated the preclinical effects of a Cdc42 inhibitor in the G93A mutant hSOD1 mouse model and in NSC34 motor neuronal-like cells. In vitro, NSC34 cells were transfected with G93A mutant hSOD1 or wild-type hSOD1 and differentiated to promote neurite outgrowth. Cells were then treated with the Cdc42 inhibitor or vehicle control. G93A mutant hSOD1-expressing cells displayed retracted neuronal processes, an effect that was largely reversed by Cdc42 inhibition. In vivo, G93A mutant hSOD1 mice were dosed i.p. with 12.5mg/kg or 25mg/kg of Cdc42 inhibitors every other day from the beginning of disease onset (90 days-old) until end-stage. Disease progression was tracked by body weight changes, paw grip endurance, and accelerated rotarod. Sections of spinal cord were immunostained for astrogliosis and microgliosis. A significant extension in survival, enhanced grip strength and rotarod performance, and decreased gliosis were observed in Cdc42 inhibitor-treated mice as compared to their untreated littermates. Future studies will explore the effects of Cdc42 inhibitors on spinal motor neuron survival and neuromuscular junction integrity and innervation. Our findings indicate that dysregulation of Cdc42 function may play a pathogenetic role in ALS. Supported by NIH Director's Transformative Research Award ALS2 Initiative R01GM146257/ R01OD031672.

Disclosures: **M.E. Spaeth Herda:** A. Employment/Salary (full or part-time):; NIH Director's Transformative Research Award ALS Initiative. **S. Malasala:** None. **M.A. McGarr:** None. **A.G. Weaver:** None. **A.E. Koly:** None. **C. Pena:** None. **V.D. Myhre:** None. **Y. Chen:** None. **Q. Lu:** None. **D.A. Linseman:** None.

Nanosymposium

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

Support: the Fujita Mind-Brain Research & Innovation Center for Drug Generation (Fujita Mind-BRIDGe) of the Japan's Peak Research Universities (J-PEAKS) Program funded by the Japan Society for the Promotion of Science (JSPS)
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Title: Rac1 Activation Improves Cognitive Deficits in an Alzheimer's Disease Mouse Model

Authors: *Y. YAMAHASHI[1], M. RIEDL[1], M. HASEGAWA[2], A. MOURI[3], K. KAIBUCHI[1]; [1]Intl. Ctr. for Brain Science, Fujita Hlth. Univ., Toyoake, Aichi, Japan; [2]Intl. Ctr. for Brain Science, Fujita Hlth. Univ., Aichi, Japan; [3]Intl. Ctr. for Brain Science, Fujita Hlth. University/ Fujita Hlth. Univ. Grad. Sch. of Med. Sciences,, Nagoya, Japan

Abstract: Dysregulated acetylcholine (ACh) signaling is implicated in cognitive deficits associated with neuropsychiatric and neurodegenerative disorders. Muscarinic acetylcholine receptor M1 (M1R) has been a therapeutic target because of its vital role in learning and memory. Targeting intracellular signaling cascades downstream of M1R has emerged as a strategy to enhance cognition while minimizing cholinergic side effects. We recently identified the M1R-PKC-Beta-PIX-Rac1-p21-activated kinase (PAK) pathway in dopamine receptor D2-expressing medium spiny neurons (D2R-MSNs) of nucleus accumbens (NAc) enhanced aversive learning in wild-type mice, based on a phosphoproteomic method developed in our lab (Yamahashi et al., Mol Psychiatry, 2022). We further found that Rac1 activation in the NAc by miRNA-mediated knockdown of Bcr, a Rac1 inactivator, enhanced aversive learning in wild-type mice (Wang et al., IJMS, 2023). Here, we investigated whether Rac1 signaling mediates M1R-dependent learning and memory in the hippocampus and represents a viable target for cognitive enhancement. Biochemical analysis revealed that M1R-specific agonist BQCA treatment (10 micromolar) activated Rac1-PAK signaling via PKC in ex vivo hippocampus slices from wild-type mice. Immunohistochemical analysis revealed the administration of M1R-specific agonist PQCA (10 mg/kg, i.p.), a BQCA analog with better brain penetration, enhanced Rac1-PAK signaling in the CA1 region of dorsal hippocampus in both wild-type mice and APP^{NL-G-F}/MAPT double knock-in Alzheimer's disease (AD) model mice. Activation of Rac1 signaling in the CA1 region of dorsal hippocampus by adeno associated virus (AAV)-mediated expression of constitutively active (CA) PAK mutant enhanced aversive learning in wild-type mice ($p<0.05$ vs control group). In APP^{NL-G-F}/MAPT double knock-in model mice, in which PAK is inactivated in the dorsal hippocampus ($p<0.05$ vs wild-type mice), Rac1 signaling activation recused impaired aversive learning ($p<0.01$ CA PAK non-expressing AD group vs CA PAK expressing AD group).The above findings identify Rac1-PAK signaling as a critical effector of M1R activation in the hippocampus. Given that aversive learning is commonly used to assess the effect of therapeutic drug for learning and memory deficits, Rac1 activation could be used as a therapeutic strategy for treating cognitive dysfunction in AD and schizophrenia.

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

Support: NIH Director’s Transformative Research Award R01GM146257

Title: Small molecule modulators targeting the ARF1-C9ORF72:SMCR8:WDR41 interaction in ALS/FTD

Authors: F. AZIMIAN[1], **A. JOBY CHACKO**[1], *S. NIK AKHTAR[2], Y.-H. CHEN[1], Q. LU[3]; [1]Univ. of South Carolina, Columbia, SC; [2]Univ. of South Carolina Columbia, Columbia, South Carolina, Unit, SC; [3]Dept of Chem. and Biochem., The Univ. of South Carolina, Columbia, SC

Abstract: A GGGGCC hexanucleotide repeat expansion in the *C9ORF72* gene is the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). The C9ORF72 protein assembles with SMCR8 and WDR41 into the C9ORF72-SMCR8-WDR41 (CSW) complex, a GTPase-activating protein complex that regulates small GTPases involved in membrane trafficking [1]. Loss of C9ORF72 function due to the repeat expansion can disrupt ARF1 regulation by the CSW complex, leading to hyperactivation of ARF1, Golgi apparatus fragmentation, and neurodegenerative pathology [2]. Therefore, targeting ARF1 signaling is an important strategy towards ALS/FTD treatment. However, no pharmacological approaches are currently available to directly modulate the ARF1-CSW protein-protein interaction in ALS/FTD. Here, we conducted a virtual screen of >40 million compounds from the MCULE library against the ARF1-CSW interface to identify the top hits. Based on molecular docking and MM-GBSA binding free energy algorithm, ADME/T predictions, and interaction pattern analysis, MCULE-5095997944 emerged as a promising candidate. In cell-based assays, MCULE-5095997944 significantly reduced the level of GTP-bound ARF1 upon stimulation, indicating that it antagonizes ARF1 activation. Treatment with this compound also dispersed the perinuclear Golgi organization in cultured HEK293 cells, consistent with the inhibition of ARF1 signaling. These results validate that MCULE-5095997944 can modulate ARF1-CSW signaling in the context of living cells. Through lead optimization, several MCULE-5095997944 analogs were identified, which retain the crucial quinolinone core and key interaction features while enhancing predicted binding interactions at the ARF1-CSW interface. Molecular dynamics (MD) simulation analysis confirmed that the lead compound remains stably bound at the interface over 200 ns, supporting a favorable binding pose and pharmacological profile. MCULE-5095997944 and its optimized analogs thus represent a new chemical approach to target ARF1/CSW signaling, potentially addressing a key pathogenic mechanism downstream of the *C9ORF72* mutation. Supported in part by NIH Director’s Transformative Research Award R01GM146257 for Accelerating Leading-edge Science in ALS (ALS^2) Initiative

Disclosures: F. Azimian: None. **A. Joby Chacko:** None. **S. Nik Akhtar:** None. **Y. Chen:** None. **Q. Lu:** None.

Nanosymposium

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

Support: Michael J. Fox Foundation
NIH/NINDS R01-NS064934

Title: Phosphorylation of Rab proteins predicts disease severity in neurodegeneration

Authors: *A. B. WEST[1], T. MALANKHANOVA[1], W. WANG[2];
[1]Duke Univ., Durham, NC; [2]Duke university, Durham, NC

Abstract: Mutations in the *LRRK2* gene, encoding the Rab-like GTPase and serine/threonine kinase known as LRRK2, represent a common cause of familial Parkinson's disease. LRRK2 phosphorylates nearby its own GTPase domain in *cis*, but also phosphorylates other Rab small GTPases in *trans*. Among the few known LRRK2 Rab substrate pairs, Rab10 presents highly expressed in biofluids and different types of cells. While inhibitors of the LRRK2 enzyme are sought to block idiopathic neurodegenerative diseases including Alzheimer's disease and Parkinson's disease, a paucity is yet known in how LRRK2 activity, manifested through phosphorylated Rab proteins, might predict disease progression or change in different disease states. Our recent efforts have led to the development of robust sensitive and specific high-throughput assays for the assessment of LRRK2 expression and Rab substrate phosphorylation in deeply-phenotyped longitudinal cohorts. These ongoing assessments may shed light into disease pathways linked to alter Rab function and how these changes modify important disease phenotypes. With better descriptions of Rab changes in disease, it is anticipated that additional precision may emerge to help guide successful therapeutic leveraging of aberrant Rab function in different neurodegenerative disease outcomes.

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Nanosymposium

NANO015: Cutting-Edge Therapeutic Strategies for Neurodegenerative Disorders

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Presentation Number: NANO015.13

Topic: J.04. Physiological Methods

Support: NIH R35GM147112

Title: Quantitative Subcellular Imaging Tools for Investigating Chloride Pathophysiology and Intracellular Trafficking Dysregulation in Neurodegenerative Diseases

Authors: *K. LEUNG;
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Abstract: As the most abundant anions in cells, chloride ions play crucial roles in numerous cellular functions, with intracellular chloride concentrations ranging from 10 mM to 120 mM across different organelles. Dysregulation of chloride homeostasis has been implicated in a variety of diseases, including cystic fibrosis, kidney stones, osteoporosis, low QT syndrome, and

neurodegenerative disorders, collectively contributing to a substantial healthcare burden. Despite its fundamental biological significance, chloride physiology remains comparatively underexplored relative to other ions, such as sodium, potassium, and calcium. In this presentation, I will discuss our recent advancements in the development of subcellular imaging tools that enable the precise visualization of the intracellular chloride landscape and dynamics. These imaging tools have significantly enhanced our understanding of chloride pathophysiology, including investigations into the physiological role of chloride in the cGAS-STING pathway, which is a critical component of the innate immune system driving aging-related inflammation and neurodegeneration. Additionally, I will highlight how these subcellular chloride imaging technologies visualized the gamma-aminobutyric acid stimulated astrocytic chloride efflux in C8-D1A astrocyte. GABA-induced astrocytic Cl– efflux plays a critical role in regulating the excitability of neurons. Using our imaging tools, we found that in cell models of Niemann-Pick disease type C, the GABA-mediated astrocytic Cl– efflux is reduced. Interestingly, we discovered that this reduction can be restored by removing abnormal cholesterol accumulation with β-cyclodextrin treatment. These findings may give us new insights into the molecular mechanisms driving neuronal hyperexcitability in these diseases.

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Nanosymposium

NANO016: Photoreceptors and Retinal Circuitry

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Title: Deep conservation of amacrine cell diversity across the vertebrate phylogeny

Authors: *D. TOMMASINI[1], A. MONAVARFESHANI[2], V. DINESH[3], J. HAHN[4], O. MARRE[7], T. PUTHUSSERY[5], J. R. SANES[8], K. SHEKHAR[6];
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Abstract: Background: Amacrine cells (ACs) are a highly diverse class of inhibitory interneurons in the retina, which canonically release GABA or glycine. Mice have over 60

distinct AC types. While previous work showed that other retinal cell classes are highly conserved across vertebrates, the conservation of ACs remains largely uncharacterized. Approach: We performed an integrative analysis of single-cell transcriptomic profiles of ACs across 22 species: human, three non-human primates, five rodents, three ungulates, opossum, ferret, tree shrew, a bird, a reptile, three teleost fish, a cartilaginous fish, and a lamprey. Using cross-species integration, we identified 42 clusters representing putative orthologous types. Following previous convention (Hahn et al., Nature, 2023), we term these orthotypes. A subset of these orthotypes were validated by immunolabeling for conserved molecular markers in various species. Results: Most AC types show tight molecular correspondence across tetrapods. AC type abundance and specialization vary substantially across species, likely reflecting adaptations to different visuo-ecological niches. AC type diversity scales with the diversity of retinal ganglion cell (RGC) types. Several known and novel AC types (e.g., starburst, A2, VG3, PDGFRA+) are conserved from mammals to fish. A conserved transcription factor code governs AC identity and/or maintenance. Comparison across species lead to the hypothesis that an ancestral cell with AC/RGC hybrid properties gave rise to glycinergic ACs and a cell with GABAergic AC/RGC properties; the latter then diversified into modern-day AC and RGC types. Conclusion: Amacrine cell types are deeply conserved across vertebrates, with evolution acting primarily on the abundance and subspecialization of an ancestral cellular repertoire. Our findings provide unique insight into the evolution of neural circuits.

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Nanosymposium

NANO016: Photoreceptors and Retinal Circuitry

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Topic: E.06. Vision

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Title: Adaptive mechanisms allow retinal ganglion cell selectivity to homogeneous stimuli in natural scenes

Authors: *B. LORENZI, S. VIRGILI, D. VARRO, O. MARRE;
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Abstract: Adaptation is ubiquitous in sensory systems. Sensory neurons change their activity depending on the past stimulus history at various timescales. Short- and long-term adaptations have been mostly studied in the retina using simple, artificial stimuli. It is thus unclear how these adaptive processes impact ganglion cells and how they change their feature selectivity to natural scenes as a function of the recent stimulus history. Adaptation could simply normalize the input,

or substantially reshape the functional selectivity of ganglion cells. Here we measured and modeled how short-term adaptation impacts ganglion cell selectivity to natural scenes in the mouse retina. We recorded ganglion cells while stimulating them with natural images perturbed with noise patterns of small amplitudes. We then presented new stimuli where the same perturbed images were now preceded by another flashed stimulus (400 ms before), to trigger short-term adaptation. Short-term adaptation impacted ganglion cell responses as well as their selectivity to perturbations in the image. We designed deep learning models that could reproduce quantitatively ganglion cell responses. A two-layer neural network could predict the responses to flashed natural images, but a gain control mechanism was necessary to generalize and predict the responses to two images presented in rapid succession. In a subset of ganglion cell types, this gain control revealed a sensitivity to homogeneous objects on natural images, enabling detection of large objects despite the presence of natural backgrounds. Adaptive mechanisms need to be included in models to achieve robust generalization, and are essential to uncover the full functional selectivity of retinal ganglion cells. This work advances our understanding of neural computation in early visual processing and sensory encoding principles.

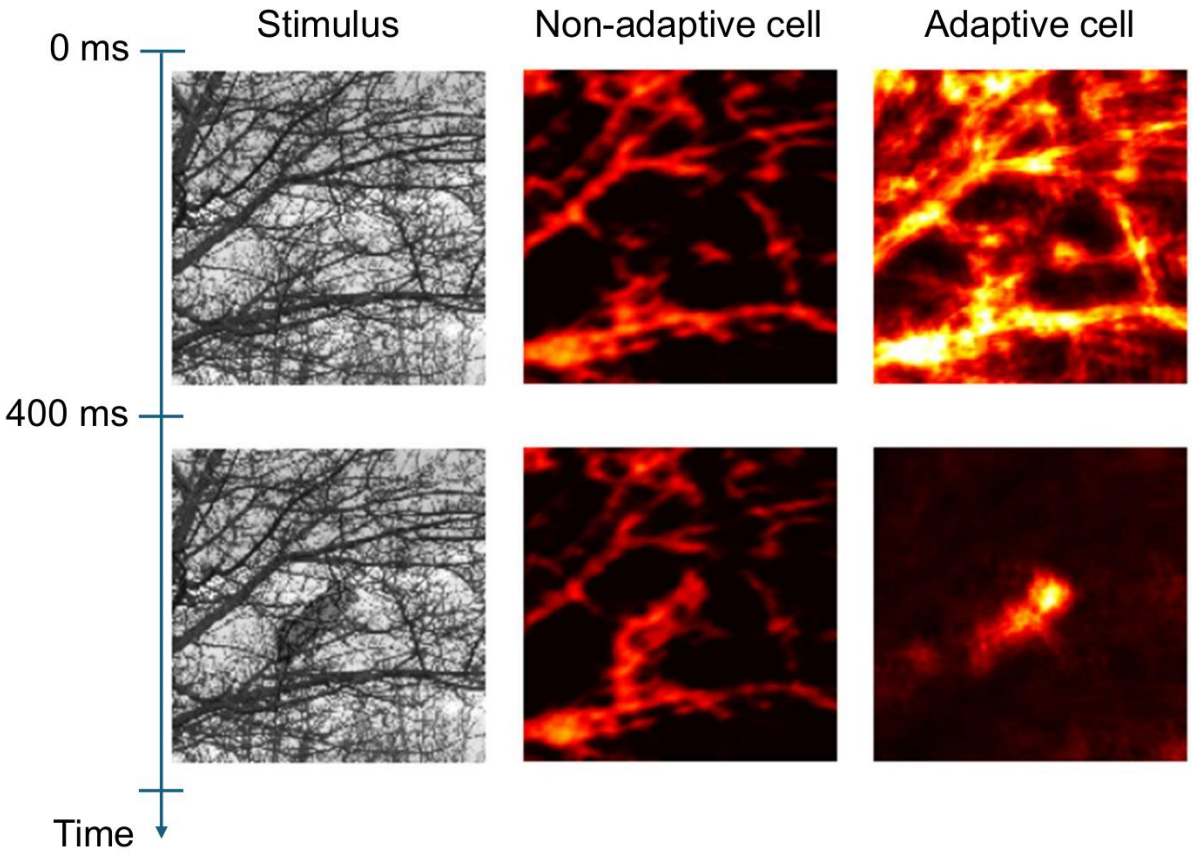


Figure 1. Model-predicted neural activity in selected non-adaptive and adaptive cell populations across two consecutive frames

Disclosures: B. Lorenzi: None. **S. Virgili:** None. **D. Varro:** None. **O. Marre:** None.

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Title: Afadin-deficient retinas exhibit severe neuronal lamination defects but preserve visual functions

Authors: *A. UENO[1], K. SAKUTA[2], C. KOIKE[3];
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Abstract: Neural lamination is a common feature of the central nervous system (CNS), with several subcellular structures, such as adherens junctions (AJs), playing a role in this process. The retina is also heavily laminated, but it remains unclear how laminar formation impacts retinal cell morphology, synapse integrity, and overall retinal function. In this study, we demonstrate that the loss of afadin, a key component of AJs, leads to significant pathological changes. These include the disruption of outer retinal lamination and a notable decrease as well as mislocalization of photoreceptors, their outer segments, and photoreceptor synapses. Interestingly, despite these severe impairments, we recorded small local field potentials, including the a- and b-waves derived from photoreceptor and bipolar cells, respectively. Additionally, we successfully characterized the receptive fields of certain retinal ganglion cells. Overall, these findings provide the first evidence that retinal circuit function can be partially preserved even when there are significant disruptions in retinal lamination and photoreceptor synapses. Our results indicate that retinas with severely altered morphology still retain some capacity to process light stimuli and visual function.

Disclosures: A. Ueno: None. **K. Sakuta:** None. **C. Koike:** None.

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Presentation Number: NANO016.04

Topic: E.06. Vision

Support: BBSRC G4001-04

Title: The role of inhibition in the diurnal modulation of information transfer through the retina of zebrafish

Authors: *E. W. BIRKETT, K. KUPCZYK, L. LAGNADO;
Sch. of Life Sci., Univ. of Sussex, Brighton, United Kingdom

Abstract: Visual processing in the retina depends on inhibition from horizontal cells to cones and from amacrine cells to bipolar cells (BCs). But inhibitory synapses also inject noise into the circuit, which can degrade information transmission. We aimed to understand the role of inhibition in adjusting the flow of information about the most basic property of a visual stimulus - temporal contrast. Does this change during the diurnal modulation of retinal function? To investigate, we used multiphoton imaging of glutamate in larval zebrafish sparsely expressing the glutamate reporter iGluSnFR in BCs. A rotating scan path simultaneously recorded glutamate transients on BC dendrites and axonal compartments (1 kHz) to estimate the efficiency with which information was transmitted about a set of 11 contrasts (0-100%, full-field, 5 Hz). Measurements were made before and after pharmacologically blocking inhibition using a combination of strychnine, gabazine and TPMPA, injected into the anterior chamber of the eye. The stimulus provided an information rate of 17.3 bits s^{-1}. The mutual information was calculated between i) the iGluSnFR signal at individual cone synapses and the stimulus; and ii) the BC output and total excitatory input it receives from cones. The efficiency with which BCs transmitted information arriving from cones was increased by blocking inhibition (83% increase in the morning and 52% in the afternoon). In cones, blocking inhibition increased the efficiency of information transmission in the morning (63%) but had no effect in the afternoon. On average, inhibitory synapses *reduce* the transmission of information about temporal contrast, likely reflecting the noise they introduce into cones and BCs. This reduction was greatest in the morning and occurred at both the first and second synaptic stages. In the afternoon, however, inhibition from horizontal cells did *not* affect the efficiency of information transmission through cones. It may, however, be that inhibition in the inner retina increases the information transmitted about other properties of a visual stimulus, such as speed or direction of motion.

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Nanosymposium

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Presentation Number: NANO016.05

Topic: E.06. Vision

Support: Knights Templar Career Starter Award
Holland Trice Scholars Award

Title: Potential mechanism of homeostatic plasticity for synapse maintenance during photoreceptor degeneration

Authors: T. RAU[1], M. RASPER[3,2], A. BHAKHRI[3,2], ***M. L. SCALABRINO**[2];
[2]Ophthalmology & Visual Sci., [1]Med. Col. of Wisconsin, Milwaukee, WI; [3]

Abstract: Photoreceptor (PR) degeneration causes impairment in the visual system through death of the light sensing neurons, leading to blindness once a critical number of PRs are lost. Prior to the loss of that critical number, the system uses homeostatic mechanisms to compensate for reduced light signal, where second order glutamatergic neurons, bipolar cells (BCs), boost the signal through undefined mechanism(s). At a time point where ~70% of PRs are lost, visual signal transmission is only ~30% reduced from undamaged. While BCs are known to rewire significantly as PRs die, how rewiring impacts retinal function remains poorly defined. Using a mouse model of the inherited PR degeneration retinitis pigmentosa (*Cngb1*$^{neo/neo}$), we discovered several genes associated with synaptogeneis are downregulated only in late-stage disease when visual outcomes are worse and gene replacement is less successful at restoring function. Transcript levels of *Lin7b*, a protein associated with channel/receptor localization in brain, were corrected with early gene replacement. However, *Lin7b* remained at levels similar to untreated following late gene replacement. We hypothesize downregulation of *Lin7b* contributes to impaired signaling between PRs and ON bipolar cells.To test this hypothesis, we created two CRISPR constructs delivered to ON BCs using AAV. One construct inhibits transcription using catalytically inactive Cas9 paired with a KRAB repressor element (CRISPRi) in healthy, early disease, and early treatment to assess whether induced downregulation impairs function compared to uninjected contralateral eyes. The second construct amplifies expression using a VP64 transcriptional activator (CRISPRa), delivered in late disease and late treatment. Changes to retinal structure and function were assessed using in vivo full field electroretinography, ex vivo multielectrode array recordings, immunohistochemistry, and expansion microscopy. Manipulation of *Lin7b* was confirmed in both HEK293T cell culture and in mouse retinas injected with either AAV-CRISPRi or AAV-CRISPRa. In eyes injected with the CRISPRi virus, we found significant dendritic remodeling and impaired b-wave function from ERGs, indicating this protein is essential to PR-BC structure and signaling. Ongoing work is assessing the extent to which upregulation rescues impaired function in late disease and late treatment and the changes to whole retinal circuits. This is the first report of the role of Lin7b in mammalian retina. These findings may present an avenue toward extending visual function in patients with photoreceptor degeneration or as means to improve synaptogenesis in cell replacement therapy.

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Presentation Number: NANO016.06

Topic: E.06. Vision

Support: NIH Grant EY034652
NIH Grant EY012793

Title: Physiology of noncanonical ON bipolar cells innervating the accessory ON layer of mouse retina

Authors: *Y. XUE[1], S. LEE[2], D. M. BERSON[3], Z. ZHOU[4];
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Abstract: ON and OFF pathways in the vertebrate visual system arise from ON and OFF retinal bipolar cells (BCs), which express metabotropic (mGluR6) and ionotropic (KA/AMPA) glutamate receptors, respectively. In the mouse retina, 9 ON and 6 OFF BC types have been identified, with axon terminals stratifying in the inner and outer halves of the inner plexiform layer (IPL) to relay ON and OFF glutamate signals to ganglion cells (GCs) and amacrine cells (ACs). Here, we report the existence and receptive field properties of novel, nonconventional bipolar cell (NBC) types that exhibit ON light responses but stratify at the IPL's outer margin, within a distinct layer known as the accessory ON layer (AOL). Two-photon imaging of whole-mount retinas of mGluR6-Cre:GCaMP6s(Ai62) mice identified mGluR6-driven GCaMP6s expression in sparse populations of BC arbors within the AOL. Patch clamp recording from cell bodies traced to these axons revealed three distinct cell populations. One type (NBC1) had small non-tiling dendritic and axonal arbors that stratified narrowly in the AOL. The other two, termed BC9oa and BC9ob, resembled previously reported BC9o cells with thin, expansive axons in the outer IPL. BC9oa axons were unistratified in the AOL (S1), whereas BC9ob axons were bistratified, in AOL and S4/5. NBC1 was distributed throughout the retina, but BC9oa and BC9ob were largely restricted to dorsal regions. Imaged in AOL, axon arbors of all three types displayed robust ON GCaMP6s responses to center light stimulation, which were blocked by LAP4, but not by ACET or CNQX. Under whole-cell patch clamp, NBC1, BC9oa, and BC9ob showed light-evoked sustained LAP4-sensitive inward currents (voltage-clamped at -70 mV) and membrane depolarizations (current-clamped at rest), confirming their identity as ON BCs expressing mGluR6. Each exhibited distinct excitatory and inhibitory receptive fields, different from those of BC6 cells recorded in the same retinas. Connectomic analysis of published SBEM datasets (Ding et al., 2016; Yu et al., 2023) identified putative NBC1s forming ribbon synapses with M1 and M6 ipRGCs, sOFF alphas, dopaminergic amacrine cells, and amacrine types linked to rod function (A2, A17, nNOS1) among others. This study identified novel, unconventional ON cone bipolar cells that specifically innervate the AOL in the mouse retina.

Disclosures: **Y. Xue:** A. Employment/Salary (full or part-time):; Yale University. **S. Lee:** A. Employment/Salary (full or part-time):; Yale University. **D.M. Berson:** A. Employment/Salary (full or part-time):; Brown University. **Z. Zhou:** A. Employment/Salary (full or part-time):; Yale University.

Nanosymposium

NANO016: Photoreceptors and Retinal Circuitry

Location: SDCC Rm 23A

Time: Sunday, November 16, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO016.07

Topic: E.06. Vision

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Title: Bipolar Cell Polarity Switch Generated through Rod Signaling Pathway

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Abstract: The visual system uses two primary pathways to propagate a light signal through the retina: the ON and OFF pathways. ON cells are characterized by depolarization to light onset, and OFF cells by hyperpolarization. ON and OFF cells also exhibit morphological differences. Here, we found that this morphological-physiological rule is violated in some starburst amacrine cells (SACs) and bipolar cells (BCs) when recorded in mesopic light conditions. We used transgenic mouse models and applied pharmacology to examine the role of rods and cones to this polarity switch. We made whole cell patch clamp recordings in wholemount retina of C57BL/6J (WT), Cnga3$^{(-/-)}$ or Gnat2$^{(-/-)}$ cone-KO, and Gnat1$^{(-/-)}$ rod-KO mice. We stimulated SACs or BCs with a spot of green or UV LED light at either mesopic or photopic light conditions and recorded the voltage response. Cell types were identified by intracellular fluorescent dye labeling. We found approximately half of ON SACs showed an OFF-light response in mesopic conditions. Blocking GABAergic and glycinergic inhibition did not prevent the polarity switch. Blocking the ON pathway with L-AP4 removed all light responses, suggesting that the OFF signaling crossover was not the reason for the polarity switch. However, the polarity switch was reliably corrected at photopic light levels, indicating that rod-signaling caused the switch. When we recorded from presynaptic BCs, we similarly observed that subsets of OFF and ON cone BCs exhibit a polarity switch in mesopic light conditions. The polarity switch was not observed in the rod-knockout mouse model (Gnat1). However, in two cone-KO models (Cnga3 and Gnat2), the switch was still present in some BCs. Blocking electrical coupling by MFA failed to correct the polarity switch. Furthermore, iGluR antagonists (CNQX or NBQX) were applied to block horizontal cell signaling in cone-KO and WT mice, which failed to block the polarity switch. Finally, we applied TBOA to block the excitatory amino acid transporters (EAATs), which removed the polarity switch, suggesting that EAAT5 depolarized cones in response to light through its chloride conductance. In conclusion, we found that rods originate the polarity switch signals seen in some cone BCs and downstream in SACs, and EAATs in photoreceptor terminals generate the polarity switch. Our results indicate that retinal interneurons continuously adjust their polarities during the mesopic conditions.

Disclosures: T. Ichinose: None. **D.L. Beaudoin:** None. **A. Hassan:** None. **C.B. Hellmer:** None.

Nanosymposium

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Location: SDCC Rm 24A

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Presentation Number: NANO017.01

Topic: F.04. Voluntary Movements

Support: BRAIN BBQS R34 - DA059509
US-Israel Binational Science Foundation, CRCNS R01 - DA059993

Title: Neural population dynamics during naturalistic versus task-related behavior

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Abstract: Motor activity in cortex and striatum is present in trained and naturalistic contexts; however, the representational dynamics associated with activity in these areas across contexts is unknown. We therefore compared concurrently recorded cell- and layer-specific activity across these brain areas in a goal-directed task and open field. In the open field, mice produce a broad repertoire of behaviors, including locomotion, exploration, grooming, orienting, and rearing. In our head-fixed, joystick reaching task, mice produce a narrow set of repeated reaches to obtain rewards for correct responses. In both behavioral contexts, the behavioral syllabi and sub-actions were readily decoded from both single unit and population activity. At the population level, spontaneous naturalistic behavior demonstrated a relatively high dimensional neural representation, while reaching behavior was associated with a relatively low dimensional representation. Superficial and deep layers of primary motor cortex (M1) were better described by a linear manifold, while both dorsal striatum and ventral striatum were better described by a nonlinear manifold. We also determined ongoing measures of neural population state and information processing capacities across behavioral context. Metastability refers to brief sequences of population patterns. Each brain region we tested revealed a unique set of metastable population sequences, and these sequences differed across behavioral context. Criticality refers to spatiotemporal scale invariance beneficial for maximizing information processing capacity and computations across a wide dynamic range. Each brain region revealed a unique pattern of criticality metrics. Contrary to previous reports, we found activity in L5/6 was closer to criticality than L2/3 when mice are engaged in a skilled motor task. Additionally, L5/6 was closer to criticality than dorsomedial striatum during skilled motor activity, whereas dorsomedial striatum was closer to criticality than L5/6 during naturalistic behavior. These metastability and criticality patterns suggest differing roles of corticostriatal pathways in different behavioral contexts. Together, these results begin to elucidate how different behavioral contexts reveal

signatures of unique neural population dynamics across the motor system and the wide variety of behaviors that it parses.

Disclosures: P. Middlebrooks: None. **A. Eagle:** None. **M.A. Nicholas:** None. **A. Hsu:** None. **E.A. Yttri:** None.

Nanosymposium

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Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO017.02

Topic: F.04. Voluntary Movements

Support: French National Research Agency ANR OPTIMAGE

Title: Cognitive decline disrupts motor adaptation to gravity in older adults

Authors: G. POIRIER[1], C. PAPAXANTHIS[3], L. LORDON[4], Y. BÉJOT[5], P. MANCKOUNDIA[1], F. MOUREY[1], ***J. GAVEAU**[2];
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Abstract: Multiple studies suggest that an internal representation of gravity allows individuals to anticipate its effects and thus produce movements that minimize muscular effort in a gravitational environment. This is supported, for instance, by a shorter time to peak velocity (rD-PV) for upward movements compared to downward ones. This difference is referred to as directional asymmetry (ΔrD-PV $\neq 0$) and has been shown to reflect the optimality of motor planning in a gravitational environment (Berret et al., 2008; Gaveau et al., 2014, 2016, 2021). This capacity for optimal movement planning is preserved during physiological aging (Poirier et al., 2020; 2023). However, with the onset of cognitive disorders, a recent study reports that anticipatory processes in motor control deteriorate (de Nobile et al., 2025). This study aimed to test whether the adaptation of motor planning to the gravitational environment is affected in old individuals with cognitive impairments. Twenty-seven old patients with cognitive impairments (79±10 years old, MMSE score = 23±5), and thirty healthy old participants (71±6 years old, MMSE score = 30±0) were recruited. They were asked to perform single-degree-of-freedom arm pointing movements in two directions (upward and downward, amplitude = 30°). Finger position was recorded using a motion capture system (VICON, 100Hz). We then calculated the relative duration to peak velocity (rD-PV) and quantified movement smoothness with the Log dimensionless Jerk (LDLJ). Although movement duration was similar between the two groups, our results reveal a decrease in directional asymmetry (ΔrD-PV) in patients compared to healthy participants (Age x Direction effect: $F = 4.58$; $p = 3.69e\text{-}2$; $\eta p^2 = 0.09$). This suggests that their movements are less well adapted to the gravitational environment and thus less efficient. Movement smoothness was also reduced in patients compared to healthy old adults (Age effect:

F = 12.98; p = 6.77e-4; η_p^2 = 0.19). Patients' cognitive state (MMSE score) correlated with movement efficiency but not with movement smoothness (partial correlation accounting for age differences: ΔrD-PV, r = 0.43; p = 2.75e-2; LDLJ, r = 0.08; p = 6.93e-1). Our results confirm that the strategy of effort minimization persists during physiological aging. However, the loss of cognitive abilities in frail old individuals impairs this adaptive motor capacity, suggesting a compensatory role of cognitive functions during aging (Seidler et al., 2010). In line with the findings of de Nobile et al. (2025), our results support the idea that parameters indicative of motor anticipation could serve as a biomarker of the development of neurocognitive disorders.

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Nanosymposium

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Presentation Number: NANO017.03

Topic: F.04. Voluntary Movements

Support: NIH NINDS R35NS122333
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Title: Evolution of object identity information in sensorimotor cortex throughout grasp

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Abstract: The transition from opening your hand to grasping a coffee cup happens smoothly, yet the neural systems supporting this behavior go through a significant state shift at the onset of grasp. At this moment, the motor system begins to control contact forces and the somatosensory system receives a barrage of cutaneous signals that convey information about contact forces and the object's local features (e.g., edges, texture, and curvature). These cutaneous signals supplement the ongoing flow of proprioceptive input that encodes hand posture. It is unclear whether the neural signals representing object identity persist unchanged through this transition. In the present study, we sought to quantify the object-specific neural signals in the activity of individual neurons of the primary motor cortex (M1) and Brodmann areas 3a, 3b/1, and 2 of the somatosensory cortex in macaque monkeys. We found that, although the firing rates of individual neurons in each area declined rapidly after the object was grasped, this did not reduce the information they carried about the grasped object. On the contrary, it reflected a consolidation of object-specific information within sensorimotor cortex. The exception was proprioceptive area 3a, whose neurons were most informative right before contact and slightly less so after the object was securely held. In contrast, both individual neurons and population

activity in cutaneous areas 3b and 1 carried little object-specific information before contact but became highly informative afterward. Population-level analyses further revealed that multimodal area 2, which receives both cutaneous and musculotendinous inputs, does not represent object identity stronger than areas 3b and 1. Although area 2 showed higher activity before contact, this activity lacked task-relevant information, suggesting that uninformative cutaneous inputs may have interfered with informative proprioceptive signals. Following object contact, however, area 2 became highly informative, consistent with its proposed role in haptic object perception. In contrast, only M1 and area 3a carried object-specific information before contact. However, neither region appeared to encode a generalizable object representation, indicating a likely integration of force-related signals with postural information. Together, these findings underscore the profound effects of object contact on the neural representation of object identity in sensorimotor cortex.

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Nanosymposium

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Presentation Number: NANO017.04

Topic: F.04. Voluntary Movements

Support: PAPIIT BG200424

Title: The neurophysiological underpinnings of the invariant representation of rhythmic categories in rhesus monkeys

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Abstract: The capacity to perceive and maintain rhythm is a universal aspect of human cultures, suggesting a biological foundation for rhythm perception and synchronization (Merchant 2015). While humans demonstrate a bias towards simple integer ratios (1 to 4) during rhythm reproduction (Jacoby&McDermott 2017), macaques exhibit an impressive ability to synchronize with visual and auditory metronomes in a predictive and precise manner, showing a preference for visual synchronization (Castillo-Almazán 2025, Gamez 2018). Through controlled behavioral experiments, we provide evidence that macaques can not only synchronize to short-long or long-short rhythms but also learn representations that generalize across a wide range of rhythm ratios and total durations, indicating that monkeys possess the ability to flexibly represent ratios within

a relative timing framework. In addition to this, we explored the neurophysiological basis of beat extraction that enables macaques to represent rhythmic categories within the Medial Premotor Cortex, allowing for accurate synchronization and a categorical representation of rhythm. Together, the results support the notion that monkeys can perceive and synchronize to simple metric beats, and highlight the enormous potential of using monkeys to study the neurophysiological basis of complex rhythm perception.

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Presentation Number: NANO017.05

Topic: F.04. Voluntary Movements

Support: R01-EY035826
UF1 – NS122123

Title: Modular structure and depth-dependent heterogeneity in the primate premotor cortex

Authors: *A. DUBEY[1], Q. XU[2], A. ESTRADA BERLANGA[1], K. WINGEL[1], J. CHOI[4], B. PESARAN[3];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA, ;
[3]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [4]New York Univ., New York, NY

Abstract: Dorsal premotor cortex (PMd) is implicated in various aspects of motor control, including motor preparation, sequence generation, and the integration of sensory information for action. While the functional roles of PMd neurons are well-studied, the underlying organization of neurons involved in preparatory and movement-related activity at different depths remains poorly understood due to technical limitations of traditional microelectrode recordings and more recent population recordings using implanted electrodes. A detailed understanding of PMd functional organization is critical for understanding core principles of cortical motor processing and developing neurotechnologies to support brain-machine-interface (BMI) applications. Here, we investigated the structure of neuronal activity in PMd across cortical depth using high-density Neuropixels probes in two Rhesus macaque monkeys. We recorded spiking activity and local-field potential (LFP) during the performance of center-out reaching tasks across multiple experimental sessions. Each session targeted a different spatial location within the PMd with probe insertions typically ranging from 3 to 7 mm in depth. Each experiment yielded recordings from 50-200 isolated single-units. Consistent with prior work, the spiking activity of single units revealed two major groups of neurons: preparatory neurons which increased firing around 200-300 ms before reach onset, and movement neurons which increased firing around reach onset. Critically, however, our high-resolution recordings, leveraging Neuropixels probes with 384

contacts densely sampled at 20 micrometers, revealed significant heterogeneity in the depth-organization of these PMd neurons across different spatial locations. We observed either a modular or salt-and-pepper organization. At approximately 40% of sampled locations, a modular organization was present, with preparatory neurons clustering together and movement neurons clustering together. In contrast, neurons recorded at the remaining 60% of spatial locations exhibited a salt-and-pepper distribution, with both neuron groups intermixed across depth. Interestingly, High-gamma (70-200 Hz) LFP activity during the task strongly correlated with the spiking activity at spatial locations showing modular organization, but this correlation was markedly weaker at spatial locations with salt-and-pepper organization. Together, these findings reveal a striking heterogeneity in functional organization of PmD, providing crucial insights for understanding cortical motor processing and advancing next-generation brain-machine interfaces.

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Topic: F.04. Voluntary Movements

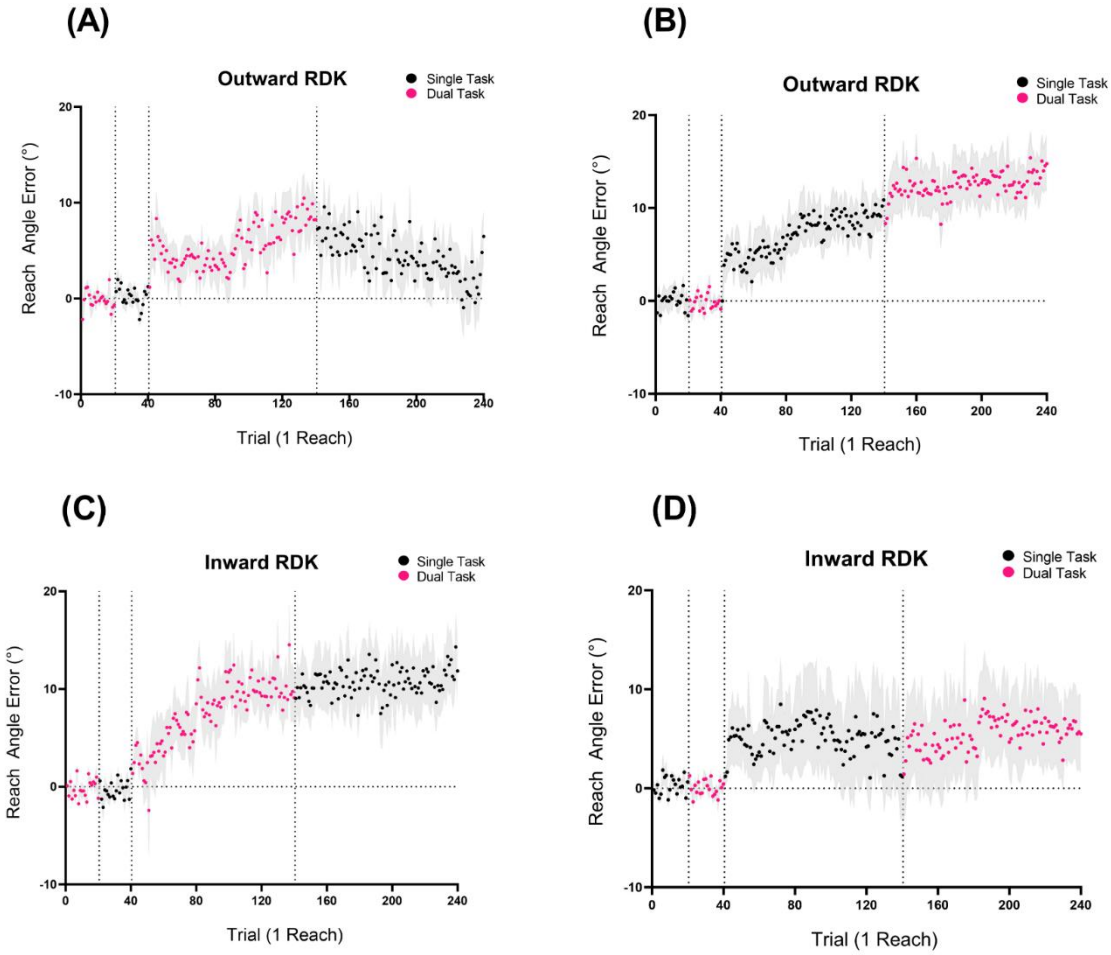
Support: Funded by the Australian Research Council - DP230102179

Title: Benefits of dual tasking on implicit sensorimotor adaptation

Authors: *B. MILLER-MILLS[1], T. KWAN[2], T. J. CARROLL[3], E. POH[4];
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Abstract:

Figure 1



Attention plays a crucial role in maintaining precision and effectiveness in goal-directed actions. There is evidence that dividing attention across tasks impairs performance in various domains. However, the impact of attention on sensorimotor adaptation remains inconclusive, with some studies reporting deficits and others showing no effects. Because sensorimotor adaptation can involve both explicit and implicit processes, the conflicting findings may reflect different effects of attention on each process. Here, we investigated how divided attention influences implicit sensorimotor adaptation using an error-clamp paradigm, coupled with a random dot kinematogram (RDK) motion coherence discrimination task. Each participant (n=12 / group) adapted to the error clamp with and without the dual task, in counterbalanced order. We also assessed the effect of the timing of the secondary task by presenting the RDK either during the outward movement (coinciding with error feedback), or the inward movement (following error feedback). The grand mean for RDK performance accuracy was ~66% (chance = 50%), suggesting a substantial attentional demand (main effect of groups: $f(3,44) = 0.506$, $p = 0.68$). For the outward group, sensorimotor adaptation was greater for participants when they performed the dual-task condition than when they performed the single task condition,

irrespective of order (dual task first, $t(11) = 3.24$, $p = 0.004$, Figure 1A; dual task second, $t(11) = 3.38$, $p = 0.003$, Figure 1B). There was no effect of the attention task when it was performed during the inward movement back to the home position (main effect of Task, $f(1,22) = 0.004$, $p = 0.946$, Figures 1C,D). Thus, sensorimotor adaptation was enhanced when attention was divided (i.e. RDK was presented on the outward movement), compared to when attention was focused entirely on the adaptation task (RDK during the inward movement). This suggests that implicit sensorimotor adaptation is sensitive to attentional demand, with effects from attention possibly being restricted to a critical time window where error feedback is received.

Disclosures: B. Miller-Mills: None. **T. Kwan:** None. **T.J. Carroll:** None. **E. Poh:** None.

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Topic: F.04. Voluntary Movements

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Title: Unraveling the cognitive and sensorimotor contributions to manual dexterity: insights from healthy individuals and people with multiple sclerosis

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Abstract: Manual dexterity is a skill involving fine motor movements, usually associated with the sensorimotor domain. However, it also involves cognitive components, potentially involving prefrontal circuits. The Nine-Hole Peg Test (9-HPT) is used for assessing manual dexterity in clinical settings, including in Multiple Sclerosis (MS). The standard 9-HPT measures the total time taken to complete the task, but this single metric may not fully capture performance complexity. To explore sensorimotor and cognitive contributions, we recently used functional near-infrared spectroscopy (fNIRS) to assess cortical activity during the 9-HPT in healthy participants. They performed with the dominant (right) hand the 9-HPT and a novel control task, the One-Hole Peg Test (1-HPT), which isolated sensorimotor components by requiring peg placement and removal using a single hole. Participants were slower on the 9-HPT than the 1-HPT. Both tasks activated sensorimotor areas involved in reaching and grasping, but participants with higher execution time difference (ΔHPT) showed greater prefrontal activation (right Brodmann Area (BA) 10 and BA11) than those with lower ΔHPT. A linear correlation between ΔHPT and right BA10 activity indicated that individuals with larger performance differences recruited prefrontal regions, likely for planning and control, but without improving speed. Here,

we conducted a similar fNIRS study (9-HPT vs 1-HPT) in 25 people with MS (PwMS). The 9-HPT was slower than the 1-HPT (23.80±0.96 vs. 21.83±0.86 s, $p<0.001$) and evoked higher activity in the left BA11, BA44, and BA43 (i.e., areas associated with executive functions and planning). Higher activity during the 1-HPT was found in the left BA3 and right BA4 (i.e., key areas for somatic sensation and motor control). Further, ongoing work adopts a newly developed engineered 9-HPT, capable of automatically measuring additional temporal parameters. Data from 10 PwMS and 10 healthy controls demonstrated its reproducibility, with total time measurements correlating with manual stopwatch timings ($r=0.99$, $p<0.001$). PwMS showed longer times for total execution (21.95±4.21 vs. 18.14±1.57 s, $p=0.01$), insertion, and removal than healthy controls. They also exhibited longer intervals between the insertion and removal phases (1.31±0.38 vs. 1.01±0.17 s, $p=0.03$) and longer inter-peg intervals. This suggests increased time needed for planning single finger movements in PwMS and highlights the potential for this tool to provide a more detailed assessment, including currently unexplored aspects, particularly useful in clinical populations where both motor and cognitive functions can be affected.

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Nanosymposium

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Topic: F.04. Voluntary Movements

Support: NIH Grant R21HD111748
Rehabilitation Science New Student Award

Title: Do younger and older adults employ distinct strategies for online motor control during unexpected perturbations?

Authors: *P. THAPA[1], S. A. JAYASINGHE[2];
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Abstract: Online control of goal-directed reaching is crucial for efficient movement during activities of daily living. Although previous studies have provided evidence of impaired motor control to expected perturbations in older adults, their motor behavior to unexpected perturbations is unclear, particularly in comparison with younger adults. Interlimb differences in reaching strategies between older and younger adults during unexpected perturbations also remains unknown. We hypothesize that younger and older adults employ distinct reaching strategies, such that younger adults prioritize speed and older adults prioritize accuracy, when updating their motor plans during unexpected perturbations. We used a double-step paradigm (99 trials) in which the target remained unperturbed or was displaced pseudorandomly to 1 of 2

locations to the left or right of its original vertical position, at movement onset. We recruited 10 younger (4 females, 6 males; age 28.9 years +/- 1.52 SEM) and 10 older adults (8 females, 2 males; age 68.9 years +/- 2.62 SEM). Participants completed the task with each hand. Our results showed that in no perturbation conditions, younger adults moved significantly more efficiently compared to older adults ($p = 0.042$). However, there were no differences between groups during perturbation. In general, younger adults moved faster than older adults during perturbation ($p = 0.04$), irrespective of tested hand. With respect to the onset of movement correction, we found that older adults took longer ($p = 0.023$) and made larger accuracy errors ($p = 0.045$) compared to younger adults. Interestingly, despite those differences in early correction strategies, there were no differences in endpoint accuracy between the groups ($p = 0.368$). These results suggest that during an unexpected perturbation, younger adults prioritized speed at the cost of spatial efficiency while older adults maintained endpoint accuracy despite larger errors and delayed time to early correction. Our results indicate the preserved ability to utilize feedback information in older adults during online corrections, despite age-related declines in sensorimotor function. Future work will examine muscle coordination strategies that give rise to these kinematic behaviors in older and younger adults.

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Nanosymposium

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Presentation Number: NANO017.09

Topic: F.04. Voluntary Movements

Title: Disconnection syndromes and injury to neural systems after stroke

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Abstract: Background: Stroke-related impairments present in wide-ranging combinations, including cognitive and sensorimotor deficits, complicating an understanding of their relationship with the anatomy of injury. Here, we hypothesized that upper extremity (UE) sensory impairment, UE motor impairment, abnormalities of mood, and cognition deficits would each be associated with distinct patterns of neural injury, and explored whether outcome measures that rely on multiple behaviors are more vulnerable to injury-related disconnection. **Methods:** Stroke subjects were assessed for deficits in [1] elementary UE sensorimotor behaviors that require limited cognitive control (shoulder abduction and finger extension strength [SAFE], Fugl-Meyer Assessment [FMA], and wrist proprioception sense test [WPST]), [2] complex sensorimotor behaviors that require substantial cognitive control (Box and Blocks Test [BBT] and Trail Making Test A [TMT A]), [3] cognition (Montreal Cognitive Assessment), and

[4] mood (Geriatric Depression Scale). Infarcts were outlined on clinical scans, normalized to standard brain space and used to compute lesion volume, injury to the corticospinal tract (CST) and thalamocortical sensory tract (TST), and measures of large-scale neural network disconnection. Associations between lesion-related neuroimaging measures and behavior were examined using voxel-lesion-symptom mapping (VLSM) and correlation analysis. **Results:** Sixty subacute stroke patients (mean age 69.2, 42% females) were included, with stroke lesion volumes ranging from 0.1 to 354.9 (mean 30.9) ml that affected the CST in 73% and TST in 62% of the subjects. CST and TST injury were significantly associated with UE sensorimotor behaviors (CST with SAFE r = -0.45, FMA r = -0.44, BBT r = -0.44, WPST r = 0.37, and TMT A r = 0.28) but not with cognition or mood, underscoring the specificity of these location-behavior associations. Two tests that rely substantially on both motor and cognitive function (i.e. psychomotor speed), the BBT and the TMT A, were significantly associated with widespread disconnections between brain networks, such as the frontoparietal, ventral and dorsal attention, and the default mode network. **Conclusion:** These findings indicate that elementary UE sensorimotor behaviors such as strength are related to the integrity of motor system structures (e.g., the CST), but that complex UE motor behaviors that include cognitive demands (BBT, TMT A) additionally require integration across multiple brain networks that are important for directing and retaining attention, as well as planning and coordinating hand activities such as grasping.

Disclosures: A. Schwarz: None. **C. Holl:** None. **S. Cramer:** None.

Nanosymposium

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Time: Sunday, November 16, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO017.10

Topic: F.04. Voluntary Movements

Support: NSF CMMI-2133084

Title: Effect of reaching height and initial hand position on upper limb kinematics in infants

Authors: *I. SAHIN[1], A. STEPANIAN[2], V. N. CHRISTOPOULOS[3], E. KOKKONI[1]; [1]Bioengineering, [2]Neurosci., Univ. of California, Riverside, Riverside, CA; [3]Bioengineering, USC, Los Angeles, CA

Abstract: Prior studies in controlled lab environments have shown that adults adjust their arm movements and muscle activation when reaching for objects at different heights. Nevertheless, the developmental origins of this adaptive motor behavior remain largely unknown. This study examines how varying the presented object height affects reaching kinematics in infants within natural environments, also mirroring natural object presentation from an ecological perspective. Information from this study contributes to a better understanding of the adaptive mechanisms of early motor control, which can also inform the design of controllers for devices to assist reaching

in infants with motor impairments. Video-recordings from 35 infants (M_{age}=7.71±1.82 months) performing reaching actions toward stationary objects were examined. These objects were presented across different vertical locations, which, along with the infants' hand location at reaching onset, were classified by height into two groups: high (at abdomen and eye levels) and low (at the hip joint and ground level). A total of 228 reaching segments were analyzed. The wrist joint was digitized to obtain its 2D position in every frame. The following kinematic variables were computed: total number of movement units (MUs), straightness index (SI), path length (PL), and average speed. To assess differences in these variables across heights, non-parametric Mann-Whitney U tests were performed. The results confirmed our hypothesis that infant reaching kinematics would differ for objects presented at different heights. When the object was placed high, MUs (4.81±3.69) and PL (16.60±10.11 cm) were significantly greater (U_{MUs}=4664, p_{MUs}<0.001; U_{PL}=4674, p_{PL}<0.001) than when the object was placed in the low level (MUs: 3.25±2.61; PL: 12.93±8.94 cm), but the average speed was not affected (U=0.074, p=0.786). In contrast, the SI was significantly smaller (U_{SI}=5327, p_{SI}=0.048) when the object was placed high (1.97±1.51) than low (2.12±3.78). Furthermore, when combined with the initial hand location, the SI was significantly greater (USI=4070, p_{SI}=0.036) when the hand's initial vertical location differed from the object's height (2.41±3.41) as opposed to starting at the same level (1.94±2.92). These findings suggest that when objects are placed higher and/or at a greater distance from the initial hand location, infants exhibit longer, less direct paths and make more corrective adjustments along the way as they reach for the object. This indicates that reaching in these cases requires sophisticated motor control, involving compensation for gravity, integration of visual feedback, and postural stability.

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Presentation Number: NANO017.11

Topic: F.04. Voluntary Movements

Title: Reduced Heart-Brain Coherence Enhances High-Intensity Performance Under Altruistic Motivation

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Abstract: Recent research on heart-brain coherence suggests that cardiac signals may actively influence neural and motor functions beyond their traditional autonomic roles. This study

investigated how altruistic motivation affects heart-brain coherence (HRV-EEG synchronization) during a near-maximal grip force task (95% MVC) performed to failure. Thirty-one healthy, physically active adults (mean age: 30.6 ± 8.2 years; 15 men, 16 women) were randomly assigned to altruistic, extrinsic, or neutral motivational conditions before performing two trials. Participants received motivational framing specific to their assigned condition before the second trial. Heart rate variability (HRV) and electroencephalography (EEG) signals were continuously recorded to assess coherence changes. Contrary to predictions, participants in the altruistic group significantly increased their endurance by 68% ($p = 0.004$), accompanied by marked reductions in low-frequency (LF) HRV-EEG coherence across Delta, Theta, and Alpha EEG frequency bands. Correlation and regression analyses demonstrated that decreased LF HRV coherence with Delta, Theta, and Alpha bands, particularly within the Default Mode, Frontoparietal, and Sensorimotor brain networks, strongly predicted improved motor performance ($R^2 = 0.93$). These findings challenge the assumption that increased heart-brain coherence invariably enhances performance, suggesting instead that altruistic motivation strategically "decouples" autonomic-cortical synchronization to facilitate greater motor endurance. The results provide insight into the motivational modulation of physiological systems, offering implications for clinical rehabilitation adherence, athletic performance enhancement, and high-demand occupational training.

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Topic: F.04. Voluntary Movements

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Title: The relevance of mechanical impedance when interacting with dynamically complex and uncertain objects

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Abstract: Manipulating complex objects is ubiquitous in our daily activities, such as tying shoelaces, donning a jacket, or carrying a cup of coffee. However, such non-rigid objects present dynamical challenges: When carrying a cup of coffee, the coffee develops unpredictable dynamics that can lead to spilling the coffee - instability. Previous work utilized a human-inspired impedance controller to show that dynamic stability of the object is ensured by

preparing the system's initial conditions and selecting appropriate interaction frequencies. However, it remains unclear if or how mechanical impedance is modulated in the case where the physical properties of the object are uncertain or difficult to estimate. Extending our previous work, we used the same task of transporting a ‘cup of coffee’ simplified to moving a cup with a rolling ball inside. Participants translated the virtual cup and ball on a horizontal line displayed on a screen via a robotic manipulandum. Participants were instructed to 'jiggle' the cup to prepare the cup and ball's states for the ensuing continuous rhythmic movement at their preferred frequency. The isometric grip force on the robot handle served as a proxy for the mechanical impedance of the arm. To introduce uncertainty regarding object properties, we manipulated the curvature of the cup in two conditions: i) random condition, the curvature changed randomly from trial to trial without explicit cues, and ii) blocked condition, the curvature remained constant across trials. Using stochastic open-loop optimal control simulations we solved for time-dependent impedance and force under the two conditions of the experiment. For both random and blocked conditions, participants controlled the relative phase to either in-phase (0°) or anti-phase (180°) before starting the rhythmic task. Despite the uncertainty in dynamics, they maintained comparable stability of relative phase in both conditions. While the ball's initial angle and the cup's frequency varied among participants, the nonlinear covariation of the two variables stabilized the cup-ball dynamics in both conditions, as predicted by the forward simulations. While the net force applied on the cup was similar between the conditions, the grip forces, indicative of mechanical impedance, were higher in the random condition. Optimal control simulations corroborated these findings, revealing that participants selected preparation and interaction frequencies that effectively minimized mechanical impedance.

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Presentation Number: NANO017.13

Topic: F.04. Voluntary Movements

Title: Cortical Excitability During Brain-Computer Interface Stroke Treatment Predicts Functional State

Authors: *S. SIEGHARTSLEITNER, K. MAYR, C. GUGER;
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Abstract: Brain-Computer Interface (BCI)-based neurorehabilitation has been shown to support motor function recovery in both upper and lower extremities following stroke. In particular, BCIs that incorporate functional electrical stimulation (FES) as a feedback mechanism are considered especially effective. Beyond their therapeutic role, such systems provide a unique opportunity to capture brain activity during the treatment process, offering session-by-session insights into patients’ cortical activation patterns. In this study, we investigated whether cortical

excitability during motor imagery BCI-FES therapy (recoveriX, g.tec medical engineering) can predict patients' functional state. To this end, we estimated event-related (de)synchronization (ERD/S) in 83 stroke patients undergoing upper extremity BCI treatment. ERD/S was averaged across the first four therapy sessions and used as input features to a support vector machine with a Gaussian kernel, trained to predict each patient's pre-treatment functional state. This state was defined as the first principal component derived from their Upper Extremity Fugl-Meyer Assessment and Barthel Index scores. Prediction performance was assessed using 10 repetitions of 10-fold cross-validation. Our results show that ERD/S patterns during motor imagery of the affected hand can robustly predict patients' functional state, achieving an average Pearson's r of 0.62 (SD = 0.02). The model's performance proved stable across folds, indicating that the findings reflect meaningful neural markers rather than statistical noise. The patient cohort was diverse in terms of lesion location and baseline impairment, supporting the generalizability of this approach. While clinical scales remain the gold standard for assessing recovery, this method offers an additional, neurophysiological window into patient status, enabling more continuous and individualized monitoring throughout the rehabilitation process.

Disclosures: S. Sieghartsleitner: A. Employment/Salary (full or part-time):; g.tec medical engineering. **K. Mayr:** A. Employment/Salary (full or part-time):; g.tec medical engineering. **C. Guger:** A. Employment/Salary (full or part-time):; g.tec medical engineering.

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Topic: I.03. Decision Making

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Title: Identifying cross-task sex differences in cognitive flexibility in mice mediated by the explore-exploit tradeoff

Authors: *N. M. GRISSOM;
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Abstract: Cognitive flexibility is a transdiagnostic focus of impairment in multiple neuropsychiatric disorders. Effective modeling of cognitive flexibility across species is thus essential for effective preclinical treatment development. However, it is inevitable that changes in task design need to be made across species to better match their ethological niche, but it is unclear to what extent changes in task design impacts the assessment of cognitive flexibility, potentially limiting effective translation. To begin to address this problem, we have recently developed touchscreen methods for assessing both an operant set shift task and a spatial restless bandit task in the same mice over the course of several weeks of testing. 32 mice (16 male/16

female, B6129SF1/J wildtypes) were first assessed on operant set shift, then transitioned to restless bandit testing. Overall, we find that performance across days was largely similar within animals on either task. We have previously used a hidden Markov model (HMM) approach to identify whether animals differ in their explore-exploit balance in the restless bandit task, identifying greater engagement of exploit states and persistence of choice in females. A modified HMM again identified greater exploit state attainment in females in the operant set shift task, which was associated with improved performance on set shift. Next, we transitioned these animals to the restless bandit task, and found that explore-exploit balance was highly correlated between tasks on an individual animal basis, with similar overall sex differences across both tasks. These data suggest that the cognitive flexibility construct measured in operant set shift tasks is highly overlapping with explore-exploit state constructs measured in bandit tasks. Current analysis is exploring what patterns of cognitive flexibility in set shift are most predictive of later restless bandit performance, and whether we can also model these constructs effectively in longer term, in-cage testing. We will discuss implications for conserved neural mechanisms governing cognitive flexibility within individuals across tasks, and across species, including both potential cortical and subcortical mechanisms.

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Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.02

Topic: I.03. Decision Making

Title: Neuromodulatory regulation of cognitive flexibility in the ACC.

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Abstract: Cognitive flexibility and attentional persistence depend upon the functional integrity of the anterior cingulate cortex (ACC). Dysfunction in this region and its related circuits are hypothesized to underlie cognitive impairments in many neuropsychiatric disorders, including but not limited to depression, schizophrenia, and addiction. Presently, these impairments are not readily remediated with current pharmacotherapies. To better understand the neurochemical basis of these cognitive impairments, the current work uses a rat model of cognitive flexibility in conjunction with a series of neurochemically specific lesions of norepinephrine, acetylcholine, dopamine, or serotonin in the anterior cingulate cortex. Long-Evans rats who were young adults at the time of surgery were infused with neurochemically selective toxins in the ACC two weeks prior to cognitive testing. Subjects were evaluated in an attentional set-shifting task (ASST) that assesses the formation and maintenance of an attentional set, distractibility, and cognitive flexibility. The cognitive effects of each lesion were highly specific. Loss of acetylcholine or

dopamine in the ACC increased distractibility to salient distractors and impeded the formation of an attentional set. Dopaminergic lesions also impaired cognitive flexibility as measured by reversal learning and shifting of an attentional set. Serotonergic lesions of the ACC produced impairments in the initial test of reversal learning but facilitated performance on subsequent tests of reversal learning. Noradrenergic lesions of the ACC did not impair any aspect of ASST performance. Together, these findings support the hypothesis that each of the neuromodulatory systems assessed contributes to unique aspects of cognitive flexibility. Acetylcholine is necessary to filter irrelevant distractors. Dopaminergic lesions produce widespread impairments, supporting the hypothesis that this system is critical to learning task constraints and forming cognitive sets. Serotonergic lesions produce significant but transient impairments in reversal learning, while sparing the formation of attentional sets. These results highlight the complex contributions of neuromodulatory systems in the ACC to cognitive flexibility.

Disclosures: J.A. McGaughy: A. Employment/Salary (full or part-time):; University of New Hampshire. **D. Sarubin:** A. Employment/Salary (full or part-time):; University of New Hampshire. **T. Kimball-Sabatella:** A. Employment/Salary (full or part-time):; University of New Hampshire. **A.T. Brockett:** A. Employment/Salary (full or part-time):; University of New Hampshire.

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Topic: I.03. Decision Making

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Title: Premotor cortical circuits support contextual decision-making through flexible stimulus-response mapping

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Abstract: Contextdependent decision-making enables organisms to extend their behavioralrepertoire beyond fixed stimulus-response (S-R) associations and is thusconsidered a cornerstone of cognition. The absence of such flexibility is ahallmark of many psychiatric and neurological disorders. We have developed aparadigm to study the neural mechanisms of a context dependent decision inlaboratory mice, trained to deploy a different stimulus-response associationdepending on a contextual cue. Mice were trained to lick a left or right portin response to a “test” stimulus (odor A or odor B). A context cue waspresented at the beginning of

the trial (odor C or D), followed by a 1-2 sdelay. In context 1 (Cx1) the mouse was rewarded for licking left in responseto A and right in response to B. In Cx2, the stimulus-response (S-R)association was reversed. Optogenetic silencing of neurons in the anteriorlateral motor area (ALM) during presentation of the context cue and delayperiod impairs performance. Using 2p Ca^{2+} imaging, we found neurons inLayer 2 of ALM that respond selectively to one of the context odors (C or D).The population maintained this information through the delay period. Whiledirected-lick neurons were not active in this epoch, we found that apical tuftdendrites of these neurons exhibited Ca^{2+} signals that were also selectivefor C or D. We hypothesize that this signal plays a role establishing flexibleS-R mapping. We report on our progress conducting an optical dissection of thiscircuit, including the three major types of interneurons in the ALM. We foundthat the somatostatin (SOM)-, but not parvalbumin- or vasointestinalpeptide-positive neurons, tuned to context cues. Finally, combining Ca^{2+} imaging with holographicphotostimulation, we are examining the causal relationship between the activityof context selective neurons and the dendritic gating computation on thedirected-lick neurons as well as behavior outcomes

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Title: Intracortical microstimulation in dACC disrupts the onset of exploration

Authors: *R. SINGHAL[1], G. DUBOIS[2], B. Y. HAYDEN[3], B. A. EBITZ[2];
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Abstract: Uncertain environments demand a balance between exploring new options and persistently exploiting the ones known to maximize reward. The dorsal anterior cingulate cortex (dACC) is thought to be involved in the flexible switching between persistent and exploratory behavior, due to correlations between dACC activity and cognitive variables linked to exploration. However, it remains unclear whether dACC is causally involved in this switch or instead, merely tracks these variables as part of its role in performance monitoring. Here, we ask whether intracortical microstimulation in dACC causally alters the likelihood of exploratory decisions.
Methods: Two rhesus macaques performed a saccadic 3-armed bandit task. Although the 3

targets were visually identical, they had dynamic reward probabilities that encouraged the monkeys to balance exploiting good options with exploring alternatives for better long-term gains. We stimulated dACC (target) and dorsolateral prefrontal cortex (dlPFC; control) during inter-trial intervals on random trials. A second control experiment assessed whether dACC stimulation was rewarding by offering the monkeys a choice between stimulation and variable levels of juice reward. To investigate how dACC stimulation modulates behavior, we also recorded neural activity in dlPFC, a downstream region involved in choice specification, during dACC stimulation in the bandit task.
Results: Stimulation in dACC selectively disrupted the onset of exploration independent of reward history. This effect was exclusive to dACC stimulation and was not observed with stimulation in dlPFC. Stimulation in dACC stimulation did not function as an illusory reward because neither monkey had any preference for stimulation in the choice task. In neural activity recorded in dlPFC, there was more variance in the choice-predictive neural subspace during exploration than exploitation. Stimulating dACC transiently pushed the activity in dlPFC out of the task subspace. Activity then slowly relaxed back as the trial progressed. Although dACC stimulation had persistent effects on neuronal firing rates in dlPFC, it did not significantly modulate the variance or make stimulated trials look more exploitative. These results suggest that stimulating dACC is sufficient to alter control over exploratory choices, perhaps by affecting persistent changes in dlPFC activity. These insights could be instrumental in guiding the development of neural prosthetics for therapeutic use in disorders marked by deficits in cognitive flexibility.

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Title: Neural circuit mechanisms of bottom-up reward learning

Authors: *Z. R. ZEISLER[1], F. M. STOLL[1], L. LONDON[3], D. FOLLONI[3], M. G. PERICH[4], P. H. RUDEBECK[2];
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Abstract: Synaptic mechanisms have long been thought to underlie associative learning. However, humans and animals often make associations between actions and outcomes that exceed the timescale of such mechanisms. Prior work has implicated frontal cortex and limbic system in binding together temporally separated events, but the specific pathways and neural

mechanisms involved are unclear.
Here, we trained rhesus macaques in a three-choice probabilistic reversal learning task that incorporated trace intervals between subjects' choices and contingent outcomes. Each session was comprised of 18 blocks of 35-50 trials, where we varied the stimulus-reward probability contingencies after each block and the length of the trace interval every two blocks. Monkeys were slower to learn which stimulus was associated with the highest probability of reward when the trace interval was longest. Further, reinforcement learning models fit to subjects' choices revealed that long trace intervals were associated with lower learning rates compared to shorter intervals.
We recorded nearly 5,000 neurons across ventral frontal cortex, amygdala, and striatum using semi-chronic microdrives. Notably, the stability of single neuron encoding across delay conditions was not uniform across brain areas. Reward coding was most stable in the agranular insula (aINS), with nearly 80% of neurons that significantly encoded reward doing so across multiple trace conditions. Stimulus identity coding, by contrast, was most stable in amygdala. Interestingly, stimulus value neurons in all recorded areas were less likely to maintain their encoding across trace conditions.
Next, we took a population level approach to the neural activity. Using principal component analysis, we observed larger differences in population state trajectories between no trace and long trace conditions than between short trace and long trace. This difference was largest in aINS and ventrolateral prefrontal cortex (vlPFC), suggesting a significant reorganization of neural activity, particularly in these areas, when subjects had to hold a memory of their choice until feedback was delivered.
Ongoing work utilizing local field potential seeks to characterize inter-area communication, and preliminary results indicate an increase in beta band coherency between amygdala and vlPFC during long trace intervals. In parallel, we are training data-constrained RNNs to further probe the cross-area mechanisms that may be differentially employed to bridge long trace intervals. Taken together, our results highlight that distinct learning and neural mechanisms are recruited when animals' actions and outcomes are separated in time.

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Title: Flexible use of stimulus vs. spatial information in learning: Probing the role of an amygdala-to-orbitofrontal pathway with single cell imaging and chemogenetics

Authors: J. L. ROMERO SOSA[1], A. YEGHIKIAN[1], A. SOLTANI[2], *A. IZQUIERDO[1]; [1]UCLA, Los Angeles, CA; [2]Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Adaptive learning and choice under uncertainty often requires the brain to estimate the predictive values and reliability of different sources of information in order to determine their relative contributions to decision making (i.e., arbitration). Previous work has shown that the basolateral amygdala (BLA) is critical for learning whether stimulus and spatial information should be used in uncertain reward conditions, in both macaques and rats (Taswell et al, 2021; Aguirre, Woo et al., 2024). Moreover, OFC may also play a prominent role given its involvement in learning of probabilistic reward conditions, or "expected uncertainty." Therefore, BLA may contribute the arbitration process through its connections to orbitofrontal cortex (OFC). To test this hypothesis and probe the role of the BLA-to-OFC pathway, we prepared male and female Long-Evans rats (n=5) with transsynaptic anterograde Cre in BLA and Cre-dependent GCamp6f in ventrolateral OFC, with a GRIN lens in the latter for miniscope imaging. Rats were tested on a stimulus discrimination task involving150 trials per session where the better (more rewarding) stimulus could appear pseudorandomly on the left or right side of a touchscreen. The rats were required to choose (i.e., nosepoke) the better stimulus while ignoring the spatial location in which it appears. The reward probabilities for the two options varied once the rats reached a performance criterion of 75% on two consecutive sessions for each stage: 100:0, 90:10, 80:20, and then 80:20, 90:10, 100:0. We measured the extent to which the animals used stimulus and spatial information in each session and found that in the 100:0 learning condition, rats significantly increased their use of the stimulus (Bsession = 0.02, p=1.4e-5), but not spatial information (Bsession = 0.004, p=0.59) across sessions. Yet, the spatial information was still used throughout learning (mean use, p=0.64) and did not significantly decrease. Ongoing analyses are directed at training two separate SVM decoders to predict stimulus choice vs. spatial choice, along with trial outcome. Additionally, a separate group of animals (n=6) with transsynaptic anterograde cre in BLA and cre-dependent DREADDs in OFC are currently being tested on the same task, to determine the functional significance of this pathway on the arbitration of stimulus and spatial information in learning under uncertainty.

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Presentation Number: NANO018.07

Topic: I.03. Decision Making

Title: Anterior Cingulate Cortex Represents a Cognitive Graph of Behavioral Strategies and Arbitrates Between Them

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Abstract: The anterior cingulate cortex (ACC) is critical for flexible behavior in changing environments. To examine how the ACC contributes to this behavioral flexibility, we tested whether 1) the ACC first constructs an internal representation—a cognitive graph—of action sequences and second, and 2) if the ACC causally arbitrates the strategic decision to persist with a current strategy or switch to an alternative. To test the first hypothesis, we trained rats to perform sequences of left and right choices in an operant box. Rats were able to learn long arbitrary sequences with only a terminal reward as a cue. When the environmental rule switched to reward a different action sequence, rats rapidly converged onto the new sequence. Using wireless recording, we found that neural ensemble activity in ACC develops a highly structured, yet compact, scalable representation anchored at the start and end of these action sequences. We furthermore found that graphs are organized to reflect relational similarities across contexts, and individually, permit flexible refinement of component states. To test if ACC instructs switching between strategies, we trained rats in a two-armed bandit task, where the probability of reward changed randomly throughout behavioral sessions. We found through optogenetic inactivation experiments that the intratelencephalic and pyramidal tract promotes exploitation and exploration, respectively. Together these observations argue that the cingulate learns a representation of environmentally strategies and through the intratelencephalic and pyramidal tracts arbitrates between them.

Disclosures: D.G. Tervo: None. **A.Y. Karpova:** None. **M. Proskurin:** None. **S. Druckmann:** None. **E. Kuleshova:** None. **M. Manakov:** None. **H. Wang:** None.

Nanosymposium

NANO018: Adaptive Choice

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.08

Topic: I.03. Decision Making

Support: NSF Grant 2020844
NSF Grant 2336466

Title: Reward function compression in human reinforcement learning

Authors: *G. MOLINARO, A. G. E. COLLINS;
Univ. of California, Berkeley, Berkeley, CA

Abstract: The study of reward-based learning has traditionally focused on primary, innately reinforcing rewards and secondary rewards that derive value through learned associations. However, humans can pursue complex goals whose value must be assigned deliberately. Here,

we investigate how people leverage the natural reward system to attain abstract goals. We propose that humans engage in “reward function compression” - the process of building simplified mappings from a set of outcomes to values by identifying a subset of features to guide learning. Across six experiments (N = 390), participants learned stimulus-response associations through trial and error, receiving either numeric points or abstract visual goals as feedback. When goals changed on each trial, learning was significantly impaired compared to points (Experiment 1), but this difference diminished when using consistent goals (Experiment 2). Critically, learning efficiency did not improve simply with goal repetition (Experiment 3), suggesting that familiarity or learned associations alone cannot explain the benefits of consistent goals. By contrast, learning was disrupted when the same outcomes could serve as both goals and non-goals (Experiment 4), indicating that people struggle to build consistent reward functions when outcome values conflict. Learning also declined rapidly with each additional unique goal per block (Experiment 5), suggesting that reward function compression becomes increasingly challenging as the space of goal outcomes expands beyond a small number. Finally, learning performance improved when goal features could be compressed into lower-dimensional spaces compared to when they could not (Experiment 6). These results suggest that humans actively attempt to build simple yet informative reward functions when pursuing novel goals. Our findings highlight the computational and cognitive challenge of reducing vast outcome spaces to simple scalar rewards, providing initial evidence for reward function compression in human reinforcement learning.

Disclosures: G. Molinaro: None. **A.G.E. Collins:** None.

Nanosymposium

NANO018: Adaptive Choice

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.09

Topic: I.03. Decision Making

Support: HHMI

Title: Cortical control of innate behavior from subcortical demonstration

Authors: J. A. KELLER[1], A. KEMTUR[2], R. JOHNSON[3], ***J. T. DUDMAN**[4];
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Abstract: Motor control in mammals is traditionally viewed as a hierarchy of descending spinal-targeting pathways, with frontal cortex at the top. Cortical control is thought to enable remarkable flexibility and adaptability to the deployment of motor programs to achieve complex goals. However, many redundant muscle patterns can solve a given task, and this high dimensionality is critical for flexibility it also poses a problem for efficient learning. For example, it does not seem possible to search through this space to find solutions efficiently. A

feasible solution invokes subcortical innate motor patterns, or primitives, to reduce the dimensionality of the control problem. How cortex learns from or to utilize such primitives remains an open question. To address this, we studied cortical and subcortical interactions as head-fixed mice learned contextual control of innate hindlimb extension behavior. Naïve mice performed reactive extensions to turn off a cold air stimulus within seconds and, using predictive cues, learned to avoid the stimulus altogether in tens of trials. Optogenetic inhibition of large areas of rostral cortex completely prevented avoidance behavior, but did not impair hindlimb extensions in reaction to the cold air stimulus. Remarkably, mice covertly learned to avoid the cold stimulus even without any prior experience of successful, cortically-mediated avoidance. These findings support a dynamic, heterarchical model in which the dominant locus of control can change, on the order of seconds, between cortical and subcortical brain areas. We propose that cortex can leverage periods when subcortex predominates as demonstrations, to learn parameterized control of innate behavioral primitives. In ongoing work we have developed computational models of the complete embodied task to allow us to learn detailed mechanistic models of cortical learning from subcortical demonstration.

Disclosures: J.A. Keller: None. **A. Kemtur:** None. **R. Johnson:** None. **J.T. Dudman:** None.

Nanosymposium

NANO018: Adaptive Choice

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Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.10

Topic: I.03. Decision Making

Support: RO1 MH126971-01A1

Title: Mental representations of latent states in the human brain

Authors: *F. M. BOUCHACOURT[1], A. ZHUNUSSOVA[4], S. BRUINSMA[5], C. DERWAY[2], D. HÄMMERER[6], M. R. NASSAR[3];
[1]Neurosci., [3]Metcalf Hall, [2]Brown Univ., Providence, RI; [4]Inst. of Psychology, Univ. of Innsbruck, Innsbruck, Austria; [5]Columbia Univ., New York, NY; [6]Univ. of Innsbruck, Innsbruck, Germany

Abstract: Behavioral policies need not always be relearned from scratch when the environment changes. When appropriate, we build mental representations of hidden contingencies (“latent states”), and flexibly use these representations to retrieve a previous behavior more rapidly. While the prefrontal cortex and hippocampus have been proposed as key regions involved in representing latent states, their precise neural implementation remains incompletely understood. To examine the formation and reuse of latent states in the brain, we designed a human task that dissociates latent states from action-outcome contingencies. This allowed us to quantify when participants behaviorally reused a latent state. In the task, participants learned three latent states, each defined by three color-location associations. Behaviorally, we observed gradual learning of

latent states over time. Critically, after an initial learning phase—which varied in length across individuals—participants began to show single-trial reuse of latent states. To account for this behavior and the individual variability, we developed a network model in which latent states emerged through chunking of conjunctive neurons selective to colors and locations activated in temporal contiguity between trials. When repeated, a latent state could be retrieved from a single color-location association by reactivation of the whole chunk. The model predicts abrupt behavioral shifts following latent state transitions—a pattern that was confirmed in participants' responses.
To identify the neural and computational substrates of latent state learning and inference, we are combining fMRI and pupillometry. Our first analyses, using both univariate and multivariate methods, reveal that latent state representations are distributed across several brain regions, including the prefrontal cortex and the temporal lobe. Ongoing work is exploring how transitions between latent states relate to fluctuations in arousal and norepinephrine neuromodulation.

Disclosures: F.M. Bouchacourt: None. **A. Zhunussova:** None. **S. Bruinsma:** None. **C. Derway:** None. **D. Hämmerer:** None. **M.R. Nassar:** None.

Nanosymposium

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Presentation Number: NANO018.11

Topic: I.03. Decision Making

Support: R21MH127607
K23DA050909
University of Minnesota's MnDRIVE

Title: Dynamic prefrontal coupling coordinates adaptive decision-making

Authors: *X. YAN[1], S. KOENIG[2], B. A. EBITZ[4], D. P. DARROW[3], A. B. HERMAN[5]; [1]Univ. of Minnesota, Twin Cities, Roseville, MN; [2]Psychiatry and Neurosurg., [3]Univ. of Minnesota, Twin Cities, Minneapolis, MN; [4]Neurosciences, Univ. de Montréal, Montréal, QC, Canada; [5]Univ. of Minnesota, Minneapolis, MN

Abstract: Adaptive decision-making requires flexibly maintaining or changing behavior in response to uncertainty. While the dorsomedial (dmPFC) and dorsolateral (dlPFC) prefrontal cortex are each essential for this ability, how they coordinate to drive adaptation remains unknown. Using intracranial EEG recordings from human participants performing a dynamic reward task, we identified distinct, frequency-specific computations: dmPFC high-gamma activity encoded uncertainty before stay decisions but transitioned to prediction error before switches, while theta activity shifted from uncertainty to value representation. In contrast, dlPFC theta activity signaled both value and uncertainty before stays, but predominantly value before switches. Crucially, these regions coordinated through two temporally specific coupling

mechanisms that predicted behavioral changes: theta-theta amplitude coupling during feedback processing and theta-gamma phase coupling before decisions. Both coupling mechanisms strengthened before switches, suggesting that changing behavior requires greater dmPFC-dlPFC integration than maintaining. These findings reveal how the dorsal prefrontal cortex employs frequency-specific computations and precise temporal coordination to guide adaptive behavior.

Disclosures: X. Yan: None. **S. Koenig:** None. **B.A. Ebitz:** None. **D.P. Darrow:** None. **A.B. Herman:** None.

Nanosymposium

NANO018: Adaptive Choice

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Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.12

Topic: I.03. Decision Making

Support: NIH/NIMH R01MH124849

Title: Neural dynamics underlying divergent influences of reward and punishment on control allocation

Authors: *X. LENG[1], R. FRÖMER[2], A. SHENHAV[1];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]Univ. of Birmingham, Birmingham, United Kingdom

Abstract: To decide how to allocate cognitive control in a task, people must weigh the cost of mental effort against potential consequences for success and failure. Our prior work showed that people allocate control differently depending on expected rewards for success (correct responses) versus punishments for failure (errors), prioritizing attentional focus in the former case and caution in the latter. An outstanding goal of this work is to understand the neural mechanism by which these different incentives lead to distinct control strategies (e.g., attention vs. caution). Specifically, it remains unclear how reward and penalty are differentially represented over the course of incentive presentation and up through eventual task performance. We recorded EEG from participants (N=40) performing self-paced intervals of an incentivized cognitive control task. Before each interval, participants were cued with the amount of monetary reward for correct responses and penalty for errors. We analyzed ERP components time-locked to the cue and found that fronto-central N2 (a proxy for early monitoring) selectively increased with higher penalty, whereas centro-parietal P3b (a proxy for cue evaluation) and fronto-central CNV (a proxy for control allocation) demonstrated interactions between reward and penalty, with the lowest amplitude when both incentives were low. Using temporal-spectral EEG profiles, we trained classifiers to decode temporally sustained neural representations of each incentive type from activity locked to task stimuli. We found that neurally decoded incentive representations were associated with corresponding influences of a given incentive on task performance, with reward decoding predicting speeded responding and penalty decoding predicting increased

accuracy. We found that incentive representations could also be decoded from neural activity around the response, with penalty-related representations enhanced immediately prior to an error and reward-related representations enhanced immediately following an error. Together, our findings suggest that reward and punishment are differentially encoded during incentive evaluation, control allocation, and performance monitoring. Prior to task performance, penalty magnitude modulates early monitoring before reward and penalty converge to determine control allocation. During task performance, representations of each incentive diverge to predict distinct performance profiles. Both incentive representations also emerge at distinct points during error monitoring. Overall, this work offers new insights into the dynamics of incentive processing and how incentives guide control.

Disclosures: X. Leng: None. **R. Frömer:** None. **A. Shenhav:** None.

Nanosymposium

NANO018: Adaptive Choice

Location: SDCC Rm 30

Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.13

Topic: I.03. Decision Making

Title: The dimensionality of suboptimal perceptual decision making

Authors: *R. C. WILSON[1], J. XUE[2];
[1]Georgia Tech., Decatur, GA; [2]Georgia Tech., Atlanta, GA

Abstract: Humans and animals can be highly suboptimal, even on the simplest behavioral tasks. In this work we take an individual differences approach to investigate the dimensionality of this suboptimal behavior, building an interpretable neural network to identify the factor structure of perceptual decision making.The experiment we focus on is a simple perceptual decision-making task known as the Bernoulli Clicks Task, making use of a data set with 155 participants. In this task, participants don headphones and listen to a stream of click sounds presented over the course of 1 second. Clicks are presented every 50ms in either the left ear or the right ear and the participants' goal is to identify the side receiving the most clicks. Previous work has identified a range of suboptimalities in this task including uneven weighting of the clicks over time (integration kernel), repetition effects (choice kernel), win-stay-lose-shift effects (reinforcement learning), side biases and left-over behavioral variability characterized as noise.Our modeling approach revolves around a type of interpretable neural network that can be thought of as a combination of logistic regression and factor analysis. In the simplest version, this network has two input layers: one for the trial (the 20 clicks as well as past choices and outcomes) and one for the participant (encoded in a one-hot fashion). These two input layers project to two hidden layers that separately encode low-dimensional embeddings for trial and participant. These low-dimensional embeddings are combined via a dot product, which is then fed into a softmax to predict behavior.By varying the number of units in the hidden layers, D, we identify the dimensionality of the individual differences as the value of D that minimizes loss on a held-out

test set. By looking at the learned weights in the best fitting network, we further identify the suboptimalities captured by each of the dimensions. In this way we identify a best fitting dimensionality of five. These five dimensions include two describing the integration kernel (a "bump" shape and a primacy-recency effect), two describing order effects (a choice kernel and a reinforcement learning effect), and one reflecting a side bias. Our analysis provides a data-driven ontology for characterizing individual differences in suboptimal behavior linking the slow timescale of traits to the fast timescale of perceptual decision making.

Disclosures: R.C. Wilson: None. **J. Xue:** None.

Nanosymposium

NANO018: Adaptive Choice

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Time: Sunday, November 16, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO018.14

Topic: I.03. Decision Making

Support: C.V. Starr Fellowship
Vannevar Bush Faculty Fellowship

Title: Common control mechanisms govern task flexibility in artificial networks and human brains

Authors: *H. RITZ[1], N. D. DAW[2], J. D. COHEN[1];
[2]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ

Abstract: We can flexibly switch between tasks, e.g., switching from speaking English to Spanish, but the neural mechanisms supporting task switching remain poorly understood. Existing theories of task switching are based on verbal theories or hand-crafted neural networks; however, these theories often don't make specific neural predictions.
To fill this gap, we developed a recurrent neural network (RNN) model of task switching. RNNs were cued to perform one of two tasks, waited through a delay period, and then responded to the cued stimulus dimension. We trained pools of RNNs (N=512 per pool) under curricula that captured two major factors of existing theories. To capture active preparation, we varied whether RNNs were trained on sequences of one trial, or whether they also had experience with sequences of two trials (i.e., could learn to switch). To capture the influence of previous trials, we varied the inter-trial interval.
To quantify RNNs' latent dynamics, we fit a linear-Gaussian state space model (LGSSM) to the hidden unit activity in each network. This provided a globally linear model of the dynamics, which we could then interpret using tools from dynamical systems theory and control theory. LGSSMs revealed distinctive signatures of active preparation. When tested on two-trial sequences, only switch-trained RNNs had similar task dynamics on switch and repeat trials. This occurred due to (1) stronger convergence towards a neutral task state during the ITI and (2) enhanced task dynamics following the cue. RNNs with shorter ITIs had less time to reach a

neutral task state, offering a novel explanation for between-trial interference.
To test whether these predicted signatures of preparation were present in the human brain, we re-analyzed two EEG experiments (Arnau et al., 2024, N=26 and Hall-McMaster et al., 2019, N=30). These experiments both used a similar cued task-switching paradigm, but the ITI in Hall-McMaster was ~3 times longer than in Arnau. Fitting LGSSMs to these EEG datasets revealed patterns of task dynamics that were strikingly similar to switch-trained RNNs. EEG were quantitatively more similar to switch-trained RNNs than single-trial RNNs on the basis of switch-repeat similarity, neutral-state convergence, and post-cue task dynamics (all bootstrap $p < .05$). Moreover, differences between EEG datasets with different ITIs were closely mirrored by differences between RNNs trained under different ITIs.
Together, this experiment provides a new theory of flexible information processing across brains and machines, and offers a path towards testing richer process models of cognitive control.

Disclosures: H. Ritz: None. **N.D. Daw:** None. **J.D. Cohen:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 1R01NS136246-01

Title: Single nuclei multiplexed multiomics analysis of direct and indirect neocortical neurogenesis

Authors: *Z. LI[1,2], T. F. HAYDAR[2];
[2]Dept. of Anat. and Neurobio., [1]Boston Univ., Boston, MA

Abstract: Over 50 transcriptomically distinct types of excitatory neurons that populate the adult mouse neocortex have been identified. How developmental mechanisms generate this diversity and shape the integration of these cells into neocortical circuits remain open. At the onset of this process, apical radial glial cells (aRGCs) - the stem cells of the neocortex - generate neurons either directly via asymmetric self-renewing cell divisions or indirectly through the genesis of intermediate progenitor cells (IPCs) that express the transcription factor Tbr2 (or Eomes). Recently, researchers have identified a novel biological rationale for the direct and indirect production streams operating during cortical neurogenesis. Results show that intra-laminar electrophysiological and morphological properties of excitatory neurons are determined, in part, by whether or not they are generated by Tbr2-expressing IPCs, and that these properties are fine-tuned in a layer specific manner in the neocortex. Here, we explore whether lineage information encoded within the germinal zone is transported into the neocortex by nascent excitatory neurons and maintained by mature neurons at the molecular level. We conducted single nuclei multiomics (ATAC- and RNA-seq) experiment using a novel sample multiplexing method,

which allowed us to analyze over 80 biological samples simultaneous, ensuring scientific rigor while greatly reducing cost.

Disclosures: Z. Li: None. **T.F. Haydar:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
NIH (PO1 NS083513
NIMH award (U01MH130995)
he Chan Zuckerberg Biohub (1DP2NS122550-01

Title: Hypoxia-inducible factor pathway is essential for embryonic interneuron development and NMDAR expression

Authors: *I.-L. LU[1], J. KIM[2], A. ALVAREZ-BUYLLA[3], A. R. KRIEGSTEIN[3], M. F. PAREDES[5], D. H. ROWITCH[4];
[1]Dept. of Pediatrics, Div. of Neonatology/ Univ. of California, San Francisco, SAN FRANCISCO, CA; [2]Neurol., [3]Eli and Edythe Broad Ctr. for Regeneration Med. and Stem Cell Res., Univ. of California San Francisco, San Francisco, CA; [4]Univ. of California San Francisco, SAN FRANCISCO, CA; [5]UCSF, San Francisco, CA

Abstract: Antenatal mammalian brain development normally takes place in a hypoxic environment (e.g., 30-40 Torr O_2 in humans), and this changes abruptly at birth with the first breath (~80 Torr O_2). Prematurity exposes the developing fetus to higher oxygen levels earlier than expected in neurodevelopment, potentially dysregulating molecular mechanisms, such as hypoxia inducible factor (HIF) signalling. We report that conditional knockout (cKO) of *HIF1α* genes by *Nkx2.1-cre* in medial embryonic ganglionic eminence (MGE) results in decreased proliferation of Lhx6+ interneuron (IN) precursors. Conversely, the deletion of *von Hippel-Lindau (vHL)*, required for HIF1A degradation, resulted in increased proliferation of Lhx6+ precursors, followed by a wave of apoptosis. Single-cell transcriptomics revealed HIF gene targets in MGE for proliferation and synaptogenesis. Additionally, we found the *NMDA receptor* to be a novel and direct HIF target. In postnatal (P) day 28 *HIF1α* cKO cortex, PV+ IN numbers and GABAergic synapses on layer 2/3 excitatory neuron (L2/3 EN) soma were specifically and significantly reduced. We observed dramatically decreased numbers of Grin2b/Basson dendritic puncta (at levels comparable to *NMDAR cKO* animals) associated with increased c-Fos expression in L2/3 ENs. These results indicate that PV cortical IN and *NMDAR* synapse development for upper cortical layer excitatory neurons requires *HIF1α* function.

Disclosures: I. Lu: None. **J. Kim:** None. **A. Alvarez-Buylla:** None. **A.R. Kriegstein:** None. **M.F. Paredes:** None. **D.H. Rowitch:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: DBT M.K. Bhan Young Researcher fellowship- HRD-12/4/2020-AFS-DBT

Title: Role of histone-binding protein RBBP4 in regulating neural progenitor cell fate and neurogenesis during mouse neocortical development

Authors: *S. K. DHANYA[1,2];
[1]Bhavana Muralidharan Lab., Inst. For Stem Cell Sci. and Regenerative Med., Bangalore, India; [2]Neurodevelopment and Dis. Lab., Inst. for Stem Cell Sci. and Regenerative Med., Bangalore, India

Abstract: Cerebral cortex consists of diverse neuronal and glial subtypes arranged cytoarchitecturally in six layers. Chromatin regulation plays a critical role in cortical neurogenesis, and aberrations in chromatin modifiers are linked to neurodevelopmental disorders (Stessman et al., 2017). Retinoblastoma binding protein 4 (RBBP4) is a core subunit of several chromatin modifying complexes and has been found to be abundantly expressed in neocortex. Putative risk variants in RBBP4 have been associated with autism spectrum disorder (ASD; Firth et al., 2009). The exact molecular basis by which RBBP4 functions alter the epigenetic regulation of cortical development needs to be explored. Our previous study demonstrates that the downregulation of RBBP4 in E12.5 neocortical progenitors reduced neuronal output, specifically affecting CTIP2-expressing neurons and impairing neocortical progenitor proliferation, thereby indicating the critical role of RBBP4 in regulating deep layer neurogenesis (Dhanya et al., 2024). To gain deeper insight into the molecular mechanisms underlying how loss of RBBP4 function impacts neural progenitor cell fate and neurogenesis, transcriptomic profiling was conducted on FACS-sorted neocortical cells following knockdown of RBBP4 at E12.5, the stage at which deep-layer neurogenesis occurs.
Genome-wide occupancy analysis revealed that RBBP4 primarily binds to distal regulatory elements, and neuron differentiation is a significant GO biological pathway of RBBP4-bound genes (Dhanya et al., 2024). Interestingly, we found that RBBP4 binds to Cdon, a receptor protein in the Shh signaling pathway, and knockdown of Cdon phenocopies RBBP4 knockdown resulting in a significant reduction in neurogenesis, particularly CTIP2-positive layer V neurons. CDON overexpression could rescue the phenotype caused upon loss of RBBP4 in the neocortex, thereby suggesting the functional link between RBBP4 and its target gene CDON. Transcriptional profiling experiments will provide the molecular basis by RBBP4 regulates

neurogenesis in the developing neocortex and links chromatin regulation to cell adhesion signaling in cortical development. Our study investigating the RBBP4-CDON axis will unravel how their interaction coordinates neuronal layer specification and how dysregulated chromatin regulation impacts cellular mechanisms in neurodevelopmental disorders.

Disclosures: S.K. Dhanya: A. Employment/Salary (full or part-time):; DBT MK Bhan Young Researcher.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: NS095654
MH106934

Title: Specification and Wiring of Claustro-Amygdalar and Paleocortical Neurons in Mammalian Brain Development

Authors: *N. KAUR[1], R. KOVNER[1], T. ZHU[2], D. ANDRIJEVIC[1], M. SHIBATA[1], A. SHIBATA[1], K. PATTABIRAMAN[1], Y. S. MINEUR[1], M. PICCIOTTO[1], H. HUANG[2], N. SESTAN[1];
[1]Yale Univ. Sch. of Med., New Haven, CT; [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Our social and emotional behaviors are fundamental to our everyday interactions and well-being. These behaviors are regulated by key components of the limbic system that are located in the ventrolateral cortex (VLC), including basolateral amygdala complex (BLC), claustrum (CLA), insular cortex (IC), and piriform cortex (PIR; part of paleocortex). The excitatory neurons (ExNs) in these regions form bidirectional circuits with the prefrontal cortex (PFC) and relay multimodal information to assign salience and valence, shaping intricate social and emotional responses. These circuits develop over a protracted developmental window that extends into adolescence. During this critical period, these circuits are vulnerable to various genetic and environmental disruptions that may be associated with the onset of many neuropsychiatric disorders. Therefore, a key question at the crossroads of basic neuroscience and psychiatry is how these circuits are built. Here, we identified a gene regulatory network comprising transcription factors (TFs) SOX4, SOX11, and TFAP2D essential for VLC ExNs specification, survival, and PFC connectivity (Fig. 1). SOX4 and SOX11 absence in the ExNs results in a marked reduction in the size of BLC, CLA, and PIR. These TFs control BLC formation through direct regulation of Tfap2d expression. Cross-species analyses identified conserved Tfap2d expression in developing VLC ExNs in humans, macaques, and mice. Although the loss and haploinsufficiency of Tfap2d yield similar alterations in learned threat-response behaviors, differences emerge in the phenotypes at different Tfap2d dosages,

particularly in terms of changes observed in BLC size and BLC-PFC connectivity. This study shows that homozygous and heterozygous mutations, often mirroring psychiatric risk alleles, can cause distinct anatomical circuit changes but converge on the same behavioral outcomes. This highlights the need to uncover molecular drivers of VLC developmental alterations that lead to divergent behaviors and inform the stratification of neuropsychiatric disorders.

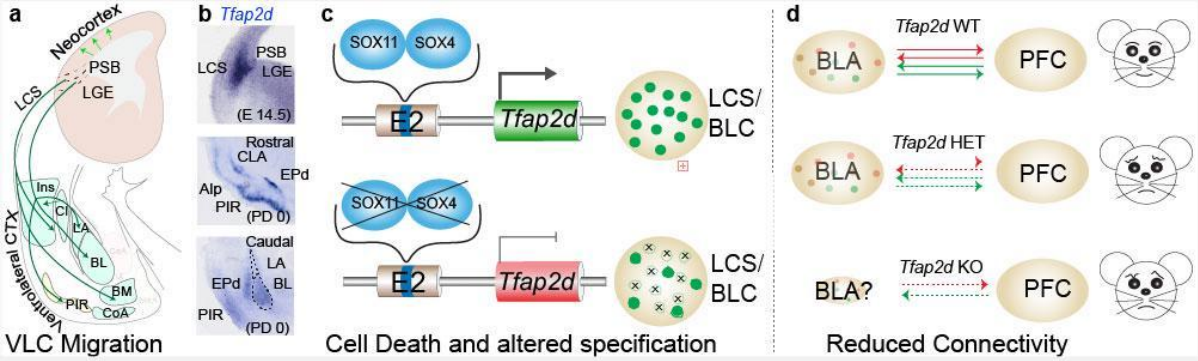


Fig. 1 Summary of the Study

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Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

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Presentation Number: NANO019.05

Topic: A.01. Neurogenesis and Gliogenesis

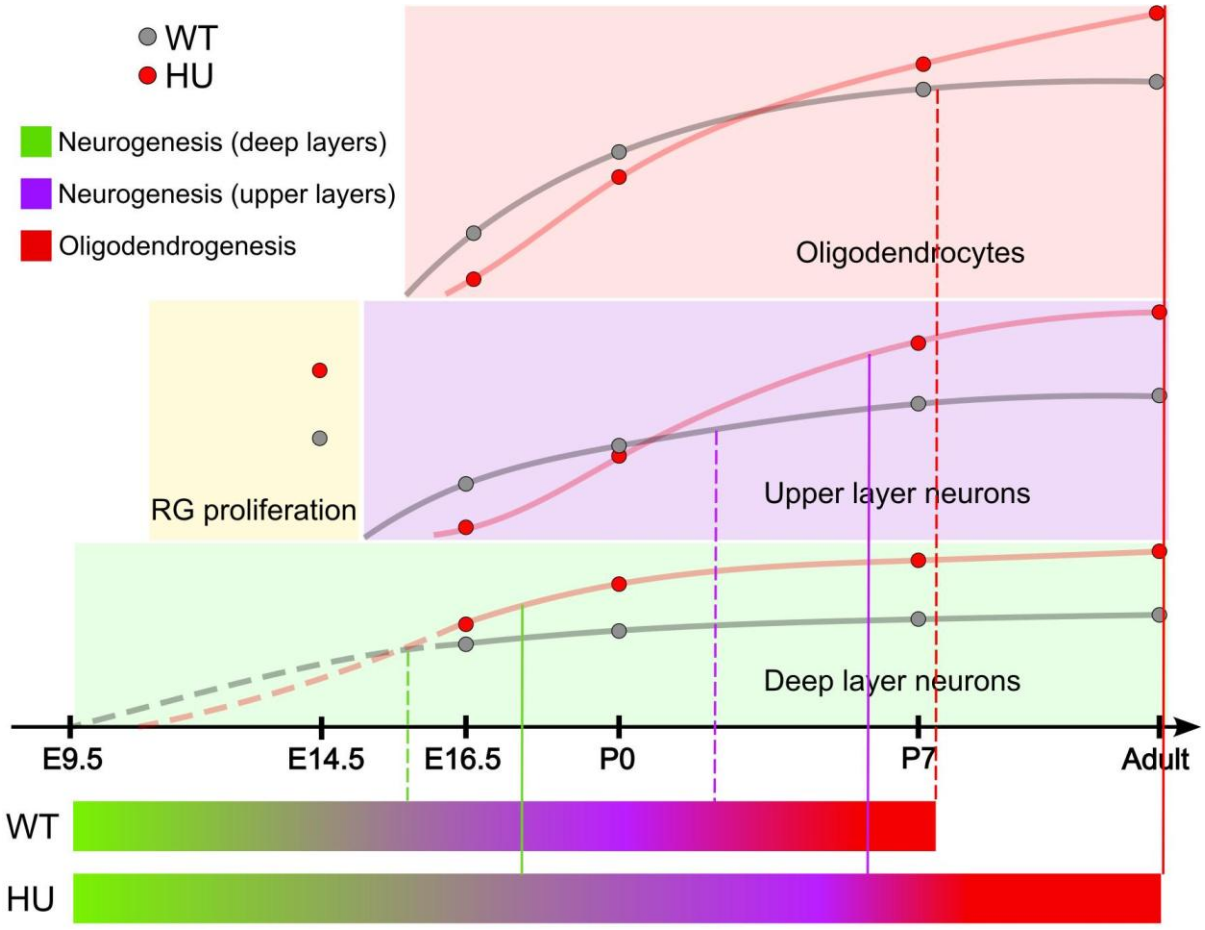
Support: James S. McDonnell Foundation #22002046
NIH HG011641
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Title: Human CLOCK regulates neoteny to extend neuro- and gliogenesis during early neocortical development

Authors: *Y. LIU[1], J. S. TAKAHASHI[2], G. KONOPKA[3];
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Abstract: The human neocortex experienced a remarkable expansion during evolution. This expansion is characterized by increased both neurons and glia. Due to the human-specific

upregulation of CLOCK in the neocortex, we generated a CLOCK humanized mouse model (HU) and discovered that human CLOCK increased neuron and oligos density in the frontal cortex. To understand the underlying mechanism, we studied the proliferation and cytoarchitecture of developing neocortex on BrdU administrated embryos. We discovered that CLOCK is expressed in radial glia rather than intermediate progenitor as early as E14.5. Consistent with the cell type-specific expression of CLOCK, human CLOCK appeared to maintain radial glia in progenitor fate and enhance their proliferation to increase the progenitor pool germinal zone. Additionally, human CLOCK also altered the dorsal-ventral organization of developing cortex. At E16.5, HU mice increased relative thickness of ventricular zone and subplate, and this thickening persisted in the germinal zones at P0, suggesting enhanced neurogenesis in HU mice. To further elucidate the developmental process leading to the increased cell density observed in adult HU mice, we quantified postmitotic neurons and oligos across developmental stages. From E16.5 to adult, upper layer neurons and oligos show less abundant in HU mice during the earlier developmental stages. Deep layer neurons, upper layer neurons, and oligos start to show higher density in HU mice from P0, P7, and adult respectively. These results suggest protracted windows of neuro- and gliogenesis. Therefore, this study demonstrates that CLOCK may regulate the neoteny of human neocortical development for an extended timeline to expand neuronal and glial populations. Future work will leverage single-nuclei omics and iPSCs-derived organoids to understand the molecular mechanisms that are regulated by CLOCK in human brain development.



Disclosures: Y. Liu: None. **J.S. Takahashi:** None. **G. Konopka:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS100007

Title: Single molecule array (Simoa) technology as a highly sensitive screening tool for transcription factor interactions

Authors: *G. CHO[1], J. A. GOLDEN[3], Y. LIM[2];
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Abstract: Transcription factors (TFs) play a critical role in gene regulatory networks that maintain neural stem cells and drive neuronal differentiation. These networks rely on the coordinated interactions of multiple TFs at enhancer and silencer regions of the genome. TF-TF interactions are typically weak and transient, which allows for the flexible assembly and disassembly of transcriptional complexes. While this dynamic nature is advantageous for rapid cellular adaptation, it poses a significant challenge for detecting and studying these interactions using conventional methods. To address this limitation, we adapted the ultrasensitive Single Molecule Array (Simoa) assay—originally developed for biomarker detection—to screen and identify TF-TF interactions. ARX (Aristaless-related homeobox) is a key transcription factor involved in maintaining dorsal neural progenitors and promoting interneuron differentiation. The diverse functions of ARX in distinct gene regulatory networks likely arise from its context-dependent interaction partners in the dorsal and ventral neural tube. Using the Simoa technique, we identified ARX interaction partners: Hes1 and Neurog2 in the dorsal neural tube, and Dlx2/5 in the ventral region. A large-scale screen of TF-TF interactions has the potential to bridge the gap in understanding cell type-specific gene regulatory networks and serve as a foundation for generating distinct neuronal subtypes.

Disclosures: G. Cho: None. **J.A. Golden:** None. **Y. Lim:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.07

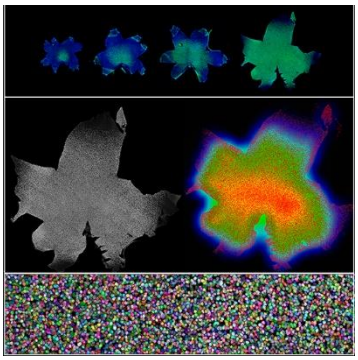
Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 EY033385-01
P30 EY08098
RPB Unrestricted Departmental Grant
Hillman Innovation Grant
ARVO/Genentech 2019

Title: Longitudinal characterization of Retinal Ganglion Cells during the chick embryonic development: peaking at the High Acuity Area

Authors: *V. VALLE[1], L. COHEN[1], K. PRICE[1], V. SOMAN[2], U. CHANDRAN[2], S. I. SILVA[1];
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Abstract: Purpose: The molecular and cellular mechanisms governing the formation of a high acuity area (HAA), also known as fovea, during retinal development remain mostly unknown. It has previously been described that retinoic acid (RA) is modulated during development of the chick HAA by exclusion of RA in the central retina, becoming necessary for a complete absence of rods and peak cellular density in the ganglion cell layer in this area. While the highest density of retinal ganglion cells (RGCs) is a well established attribute of mature HAAs, little is known about progression and acquisition of this RGCs peak density during development. Methods: To identify HAA-specific attributes regarding RGCs during retinal development, chick retinas across development were used. A pharmacological-genetic approach based on all-trans Retinoic Acid in ovo injections during the predicted "foveogenesis" period, at E4, followed by scRNAseq analysis, was performed to detect potential foveal specific gene expression responses after RA treatment. Following this, combination of HCR in situs, cell death detection and longitudinal immunofluorescence analyses with unreported RGCs antibodies were conducted over flat-mount preparations and evaluated by advanced imaging of flat-mounts reconstructions, culminating in the acquisition of RGCs isodensity maps. Results: After Retinoic Acid treatment, analysis of central retinas at E4 revealed differentially gene expression mostly related to RGCs differentiation when compared with control retinas, without obvious changes in cell death. Analysis of multiple markers of RGCs during development by immunofluorescence studies on control retinas reveal an enrichment in the centro-ventronasal region, starting at the first embryonic week and further consolidated during the second embryonic week. Conclusion: Our longitudinal evaluation of RGCs populations on flat-mounts of developing chick retinas reveal the progression and acquisition of RGCs peak density at the HAA, thus validating this particular cell type as a valid proxy to analyze foveal development.



Disclosures: V. Valle: None. **L. Cohen:** None. **K. Price:** None. **S.I. Silva:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.08

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI 23K14203
JSPS KAKENHI 21K20853
Takeda Science Foundation Grants 2024049458

Title: Asymmetric Localization of ZEB2 and ZEB1 Directs Asymmetric Daughter Cell Fates in Bergmann Glia-like Progenitors

Authors: *T. ADACHI[1], K. ICHIJO[2], K. SUYAMA[1], M. MIZUNO[1], S. ITO[1], S. MIYASHITA[1], T. OWA[1], M. HOSHINO[1];
[1]Dept. of Biochem. and Cell. Biol., Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [2]Dept. of Otolaryngology, Head and Neck Surgery, Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan

Abstract: The ability of a single progenitor to generate multiple cell types is fundamental to the development and function of multicellular organisms. In the mammalian cerebellum, three astroglial cell types arise postnatally: Bergmann glial cells (BGs) in the Purkinje cell layer (PCL), astrocytes in the inner granule cell layer (IGL), and white matter (WM) astrocytes. These originate from two progenitor types: Bergmann glia-like progenitors (BGLPs) in the PCL and astrocyte-like progenitors (AsLPs) in the white matter. AsLPs are multipolar, proliferative cells resembling astrocyte-like morphology progenitors also found in other mammalian brain regions. In contrast, BGLPs are unipolar and morphologically indistinguishable from BGs, a progenitor type unique to the cerebellum. Our previous study showed that BGLPs at postnatal day 0 (P0) give rise to all three astroglial lineages (Suyama et al., 2025, bioRxiv), but the molecular mechanisms regulating this diversification remained unclear. To address this question, we hypothesized that specific molecules asymmetrically localize during BGLP division to guide fate decisions. We first observed that 80-90% of BGLP divisions are horizontal to cerebellar layer

structure. Using spatial transcriptomics (Xenium 5k), *in situ* hybridization (RNAscope), and immunohistochemistry, we found that during horizontally divisions, the transcription factor ZEB2 localizes asymmetrically—at both mRNA and protein levels—to the pial-side daughter inheriting the unipolar process. ZEB1, a paralog of ZEB2, localizes to the IGL-side daughter lacking the process. ZEB2 is known to be enriched in unipolar astroglial cells (BGLPs and BGs), while our data newly show that ZEB1 is enriched in multipolar cells (AsLPs, IGL and WM astrocytes). To test function, we performed cerebellar surface-targeted *in vivo* electroporation to manipulate *Zeb2* and *Zeb1* expression specifically in BGLPs. By this experiment, we found *Zeb2* was necessary and sufficient for BG fate, whereas *Zeb1* was required and sufficient for IGL and WM astrocyte fate. These results were confirmed using conditional knockouts ($Glast^{CreERT2/+}$; *Ai9;* $Zeb2^{flox/flox}$ or $Zeb1^{flox/flox}$) with topical crystal tamoxifen introduction to cerebellar surface at P0 which specifically knockout these genes in unipolar astroglial cells. We also found that *Zeb2* and *Zeb1* mutually repress each other in BGLPs, and this antagonism is also present during horizontal division. Together, our results uncover a mechanism in which asymmetric inheritance of ZEB transcription factors regulates binary astroglial fate. We will also present preliminary data on upstream cues initiating this asymmetry.

Disclosures: T. Adachi: None. **K. Ichijo:** None. **K. Suyama:** None. **M. Mizuno:** None. **S. Ito:** None. **S. Miyashita:** None. **T. Owa:** None. **M. Hoshino:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.09

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 5R01MH125956-04

Title: Tracking glial cell fate decisions in the developing human brain using genetic lineage tracing

Authors: *N. KEBEDE[1], A. SING[2], S. A. SLOAN[2];
[2]Human Genet., [1]Emory Univ., Atlanta, GA

Abstract: The brain develops through a coordinated process of self-renewing proliferation and differentiation of neural progenitors. As a field, we are still uncovering new types of neural progenitors and mechanisms by which these cells differentiate into the major brain cell types. Human glial lineages are particularly difficult to investigate since gliogenesis occurs much later in development and glial cells, specifically astrocytes, have very similar transcriptomic profiles to their progenitors. In this study, we investigate the lineages of cortical radial glia and a new class of tri-potential glial progenitors (gIPCs) to determine how they give rise to differentiated astrocytes. To do this, we developed a lineage tracing approach to track primary human neural progenitors engrafted onto ex vivo slice cultures. We observed that these progenitors give rise to

all major neuronal and macroglial cells, with an enrichment of glial lineages, allowing us to better interrogate glial cell fates. Importantly, the transcribed CellTag barcodes allow us to trace the lineage relationships between our cells with simultaneous information about their cell identities. To identify clonally related cells, we developed and validated a clone calling approach that categorizes families of cells derived from the same progenitor cell with high fidelity. Using our clonal database, we identified the presence of two cortical astrocyte lineages that come from non-overlapping progenitor pools of radial glia and gIPCs. These astrocyte lineages rarely co-occur within clonal families and display distinct transcriptomic profiles, which we validate by immunohistochemistry (IHC) and fluorescent activated cell sorting (FACS). Additionally, we identify distinct transcription factors enriched in the two astrocyte populations that implicate SHH signaling and primary ciliogenesis as candidate pathways that drive lineage divergence. Our findings provide new insight into the progenitor sources and molecular specification of human astrocytes, with implications for understanding glial development and neurodevelopmental diseases.

Disclosures: N. Kebede: None. **A. Sing:** None. **S.A. Sloan:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.10

Topic: A.01. Neurogenesis and Gliogenesis

Support: Arizona Biomedical Research Centre (ABRC) Grant RFGA2022-010-16
NIH Grant R00MH125329

Title: Investigating the environmental conditions that contribute to cellular and molecular changes in early cortical development using in vitro PSC models

Authors: *A. MORALES[1], T. PENNINGTON[1], G. BAMFONGA[1], B. BARTELLE[2], H. GU[3], M. G. ANDREWS[1];
[1]Sch. of Biol. & Hlth. Systems Engin., Arizona State Univ., Tempe, AZ; [2]Arizona State Univ., Tempe, AZ, ; [3]Col. of Hlth. Solutions, Arizona State Univ., Tempe, AZ

Abstract: It has been long established that environmental conditions, such as glucose and oxygen, play a key role in the canonical development of all biological organisms, including humans. Cellular metabolism is essential for tissue formation, energy production, and systemic homeostasis. Glucose metabolism dysregulation is a common phenotype across human neurodevelopmental disorders. In the context of human cerebral cortex development, there's a limited understanding of how metabolic pathways, such as glycolysis, impact the proliferation and differentiation of discrete human cortical cell types. Due to challenges with investigating cellular and molecular regulators of the developing human brain, we will utilize human pluripotent stem cell (PSC) derived cortical organoids. Cortical organoids are a highly tractable

model system that can be used for high-throughput investigation of early stages of development and can be manipulated to study regulatory and metabolic programs. Through modifying the cell culture environment of cortical organoids from neural induction through early/mid neurogenesis, human cortical cells can be studied in an endogenous-like environment to assess the impact of metabolism on developmental programs. Initial observations suggest that cortical cell populations throughout development differentially respond to changes in glucose level, oxygen level, and even basal medium composition. Single cell RNA sequencing (sc-RNA seq) has elucidated shifts in developmental timing and expansion of different cellular populations between experimental groups. We have collected and leveraged a large dataset consisting of 36 experimental conditions, including 3 PSC lines, 4 media conditions and 2 oxygen conditions yielding 248,661 cells to assess developmental profiles. In particular, we are evaluating how cell type specific metabolism, proliferation, differentiation, and stress programs change between cell culture conditions and across time. In the future, we can benchmark against publicly available human cortical datasets to assess similarity to endogenous developmental profiles. Based on initial observations, we hypothesize that environmental factors, such as glucose and oxygen, play a key role in the developmental trajectories and lineage choices during human cortical development. Our omics dataset has the potential to provide the field with valuable information about the impact *in vitro* culture has on canonical cortical developmental programs and elucidate the alterations seen in the context of neurodevelopmental disorders and disease.

Disclosures: A. Morales: None. **T. Pennington:** None. **G. Bamfonga:** None. **B. Bartelle:** None. **H. Gu:** None. **M.G. Andrews:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.11

Topic: A.01. Neurogenesis and Gliogenesis

Support: DoD NFRP W81XWH2110585
CFC International

Title: The impact of hyperactive RASopathy genetic variants on GABAergic interneurons: a molecular, cellular and behavioral deep dive

Authors: A. M. STAFFORD, D. PACHECO CRUZ, ***D. VOGT**;
Michigan State Univ., Grand Rapids, MI

Abstract: Cortical GABAergic interneurons are a highly diverse group of cells in the brain that acquire their properties through genetic programming, local environmental cues and more recently appreciated, cellular signaling processes that may either connect the two latter processes and/or be independent of each. Cortical GABAergic interneurons facilitate local microcircuit inhibition onto other neurons to create a local code that the brain utilizes to interpret sensory cues

into actionable cues. These processes and the balance of neurons involved are extremely important. Herein, we explore the role of the MAPK cellular signaling pathway in these events and ways in which this pathway may be managed pharmacologically. We found that hyperactivating the MAPK pathway, which represents many RASopathy syndromes, led to drastic shifts in GABAergic subtype ratios. This alteration was recapitulated in other RASopahty models, suggesting this is a common phenomenon and may apply to a majority of MAPK models. Specifically, we found that elevating MAPK signaling had a direct impact upon GABAergic cortical interneuron cardinal transcription factors, including LHX6, ARX, SATB1 and GAD1/2. Independently, we found that the MAPK signaling inhibitor, selumetinib, rescued hyperactive behaviors in elevated MAPK signaling mutant mice. Herein, we further explore these findings, including whether they are related, independent of each other and the repercussions for those diagnosed with a RASopathy syndrome with potential GABAergic interneuron alterations.

Disclosures: A.M. Stafford: None. **D. Pacheco Cruz:** None. **D. Vogt:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.12

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS133564
NIH Grant NS109176

Title: Mir-9 and m6a rna methylation coordinate neural progenitor competence in the developing cortex

Authors: S. DECKER[1], A. LA TORRE[2], ***S. SIMO**[3];
[1]Univ. of California-Davis, Davis, CA; [2]Cell Biol. and Human Anat., [3]Univ. of California Davis, Davis, CA

Abstract: The central nervous system contains a myriad of different cell types that, in the right numbers and at the right positions, form functional circuits. Failure to produce the right composition of cells can result in several mental and physical diseases that range from cognitive disorders to severe brain malformations. In the neocortex, neural progenitors, also known as radial glial cells, gives rise to all projection neurons in a conserved temporal sequence. Traditionally, this temporal sequence has been thought to be controlled by an intrinsic cascade of transcription factors that restrict progenitor competence over time. However, our work, and others, reveal that post-transcriptional mechanisms also play a central role.Here, we show that microRNAs (miRNAs) regulate neural progenitor competence and projection neuron fate specification by controlling RNA epigenetics. Using miR-eCLIP, we identified mRNAs directly targeted by miRNAs in neural progenitors during cortical development. Particularly, we found

that miR-9 targets WTAP, a core member of the N6-methyladenosine (m6A) RNA methylation complex and that miR-9 expression inversely correlates with m6A RNA methylation, which progressively decline in neural progenitors during later developmental stages. Importantly, we demonstrate that altering m6A methylation levels impacts neural progenitor competence and projection neuron specification.Together, our findings reveal a novel regulatory axis involving miR-9, WTAP, and m6A methylation that governs temporal competence in neural progenitors. This work underscores the importance of post-transcriptional mechanisms in cortical development and provides a more integrated model of fate specification in the developing brain.

Disclosures: S. Decker: None. **A. La Torre:** None. **S. Simo:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.13

Topic: A.06. Developmental Disorders

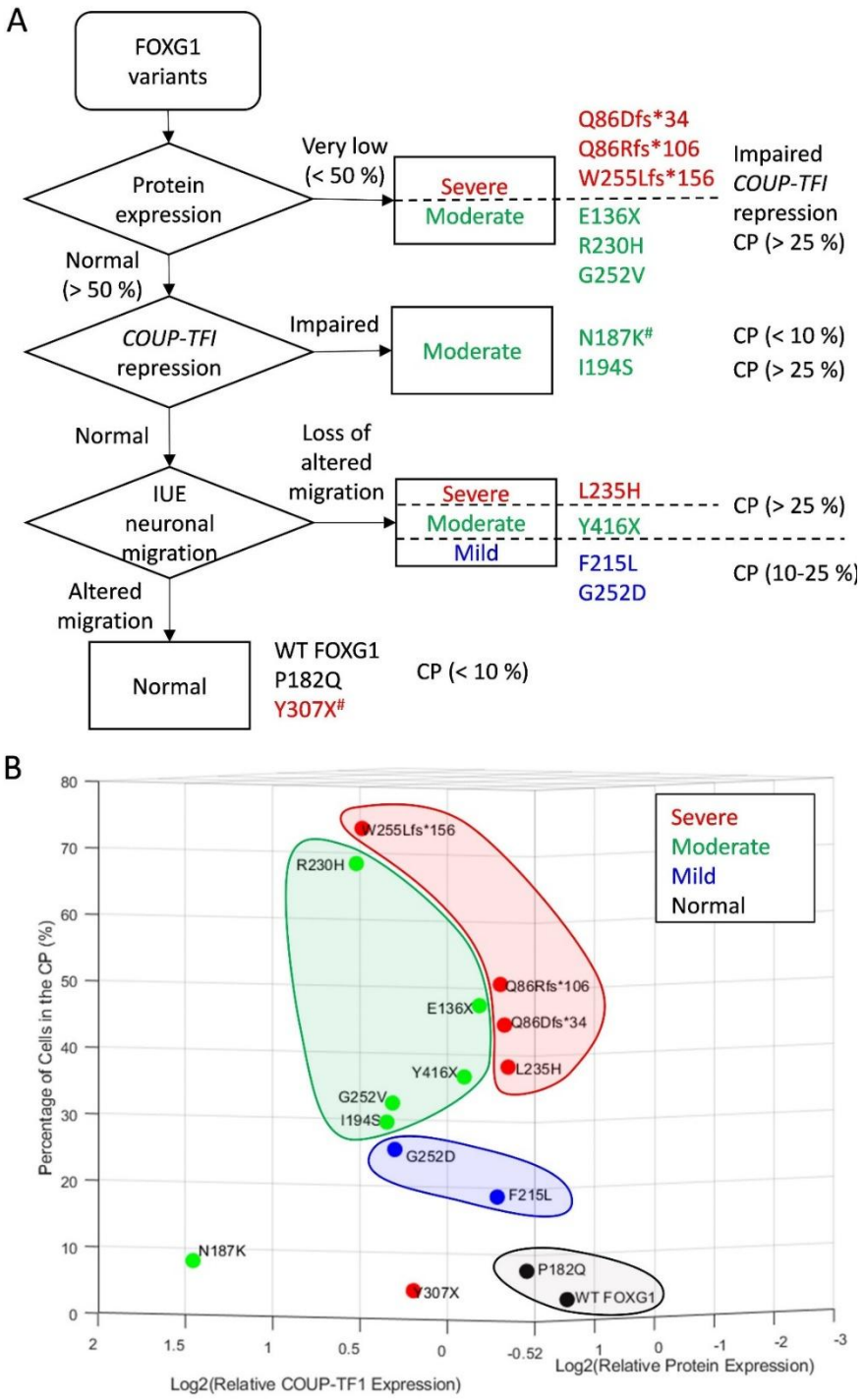
Support: NSTC-113-2628-B-A49 -017
NSTC-113-2314-B-A49 -012
NSTC-114-2622-B-A49 -004
NHRI-EX109-10904NI

Title: Functional defects in FOXG1 variants predict the severity of brain anomalies in FOXG1 syndrome

Authors: H.-Y. CHENG[1], W.-T. LEE[3], ***J.-W. TSAI**[2];
[1]Inst. of Brain Sci., [2]Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan; [3]Natl. Taiwan Univ. Hosp, Taipei, Taiwan

Abstract: FOXG1 (Forkhead Box G1) is a critical transcription factor for brain development, regulating progenitor cell proliferation, neuronal migration, and cortical circuit assembly. Pathogenic FOXG1 variants lead to FOXG1 syndrome, a neurodevelopmental disorder characterized by severe brain anomalies and cognitive impairments. Despite efforts to correlate genetic variants with clinical outcomes, the precise relationship remains elusive. Here, we analyzed clinical severity and brain anomalies in 14 individuals with FOXG1 variants, investigating how these variants impact FOXG1's properties and functions. We uncovered a strong correlation between the severity of brain anomalies in affected individuals and functional alterations of these variants. Variants with very low protein expression were associated with moderate-to-severe brain anomalies. A luciferase reporter assay was used to assess the ability of FOXG1 variants to repress COUP-TFI (NR2F1) expression-a function of FOXG1 validated through single-cell RNA-sequencing. Variants losing COUP-TFI repression ability by binding to COUP-TFI's enhancer region consistently caused moderate-to-severe brain anomalies. Furthermore, in utero electroporation (IUE) in embryonic mouse brains was employed to study

their impact on neuronal migration and differentiation. Electroporation of wild-type Foxg1 delayed neuronal migration and altered their cell fate. Remarkably, variants associated with moderate-to-severe brain anomalies impaired these functions, while those with mild brain anomalies caused partial impairment. Thus, by combining protein expression, COUP-TFI repression, and neuronal migration assays, we developed a patient stratification paradigm for predicting the severity of FOXG1 syndrome. This workflow successfully differentiated 92.3% of cases, facilitating early diagnosis and guiding future therapeutic interventions. Figure (A) A flowchart for predicting the severity of brain anomalies caused by FOXG1 variants through 3 assays. (B) Principal component analysis of FOXG1 variant severity.



Disclosures: H. Cheng: None. **W. Lee:** None. **J. Tsai:** None.

Nanosymposium

NANO019: Mechanisms of Cell Fate in the Developing Brain

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO019.14

Topic: A.01. Neurogenesis and Gliogenesis

Title: Nuclear factor one (Nfi) transcription factors regulate radial glial differentiation during early cortical development

Authors: J. W. LIM[1], E. HOSSEN[1], N. MUNDELL[1], S. PAL[1], J. BUNT[2], ***L. J. RICHARDS**[1]; [1]Neurosci., Washington Univ., Saint Louis, MO; [2]Princess Máxima Ctr. for Pediatric Oncology, Utrecht, Netherlands

Abstract: Haploinsufficiency of *NFIA*, *NFIB*, or *NFIX* results in human neurodevelopmental syndromes that are characterized by macrocephaly, intellectual disability, corpus callosum dysgenesis and ventriculomegaly. The similarities of these syndromes imply that these genes have overlapping functions during development. Previous studies using mouse models with deletion of one or two *Nfi* genes have established a basis for understanding how these phenotypes may arise, but their overlap has confounded studies dissecting the underlying cellular and molecular mechanisms. To address this, we generated Emx1-Cre-driven, *Nfia, Nfib, Nfix* triple conditional knockout (tcKO) mice and examined their phenotype using a combination of histology and single-cell multi-omics. Building upon our previous findings, we observed that NFIs regulate the transition of neuroepithelial cells into radial glial cells during early cortical development. Moreover, our tcKO mice reveal a new phenotype whereby the impaired transition of neuroepithelial cells into radial glial cells does not completely inhibit neurogenesis, but gives rise to ectopic preplate cells that occupy the marginal zone. Notably, we did not observe this phenotype when four or less *Nfi* alleles were deleted. Therefore, our mouse model reveals new insights into the function of *Nfi* genes that cannot be studied in single and double knockout mouse models.

Disclosures: J.W. Lim: None. **E. Hossen:** None. **N. Mundell:** None. **S. Pal:** None. **J. Bunt:** None. **L.J. Richards:** None.

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.01

Topic: C.01. Brain Wellness and Aging

Support: Simons Foundation SCPAB
Stanley Center at the Broad Institute
HHMI

Title: Age-related changes in prefrontal cortex drives cellular, circuit and reward-based decision making in mouse and marmoset

Authors: *K. J. MASTRO[1], W.-C. LEE[2], W. WANG[3], Y. LIN[4], M. B. JOHNSON[5], B. L. SABATINI[6], B. A. STEVENS[7];
[1]Boston Children's/Harvard Medical/Broad, Boston, MA; [2]UCSF, San Francisco, CA; [3]Harvard Med. Sch., Boston, MA; [4]Broad Inst., Cambridge, MA; [5]Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard, Cambridge, MA; [6]Neurobio., Harvard Med. Sch. Dept. of Neurobio., Boston, MA; [7]Harvard Med. Sch. Neurobio., Boston Children's Hosp., Milton, MA

Abstract: Age-related changes in behavior must be due, in part, to the changing neural architecture that occurs across the lifespan. During adolescence, there is a significant enhancement in cognitive capabilities that parallels the maturation of prefrontal cortical circuits, but how the development of prefrontal cortex (PFC) drives cognitive development is still an area of active exploration. The protracted development of the prefrontal cortex during adolescence represents a vulnerable period for genetic and environmental insults that may drive brain structure and function into the disease states. Understanding the developmental trajectory of disease-relevant circuits across this vulnerable period of development can provide tractable means for therapeutic interventions. To tackle these questions, we have established a multi-systems approach that unpacks the genetic, synaptic, circuit and behavioral changes that occur over the course of adolescence across both mice and a non-human primate, the Common Marmoset. Firstly, we have identified synaptic, cellular, and behavioral changes that occur across the neurotypical adolescent development of the mouse which extends the period for cognitive maturation from weeks to months. Most notably, there is a prolonged and significant enhancement of the inhibitory maturation that alters cognitive performance in a reward-based decision-making in the two-armed bandit task (2-ABT). Consequently, these circuit-level changes drive age-related differences in cognition far beyond the traditional window of development. Secondly, we found age-related changes in both species when performing a reward-based decision making task. Lastly, we performed single-nucleus RNA sequencing across similar periods of development and have mapped the shared cell-state changes that occur over this developmental period. In the future, these experiments will nominate shared and divergent pathways that may drive these changes and explore their relationship to age-related changes in both brain structure and function with a particular focus on the genes that have been highlighted across neuropsychiatric disease.

Disclosures: K.J. Mastro: None. **W. Lee:** None. **W. wang:** None. **Y. Lin:** None. **M.B. Johnson:** None. **B.L. Sabatini:** None. **B.A. Stevens:** None.

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.02

Topic: C.01. Brain Wellness and Aging

Title: Exploring the lateralization of language regions: structural asymmetry between sexes across the adult lifespan

Authors: O. EGBERT, ***X. CHEN**;
Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Brain division into two hemispheres is a crucial organizational feature for functional specialization in integrative tasks. Although generally asymmetric, brain lateralization is evident and begins in utero. Within an individual, the degree of asymmetry in the brain differs across regions. Particular structural asymmetry in a region can contribute to its lateralized function. Across individuals, structural asymmetry differs across sexes and ages. Particularly, language is left lateralized as a prominent feature of functional asymmetry, while the structural asymmetry in language regions is less conclusive. Language ability also tends to differ between females and males and selectively maintains in aging. To better understand the structural asymmetry of language regions and the effects of sex and age, here, we used the Dallas Lifespan Brain Study (n=465, 21-89 yrs, 96% right-handed, 61% female) and focused on examining the structural asymmetry of Broca's area (pars opercularis, pars triangularis), Wernicke's area (superior temporal gyrus), and Heschl's gyrus (transverse temporal gyrus). Overall, the brain presents a leftward anterior and rightward posterior asymmetry, consistent with previous suggestions. Our primary analysis found that Wernicke's area and Heschl's gyrus were both left lateralized in volume and area size, but right lateralized in thickness (Fig. 1a-c). Interestingly, both right lateralization differed across people: Heschl's gyrus was more symmetric in women (Fig. 1d) and Wernicke's area was more symmetric with aging (Fig. 1e). Broca's area showed mixed results across subregions and no effect of age or sex. In conclusion, Wernicke's area and Heschl's gyrus had larger area and volume in the left hemisphere, supportive of their leftward function for language, while they are thicker in the right hemisphere but more symmetric in women and older people, both groups of whom tend to have better language ability. Future studies may explore how lateralization of these regions, collectively and interactively, supports language processing in different people.

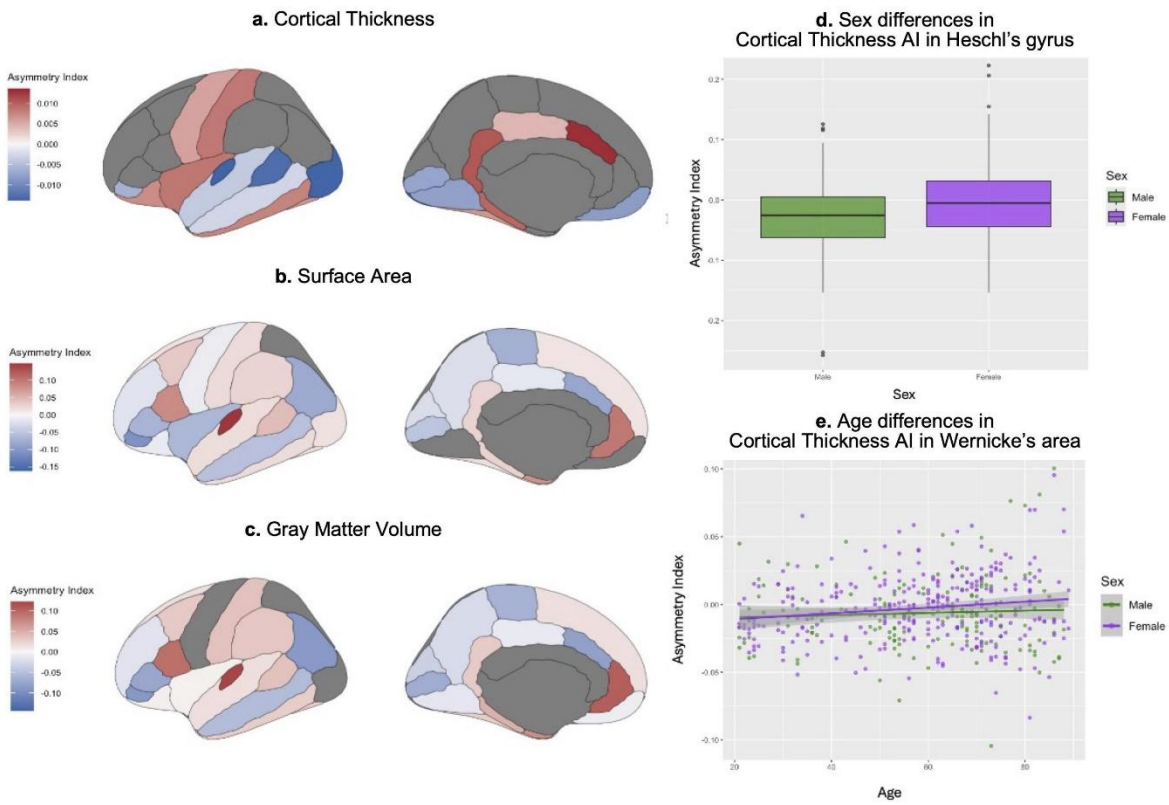


Fig. 1. Full sample averages (a-c), or "Asymmetry Index (AI)," of regional asymmetries of (a) cortical thicknesses, (b) surface areas, and (c) gray matter volumes, as well as (d) sex differences in the cortical thickness AI in Heschl's gyrus, and (e) age differences in cortical thickness AI in Wernicke's area visualized in males and females separately. AI = (L-R)/(L+R). For a-c, Color indicates FDR-corrected significance ($p \leq .05$) as well as direction. Red (positive AI) indicates greater left lateralization. Blue (negative AI) indicates greater right lateralization. For d-e, color indicates sex. Green denotes males. Purple denotes females.

Disclosures: O. Egbert: None. **X. Chen:** None.

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.03

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant T32NS096050
NIH Grant F31NS131020
NIH grant L30NS139361
Neuroscience Scholars Program

Title: Neurophysiological and Behavioral Effects of Prism Adaptation Combined with Sensory Stimulation

Authors: *F. K. ALOBA[1], M. MUTHUKUMAR[2], S. GHOSH[2], A. SLUSARENKO[3], J. PATEL[5], K. WUEST[6], M. R. BORICH[3], T. M. KESAR[4];
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Abstract: Background: Prism Adaptation (PA) is a well-established sensorimotor mechanism that recalibrates visuospatial-motor neural pathways to reduce spatial neglect (SN) post-stroke. Similarly, noninvasive, somatosensory electrical stimulation ('Stim') to the affected limb has been shown to enhance cortical motor excitability when used as an adjunct to PA training. However, the influence of healthy aging and post-stroke SN on neural and behavioral effects underlying PA is poorly understood. We tested the hypothesis that Stim modulates input-associated sensory and cognitive processing, complementing the effects of PA on the dorsal visuospatial cortical networks, and that PA- and Stim-induced neurophysiologic modulation is dampened by aging and network disruptions caused by post-stroke SN.
Purpose: We compared the effects of combining PA + Stim on upper limb motor performance, cortical excitability, and generalization of the effects of PA+ upper limb Stim to the non-stimulated lower limb in young adults (YA), older adults (OA), and post-stroke SN; and also the effects of PA+ lower limb Stim to the stimulated lower limb and its generalization to the non-stimulated upper limb.
Methods: We recruited 15 YA, 15 OA, and 1 Stroke SN (targeted N=10) using a repeated-measures crossover design, immediately before and after a single session of PA + Stim and PA + Sham. We measured visuospatial-motor behavior using upper and lower limb pointing tasks; corticomotor and intracortical excitability using motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) to M1 from left First Dorsal Interossei (FDI), Tibialis Anterior (TA), and Soleus (SOL) muscles.
Results: Preliminary results show that both PA combined with upper limb Stim and Sham Stim showed significant leftward aftereffects in YA and OA during upper limb pointing (all $p<0.01$), with a larger magnitude of behavioral change in OA compared to YA with PA + Stim ($p= 0.03$). Neurophysiologically, we found a larger magnitude of pre-post % change in OA compared to YA in PA +Sham in the FDI ($p = 0.03$). However, YA showed a larger pre-post % change following PA +Stim in the TA (0.003) and SOL (0.013).
Discussion: Our results suggest that combining PA and Stim may target neural substrates and SN mechanisms that neither therapeutic intervention can target alone, while also facilitating greater generalization to other tasks such as transfers or walking, thereby improving rehabilitation outcomes. The long-term goal of this research is to leverage an understanding of the neural mechanisms of SN and Stim to develop novel and efficacious clinical interventions for the rehabilitation of people post-stroke with SN.

Disclosures: F.K. Aloba: None. **M. Muthukumar:** None. **S. Ghosh:** None. **A. Slusarenko:** None. **J. Patel:** None. **K. Wuest:** None. **M.R. Borich:** None. **T.M. Kesar:** None.

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.04

Topic: F.06. Posture and Gait

Title: Bilateral Toe and Heel Tapping for Quantitative Assessment of Neuromotor Dysfunction and Posture Instability

Authors: *Y. WANG;
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Abstract: Objective assessment of posture instability is crucial for fall detection and prevention for patients with Parkinson's Disease (PD). In this study, we report anti-phase and in-phase toe and heel tapping as an effective biomarker for characterizing basal ganglia dysfunction, postural instability, and balance. A pair of smart insoles records the bilateral tapping motion, each has one accelerometer, and a battery-powered microcontroller unit with tiny machine learning to process the data on the edge. After collecting and analyzing the bilateral tapping data from 60 PD patients and 60 healthy controls, we discovered that bilateral tapping can capture the spatial-temporal neurocoodination and neuromuscular control. Precisely, bilateral tapping tasks reveal rhythmic and neuromuscular coordination, detect motor timing impairments in PD, and basal ganglia dysfunction. Timing accuracy reflects the integrity of internal pacing mechanisms governed by the basal ganglia. Bilateral coordination assesses the neuron synchronization and detects disorganization. Deficits in rhythmic heel movements correlate with gait disturbances, reduced step length, and postural instability in PD, which are predictors of PD progression and fall risks. Interestingly, neural oscillations, particularly elevated beta-band activity, are directly linked to impaired motor synchronization and timing, aspects critical to tasks such as toe and heel tapping, thus providing a neural basis for the motor deficits observed in PD. A recent study demonstrated that PD patients exhibit reduced and delayed beta-band modulation during rhythmic finger-tapping tasks, indicating impaired neuromuscular coordination. This finding further supports our hypothesis that functional motor deficits in PD are closely linked to disruptions in brain electrophysiology. Given that beta-band abnormalities can manifest as motor asynchrony, investigating heel and toe-tapping synchronization provides a practical and sensitive approach because these tasks reflect neural timing disruptions. With edge TinyML, bilateral toe and heel tapping provides a constant quantitative assessment for subtle neuromotor dysfunction progression and quantifying bradykinesia and other PD-related motor symptoms. Balance and posture instability detection achieves 95% classification accuracy and 0.88 correlation coefficient with pull test scores. This promotes the recognition of balance and posture instability in early-stage PD and improves diagnostic accuracy, complementing the clinical assessment.

Disclosures: Y. Wang: None.

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.05

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant 1R56AG060052-01

Title: Age Differences in Brain Activation During Switching and Updating in Working Memory Across Load and Stimulus Domain

Authors: *Y. LIU, P. SKOLASINSKA, C. BASAK;
Ctr. for Vital Longevity, The Univ. of Texas at Dallas, Dallas, TX

Abstract: Previous research has shown that older adults exhibit reduced activation of the fronto-parietal network (FPN) and insufficient deactivation of the Default Mode Network (DMN) compared to younger adults during working memory (WM) updating at the minimal load of 2 items (Qin & Basak, 2020; Skolasinska et al., 2023). However, it remains unclear how age-related differences in brain activation impact task performance under varying WM loads and stimulus domains across different types of cognitive control. In this study, we developed a novel hybrid block and event-related continuous WM updating fMRI task, the random N-Match. Cognitive control was manipulated through unpredictable switching of cue location and updating of stored information. Each of the three runs contained six blocks that crossed three WM loads (0, 2, 3) with two stimulus domains (verbal, visual). Within each block, four event types were presented: baseline, switch, update, and switch+update. This design allowed for the investigation of both sustained brain activation associated with load and domain, and transient activation related to cognitive control during switching and updating. We examined task performance and BOLD signal modulation in 24 younger adults (18-30 years) and 56 older adults (65+ years). Older adults were both less accurate and slower to respond ($p < .001$), with the performance difference between age groups increasing from 0- to 2-match and remaining relatively stable at 3-match, suggesting age differences in focus-switching but not in activated long-term memory (Basak & Verhaeghen, 2011). No age differences emerged between the stimulus domains. Mixed-effects model results from whole-brain GLM analyses revealed that younger adults, compared to older, recruited the FPN for sustained WM load, including the middle frontal gyrus, superior parietal lobule, and frontal pole. The FPN was also engaged during switching. In contrast, older adults failed to deactivate core DMN hubs (i.e., medial prefrontal cortex, posterior cingulate cortex) under higher WM loads, whereas younger adults exhibited load-dependent sustained DMN deactivation. Older adults also showed overactivation of the left inferior frontal gyrus, with greater activation of this region being associated with poorer accuracy, supporting the neural dedifferentiation hypothesis of neural aging. These results suggest that age-related difficulties with focus-switching are attributable to reduced neuromodulation capacity in older adults during WM switching. This impairment contributes to declines in WM performance across both verbal and visual domains, indicating a common underlying age effect on WM.

Disclosures: Y. Liu: None. **P. Skolasinska:** None. **C. Basak:** None.

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

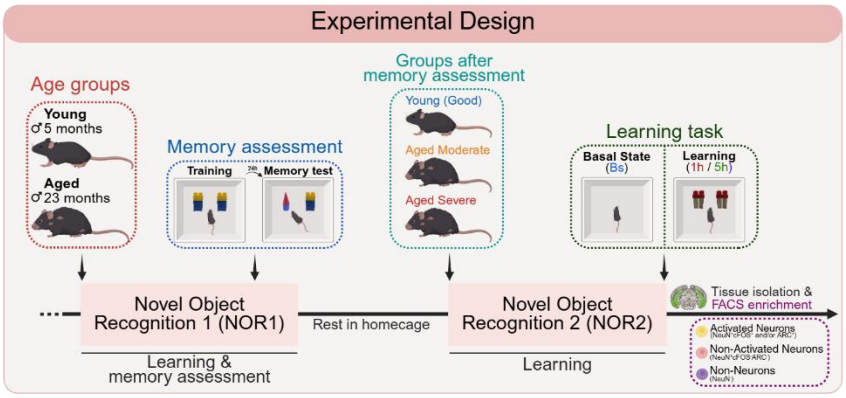
Presentation Number: NANO020.06

Topic: C.01. Brain Wellness and Aging

Title: Investigation and regional mapping of the molecular signatures underlying memory formation and age-related memory loss

Authors: *A. KOKKOSIS[1,2], K. XIONG[3], Y. BAI[3], D. VERGATA[4], T. SHAVLAKADZE[5], D. J. GLASS[5], B. ZAMBROWICZ[1], E. PAVLOPOULOS[1,2];
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Abstract: Long-term memory (LTM) formation is a learning-dependent process in the hippocampus, requiring activation of gene expression and synthesis of components critical for synapse strengthening and new synapse formation, processes actively modulated by glial cells. Despite foundational knowledge, the regulatory mechanisms underlying LTM and the impact of aging on these processes remain not well understood. To address this we employed snRNA-Seq to investigate both baseline and learning-induced molecular mechanisms in the aging mouse hippocampus in a cell-type specific manner. We developed the sequential NOR task (Figure), a paradigm that enabled the stratification of aged mice into groups with moderate and severe memory deficits, facilitating the correlation of molecular signatures with the degree of memory decline. Hippocampi were collected at 1h and 5h post-learning, capturing the critical time window for transcriptomic changes associated with LTM, and studied alongside baseline controls. We present a comprehensive single-nucleus transcriptomic atlas of the hippocampal formation, comprising ~0.7 million transcriptomes and ~180 neuronal and non-neuronal types from young and aged mice. We show for the first time learning-induced neuronal cell states defined by upregulated synaptic plasticity genes and novel learning-associated signatures. We demonstrate that aging profoundly impacts learning-induced molecular changes, with the most significant effects occurring in aged mice with severe memory deficits, particularly 5h post-learning. Notably, we report a list of genes ranked by their correlation with the severity of memory decline, allowing the identification of key molecular signatures and pathways underlying age-related memory loss. Finally, we demonstrate that aging significantly affects non-neuronal populations, especially microglia and mature oligodendrocytes, while neuron-glial interaction analysis highlights pathways linked to impaired neuronal function, reduced synaptic plasticity, neuroinflammation, and disrupted neural connectivity.



Disclosures: A. Kokkosis: A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **K. Xiong:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **Y. Bai:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **D. Vergata:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **T. Shavlakadze:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **D.J. Glass:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **B. Zambrowicz:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc. **E. Pavlopoulos:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals Inc..

Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.07

Topic: C.01. Brain Wellness and Aging

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Title: The common marmoset as a translational model for cognitive aging

Authors: C. R. VANDERLIP[1,2], S. R. DUNN[2], P. A. ASCH[2], J. H. REYNOLDS[2], ***C. GLAVIS-BLOOM**[2];
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Abstract: Aging is the predominant risk factor for cognitive decline and neurodegenerative diseases such as Alzheimer's disease, but cognitive aging in humans is highly variable; some individuals experience widespread decline, while others remain cognitively stable for decades. This variability exists not just across individuals, but also across cognitive domains, with those dependent on the prefrontal cortex and hippocampus especially vulnerable and among the earliest and most severely impacted in Alzheimer's disease (AD). Translationally relevant models are crucial for understanding the mechanisms that drive divergent cognitive aging outcomes and differentiate healthy aging from pathological decline. The common marmoset (*Callithrix jacchus*) has emerged as an advantageous non-human primate model for this work due to shared behavioral, neuroanatomical, and age-related neuropathological features with humans. Critically, their short lifespan enables a longitudinal approach to studying aging as a process that unfolds over extended durations of time, and that is highly variable amongst individuals. Despite their growing popularity as a model, robust cognitive phenotyping of marmosets, particularly as a function of age, across multiple cognitive domains, and longitudinally, is lacking. To address these major limitations for the development and evaluation of the marmoset as a model of aging, we developed MamoCog, a comprehensive touchscreen-based neuropsychological test battery for marmosets that mirrors diagnostic tests in humans. We administered MarmoCog twice per year for six years to a large cohort of marmosets ranging in age from young adult to geriatric. Our results show that marmosets exhibit marked domain-specific and individual variability in cognitive aging; some animals decline across multiple domains, others in just one, and some show no decline at all. When decline occurs, it is most severe in domains dependent on the prefrontal cortex and hippocampus. These patterns closely mirror human cognitive aging and establish the marmoset as a powerful model for identifying the age-related biological changes that drive individual differences in vulnerability to neurodegenerative disease, and for bridging the gap between normative aging and disease-related trajectories.

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Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 8:00 AM - 10:15 AM

Presentation Number: NANO020.08

Topic: C.01. Brain Wellness and Aging

Support: UKY IRC 2024

Title: The Impact of Brain Iron on Face Perception in Older Adults

Authors: ***V. ZACHARIOU**[1], M. BEHRMANN[3], Y. JIANG[4], P. E. COSKUN[2];
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Abstract: Face perception is fundamental to human social interaction, enhancing emotional well-being, fostering social bonds, and reducing isolation. Declines in this ability have been linked to increased social anxiety, impaired social interactions, and reduced quality of life. In older adults, who are particularly vulnerable to social isolation—a known risk factor for dementia—face perception is critical. However, face perception deteriorates with age, often independently of general age-related cognitive decline, increasing the risk of adverse outcomes. Our goal is to identify potential neurobiological mechanisms underlying this decline. Our recent longitudinal findings reveal localized age-related iron accumulation in specific cortical regions, including key face-processing areas in inferior temporal cortex. While iron is essential for brain health, excess brain iron that accumulates with age accelerates oxidative reactions that damage neurons and myelin, and has been repeatedly linked to cognitive decline. Given these adverse effects, we hypothesize that iron accumulation in face-processing regions may contribute to face perception decline. Eighteen healthy older adults (65-78 years) with corrected vision underwent structural MRI, quantitative susceptibility mapping to assess regional brain iron load, and functional MRI during a faces/houses discrimination task to localize face-processing regions (e.g. the fusiform face area; FFA) and control regions (e.g. the house-selective parahippocampal place area; PPA). Behavioral performance was assessed via the Cambridge Face and Car Memory Tests (CFMT/CCMT), with the car task as a control. Linear regression analyses assessed relationships between iron load in localized regions and CFMT/CCMT performance, controlling for age, sex, and NIH Toolbox measures of processing speed, working memory, and episodic memory. Higher iron load in the right FFA significantly correlated with lower CFMT performance (inverse efficiency: higher scores = lower performance; $p = 0.026$; $r^2 = 0.377$; $\beta = 1.06$; $t = 2.58$). No significant associations were observed between FFA iron and CCMT performance ($p = 0.104$) or between PPA iron and CFMT performance ($p = 0.960$). Processing speed, working memory, and episodic memory were not significantly associated with CFMT scores ($p > 0.19$). These preliminary findings implicate iron load in face-processing regions as a selective contributor to age-related face-perception decline, independent of general cognitive ability. Further research is needed to assess how brain iron impacts the functional and white-matter connectivity of brain networks supporting face perception.

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Nanosymposium

NANO020: From Neural Circuits to Cognitive Dysfunction in Aging and Neurodegeneration

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Topic: C.01. Brain Wellness and Aging

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Kanae (Y.H.)
Mitsui Sumitomo Insurance Welfare Foundation (Y.H.)

Title: Astrocyte-mediated glymphatic system impairment contributes to postoperative cognitive dysfunction in aged mice

Authors: *Y. HASEGAWA[1], R. WISEMAN[6,2], G. URSINI[7,3], Y. LI[1], X. ZHU[1], B. S. SLUSHER[6,2,3,4,5], A. KAMIYA[1,5];
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Abstract: Postoperative cognitive dysfunction (POCD) is a common complication in older adults following major surgery and is associated with impairments in learning and memory. Accumulating evidence suggests that age-related vulnerabilities in neuroimmune signaling, glial regulation, and brain clearance systems may contribute to its pathogenesis. Astrocytes regulate glutamate levels and mediate cerebrospinal fluid (CSF)-dependent glymphatic clearance via aquaporin-4 (AQP4) at perivascular endfeet. Although aging and inflammation are known to disrupt these processes, it remains unclear how surgical intervention affects astrocyte function and glymphatic flow, leading to cognitive decline. In this study, we utilized an aged mouse model to investigate the impact of abdominal surgery via histochemical, biochemical, and behavioral assays. Following intestinal manipulation under inhalation anesthesia, aged mice exhibited impairments in recognition and spatial memory. Glymphatic function was assessed by CSF tracer infusion, revealing reduced tracer penetration after surgery. Abdominal surgery also induced excess glutamate production, morphological changes of astrocytes, and altered AQP4 expression. We are currently investigating whether genetic and pharmacological inhibition of glutamate production can ameliorate these phenotypes. Our findings highlight glymphatic dysfunction as a potential pathological mechanism underlying POCD. Given that POCD is associated with an increased risk of dementia, addressing glymphatic dysfunction may provide novel molecular targets for the prevention and treatment of these age-related disease conditions.

Disclosures: Y. Hasegawa: None. **R. Wiseman:** None. **G. Ursini:** None. **Y. Li:** None. **X. Zhu:** None. **B.S. Slusher:** None. **A. Kamiya:** None.

Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.01

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

Support: STI2030-Major Projects 2022ZD0204700
STI2030-Major Projects 2022ZD0207200
National Natural Science Foundation of China 32170958
National Natural Science Foundation of China 82371255, 82071258
National Natural Science Foundation of China 323B2030
Shanghai Pilot Program for Basic Research 21TQ014
Program of Shanghai Academic/Technology Research Leader 23XD1402500

Title: Microglia replacement halts the progression of microgliopathy in mice and humans

Authors: *X. LI[1], B. PENG[2];
[1]Fudan Univ., Shanghai, China; [2]Shenzhen Inst. of Advanced Technol., Fudan Univ., Shanghai City, China

Abstract: CSF1R is primarily expressed in microglia. Its monoallelic mutation causes CSF1R-associated microgliopathy (CAMP), a major form of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) and fatal neurological disease without clinical cure. We developed mouse models harboring human hotspot mutations of CAMP and replaced CSF1R-deficient microglia with CSF1R-normal cells via microglia replacement by bone marrow transplantation (Mr BMT), which attenuated pathology in mice. We further demonstrated that, in the context of CSF1R-deficiency, traditional bone marrow transplantation (tBMT) in ALSP functions similarly to Mr BMT, efficiently replacing microglia and reducing disease progression. We then replaced CSF1R-deficient microglia in eight patients by tBMT. The disease progression was halted during the 24-month follow-up. Together, microglia replacement corrects pathogenic mutations and halts disease progression in mice and humans.

Disclosures: X. Li: None. **B. Peng:** None.

Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.02

Topic: B.09. Glial Mechanisms

Support: STI2030-Major Projects 2022ZD0204700
STI2030-Major Projects 2022ZD0207200
National Natural Science Foundation of China 32170958, 82371255, 82071258, 323B2030
Shanghai Pilot Program for Basic Research 21TQ014

Program of Shanghai Academic/Technology Research Leader
23XD1402500
The Innovative Research Team of High-Level Local University in Shanghai
Program for Outstanding Medical Academic Leader of Shanghai
2022LJ011

Title: Hematopoetic stem cell transplantation halts the progression of CSF1R-related disorder in mice and human by microglia replacement

Authors: ***J. WU**[1], Y. WANG[2], X. LI[2], P. OUYANG[2], Y. RAO[3], L. CAO[1], B. PENG[2];
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Abstract: Objective CSF1R-related disorder (CRD) is a fatal neurodegenerative disease caused by CSF1R mutations, leading to rapid motor/cognitive decline with no effective treatments. While case reports suggest hematopoietic stem cell transplantation (also known as traditional bone marrow transplantation, tBMT) may help, systematic clinical evidence are lacking and absence of reliable animal models hinders mechanism exploration. This study systematically evaluates tBMT's clinical value, establishes CRD models, and elucidates its therapeutic mechanisms. **Methods** We conducted a prospective cohort study monitoring tBMT-treated CRD patients versus controls using neurological scales, MRI, and safety assessments. CSF1R hotspot mutation (I792T/E631K) mouse models were developed and characterized. Mechanistic studies included: compare efficacy of tBMT and peripheral immune cell replacement in I792T mice to identify effector cells; competitive transplantation comparing CSF1R-normal/deficient microglia proliferation; comparing tBMT with enhanced microglia-replacing Mr BMT; evaluating influence of myeloablation methods/donor genotypes on therapeutic efficacy; and molecular analyses (immunoblotting/scRNA-seq). ^{18}F-FDG PET was explored for noninvasive microglial monitoring and to verify successful microglial replacement in patients. **Results** tBMT significantly slowed CRD progression with manageable side effects. I792T/E631K mice replicated human pathology (motor/cognitive deficits, microglial loss, myelin/axonal damage). tBMT restored microglial density (84.08% replacement), improved pathology, and rescued behaviors, which was dependent on microglial replacement rather than peripheral immune reconstruction. CSF1R-deficient microglia showed impaired proliferation, enabling donor macrophage dominance via tBMT. Mr BMT enhanced replacement (91.15%) and accelerated response. Treatment required donor genotype correction, unrelated to myeloablation methods. Donor macrophage achieved CNS engraftment via CCL2/CCR2 axis, and then normalized CSF1R signaling and oligodendrocyte transcripts. ^{18}F-FDG PET correlated with microglial density and post-tBMT patients exhibited increased signals, demonstrating successful microglial replacement in the human. **Conclusion** tBMT effectively treats CRD by replacing dysfunctional microglia, restoring CSF1R signaling and oligodendrocyte function. Our models, mechanistic insights (including Mr BMT optimization), and ^{18}F-FDG PET monitoring provide a translational framework, establishing a new paradigm for microglia-targeted therapies.

Disclosures: J. Wu: None. **Y. Wang:** None. **X. Li:** None. **P. Ouyang:** None. **Y. Rao:** None. **L. Cao:** None. **B. Peng:** None.

Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.03

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

Support: R01AG075820
NSF CAREER 1944053

Title: Pharmacological CSF1R Inhibition Attenuates LPS-induced Brain Cytokines and Akt Signaling Pathway

Authors: *S. BITARAFAN[1,2], B. R. TOBIN[1], S. RANGARAJU[3], L. WOOD[1,4,2];
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Abstract: Microglia, the brain's resident immune cells, shift from a protective "homeostatic" state into "disease-associated microglial" phenotype under chronic inflammatory conditions relevant to Alzheimer's disease and related dementias. Colony stimulating factor 1 receptor (CSF1-R) is a critical receptor in control of variety of microglial functions including phagocytosis, polarization, and proliferation via downstream signaling cascades such as Akt/mTOR and cytokine expression. In the brain, CSF1-R is primarily expressed on microglia and is widely targeted for microglia depletion. Despite the established importance of CSF1-R in basic microglial functions, its specific role in modulating brain immune responses, particularly in the context of neurodegenerative diseases remains poorly understood. Given that dysregulated microglial activation is a hallmark of many neurodegenerative disorders and that these mechanisms are in part regulated by CSF1-R, it is a promising target to modulate microglia phenotype. We hypothesized that pharmacological inhibition of CSF1-R using BLZ945 would differentially modulate Akt/mTOR signaling and cytokine expression profiles depending on microglial activation state (acute vs sustained). To test this, 2mo old female C57BL/6 mice (n=4-5/group) received intraperitoneal injection of either 1xLPS (acute) or 4xLPS (sustained) at 1 mg/kg. Next, a single dose of the clinically relevant and brain-permeable BLZ945 (40 mg/kg) was administered concurrently with the final LPS injection. Cortical tissues were flash-frozen and lysed for molecular analysis. Phospho-proteins from MAPK, Akt/mTOR, and NFκB pathways and 18 cytokines, were quantified using Luminex multiplexed immunoassays. Principal component analysis was conducted to analyze the data. LPS induced robust cytokine expression, including IP-10, Eotaxin, IL-2, RANTES, and IL-10 in both acute and chronic LPS ($p<0.05$). Interestingly, 1xLPS led to activation of pPTEN which has a negative regulatory role and inhibits Akt activity. In contrast, 4xLPS triggered phosphorylation of multiple Akt/mTOR pathway proteins (pAkt, pTSC2, pGSK3β, $p<0.05$). Notably, CSF1-R inhibition in the acute LPS model enhanced Akt (pAkt, mTOR, pGSK3β, $p<0.05$) and MAPK (pERK, pJNK, $p<0.05$) signaling activity, while attenuating cytokine levels toward saline control (IL-2, IL-10, IFNγ, IP-

10, $p<0.05$). In the sustained LPS model, BLZ945 amplified Akt pathway, but did not attenuate LPS-induced cytokines. Thus, our proof-of-concept data show that CSF1-R inhibition has distinct effects on phospho-protein signaling and cytokine expression in acute vs. sustained inflammatory models.

Disclosures: S. Bitarafan: None. **B.R. Tobin:** None. **S. Rangaraju:** None. **L. Wood:** None.

Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

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Presentation Number: NANO021.04

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NIH R01 DA063181
NIH P50 DA026306 (P5)
NIH F31 NS129462

Title: Methamphetamine modulates inflammation - implications for HIV-associated brain injury

Authors: S. TAYABALLY[1], J. KOURY[2], R. MAUNG[3], N. Y. YUAN[4], ***M. KAUL**[5]; [1]Univ. of California, Riverside, Riverside, CA; [2]Biomed. Sci. Grad. Program, ResearchHub Fndn., Menlo Park, CA; [3]UC Riverside, Riverside, CA; [4]Res. and Develop., Stem Pharm, Inc., Madison, WI; [5]Univ. of California Riverside, Riverside, CA

Abstract: Methamphetamine (METH) is a potent addictive substance with high abuse rates, particularly among people living with HIV (PLWH) on combination antiretroviral therapy (cART). The interaction between HIV and METH exacerbates HIV-associated neurocognitive impairment (NCI) and neuronal damage, potentially through inflammatory processes. Peripheral HIV-infected monocytes/macrophages infiltrate the brain, releasing neurotoxins and pro-inflammatory factors, while the virus also infects microglia. This study investigated the in vitro effects of METH on monocytic THP-1 cells and human induced pluripotent stem cell (iPSC)-derived microglia were stimulated with the HIV LTR-mimic ssRNA40 in the presence and absence of METH. Protein multiplex assays and RNA-sequencing showed that METH increased expression of inflammatory enzymes, such as MPO and MMP-9 while concurrently reducing expression of IFNβ, thus impairing antiviral responses while promoting inflammation. In addition, cell-free conditioned media of ssRNA40- and METH-stimulated microglia exerted neurotoxicity in human iPSC-derived mixed neuroglial cell cultures. Moreover, ssRNA40 and METH also compromised survival of neurons in mixed neuroglial cell cultures. These findings suggest that METH diminishes neuroprotective and antiviral immune responses while promoting pro-inflammatory pathways, potentially aggravating HIV-associated neuronal injury and NCI.

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Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.05

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

Support: National Institute on Drug Abuse (NIDA) award R01-DA052027

Title: Persistent neuroinflammation in HIV-1 infected brain organoid models treated with antiretroviral therapy

Authors: *S. MARTINEZ-MEZA[1], T. A. PREMEAUX[4], S. CIRIGLIANO[5], C. FRIDAY[4], S. MICHAEL[4], S. MEDIOUNI[6], S. VALENTE[7], L. NDHLOVU[4], H. FINE[5], R. FURLER O'BRIEN[2], D. NIXON[3];
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Abstract: HIV-1-associated neurocognitive impairment (HIV-1-NCI) is characterized by chronic neuroinflammation and a decline in neuronal function, even when antiretroviral therapy (ART) successfully suppresses viral replication. Microglia, the primary reservoir of HIV-1 within the central nervous system (CNS), plays a critical role in maintaining this neuroinflammatory state. However, understanding how chronic neuroinflammation is generated and sustained by HIV-1, or impacted by ART, is still unknown. We established an in vitro model of microglia derived from admixed hematopoietic progenitor cells (HPC) embedded into embryonic stem cell (ESC)-derived Brain Organoids (BO). HIV-1 infected microglia were co-cultured and infiltrated into BOs. We assessed inflammation through cytokine and phospho-NF-kB levels using flow cytometry and confocal microscopy. Although microglia were the primary source of pro-inflammatory cytokines, astrocytes, neurons and neural stem cells also exhibited increased phospho-NF-kB levels, indicating a broader neuroinflammatory response. ART effectively suppressed the virus to levels below detection but did not decrease neuroinflammation. Although ART significantly suppressed HIV-1, neuronal inflammation persisted in ART-treated, HIV-1 infected BOs. Together, these findings demonstrate that HIV-1

infection of microglia infiltrated into BOs provides a robust in vitro model for understanding the impact of HIV-1 and ART on neuroinflammation.

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Nanosymposium

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Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.06

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

Support: Colton Stroke Research Accelerator Award #5140

Title: Spatial transcriptomics reveals whole-brain sex differences in neuro-glial activation during post-stroke recovery in middle-aged mice

Authors: *H.-T. HUANG[1], I. GOLYNKER[2], A. YEUNG[2], S. ZHANG[2], M. LOPEZ[2], L. GLASER[2], J. BETLEY[2], C. THAISS[2], K. MORRIS-BLANCO[1];
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Abstract: Stroke is the second leading cause of neurological disability and death in the world. Aging is the greatest risk factor for stroke, with older individuals displaying higher incidence, greater severity, and poorer recovery. Stroke induces complex, regionally heterogeneous cellular responses that contribute to long-term neurological deficits after stroke. However, traditional transcriptomic approaches fail to preserve spatial context, limiting insight into how specific cell types respond within the post-stroke brain microenvironment. The goal of the current study was to delineate the sex- and cell-specific pathophysiological mechanisms across the aging brain in an experimental model of stroke. In this study, we performed comprehensive neurobehavioral analyses and applied spatial transcriptomics along with advanced immunofluorescence techniques to characterize the spatially resolved molecular landscape across injured and uninjured brain regions in an aging paradigm of stroke. Middle-aged male and female mice were subjected to middle cerebral artery occlusion (MCAO) to induce an ischemic stroke followed by a battery of motor and cognitive function tests at various time points after stroke. Female middle-aged mice exhibited higher mortality and poorer emotional memory than middle-aged males, while middle-aged males displayed worse motor function than females after MCAO. Spatial transcriptomic analysis during the subacute recovery period after stroke revealed sex-specific differences in the spatial expression of mitophagy-related genes, and neuronal genes involved in brain metabolism. Notably, we observed that female mice exhibited higher expression of genes related to inflammatory regulation across both the injured (ipsilateral) and uninjured (contralateral) hemispheres of the brain. Considering that neuroinflammation contributes significantly to long-term stroke pathology, we further analyzed sex-dependent microglial

activation patterns. Both male and female middle-aged mice exhibited stroke-induced increases in microglial activation and accumulation across the ipsilateral and contralateral brain compared to sham controls. However, middle-aged female displayed increased accumulation of hyperactive microglia in the contralateral hippocampus and more hyper-ramified microglia in the contralateral amygdala than males. Furthermore, analysis of microglial subtypes in the contralateral hippocampus also revealed significant sex-specific differences. Collectively, these findings provide new insights into the sex- and cell-specific difference governing long-term neurological recovery after stroke.

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Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.07

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

Support: NIH Grant NS132483

Title: Inhibition of Acly downstream of inflammarafts in spinal microglia alleviates chemotherapy-induced neuropathic pain

Authors: *M. ANGELIM[1], W. DUCOTE[1], A. SHARMA[1], A. ROCCA[1], T. L. YAKSH[3], Y. MILLER[4], J. NAVIA PELAEZ[2];
[2]Pharmacol. and Physiol., [1]St. Louis Univ., Saint Louis, MO; [3]UCSD Anesthesia Lab. 0818, La Jolla, CA; [4]UC San Diego, La Jolla, CA

Abstract: Microglial activation and spinal neuroinflammation are central to the development of chemotherapy-induced peripheral neuropathy (CIPN), yet the metabolic mechanisms driving microglial dysfunction remain poorly understood. In a mouse model of CIPN induced by cisplatin (2.3 mg/kg, i.p., two injections), we identified a key role for lipid-rich membrane microdomains harboring inflammatory receptors, namely “inflammarafts”, in promoting chronic pain through microglial reprogramming and a sustained proinflammatory state. Cholesterol-loaded microglia which are enriched with inflammarafts, show increased glycolytic and inflammatory programs and increased expression of ATP-citrate lyase (ACLY), a central metabolic enzyme linking glycolysis to lipid synthesis and histone acetylation. ACLY expression is increased in CIPN tissue and sorted spinal microglia from CIPN mice exhibited increased lipid droplets, cholesterol accumulation, and altered chromatin accessibility at metabolic and inflammatory gene loci. These changes were accompanied by an increase in the transcriptional coactivator p300 and H3K27 acetylation, indicating epigenetic activation of glycolytic and inflammatory pathways. Importantly, intrathecal inhibition of ACLY (Bempedoic acid, 3.5µg) in CIPN mice reduced microglial inflammaraft formation, reduced lipid droplets and significantly

alleviated mechanical hypersensitivity. These findings identify ACLY as a key metabolic checkpoint downstream of lipid-raft-associated signaling in spinal microglia. Targeting ACLY may offer a novel therapeutic approach to limit neuroinflammation and treat chemotherapy-induced neuropathic pain.

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Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.08

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

Support: R01 NS5103212
R01 NS122174

Title: Tmem119+ microglia controls blood-brain barrier disruption through MHC class I restricted antigen presentation in a CNS vascular disease model

Authors: *M. PEDRA SEADY[1], M. MAYNES[2], A. HASSANI[3], J. THELWELL[2], C. OWENS[1], H. JENSEN[1], C. LEWIS[1], M. HANSEN[2], F. JIN[2], A. J. JOHNSON[1];
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Abstract: The importance of microglia MHC class I restricted antigen presentation to brain-infiltrating CD8 T cells has been difficult to define. To address this question, our laboratory generated novel single MHC Class I conditional knockout mice in which H-2K^b or H-2D^b can be deactivated specifically in Tmem119+ microglia with tamoxifen administration. Recombinant Theiler's murine encephalomyelitis virus (TMEV) encoding the model OVA antigen enabled analysis of virus antigen-specific CD8 T cells restricted to H-2K^b class I molecules while Daniel's strain TMEV H-2D^b class I molecules enabled analysis of D^b:VP2 specific CD8 T cells. Our results revealed profound differences in the response of CD8 T cells upon deletion of MHC class I molecules on microglia. Conditional knockout of H-2K^b in Tmem119+ microglia reduced K^b:OVA epitope specific CD8 T cells in the brain compared to Cre negative littermate controls, and BrdU staining revealed differences in T cell proliferation. Meanwhile, mice with deletion of D^b in Tmem119+ microglia had reduced levels of perforin in D^b:VP2 CD8 T cells. Furthermore, in a model of CNS vascular disease, the deletion of H-2D^b of Tmem119+ microglia reduced CD8 T cell numbers in the brain, as well as the BBB breakdown observed by a FITC albumin assay. These findings demonstrate discrete roles for specific MHC Class I molecules on microglia during CNS viral infection. This data will further our understanding of brain infiltrating CD8 T cell responses in neurologic diseases as well as the regulation of BBB by microglia.

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Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.09

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

Title: Interferon-responsive microglia link cardiometabolic multimorbidity to dementia

Authors: R. SARKAR, S. PATIL, ***M. DEBERGE**;
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Abstract: Cardiometabolic multimorbidity (CMM)—the co-occurrence of multiple cardiometabolic risk factors such as obesity, hypertension, and dyslipidemia—affects an increasing proportion of the U.S. population and is a major contributor to the rising burden of dementia. Although cardiometabolic disease is known to promote systemic inflammation and vascular dysfunction, the mechanisms by which CMM leads to central nervous system injury and cognitive decline remain poorly understood. In this study, we developed a novel two-hit mouse model of CMM that combines metabolic stress and hypertension to mimic key features of human CMM. Mice subjected to this dual insult exhibited exacerbated microglial activation, white matter degeneration, and deficits in cognitive performance compared to either single-risk or control animals. Spatial transcriptomic profiling revealed a pronounced enrichment of type I interferon (IFN-I) signaling specifically in microglia from CMM brains, with upregulation of both canonical interferon-stimulated genes (*Mx1*, *Axl*) and genes involved in IFN-I production (*Ifnb1*, *cGas*, *Sting*). Notably, this interferon-responsive microglial phenotype persisted even after normalization of metabolic and vascular parameters, indicating that CMM induces a lasting inflammatory imprint in the brain. Conditional ablation of the IFN-I receptor in microglia significantly reduced neuroinflammation, preserved white matter integrity, and rescued cognitive function. Moreover, systemic blockade of IFN-I signaling or pharmacological inhibition of the upstream STING pathway also attenuated microgliosis and reversed neurological injury. Together, these findings identify interferon-responsive microglia as a key mechanistic link between cardiometabolic dysfunction and neurodegeneration and suggest that targeting the IFN-I signaling axis may represent a promising therapeutic strategy for preventing or slowing dementia progression in individuals with CMM.

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Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.10

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

Support: NSTC 113-2314-B-303-028

Title: Intracerebral Hemorrhage Induces Secondary Neurodegeneration in Alzheimer's Disease Mice via Microglial Dysfunction and Neuroinflammation

Authors: *H.-K. LIEW[1], W. HU[3], H.-Y. HUANG[1], S.-T. TSAI[2], C.-Y. PANG[4];
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Abstract: Background: Intracerebral hemorrhage (ICH) is the most lethal form of stroke, significantly increasing the risk of post-stroke dementia, including Alzheimer's disease (AD). However, the cellular and molecular mechanisms linking ICH to accelerated AD pathology are not well understood. Microglia, the brain's resident immune cells, plays a dual role in hematoma resolution and amyloid-beta (Aβ) clearance. Following ICH, however, these cells can become dysregulated and adopt a pro-inflammatory phenotype, which may impair Aβ phagocytosis and exacerbate AD progression. Objective: This study aims to investigate how ICH influences secondary neurodegeneration in an amyloidogenic AD mouse model, focusing on alterations in the microglial phenotype and neuroinflammatory responses. Methods: ICH was induced via stereotaxic injection of 0.075 U of collagenase in 0.5 µl of saline into the striatum of 5-month-old *App*^{SAA} transgenic AD mice and age-matched C57BL/6 wild-type controls. Cognitive function was assessed at 8 months old using the Y-maze spontaneous alternation task. Subsequent immunohistochemical analyses examined microglial activation, amyloid plaque morphology, and their spatial distribution. Gene and protein expression profiling was conducted to identify microglial phenotypic markers and inflammatory mediators. Results: ICH in AD mice (ICH-AD) markedly enhanced microglial activation, as evidenced by hypertrophic somata, retracted, thickened processes, and elevated expression of pro-inflammatory markers. These changes were associated with reduced interactions between microglia and plaques and diminished clearance of Aβ, leading to increased amyloid deposition in the ipsilateral cortex and hippocampus. Although only mild cognitive impairment was observed behaviorally, the substantial upregulation of neuroinflammatory markers and the altered expression of genes related to the microglial phenotype suggested that ICH accelerates AD pathology through immune dysregulation. Conclusion: These findings demonstrate that ICH exacerbates AD-related neurodegeneration via microglial dysfunction and heightened neuroinflammation. This work highlights the critical pathological link between cerebrovascular injury and AD progression and offers new therapeutic intervention targets for patients with comorbid stroke and neurodegenerative disease.

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Nanosymposium

NANO021: Microglial and Neuroinflammation: Beyond Alzheimer's Disease

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO021.11

Topic: B.09. Glial Mechanisms

Title: Microglial activation: Methods for dissecting cell signaling pathways involved in neuroinflammation

Authors: *C. B. CARLSON, M. CURTIS, R. FIENE, D. MAJEWSKI, M. DONEGAN, S. BURTON, C. SAVIC, J. LIU, B. FREITAS, S. SCHACHTELE;
FUJIFILM Cell. Dynamics, Madison, WI

Abstract: As the resident immune cells of the brain, microglia not only play a central role in the innate immune response and but are also implicated in numerous neurodegenerative diseases. Under pathological conditions, microglia can be activated to further drive neuroinflammation. Human iPSC-derived microglia have emerged as a powerful cell model to dissect the molecular mechanisms of microglial activation. In addition to signaling cascades like NFkB or DAP12/SYK that orchestrate responses such as cytokine secretion and phagocytosis, microglial activation often converges on the NLRP3 inflammasome. This multi-protein complex mediates Caspase-1 activation and maturation of IL-1β and IL-18. Furthermore, emerging evidence suggests that key genes Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) and apolipoprotein E (APOE) influence the NLRP3 inflammasome and modulate microglial activation in both homeostatic and disease contexts. In this study, we used iCell Microglia to investigate multiple cell signaling pathways with various immunoassays. We first established that this cell type could respond to traditional pro-inflammatory stimuli (e.g., LPS or IFN-γ) and release canonical cytokines like IL-6 and TNF-α using HTRF assays (Cisbio). We further tested what factors governed such a response (including stim time, culture media, co-factors, or ECM) with apparently healthy normal (AHN) microglia cells. We also used Lumit immunoassays (Promega) to measure robust release of IL-1beta and active IL-18 via activation of NLRP3 signaling with nigericin, LPS, and extracellular ATP. Finally, we demonstrated the utility of AlphaLISA technology (revvity) to detect key cellular events within the DAP12/SYK pathway, such as TREM2/DAP12 complex formation, TREM2 aggregation, and phosphorylation of DAP12 and SYK. Finally, we explored the impact of TREM2 variants and APOE isoforms on the microglial responses in these different assay readouts. This was complemented by evaluating the response to chronic neurotoxic stimuli like aggregated amyloid beta, alpha-synuclein, or Tau proteins. This abstract underscores the central role of microglia as mediators of neuroinflammation in the context of Alzheimer’s Disease, with TREM2 and APOE acting as critical modulators. Elucidating the downstream signaling mechanisms driving microglial activation offers opportunities for targeted therapeutic interventions aimed at restoring microglial homeostasis and mitigating neurodegeneration. This study also highlights the importance of implementing human cells with AD-relevant mutations into drug discovery workflows.

Disclosures: C.B. Carlson: A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **M. Curtis:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular

Dynamics. **R. Fiene:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **D. Majewski:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **M. Donegan:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **S. Burton:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **C. Savic:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **J. liu:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **B. Freitas:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **S. Schachtele:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics.

Nanosymposium

NANO022: Ensuring Robust, Long-Term Performance of Brain-Computer Interfaces

Location: SDCC Rm 25A

Time: Monday, November 17, 2025, 8:00 AM - 10:45 AM

Presentation Number: NANO022.01

Topic: F.05. Brain-Machine Interface

Support: NIH R01NS121079

Title: Characterization of a Stable Motor Manifold in Humans Despite Neural Variability and its Use in a Novel Intracortical BCI Decoder

Authors: *W. HOCKEIMER[1,2,4], B. DEKLEVA[1,2,4], N. G. KUNIGK[2,3,4], M. BONINGER[1,2,3], S. M. CHASE[4,5,6], J. L. COLLINGER[1,2,3,4,6];
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Abstract: Implanted brain-computer interfaces (BCIs) offer a promising avenue of functional restoration for millions of people living with serious motor impairments. However, neural signals can be variable over months or years, undermining the robustness of current BCI systems. Work in non-human primates identified a stable, low-dimensional manifold representation of motor intent, despite day-to-day recording instabilities (Gallego et al., 2020). Here we characterize the stability of a neural manifold associated with human BCI control and use this stable neural representation to design a novel BCI decoder for online use. Data were recorded from two people with tetraplegia who were implanted with intracortical BCIs in motor cortex (IDE NCT01894802). Participants used their BCI to complete center-out cursor control tasks over many days (n = 83 sessions across 911 days for P2; 52 sessions across 536 days for P4). Recording quality was variable across recording sessions (mean absolute change in channel SNR between successive sessions: 0.39 ± 0.31 P2, 0.84 ± 0.64 P4). Neural responses in a low-dimensional space were trial-averaged by condition to form 'templates' of motor intent. An algorithm called "Generalized Procrustes Alignment" estimated the underlying motor intent manifold and aligned each session's data to it. Data from each session aligned well to the mean manifold (average correlation: 0.73 ± 0.17 P2, 0.92 ± 0.06 P4), suggesting this method identified a stable latent structure involved with iBCI control despite day-to-day recording quality

variability. To test whether the latent neural manifold remained stable over time or drifted with increasing distance in time, pairwise correlations between daily manifolds were organized by the gap in days between their respective sessions. The correlation between templates was mostly unaffected by the gap between them (weighted least squares (WLS) intercept = 0.57, slope = 0.0001, $R2 < 0.02$ P2; intercept = 0.85, slope magnitude < $1x10^{-4}$, $R2 < 0.005$ P4). The stable manifold was used to create a novel BCI decoder that was pre-trained on historic data and required no further training during experiments, only alignment of a short calibration set to the mean manifold. This novel BCI decoder afforded proficient cursor control to P4 (mean angular error = 31.5 ± 4.6 degrees (n=2 sessions) versus 37.6 ± 4 degrees for historic performance (n=31), $p = 0.054$) and usable control for P2 (mean angular error 69.2 ± 4.7 degrees (n=5) compared to 46.4 ± 8.9 degrees for historic performance (n=26), $p < 6.3x10^{-6}$). These data demonstrate long-term representational stability in human motor cortex, with important implications for BCI decoder design.

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Nanosymposium

NANO022: Ensuring Robust, Long-Term Performance of Brain-Computer Interfaces

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Title: Stable high-accuracy speech and cursor decoding with a chronic intracortical brain-computer interface over two years

Authors: *N. CARD[1], T. SINGER-CLARK[1], H. PERACHA[1], C. IACOBACCI[2], M. WAIRAGKAR[3], X. HOU[1], Z. FOGG[1], E. OKOROKOVA[5], L. R. HOCHBERG[6], D. BRANDMAN[7], S. D. STAVISKY[4];
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Abstract: Maintaining high performance of intracortical brain-computer interfaces (BCIs) over long periods is critical for their clinical viability. However, chronic use presents challenges, including signal nonstationarity, potential electrode degradation, and a resultant need for frequent decoder calibration. Understanding and mitigating these factors are essential for long-term BCI reliability and effectiveness.

Here we report stable, high-accuracy decoding performance over more than two years in a multimodal intracortical BCI designed for speech and cursor control. For speech decoding, we developed a "brain-to-text" BCI that decodes neural signals associated with attempted speech into phonemes and then words. For cursor decoding, we developed a recurrent neural network (RNN)-based cursor BCI that enables the user to move an on-screen computer cursor and click. A 45-year-old man ('T15') with amyotrophic lateral sclerosis (ALS) enrolled in the BrainGate2 clinical trial and had four microelectrode arrays placed in his left ventral precentral gyrus, providing neural data from 256 intracortical electrodes. Spiking activity remained detectable on 95% of electrodes throughout the two-year observation period, demonstrating robust electrode longevity.

We systematically evaluated decoding accuracy in periodic benchmark sessions spanning over 620 days post-implantation. The brain-to-text speech BCI consistently achieved up to 99.2% word accuracy in a structured Copy Task. The cursor control BCI maintained over 2 bits per second in a Grid Task. Outside controlled research sessions, T15 independently used the BCI at home for over 3,650 cumulative hours (422 days, median 9.4 hours/day), generating more than 200,000 spontaneous sentences at an average rate of 56.1 words per minute. Over 66% of sentences decoded completely correctly, with an additional 28% mostly correct, highlighting sustained high accuracy. T15 used the BCI to control his personal computer for an average of 2.5 hours per day, further underscoring the practical robustness of the system. The multi-modal BCI enabled him to send text messages and emails, browse the web, participate in video calls, and more.

These results illustrate that intracortical speech and cursor BCIs can maintain stable and high-accuracy performance over chronic timescales, indicating that intracortical BCI technology has the potential to become a practical assistive technology for people living with paralysis.

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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); Wairagkar, Stavisky, Card, and Brandman have patent applications related to speech BCI owned by the Regents of the University of California., Stavisky is an inventor on intellectual property owned by Stanford University that has been licensed to Blackrock Neurotech and Neuralink Corp.. F. Consulting Fees (e.g., advisory boards); Stavisky is an advisor to Sonera..

Nanosymposium

NANO022: Ensuring Robust, Long-Term Performance of Brain-Computer Interfaces

Location: SDCC Rm 25A

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Topic: F.05. Brain-Machine Interface

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Title: Long-term Intracortical Neural activity and Kinematics (LINK): An multi-year intracortical neural dataset for developing stable brain-machine interfaces

Authors: *H. TEMMAR[1], Y. WANG[2], N. GILL[3], N. B. MELLON[4], C. LIU[4], L. CUBILLOS[5], R. PARSONS[1], J. COSTELLO[4,6], M. CERADINI[8], M. KELBERMAN[1], M. MENDER[1,6], A. HITE[1], D. M. WALLACE[5], S. R. NASON-TOMASZEWSKI[1], M. WILLSEY[6], P. G. PATIL[6,1], A. DRAELOS[1,7], C. A. CHESTEK[1,5];
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Abstract: Brain-machine interfaces (BMIs) decode neural data to restore movement and speech in people living with paralysis. While intracortical BMIs (iBMIs) have achieved the highest BMI performance to date, neural data recorded from intracortical electrodes exhibit significant instabilities over time, sometimes changing within hours or days. This limits the practicality of current iBMIs in real-world settings, as they require frequent recalibration to remain functional. Several groups have developed promising approaches for stabilizing neural activity over time (Degenhart et al., 2020; Gallego et al., 2020; Karpowicz et al., 2025; Ma et al., 2023; Sani et al., 2021; Ye et al., 2025), but a major challenge is the absence of standardized benchmarks, making it difficult to directly compare stabilization methods. Current publicly available datasets cover a variety of tasks, but are sparsely sampled over relatively short time scales (less than one year) (Karpowicz et al., 2024). To address these gaps, we present the **LINK** dataset (**L**ong-term **I**ntracortical **N**eural activity and **K**inematics), a publicly available dataset which contains

intracortical spiking activity from the hand area of the motor cortex and kinematic data from 312 sessions of a non-human primate performing a dexterous, 2 degree-of-freedom finger movement task, spanning 1,242 days. The LINK dataset (dandiarchive.org/dandiset/001201) and code (github.com/chesteklab/LINK_dataset) are freely available to the public. We also present longitudinal analyses of the dataset's neural spiking activity and its relationship to kinematics, as well as overall decoding performance using linear and neural network models. We show that, despite observing a general decrease in neural activity over time, single channels tuned to specific movement directions generally stayed tuned to that direction and appear to be partially somatotopically organized. Additionally, we demonstrate stable within-day decoding performance (measured with R^2) from linear and nonlinear decoders across the entire dataset. Across days, both linear and nonlinear decoders had sharp decreases in R^2 as soon as one day after training, followed by a slower decline that gradually stabilized around 30 days. Future goals are to compare many of the adaptation methods which have already been proposed for use in iBMIs.

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Nanosymposium

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Location: SDCC Rm 25A

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Presentation Number: NANO022.04

Topic: F.05. Brain-Machine Interface

Title: Adaptive Decoders Promote Compaction of Neural Representations During Long-Term Brain-Computer Interface Learning

Authors: *P. RAJESWARAN[1], A. PAYEUR[2], G. LAJOIE[2,3], A. L. ORSBORN[1,4,5];
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Abstract: Neural (plasticity, learning related changes, representational drift) and technological (signal loss) nonstationarities currently limit the long-term performance of brain-computer interfaces (BCIs). Adaptive decoders that retune decoder weights to stabilize control are often assumed to preserve performance without impacting the underlying neural encoding, but this assumption has not been investigated. We re-analyzed a longitudinal intracortical BCI dataset from two nonhuman primates who trained with a velocity decoder that intermittently adapted to maintain performance via closed-loop decoder adaptation (CLDA) (Orsborn et. al. Neuron 2014). CLDA periodically adjusted decoder weights to better match users' intended movements

and was used to provide sufficient cursor control across the workspace on day 1, then reapplied intermittently to maintain performance as neural signals varied over time. A 128-channel electrode array implanted in primary and premotor cortex recorded both “readout” neurons used by the decoder and simultaneously recorded nearby “non-readout” neurons. Behavioral performance improved over days of training, correlated with increases in the neural encoding of task information that was specific to readouts; consistent with *credit assignment learning of task-relevant neurons*. Comparing neural activity early vs. late in learning also revealed that task-relevant information became increasingly concentrated within a smaller subset of readout units over time, a phenomenon we term *compaction*. We did not observe this compaction in reanalysis of comparable datasets using fixed decoders (Ganguly et. al. Plos Bio. 2009). A neural network model trained under matched conditions confirmed that decoder adaptation interacts with biological learning processes to promote credit assignment to a compact sub-ensemble of readout units. Surprisingly, task-related information became embedded in low-variance population modes, indicating that compaction does not necessarily reduce population dimensionality. Together, our results reveal that adaptive decoders do more than stabilize BCI control; they reshape the underlying neural representations during learning. These findings also suggest that adaptive decoders reshape the error feedback the brain relies on to solve credit assignment. Our results demonstrate interactions between neural encoding and BCI decoding, which highlights the need for co-adaptive approaches in the design of more robust, long-lasting BCI systems.

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Topic: F.05. Brain-Machine Interface

Support: UH3 NS107714
R35 NS122333
R01 NS130302
DOD NDSEG Fellowship

Title: Robust Generalization of a Neural Decoder Across Increasingly Non-Intuitive BCI Tasks

Authors: *A. ALAMRI[1], N. G. HATSOPOULOS[1], C. M. GREENSPON[2];
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Abstract: A central challenge in brain-computer interface (BCI) design is enabling users to generalize learned control across diverse task demands. In this study, we evaluated whether a single neural decoder, trained on a simple 8-target two-dimensional center-out task, could support accurate performance across a series of increasingly non-intuitive BCI tasks without

retraining. Using intracortical recordings from a participant with paralysis, we assessed decoder generalization to three novel tasks that each required control over two continuous dimensions.: (1) a 5×5 grid-based center out target task with modified visual task components, (2) a task requiring simultaneous control of position and size, and (3) an abstract task involving concurrent control of cursor color and size. Over eight consecutive sessions, the participant progressively improved performance, demonstrating stable and accurate control in each of the increasingly non-intuitive tasks. These results demonstrate that training with a fixed decoder can enable consistent generalization even as task demands diverge from naturalistic movement representations, offering insight into the adaptability of neural control in BCI systems.

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Nanosymposium

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Tianqiao and Chrissy Chen Neuroscience Center

Title: Long-term stability and performance of stimulation and recording in human participants up to 2,800 days

Authors: *D. A. BJANES[1], S. S. DARCY[1], K. PEJSA[1], B. LEE[2], C. LIU[2], R. A. ANDERSEN[1]; [1]Caltech, Pasadena, CA; [2]Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: The long-term stability of recording and stimulation performance of implanted electrodes is a fundamental requirement for the viability of brain computer interfaces (BCI) as clinical and assistive devices. BCIs have documented lifetimes of up to 4 years in human participants; however, the timeline of device material failure and stability of the underlying neural population are unknown for a multi-decade participant lifetime. In this work, we show 1) performance data (both stimulation and recording) from participants up to 0.8 decades and 2) correlation between physical state and electrophysiological performance of 980 explanted electrodes after +5 years in human cortex. Four participants were consented and chronically implanted with Utah arrays, for the purpose of restoring dexterous hand function. Platinum (Pt)

tipped arrays were placed in a variety of cortical locations: anterior intraparietal area (AIP), supra-marginal gyrus (SMG), Brodmann's area 5 (BA5), and primary motor (M1). Arrays tipped with sputtered-iridium oxide film (SIROF) were implanted in primary somatosensory cortex (S1). For each electrode, quantitative measurements of electrophysiological recording quality were obtained at regular intervals: RMS noise, 1 kHz impedance, and SNR. For the longest implanted participant (P2), we used spatiotemporal patterns of intra-cortical micro-stimulation (ICMS) of S1 to evoke naturalistic somatosensory percepts. We quantified reported descriptions and projected fields (the somatotopic location). Ten arrays were explanted from three participants and physical changes in the electrode metallization and insulation were characterized with scanning electron microscopy (SEM). Our data shows a stable electrical interface is possible for nearly a decade of chronic implantation. The SNR of SIROF electrodes was significantly better than Pt electrodes, and after 2800 days of chronic implantation, nearly 50% of the SIROF electrodes continued to elicit somatosensory percepts. Not only did projected fields significantly overlap across the measured time points (suggesting robust, longitudinal stability), but the somatotopic coverage expanded with time. Additionally, the physical state of the explanted electrodes significantly correlated with both stimulation and recording performance. Our findings link quantitative measurements to the physical condition of the microelectrodes and their capacity to record and stimulate and demonstrates performance data nearly twice as long as previously reported in the literature. These significant advances validate long term performance over multi-year BCI clinical trials.

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Nanosymposium

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Title: Factors in control performance and stability of implantable BCI in people with ALS

Authors: *N. F. RAMSEY[1,2], M. RAEMAEKERS[3], M. P. BRANCO[4], E. J. AARNOUTSE[5], Z. V. FREUDENBURG[6], S. GEUKES[7], A. SCHIPPERS[8], N. E. CRONE[10], M. VANSTEENSEL[9]; [1]UMC Utrecht Brain Center, Univ. of Utrecht, Utrecht, Netherlands; [2]Sci. Fac., Radboud Univ., Nijmegen, Netherlands; [4]UMC Utrecht Brain Ctr., [3]Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; [5]Neurol. and Neurosurg., UMC Brain Center,University Med. Ctr. Utrecht, Utrecht, Netherlands; [6]UMC Brain Center, Univ. Med. Center, Utrecht, Utrecht, Netherlands; [7]Neurol. &

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Abstract: A considerable number of people with ALS have participated in implantable BCI research, overall showing feasibility of decoding hand movements and even speech. Reports on stability of decoders are rather sparse due to the research nature of studies encouraging explorations of various decoding concepts (eg 2D control, hand writing, control of consumer devices). Stability is of importance when considering BCI as an assistive technology for clinical indications. For people with ALS entering locked-in state, reports on iBCI performance are few. We report on results of assessment of people with ALS in locked-in state for eligibility to obtain an experimental electrocorticography-based BCI implant, BCI performance in those who were implanted and stability of performance. All candidates were included in one of two studies after an initial screening, followed by more in-depth screening for medical eligibility and finally an MRI scan to assess anatomical and functional requirements for subsequent implantation.
Seven people were considered in the period 2013-2020. After passing general screening for inclusion requirements, one passed away before an MRI scan could be performed, one after a favourable MRI scan. A third candidate was excluded due to a lack of brain activity in the functional MRI scan, and a fourth due to insufficient activity combined with age-excessive atrophy. Two were implanted with a fully implanted 4-amplifier system and one with a 128-electrode system with pedestal and external amplifiers. Of these three, one obtained good BCI control, one showed moderate control and one exhibited poor performance. For the first two, signal strength declined steadily over the years, to a point where no difference between movement attempt and rest could be detected (7 and 2 years after implantation respectively). BCI control over that period, however, remained stable for 6 and 1 years respectively, after which it declined over a period of 1.5-2 years. During the steady performance years, decoding parameters were only modified a few times to adjust for the loss of signal strength. Analyses of MRI scans in 5 participants suggests that signal strength can be predicted by an accurate measure of thickness of grey matter in the primary motor cortex.
In conclusion we find that iBCI control performance is affected when ALS has reached a late stage, due to the progressive loss of brain signal in sensorimotor cortex, which (at least for electrocorticography) cannot be countered by altering decoder parameters. Furthermore, findings indicate that MRI and functional MRI may be good indicators of signal strength and thereby of successful iBCI control.

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Nanosymposium

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Topic: F.05. Brain-Machine Interface

Support: UHSNS114439 (NINDS)

Title: A stable self-paced silent speech brain-computer interface for device control

Authors: ***S. LUO**[1], M. ANGRICK[4], C. COOGAN[1], D. CANDREA[2], K. WYSE-SOOKOO[6], A. SCHIPPERS[7], G. W. MILSAP[3], C. GORDON[1], D. TIPPETT[1], N. J. MARAGAKIS[9], L. CLAWSON[1], M. VANSTEENSEL[8], F. TENORE[10], M. S. FIFER[11], N. F. RAMSEY[12], N. E. CRONE[5];
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Abstract: Brain-computer interfaces can potentially give people with amyotrophic lateral sclerosis (ALS) the freedom to interact with smart devices in their homes. However, maintaining reliable BCI performance over extended implantation periods remains challenging, and previous applications required commands issued in response to visual or auditory cues. Until now, it was unclear whether silent attempted speech could be accurately detected and decoded chronically to support device control without external cues. In this study, a clinical-trial participant with ALS used a chronically implanted electrocorticographic (ECoG) BCI to control smart devices over multiple months. Across 18 online closed-loop experiment sessions conducted in a 63-day span, silently mimed speech commands were consistently detected with a median false positive and negative rate of 0 per minute and decoded with a median accuracy of 97.1% (chance: 7.14%). Performance remained stable throughout the chronic recording period, with no significant degradation in detection or decoding accuracy over time. These results demonstrate that silently attempted speech can be reliably decoded from chronically implanted arrays without external timing cues, supporting the potential clinical viability of BCI-supported device control for individuals with ALS.

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Topic: F.05. Brain-Machine Interface

Support: NIH R01 HD111562

Title: Sampling representational plasticity of simple imagined movements across days enables long-term neuroprosthetic control

Authors: *H. YAN[1], N. NATRAJ[2], S. SEKO[4], R. ABIRI[5], R. MIAO[4], Y. GRAHAM[4], E. F. CHANG[3], K. GANGULY[4];
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Abstract: The successful adoption of brain computer interfaces (BCIs) requires long-term stability without excessive calibration. This is especially true for high degree of freedom (hDoF) BCIs, such as a robotic arm and hand, where instability due to the increased complexity might hinder clinical translation. Also, of importance is 'neural drift' i.e., changes in the relationship between activity and behavior over time. Excessive drift can destabilize consolidated BCI control maps. In our recently published study (Natraj et. al., 2025), we sought to overcome these issues. We examined the stability and plasticity of neural representations in two paralyzed participants who were implanted with electrocorticographic (ECoG) arrays over contralateral sensorimotor cortex. We focused on a repertoire of simple well-rehearsed imagined movements, such as finger flexion and tongue protrusion, and examined their representational stability and plasticity when learning to operate a hDoF BCI. We find evidence for both within and across-day plasticity with BCI training. Specifically, within any single daily closed-loop (CL) BCI session - with feedback, there were rapid increases in the pairwise separation between movements. Across-days, pairwise separation during CL control continued to steadily increase. In contrast, the open-loop responses - without feedback - remain stable; this indicated that the observed changes during BCI control were highly contextually specific. We also found evidence for representational "drift"; specifically, there was drift in the centroids of each day's neural distribution. However, this across-day distributional drift and the changes during BCI control were constrained to a stable regime, characterizing a mesoscale meta structure in representations with separable boundaries for the repertoire that generalized across-days. Sampling the plasticity and drift thus allowed long-term (~7 months) plug-and-play neuroprosthetic control - i.e., without further decoder updates - of a robotic arm and hand. Importantly, degradation of performance over extended periods for tasks requiring high precision could be addressed using brief recalibration. Our study shows how ECoG can both track representational statistics and permit long-term neuroprosthetic control.

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Nanosymposium

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Topic: F.05. Brain-Machine Interface

Support: NIH NINDS U01NS123125
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Title: Ten-year safety profile of intracortical microstimulation in the human somatosensory cortex

Authors: *R. A. GAUNT[1], C. M. GREENSPON[5], T. HOBBS[2], C. VERBAARSCHOT[3], A. ALAMRI[6], N. SHELCHKOVA[6], R. LIENKAMPER[4], J. YE[7], D. SATZER[6], G. VALLE[8], L. E. MILLER[9], N. G. HATSOPOULOS[6], J. E. DOWNEY[6], M. BONINGER[10], J. L. COLLINGER[4]; [2]Bioengineering, [3]Rehab Neural Engin. Labs, [1]Univ. of Pittsburgh, Pittsburgh, PA; [4]Univ. of Pittsburgh, Pittsburgh, PA, ; [5]Dept. of Organismal Biol. & Anat., Univ. of Chicago, Chicago, IL; [6]Univ. of Chicago, Chicago, IL, ; [7]Carnegie Mellon Univ., Pittsburgh, PA, ; [8]Chalmers Univ. of Technol., Gothenburg, ; [9]Northwestern Univ., Chicago, IL, ; [10]

Abstract: Intracortical microstimulation (ICMS) of the somatosensory cortex can create artificial tactile percepts in individuals with spinal cord injury (SCI) that can improve control of brain-computer interface controlled prosthetics. Despite this potential, there are limited human safety data, which presents a barrier to continued clinical translation. In an ongoing clinical trial, five participants with cervical SCI have microelectrode arrays implanted into Brodmann's Area 1. This is the longest current study in humans, with a combined 23 implant-years and more than 160 million ICMS pulses.

We retrospectively assessed ICMS-related adverse events, signal quality, and electrode health. Across nearly 2,000 stimulation sessions, 52 stimulation-related adverse events were reported—almost all were categorized as transient "persistent sensations", typically lasting <10 seconds in duration that never painful. Persistent sensations were more frequently observed with multi-electrode stimulation.

Electrode integrity metrics, including signal-to-noise ratio, peak-to-peak voltage, and impedance, declined modestly over time. Importantly, no relationship was observed between cumulative charge and signal degradation. Comparisons between stimulated sensory and unstimulated motor electrodes showed no consistent evidence of ICMS-specific damage. These findings demonstrate that ICMS is safe over long durations, with minimal adverse events and stable electrode health. This addresses a key gap in the human microstimulation safety, supporting the continued investigation of this technique.

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Nanosymposium

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Topic: F.05. Brain-Machine Interface

Support: NIH NINDS 1R01NS130302
NIH R35 NS122333

Title: Multi-electrode ICMS in somatosensory cortex provides reliable tactile perception

Authors: *M. M. ISKAROUS, A. H. ALAMRI, C. M. GREENSPON, J. E. DOWNEY; Organismal Biol. & Anat., Univ. of Chicago, Chicago, IL

Abstract: Intracortical microstimulation (ICMS) delivered in somatosensory cortex (SC) generates tactile percepts that convey contact location and force, ultimately providing brain-controlled robotic prostheses with tactile feedback. Traditional ICMS ties one sensation to one electrode, but recently multi-electrode stimulation has been used to generate stronger, more recognizable sensations. Until now, the relationship between traditional and multi-electrode ICMS regarding perceptual reliability has not been systematically investigated.
We conducted a six-month study to characterize multi-electrode ICMS in two participants (C1 and C2) each implanted with two Utah microelectrode arrays in SC (64 electrodes total). Groups of four electrodes (quartets) were defined at the beginning of the study. The projected fields and detection thresholds of the quartets were tested approximately monthly. For the individual electrodes in the quartets, these attributes have been regularly recorded for C1 and C2 over the last four years and two years, respectively.
For projected field mapping, each electrode produces a charge-balanced square-wave electrical stimulus for 1 s at 100 Hz with a 200 µs cathodic pulse, 100 µs inter-pulse interval, and 400 µs anodic pulse. The amplitude is 60 µA for the cathodic phase and 30 µA for the anodic phase (going forward, the stimulation patterns are described by the cathodic amplitude). A transformed staircase protocol is used to determine detection thresholds with a 2 µA resolution in stimulation amplitude and a safety limit of 100 µA (per electrode). An electrode (or quartet) is considered undetected if no amplitude below the maximum of 100 µA is reliably detected.
Participant C1 has high sensitivity to ICMS and was able to detect stimulation for 1566/1601 single electrode trials and 58/58 quartet trials (2-tailed 2-proportion z-test: $p = 0.18$; Cohen's h: $h = 0.30$). Participant C2 was able to detect stimulation for 386/713 single electrode trials and 144/155 quartet trials (2-tailed 2-proportion z-test: $p = 3e\text{-}19$; Cohen's h: $h = 0.95$). This initial result indicates that for people with less ICMS sensitivity, stimulation with multiple electrodes significantly improves reliability of stimulation detection. Further analysis will be done on this dataset to understand the relationship of the detection thresholds and projected field maps between multi-electrode ICMS groups and ICMS from their constituent electrodes. This project will serve as a foundation to better understand the effect of multi-electrode ICMS for use in sensory feedback applications.

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Nanosymposium

NANO023: Prefrontal Mechanisms of Executive Function and Multitasking in Humans and Animals

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Title: Adaptive information transfer in cortical neurons under volatile task environments

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Abstract: In our daily lives, we often adjust our behavior according to changing situations or demands; the amount of luggage we pack for a trip depends on the variability in weather conditions we expect. Responding appropriately to a changing environment requires using different information to guide behavior in different contexts. To test the hypothesis that flexible interactions between neurons mediate different information flow in different environments, we used a combined approach of electrophysiology and modeling. We trained rhesus monkeys on a two-feature visual discrimination task in which they were required to infer which feature to discriminate based on their history of stimuli, choices, and rewards. They were sequentially presented with two Gabor patches, the second of which differed in both spatial location and spatial frequency. The relevant feature was uncued and could switch between location and frequency with variable volatility, leading them to choose one option out of four. After training, the monkeys successfully reported the feature change they believed to be relevant and generally ignored the other feature. During the task, we made simultaneous population recordings from visual cortical area V1 and parietal cortical area 7a. This task requires balancing two demands: figuring out which task to perform and making a perceptual discrimination. We previously demonstrated that performing this task involves integrating information about visual perception from V1 and belief about the task from 7a[1]. Expanding on this work, we tested the hypothesis that interactions between neurons involved in figuring out which task is relevant (e.g. in area 7a) and in the perceptual discrimination (e.g. in V1) depend on the task volatility and the difficulty of perceptual discrimination. To quantify, we calculated mutual information between V1 and 7a, as well as calculating how much information V1 has about the visual stimulus and how much information 7a has about task belief across different environment volatilities. We found that the way neural populations encode information related to perceptual decision-making and task belief, as well as the way that neurons interact with each other, depends on both volatility and perceptual difficulty. These approaches could have implications in understanding the neural

mechanisms of cognitive flexibility and the decline of this flexibility in patients with neurodegenerative diseases.
References: [1] Xue, Kramer, Cohen, 2022, Neuron.

Disclosures: S.C.L. Durian: None. **C. Xue:** None. **S.E. Palmer:** None. **M.R. Cohen:** None.

Nanosymposium

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Title: Dorsal Anterior Cingulate Cortex (dACC) monitors learnability

Authors: *Y. JIN;
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Abstract: To survive, humans and animals are constantly learning novel predictable relationships to better adapt to the environment. However, such “learnable” patterns are often intermixed with noisy “unlearnable” randomness. It is not known if, when they are presented together, organisms are capable of monitoring the learnability of each, so that more energy can be invested in learnable rules. Here, we exposed two primates to two pictorial sets: a “learnable” set in which the stimuli were implicitly ordered and the correct response was always to choose the higher-rank stimulus, and an “unlearnable” set in which stimuli were unordered and feedback was random regardless of the choice. Interestingly, while monkeys consistently succeed in inferring the learnable order, the behavior patterns under the unlearnable set were significantly polarized to either imposing arbitrary ordering similarly to the L set, or random choices. Neural recordings in the dorsal anterior cingulate cortex (dACC, 24c, N=1072) showed that neurons responded predominantly during choice selection or after feedback delivery. Meanwhile, activities embodied information of multiple informative task variables including learnability, reward value, their interactions as well as the rank of the chosen stimulus, the latter two of which significantly correlated with the strength of the behavioral decoupling between learnable and unlearnable sets. Our results suggest the pivotal role of dACC in monitoring between real patterns as opposed to random reinforcement, which contributes to deeper understanding of the mechanism of learning under uncertainties and superstitious reasoning.

Disclosures: Y. Jin: None.

Nanosymposium

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Location: SDCC Rm 30

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Topic: I.04. Executive Functions

Title: Neural dynamics during the retrieval of sequence working memory

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Abstract: Previous studies found that monkeys exploited orthogonal neural subspaces to store items of different ordinal ranks in sequence working memory (SWM). Nonetheless, it remains unclear how these orthogonal memorized items are retrieved from SWM. In this study, we examined the retrieval process in monkeys in SWM tasks (Chen et al. 2024). We found a chained rotational dynamics over memory subspaces and the readout subspace. Specifically, upon go cue, items spontaneously rotate from lower-ranked to higher-ranked memory subspaces (e.g., from rank 2 to rank 1), and finally into the readout subspace, forming a temporal sequence of items within it. Importantly, we demonstrated that additional temporary subspaces were necessary to mediate the chained rotation, thereby preventing information interference. Thus, these findings offer a clear picture of the SWM retrieval dynamics and highlight the role of temporary subspace as a dynamical motif for robust mental manipulation.

Disclosures: X. Li: None. **J. Li:** None. **J. Chen:** None. **C. Zhang:** None. **Y. Xie:** None. **B. Min:** None. **L. Wang:** None.

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Title: Neural Substrates of a Symbolic Action Grammar in Primate Frontal Cortex

Authors: *L. Y. TIAN[1], K. GARZÓN[3,2], D. HANUSKA[2], Y. LIU[4,6], J. TENENBAUM[7], X.-J. WANG[5], W. FREIWALD[2];
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Abstract: At the core of intelligence is the capacity to solve new problems. In turn, problem-solving has been hypothesized to depend on cognitive operations resembling symbolic grammars, with two core components: discrete units (symbols) and rules for recombining symbols into new composite representations (syntax). Whether and how symbolic grammars are implemented in neuronal substrates remains unknown. Here, we establish a research program to elucidate the neural basis of action grammars. In a drawing task, macaque monkeys learn action grammars, consisting of discrete units of motor behavior (action symbols) and abstract sequencing rules (action syntax). Behavioral tests show that subjects can generalize learned grammars to draw new images. In order to understand how these grammars are implemented in neural representations and dynamics, we recorded activity of neurons across motor, premotor, and prefrontal areas. Here, we report the discovery of an action symbol representation in ventral premotor cortex (PMv, area F5). Specifically, we found that PMv encodes planned stroke primitives, and does so in a manner exhibiting three symbolic properties: abstraction, categorical structure, and recombination. Thus, we have established an experimentally-tractable paradigm to study compositional generalization using action grammars, and have identified a representation of action symbols in PMv. In ongoing work, we are studying how neural processes, in PMv and interconnected areas, enable the systematic composition of action symbols using syntactic rules.

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Topic: I.04. Executive Functions

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Title: Flexible Recombination of Shared Neural Subspaces to Build Compositional Tasks

Authors: *S. TAFAZOLI[1], F. BOUCHACOURT[4], A. ARDALAN[5], N. T. MARKOV[6], M. UCHIMURA[2], M. G. MATTAR[7], N. D. DAW[2], T. BUSCHMAN[3];
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Abstract: Cognition is remarkably flexible; we can perform many different tasks and continually adapt our behavior to changing demands. Artificial neural networks trained to perform multiple tasks will re-use representations and computational components across tasks. By composing tasks from these sub-components, an agent can flexibly switch between tasks and rapidly learn new tasks. Yet, how these subcomponents are recombined in a compositional task in the brain is not known. To address this, we recorded from frontal, parietal, temporal cortices and basal ganglia while monkeys switched between three compositionally-related categorization tasks. In Task S1, the animals categorized a stimulus based on its shape and responded with a saccade to the upper-left or lower-right location. In Task C2, the same stimulus was categorized by its color and the animal indicated their decision with an upper-right or lower-left saccade. In Task C1, the monkeys categorized by color (as in Task C2) but responded with a saccade to the upper-left or lower-right (as in Task S1). Thus, the three tasks could be thought of combining a categorization sub-task (shape or color) and a response sub-task (upper-left/lower-right or upper-right/lower-left).Analysis of neural recordings found that task-relevant information was encoded in the activity of neurons in prefrontal cortex. Specifically, there were ‘subspaces’ within the high-dimensional space of neural activity that represented the shape and color category of the stimulus and the motor response. Consistent with the hypothesis that tasks are compositional, we found the same subspaces were shared across tasks. Task C1 and Task C2 used the same subspace of neural activity to represent the color category of the stimulus, and Task C1 and Task S1 used the same subspace to represent the upper-left/lower-right motor response.These shared subspaces were flexibly recombined to build each task. To visualize the neural dynamics of this recombination, we used Targeted Dimensionality Reduction (TDR) to project neural activity onto dimensions encoding the color category and the motor response along both Axis 1 and Axis. Neural activity during the C1 and C2 tasks initially evolved along the color axis according to the stimulus’ category before transforming to move along the Axis 1 or Axis 2 dimensions for the C1 and C2 tasks, respectively. In this way, each task sequentially engaged shared subspaces, selectively transforming stimulus representations into motor representations in a task-specific manner.In sum, our findings suggest that the brain can flexibly perform compositional tasks by flexibly recombining subtask-relevant neural representations.

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Nanosymposium

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Topic: I.04. Executive Functions

Support: NIH R01MH129492

Title: Neural Geometry Changes During the Learning of Compositional Tasks

Authors: ***Q. HE**[1], S. TAFAZOLI[2], J. E. ROY[3], T. BUSCHMAN[1];
[1]Princeton Univ., Princeton, NJ; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, PA;
[3]Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Humans and other animals are efficient learners, leveraging prior task knowledge to accelerate the acquisition of new tasks. We have previously shown that the brain can flexibly perform multiple tasks by compositionally combining task-relevant, shared neural representations across tasks. This compositionality enhances learning efficiency by enabling neural circuits to be repurposed across contexts. However, this reuse also raises a critical challenge: how does the brain harness existing knowledge to learn new tasks without causing catastrophic interference with previously acquired behaviors? To address this question, we conducted chronic recordings from the prefrontal cortex of rhesus macaques as they learned a novel task (S2) while continuing to perform three previously acquired tasks (S1, C1, and C2). All four tasks required categorizing visual stimuli based on either shape or color and indicating the decision with saccadic eye movements along one of two response axes. The tasks were designed to share compositional structure: S1 and S2 involved shape-based categorization on different axes, while C1 and C2 involved color-based categorization on the same two axes. Monkeys had mastered S1, C1, and C2 before learning S2. We recorded daily from stable populations of neurons throughout the learning of S2. Our analysis of neural population geometry reveals dynamic changes in task and stimulus representations throughout learning. As performance improved, the geometry gradually converged toward a low-dimensional, compositional structure, in which representations of individual tasks were organized according to their shared components. This emerging structure was accompanied by increasing stability of stimulus representations across days, suggesting consolidation of task-relevant neural codes. As training progressed and animals encountered more challenging stimuli, the geometry remained largely compositional but exhibited subtle refinements, indicating an ongoing process of representational adjustment. Notably, the new task (S2) remained embedded within the plane defined by previously learned tasks, reflecting stable reuse of shared neural subspaces alongside flexible adaptation. Together, these findings reveal how population-level neural representations evolve with experience, supporting both compositional encoding and context-specific modifications during learning.

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Title: Representational geometry of context-dependent working memory in the primate prefrontal and parietal cortex

Authors: *P. CHEN, W. DANG, C. CONSTANTINIDIS;
Biomed. Engin., Vanderbilt Univ., Nashville, TN

Abstract: Working memory flexibly routes, stores, and manipulates information across contexts, yet the underlying neural mechanisms in the prefrontal cortex (PFC) and posterior parietal cortex (PPC) remain unclear. We recorded neural activity from the PFC (N=198) and PPC (N=235) of two adult male macaques trained to perform a context-dependent working memory task (Remember 1st- Remember 2nd). Monkeys viewed two spatial locations sequentially with delay periods in between and made a saccade to either the first or second location based on the color of the fixation point (white or blue). Inspired by prior literature, we proposed three cognitive models with the corresponding representational geometry to support this context-dependent working memory: (1) Ordinal: two stimuli stored in separate subspaces determined by sequential order; (2) Pre-allocate: two stimuli stored in separate subspaces determined by the context; and (3) Overwrite: one shared subspace with dynamic gating and overwriting. We computed cross-temporal, cross-order, and cross-task decoding profiles and compared them to model predictions. PFC activity matched the “Overwrite” model most across all profiles ($p<0.01$, with two-sided paired t-test), suggesting dynamic reuse of a common subspace. PPC exhibited similar overwrite-like neural representation ($p<0.01$ for cross-temporal and cross-task; $p>0.05$ for cross-order decoding, with two-sided paired t-test). Intermittent electrical stimulation of the nucleus basalis, a key source of cortical acetylcholine, disrupted this default PFC mechanism, recruiting independent neural populations for different contexts. Finally, we trained recurrent neural networks (RNNs) to perform the same task. Richly trained RNNs which generate low-dimensional and informative representations successfully replicated the “Overwrite” representational geometry observed in neural data. Further evaluation revealed that "Overwrite" RNNs exhibited strong robustness to variations in delay duration. Together, this study reveals that both the PFC and PPC, as well as richly trained RNNs, support context-dependent working memory through a low-dimensional subspace characterized by dynamic gating and overwriting.

Disclosures: P. Chen: None. **W. Dang:** None. **C. Constantinidis:** None.

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Title: Decoding decision dynamics from independent spatial and value workspaces in the primate PFC

Authors: *N. MUNET, J. D. WALLIS;
Neurosci., Univ. of California, Berkeley, Berkeley, CA

Abstract: Deliberative decision making is a dynamic process in which the decision maker identifies the available options, evaluates them with respect to the task objectives, and ultimately selects the higher value option. Physiological evidence suggests that, over the course of a decision, choice options and their relevant features are represented discretely and serially in the prefrontal cortex (PFC). Thus, when two or more options are presented, the PFC switches back-and-forth between the competing options in order to compute a decision between them. Prior work from our lab has revealed this motif of transient state switches in the orbitofrontal cortex (OFC), which alternates between representing the values of the chosen and unchosen options presented with each choice. Given this observation, we aim to determine whether these alternating value states can be better understood as a hallmark of the attentional dynamics involved in visually sampling choice options or as a unique signature of value-based deliberation occurring independently of attention. To test these hypotheses, we performed Neuropixels recordings in the macaque OFC and lateral PFC (LPFC), a region central to attentional control, during a novel experimental paradigm designed to identify and isolate distinct neural signals for attention and valuation at single-trial resolution. We found a double dissociation of task-general latent state subspaces in each region, suggesting functional specialization within the PFC: OFC activity encoded the option values but not spatial information, while LPFC activity primarily encoded the spatial location of each option. By analyzing correlations between the space and value decoder states, we find that the alternating dynamics of these two codes occur independently of each other. Together, our findings provide evidence for two anatomically and functionally independent visuospatial and value systems within the PFC that may enable separate attentional and evaluative processes to occur simultaneously but without interference during choice.

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Support: R01 MH121480

Title: Distinct structured representations with mnemonic chunking strategy in primate lateral prefrontal cortex

Authors: *F.-K. CHIANG, E. RICH;
Ctr. for Neural Sci., NYU, New York, NY

Abstract: Self-organized behaviors often rely on working memory to maintain and update information in mind step-by-step. Although working memory is limited by capacity constraints, cognitive strategies such as mnemonic chunking can be used to mitigate this constraint. Here, we investigated how neural ensembles in primate lateral prefrontal cortex (LPFC) structure information in sequential behaviors guided by chunking strategies. To do this, we trained two monkeys to perform a spatial self-ordered target selection task. Monkeys were trained to saccade to eight identical targets on a screen, one at a time in any order, to collect a one-time reward from each target. This required them to use working memory to update reward-target contingencies and prepare for the next target selection. From target selection patterns, we quantified chunking tendencies with modularity index (MI), which is the strengths of subgroup segregations when targets are assigned to two chunks. Behaviorally, we found that reaction times were longer when transitioning between subgroups than within subgroups, suggesting an abstract chunking boundary can be identified. Four types of chunking patterns were found for the 8 targets: 2 vs. 6, 3 vs. 5, 4 vs. 4, and 5 vs. 3 chunks. Error rates were lower within the same chunk compared to those transitioning between chunks, and negatively correlated with MI, suggesting that stronger chunking improved task performance. Neurons were recorded from four 64-channel Utah arrays implanted along with LPFC, including dorsal principal sulcus and prearcuate gyrus. Using a liner target decoder we found that neural representations of the targets were more commonly confused within the same chunk. We then assessed how neural representation in chunks correlated to MI. We found that chunking significantly enhanced representations of targets within the second chunk, but not the first. Further investigation revealed that targets in the first chunk tended to be selected in a reliable order, and stronger sequencing enhanced the representations of targets within the first chunk. Overall, our results suggest that chunking strategies alter representations in working memory, but this can result in different structured representations in LPFC.

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Title: The neural basis of multidimensional attention control

Authors: *D. GHEZA[1], W. KOOL[2];
[2]Psychological & Brain Sci., [1]Washington Univ. in St Louis, St Louis, MO

Abstract: Humans can manage and switch between multiple, conflicting sources of information. However, models of cognitive control assume that conflict is detected between one task and one source of distraction, and are agnostic on how the brain manages multiple distractors simultaneously. To address this gap, we developed a multi-dimensional task-set interference paradigm, requiring individuals to manage distraction from three independent dimensions. Behavioral and modeling work based on this paradigm highlighted the striking human ability to adjust attention in a distractor-specific fashion, and has generated testable predictions of the neural mechanisms that support multidimensional conflict monitoring. To test them, we decode task representations with a combination of multivariate encoding analyses and representation similarity analyses that aim to integrate EEG and fMRI imaging modalities. In a first EEG study, we have confirmed the selective suppression of multivariate representations of distractors. Trial-by-trial control adaptation regulates the strength of a fast and reactive suppression mechanism, which operates primarily on attentional orienting, and partially on the strength of feature representations. However, this selective suppression highlights the need to scale conflict-control loops to three, and possibly more, simultaneous dimensions, posing a challenge to classic theories of control. With an EEG-fMRI fusion approach, we aim to test the novel hypothesis that neural signals of conflict emerge, and are resolved, from the integration of diverse task variables within the medial prefrontal cortex. Specifically, we hypothesize that the multivariate pattern of activity in this region integrates target- and distractor-related representations, whose coding axes shift as a function of conflict history.

Disclosures: D. Gheza: None. **W. Kool:** None.

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Topic: I.04. Executive Functions

Title: Discovering novel circuit mechanisms in higher cognition through interpretable recurrent neural network training

Authors: *B. MIN[1], Y. ZHANG[2,3];
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Abstract: Training recurrent neural networks (RNNs) has revolutionized the way how systems neuroscientists form hypothesis when studying circuit mechanisms in various problems. However, the trained RNNs oftentimes are difficult to be interpreted, inconsistent with neural data or not necessarily comprising the full set of biological solutions. Here, we developed an interpretable RNN training framework, namely Restricted-RNN, capable of generating interpretable circuit hypothesis through a multilevel proposing-and-testing procedure that seamlessly integrates computational-, collective- and implementational-level descriptions. The validity of Restricted-RNN is demonstrated through the identification of novel circuit mechanisms underlying sequence working memory control and the counter-intuitive firing rate reversal in perceptual decision-making, with the key predictions being confirmed by monkey prefrontal and parietal neurophysiological data. Critically, the interpretable nature of Restricted-RNN endowed us with a unified theory to explain the seemingly disparate phenomena across different tasks with a novel neural control state space, providing an intriguing geometric understanding for the ubiquitous control in cognitive processes.

Disclosures: B. Min: None. **Y. zhang:** None.

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Topic: I.04. Executive Functions

Support: National Institute of Mental Health of the National Institutes of Health Award Number R01MH129641

Title: Multi-task assessment of cognitive control factors in nonhuman primates

Authors: *X. WEN[1], A. NEUMANN[2], L. MALCHIN[3], T. WOMELSDORF[3];
[1]Vanderbilt Brain Inst., Nashville, TN; [3]Dept. of Psychology, [2]Vanderbilt Univ., Nashville, TN

Abstract: Cognitive control is composed of the four cognitive domains Inhibitory-Control, Updating, Working Memory, and Set-Shifting. These domains are partially separable in humans, but more unified in children and other subpopulations. How these cognitive domains separate or unify to a common cognitive control factor in nonhuman primates (NHPs) is unknown. We addressed this question with a multi-task cognitive assay for NHPs that tested all four cognitive control domains. Six monkeys performed four tasks on a touchscreen kiosk over forty experimental sessions: an Antisaccade (AS) task measuring Inhibitory-Control; a delayed match-to-sample (DMTS) task that varied the delay to measure Working Memory (WM), and the

number and perceptual similarity of test objects to measure Inhibitory-Control; a continuous updating task requiring subjects to find the novel object among an increasing array of objects to measure WM-Updating and Inhibitory-Control; and a feature-reward learning task measured Set-Shifting. We found, first, that NHPs showed robust cognitive control costs within and across sessions. Second, we tested for construct validity of the Inhibitory-Control construct and found positive correlations across metrics quantifying inhibitory control in different tasks. For example, better Antisaccade performance was associated with a reduced spatial congruency effect and with a reduced negative impact of target-distractor similarity in the DMTS task. These results suggest that there is a common cognitive ability to inhibit interfering information. Third, we tested whether there is a common cognitive control factor that becomes evident in correlations among performance metrics from the Inhibitory-Control, WM-Updating and Set-Shifting domains. After removing subject-specific variances we found a positive correlation structure. Faster Set-Shifting correlated with better Inhibitory-Control in the AS task and the WM task, and with better WM-Updating in the continuous updating task. These correlations were moderate (r's < 0.5) but were robust against variability between subjects. In summary, our study documents the versatility to reliably track all three major cognitive control factors with a multi-task assay in NHPs. The Inhibitory-Control factor showed construct validity as it was measurable in different tasks and metrics. Most notably, performance metrices indexing Inhibitory-Control, WM-Updating, and Set-shifting were correlated, consistent with a common cognitive control factor in NHPs. Together, this study characterizes the unity and diversity of cognitive control functions in NHPs.

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Title: Connectomic dissection of a circuit for locomotory behavior in Hydra vulgaris

Authors: *C. DUPRE, J. W. LICHTMAN, F. ENGERT;
Harvard Univ., Cambridge, MA

Abstract: Hydra is a useful animal model to study basic behavior, representing the first instances in evolution to have a nervous system. A freshwater polyp mostly found in ponds and lakes, it is radially symmetric with a body essentially made of a cylinder with a head and tentacles at one extremity and a foot at the other extremity. It can move its body column in

various directions in order to explore its surroundings and contract it to hide and escape threats. Using these simple motion primitives Hydra spends most of its time foraging, defined as an exploration of the local environment for food items. The number of components that make foraging behavior is not known, and the temporal structure of this behavior still needs to be elucidated. Hydra is most of the time sessile, i.e. it stays attached with its foot to a substrate. However, it can sometimes move to a different location by somersaulting, which is done by attaching its head to the substrate and detaching its foot in order to reattach it somewhere else.To understand how this feat can be accomplished, we are combining connectomics and behavior analysis to reconstruct the circuitry that generate motion.

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Title: A Complete Brain and Nerve Cord Connectome Reveals Body-Wide Sensorimotor Transformations in Adult Drosophila melanogaster

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Washington, Seattle, WA; [12]The Salk Inst., La Jolla, CA, ; [13]Univ. of Nevada Reno, Reno, NV; [14]Vanderbilt Univ., Nashville, TN; [15]Max Planck Inst. for Biol. Intelligence, Martinsried, Germany; [16]Univ. of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico; [17]Zetta AI LLC, Sherril, NY; [18]Univ. of Edinburgh, Edinburgh, United Kingdom; [19]Japan Advanced Inst. of Sci. and Technol. (JAIST), Nomi, Japan; [20]West Virginia Univ., Morgantown, WV; [21]Allen Inst. for Brain Sci., Seattle, WA; [22]Univ. of Bonn, Bonn, ; [23]Leipzig Univ., Leipzig, Germany; [24]Virginia Tech., Blacksburg, VA; [25]HHMI Janelia, Ashburn, VA

Abstract: Behavior relies on coordinated signaling across the nervous system - from the sensory input to the control of motor output. Connectomes, or wiring diagrams at synaptic resolution, map these pathways by detailing the connections between individual neurons. For the key model organism Drosophila melanogaster, volume electron microscopy has enabled several connectomes that illuminate regional circuit functions. Yet, a complete connectome spanning the entire central nervous system—including the brain, ventral nerve cord, and the neck connective that houses critical ascending and descending neurons—has remained unavailable. Here, we present the Brain And Nerve Cord (BANC): a comprehensive map of approximately 150,000 neurons, complete with reconstructions of nuclei, mitochondria, and dense core vesicles, with synapses annotated by neurotransmitter type. By integrating existing datasets, we assigned cell-type identities across the system and identified novel cell types in the neck connective. To examine how sensory input is transformed into motor output, we developed a circuit analysis framework based on a linear, indirect connectivity metric ('influence') to trace information flow from arbitrary source neurons to targets. Using this metric, we find that descending and ascending neurons form modular, functionally distinct groups that support related behaviors. Focusing on representative circuits, we highlight cooperative interactions between ascending and descending pathways that underlie specific behaviors. These analyses offer only an initial glimpse into the insights enabled by the BANC, which we anticipate will serve as a valuable public resource for dissecting the neural basis of sensorimotor transformation and behavior.

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Topic: J.03. Anatomical Methods

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Title: Carving circuits from whole-brain correlative connectomics in larval zebrafish

Authors: *M. PETKOVA[1], F. ENGERT[2], J. W. LICHTMAN[1];
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Abstract: Tracing the wiring diagram of a neural circuit is a powerful approach for evaluating models of brain function. Connectomics—the mapping of neurons and their connections—presents both technical and conceptual challenges. The technical challenge involves acquiring and condensing vast electron microscopy datasets into connectivity matrices, typically through multi-year, multi-team collaborations. The conceptual challenge lies in coarse-graining these matrices into neural circuits that give rise to neural activity and animal behavior. I will describe both challenges in the context of whole-brain mapping in the larval zebrafish. To bridge structure and function, we integrate electron microscopy with complementary light microscopy in two animals: in one, excitatory and inhibitory neurons are molecularly labeled; in the other, whole-brain functional imaging reveals activity across the nervous system. Together, these approaches allow us to interrogate neural circuit models spanning sensory input to motor output.

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Title: Connectomic Profiling of Synaptic Inputs to Hypothalamic Parenting Microcircuits

Authors: *X. HAN[1], P. H. LI[2], R. SCHALEK[1], Y. WU[1], V. JAIN[2], J. W. LICHTMAN[1], C. G. DULAC[1];
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Abstract: Parenting behavior in mice is regulated by Galanin^{+}/Calcr^{+} neurons in the hypothalamic preoptic area (POA), a population active in mothers, fathers, and parental virgin females, but inactive in infanticidal virgin males. Although these neurons are present in similar numbers across sex and physiological state, they exhibit gene expression differences related to synaptic function, suggesting state-dependent circuit reorganization. To uncover the synaptic basis of this modulation, we applied volumetric correlated light and electron microscopy (vCLEM) to generate a connectomic dataset from a virgin female, enabling detailed reconstruction of presynaptic boutons onto POA Galanin^{+}/Calcr^{+} neurons. Using SegCLR, a self-supervised deep learning framework, we generated high-dimensional embeddings of boutons and performed unsupervised classification based on ultrastructural features and spatial context. This connectomic approach enables unbiased identification of input diversity and structural motifs potentially linked to parenting behavior. Building on prior functional and transcriptomic evidence of dynamic input recruitment, we aim to reveal how structured, compartment-specific synaptic architecture supports behavioral flexibility. Ongoing work is extending this analysis to mother mice to investigate how reproductive experience reshapes input organization, providing insight into how experience and internal state remodel hypothalamic circuits critical for survival behaviors.

Disclosures: **X. Han:** A. Employment/Salary (full or part-time):; Harvard University. **P.H. Li:** A. Employment/Salary (full or part-time):; Google Research. **R. Schalek:** A. Employment/Salary (full or part-time):; Harvard University. **Y. Wu:** A. Employment/Salary (full or part-time):; Harvard University. **V. Jain:** A. Employment/Salary (full or part-time):; Google Research. **J.W. Lichtman:** A. Employment/Salary (full or part-time):; Harvard University. **C.G. Dulac:** A. Employment/Salary (full or part-time):; Harvard University.

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Title: Dissection of a neuronal integrator circuit through correlated light and electron microscopy in the larval zebrafish

Authors: *G. F. SCHUHKNECHT[1], J. BOULANGER-WEILL[2], F. F. KÄMPF[3], M. PETKOVA[4], R. TILLER[4], M. R. STINGL[4], A. HEBLING[5], M. JANUSZEWSKI[6], J. W. LICHTMAN[4], F. ENGERT[4], A. BAHL[3];
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Abstract: Making behavioral decisions based on noisy sensory information in the environment is a critical computational task for the brains of all animals, yet precise circuit implementations at the synaptic level remain poorly understood. Dissecting the underlying neural circuits in vertebrates requires precise knowledge of functional neural properties and the ability to directly correlate neural dynamics with the underlying wiring diagram in the same animal. Here, we combined functional calcium imaging with large-scale electron microscopy (EM) in two separate datasets to uncover the wiring logic of visual evidence accumulation in the larval zebrafish. The first dataset contained only the anterior hindbrain and was functionally imaged while animals performed the optomotor response. This allowed for the connectomic analyses of functionally identified cells in the same animal, which revealed that functional response types could be assigned to corresponding anatomical morphotypes. In parallel, we validated the same morphotypes across many animals by photo-converting functional response types and reconstructing their 3D morphologies using light microscopy. These augmented data were used to train and validate a classifier that then allowed us to identify these functional morphotypes

across animals.This classifier could then be used on the second EM dataset, which had not been functionally imaged, but spanned the whole brain and contained labels for excitatory and inhibitory neurons.Complementary circuit reconstructions across these two datasets revealed that bilateral inhibition, disinhibition, and recurrent connectivity are prominent motifs for sensory evidence accumulation in the anterior hindbrain. We used these data to constrain a computational network model of how integration and decision-making are implemented in the vertebrate brain. Importantly, this model generated a set of new predictions that we could validate independently with new experiments. In summary, our work demonstrates how hypothesis-driven functional connectomics can be used to gain mechanistic insights into how neuronal circuits give rise to computation. Moreover, our classifier provides a community resource for targeted circuit reconstructions across EM resources in the larval zebrafish.

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Presentation Number: NANO024.06

Topic: J.03. Anatomical Methods

Support: Otto Hahn Group by Max Planck Society

Title: Connectomics of mammalian navigation

Authors: *H. SCHMIDT;
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Abstract: Our ability to navigate complex environments has fascinated neuroscientists for centuries. With the discovery of the place-and grid-cell system in rodents and other mammals, the ambition to mechanistically understand the formation of a representation of space has driven modeling and circuit tracing work in the hippocampal-entorhinal system. Still, our understanding of this circuit, in particular the interactions between grid-and place cell circuits, is limited. We are using state-of-the-art 3D EM connectomics techniques to map the relevant navigational circuits, and chose to focus on the smallest terrestrial mammal, the Etruscan shrew. By comparison with rat, mouse (and in the future primate) data, we are starting to explore the systematic properties of the navigational circuitry in mammals, and their evolutionary variability within mammalian species.

Disclosures: H. Schmidt: None.

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Presentation Number: NANO024.07

Topic: J.03. Anatomical Methods

Title: Volumetric reconstruction of molecularly defined interneurons in the mouse visual cortex

Authors: *E. SJOSTEDT[1,3], X. HAN[2], R. SCHALEK[1], J. W. LICHTMAN[1];
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Abstract: Interneurons play a critical role in cortical connectivity, with diverse subtypes distinguished by differences in connectivity, morphology, and gene expression profiles. Common classification approaches include electrophysiological characteristics, spatial localization, molecular identity, and synaptic connectivity. However, integrating these categories in connectomics data remains challenging due to limitations in overlapping data across modalities.To address this, we generated a correlative light and electron microscopy (CLEM) dataset from the mouse visual cortex, incorporating four widely used interneuron markers: Pvalb, Sst, Calb1, and Calb2. Using a 4-plex staining strategy, with one polyclonal antibody (Calb2) and three single-chain variable fragments (targeting Pvalb, Sst, and Calb1), we acquired confocal images followed by serial electron microscopy of the same volume. The EM volume spans all cortical layers, covering 730 µm from the pial surface to the white matter, 600 µm in width, and 96 µm in thickness. The confocal image overlaps most of this region, though it is limited in depth.This multimodal dataset links molecular identity with 3D ultrastructural morphology, providing a unique opportunity to examine structural distinctions among molecularly defined interneuron sub types. The volume contains over 8,000 cells (neurons, glia, and vascular cells) and includes approximately 200 molecularly labeled neurons. Morphological analyses focus on the central region, where high-quality reconstructions allow detailed comparisons of soma shape and volumetric features across different molecularly defined cell types.While spatially constrained and limited to local connectivity, the dataset's primary value lies in the potential of training machine learning models (such as SegCLR embeddings) to identify interneuron types in large-scale volumetric EM datasets based on structural correlates of molecular identity. This dataset represents a foundational step toward enabling molecular annotation of large-scale EM reconstructions through machine learning, with potential for future expansion.

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Title: Multimodal multiplexing with expansion microscopy as a path to molecular connectomics

Authors: S. PARK[1], A. SHERIDAN[1], ***S. TRUCKENBRODT**[3], K. LEEPER[1], J. WINNUBST[1], H. DAMSTRA[1], J. M. MICHALSKA[1], J. LYUDCHIK[2], C. MAGNO[1], S. CHAN[1], A. MEERAN[1], J. AXUP[1], E. S. BOYDEN[4], J. KORNFELD[3], S. RODRIQUES[5], A. PAYNE[1];
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Abstract: The field of brain mapping has long largely relied on morphological information to infer the complex molecular makeup of cells and synaptic connections. Recent advances in expansion microscopy, multimodal multiplexing, optical morphological readouts (LICONN), and neuronal barcoding now present a new path towards integrating multidimensional molecular information. This progress is exemplified in PRISM, a molecular connectomics platform under development at E11 Bio, which integrates the readout of virally delivered protein barcodes, endogenous synaptic and cellular markers, and neuronal morphology. In this talk, I highlight the developments in expansion microscopy and multimodal multiplexing that facilitate the readout of dozens of virally expressed protein epitopes and endogenous molecular targets at synaptic resolution.

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OEAW DOC fellowship 26137

Title: Liconn enables molecularly-informed connectomic reconstruction

Authors: *M. R. TAVAKOLI;
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Abstract: Light microscopy-based (super resolution) technologies offer unprecedented opportunities to study biological systems as complex as the brain across scales, from the centimeter-sized whole organ down to its nanoscopic features. The advantage of fluorescence light microscopy, the molecular specificity and its broad accessibility, enables scientists across the Globe with a wide range of molecular tools to tailor experimental conditions for manipulating and interrogating cellular outputs, enabled by complex subcellular and molecular machineries. Cellular entities of the brain (neuron and glial cells) are physically wired and form circuits, which together with their molecular and functional characteristics enable the brain's information-processing capability. Thus, a comprehensive understanding of the brain requires to study the brain at multiple levels, from the molecular machineries to the synapses, circuits and the behavior. Multi-level investigation of the brain (e.g. molecular, cellular and circuit levels) with light microscopy has been hampered by its limitation to provide structure contrast at the synapse-level. Hence, electron microscopy is the technology of choice to reconstruct the brain circuit, as this technology provides a comprehensive structural contrast and nanoscale resolution. However, linking structural, and functional characteristics of brain's cellular network to the intracellular compartments and their respective molecular machineries is extremely challenging with electron microscopy, because sample preparation and readout are not usually compatible with direct visualization of specific molecules, requiring correlation with light microscopy. In this talk, I will discuss our efforts on developing readily adoptable optical imaging and molecular tools enabling multi-modal investigation of the brain. We have developed the LICONN technology, for light microscopy-based connectomics, which combines the power of hydrogel-based expansion microscopy, optical imaging and automated reconstruction algorithm. Furthermore, I will discuss its application across species, and its future prospects.

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Topic: J.03. Anatomical Methods

Support: Shanahan Foundation Fellowship

Title: Local circuit properties of pyramidal neurons in mouse primary visual cortex

Authors: *S. DORKENWALD[1,2], F. C. COLLMAN[1], S. MIHALAS[1], E. T. SHEA-BROWN[2], D. W. TANK[3], A. A. WANNER[4];
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Abstract: Developmental, genetic programs, and experience-dependent synaptic plasticity shape cortical circuits. Much of the connectivity statistics in the cortex have been explained by global network properties such as cell type identity and spatial arrangement, but these cannot account for higher-order network motifs. Networks of cortical pyramidal cells are thought to consist of 'a skeleton of stronger connections in a sea of weaker ones', but the architecture of these subnetworks is not known yet. Here, we used dense electron microscopy-based neuronal circuit reconstructions of hundreds of pyramidal cells in layer 2, 3, and 4 of the mouse primary visual cortex to explore how the subnetworks of strong and weak connections are organized. We found that subnetworks of strong connections form more reciprocal connections between neuron pairs, and more feedforward assemblies between groups of neurons. The consistency of our findings across layers of cortex and in two connectomic reconstructions suggests a general principle of cortical circuit organization. Such an organization likely plays an important role in circuit computations, and we investigate its functional implications.

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Presentation Number: NANO024.11

Topic: J.03. Anatomical Methods

Title: Pathfinder: a multi-stage AI for near-automated reconstruction of neural circuits

Authors: *M. JANUSZEWSKI[1], T. TEMPLIER[2], K. J. HAYWORTH[3], D. PEALE[4], H. HESS[5];
[1]Google Res., Zurich, Switzerland; [2]HHMI / Janelia Res. Campus, Ashburn, VA, ; [3]Janelia Res. Campus, Ashburn, VA, ; [4]Integral Physics to Engineering, LLC, San Diego, CA, ; [5]HHMI, Janelia Res. Campus, Leesburg, VA.

Abstract: Comprehensive mapping of neural connections is fundamental to neuroscience, but progress is hindered by the immense cost and time required to manually correct errors from automated reconstruction algorithms. This proofreading bottleneck makes connectomic projects on the scale of a full mouse brain economically infeasible with current technology. PATHFINDER is a novel, multi-stage AI system designed to address this challenge by dramatically improving reconstruction accuracy. PATHFINDER uses specialized models (FFN v1.5, SENSE, SHAPE) to process data across progressively larger spatial scales. FFN v1.5

segments the volumetric images of brain tissue. SENSE defines the agglomeration space. Finally, SHAPE guides an efficient combinatorial search for optimal, morphologically plausible reconstructions. We evaluated PATHFINDER on all axons within a 0.7M μm³ volume of mouse cortex imaged with IBEAM-mSEM. The system achieved a normalized expected run length (nERL) of 94.2% for axons, reducing the overall error rate by an order of magnitude compared to previous state-of-the-art methods. This translates to an estimated 84-fold increase in proofreading throughput.By drastically reducing the need for manual correction, PATHFINDER marks a turning point where the cost of proofreading becomes comparable to that of image acquisition. This advance unlocks the potential for both routine analysis of smaller volumes and large-scale brain mapping.

Disclosures: M. Januszewski: A. Employment/Salary (full or part-time):; Google.

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Austrian Academy of Sciences DOC fellowship 26137
European Union's Horizon 2020 research and innovation programme and European Research Council (ERC) grant 101044865 'SecretAutism'

Title: Scalable image analysis for brain reconstruction with light microscopy

Authors: *J. LYUDCHIK[1], M. R. TAVAKOLI[2], M. JANUSZEWSKI[3], J. DANZL[4];
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Abstract: As light microscopy emerges as a viable modality for large-scale connectomic mapping, there is a growing need for scalable image analysis tools that can extract connectivity information from these complex, multichannel datasets. The integration of synapse-level structural data with multiplexed molecular labeling not only reduces the need in manual annotations for deep learning-based analysis but also enriches the amount of information that can be extracted from brain tissue images.In this work, I present image and data analysis pipelines developed for the visualization and reconstruction of brain tissue imaged with light microscopy. I would like to focus particularly on synapse detection and connectivity analysis, and discuss how immunolabeling enables more efficient and informative brain mapping—offering a promising path toward scalable connectomics across species.

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Title: New Imaging Technologies for Multiscale Connectomics

Authors: *A. KUAN[1], S. PHAN[2], S. KAYANI[1], Z. ZHANG[1], L. BENOIT[3], J. LIAO[1], K. KIM[4], M. KIM[5], K. DELGADO[6], X. CHEN[1], M. ELLISMAN[7], W.-C. A. LEE[8], A. PACUREANU[3]; [1]Neurosci., Yale Sch. of Med., New Haven, CT; [2]Natl. Ctr. for Microscopy and Imaging Res., UCSD, La Jolla, CA; [3]ESRF, the European Synchrotron, Grenoble, France; [4]Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA; [5]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [6]Neurobio., Harvard Med. Sch., Boston, MA; [7]UCSD Dept. of Neurosciences, SOLANA BEACH, CA; [8]F.M. Kirby Neurobio. Ctr., BCH / Harvard Med. Sch., Boston, MA

Abstract: A major goal of neuroscience is understanding how neuronal circuits produce cognition. The growing field of connectomics promises to revolutionize this pursuit by providing comprehensive wiring diagrams of the brain. Connectomes of smaller insect nervous systems and isolated microcircuits in mammalian brains have been transformative for the field, but scaling connectomic imaging to brain-wide volumes in mammalian brains remains difficult with current volume electron microscopy (vEM) technologies. Here we present multimodal imaging approach that leverages synchrotron-based X-ray Nano-Holography (XNH) and serial section electron tomography (ssET) to enable multiscale mapping of mammalian brains. Using intermediate voltage electron tomography, we show semi-thin brain sections (>500 nm) can be reconstructed at synapse resolution from a moderate number of projections (<30), and that successive sections can be stitched together to form large contiguous volumes. Imaging >10x thicker samples not only replaces the precarious process of ultra-thin sectioning with routine histological sectioning, but also vastly reduces the number of sections that need to be collected. We also show that non-destructive XNH can resolve individual myelinated axons in dense white matter within thick (~1 mm) tissue samples, enabling rapid mapping of long-range projections between brain regions. By stitching together XNH datasets, we map dense axonal projections across mesoscale regions of cortical white matter in the mouse brain. Moreover, we show that XNH and vEM are compatible on the very same samples, making it possible to efficiently obtain multiscale wiring diagrams characterizing the hierarchical structure of mammalian brains.

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Title: Two neural circuit motifs extracted from volume EM images of the rhinophore ganglion of the gastropod mollusc, Berghia stephanieae

Authors: H. H. SANT[1,2], A. COOK[1], S. J. DEAMICIS[1], K. DHIMAN[1], Y. WU[3], R. SCHALEK[3], J. W. LICHTMAN[3], ***P. S. KATZ**[1];
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Abstract: The small number of large, identifiable neurons of gastropod mollucs, which are accessible for intracellular electrophysiology, have facilitated the elucidation of neural circuits. However, the much larger number of small neurons have been ignored because of the inability to determine their synaptic connectivity. Here we used a volume electron microscopy (vEM) dataset to determine the features of neural circuits in the rhinophore ganglion (*rhg*) of the gastropod, *Berghia stephanieae*. The *rhg* sits at the base of the rhinophore, the main olfactory tentacle, and thus presumably plays a role in olfactory processing. The neurons and circuitry of the *rhg* have been enigmatic because the neurons are smaller (<10 μm diameter) than in other ganglia. Using Volume and Annotation Segmentation Tool (VAST), we reconstructed neurons and axons-of-passage in the *rhg* to uncover two distinct circuit motifs: 1) sensory axon convergence onto projection neurons (PNs) and 2) a peptidergic feedback circuit. The vEM dataset consisted of 4700 sections at 33 nm thickness and 4nm x-y resolution. We traced over 150 axons from the distal rhinophore nerves to the proximal connective leading to the cerebral ganglion (*ceg*) and processes emerging from ~20 somata, which had axons projecting through the connective to the *ceg*. Six of these PNs were reconstructed in 3D. They received convergent input from ~120 afferent axons. This convergent input onto PNs is a hallmark of an olfactory glomerulus seen in insects and vertebrates, but not previously found in molluscs. We also 3D reconstructed efferent axons that projected from the *ceg* to the lateral rhinophore ganglion neuropil (L*rh*n), which was previously shown to contain efferent peptidergic axons but be devoid of afferent axons. We found a PN near the L*rh*n that had an axon that projected back to the connective and also branched into the L*rh*n. In addition, this PN had dendritic branches extending from the soma, which arborized in the L*rh*n. Thus, using vEM, we determined that the

rhg has two distinct neural circuit motifs, one in which PNs receive convergent afferent input and another in which at least one PN receives efferent peptidergic input, but no afferent input. This suggests the rhinophore ganglion does not have a single processing function.

Disclosures: H.H. Sant: None. **A. Cook:** None. **S.J. DeAmicis:** None. **K. Dhiman:** None. **Y. Wu:** None. **R. Schalek:** None. **J.W. Lichtman:** None. **P.S. Katz:** None.

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Topic: B.08. Epilepsy

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Title: Astrocytic FABP7 Regulates Circadian Seizure Susceptibility and Protein Expression Pathways Implicated in Neural Excitability

Authors: A. BERG[1], S. H. TARIQ[1], C. C. FLORES[1], M. LEFTON[1], Y. OWADA[3], C. J. DAVIS[1], T. N. FERRARO[4], J. M. JACOBS[5], Y. LEE[1], W. L. SCHROEDER[2], *J. R. GERSTNER[1];
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Abstract: Seizure susceptibility in epilepsy exhibits notable diurnal variation, suggesting underlying mechanisms involve circadian regulation. Building upon prior work demonstrating that astrocyte-expressed fatty acid binding protein 7 (Fabp7) modulates nocturnal seizure threshold and activity-dependent gene expression, this study explores the molecular mechanisms by which Fabp7 influences seizure activity. Using mass spectrometry-based proteomics, we examined cortical-hippocampal tissue from wild-type (WT) and Fabp7 knockout (KO) mice under maximal electroshock seizure threshold (MEST) and sham conditions. Differentially expressed proteins (DEPs) revealed that in WT mice, seizure induction downregulated mitochondrial proteins involved in oxidative phosphorylation, aligning with neuroprotective mitochondrial adaptations seen in epilepsy. Conversely, Fabp7 KO mice exhibited significant upregulation of proteasomal subunits, particularly components of the 20S core particle, suggesting enhanced proteasomal activity independent of seizure induction and implicating glial lipid signaling pathways in regulating neuronal excitability. Notably, in KO sham mice, elevated proteasomal proteins may contribute to altered dendritic spine dynamics and synaptic plasticity, supporting prior findings linking proteasome regulation to neuronal structure and function in epilepsy. These proteomic alterations correspond with evidence that astrocytic Fabp7 influences lipid metabolism, membrane repair, and oxidative stress responses, potentially coordinating sleep-wake rhythms and seizure vulnerability. Our results underscore Fabp7's multifaceted role

in modulating neural circuit stability through cellular and molecular pathways involving mitochondrial function, proteostasis, and glia-neuron interactions, with implications for understanding circadian influences on epileptogenesis.

Disclosures: A. Berg: None. **S.H. Tariq:** None. **C.C. Flores:** None. **M. Lefton:** None. **Y. Owada:** None. **C.J. Davis:** None. **T.N. Ferraro:** None. **J.M. Jacobs:** None. **Y. Lee:** None. **W.L. Schroeder:** None. **J.R. Gerstner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blood Brain Biotechnology, LLC.

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Topic: B.08. Epilepsy

Title: Neorhythm: a neurocardiac electrophysiology based explainable eeg and ecg system for detection of neonatal seizures

Authors: *R. AMURTHUR;
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Abstract: Neonatal seizures go frequently undetected since an estimated 85% lack obvious clinical signs. This poses significant risk of severe neurological damage or mortality if not promptly diagnosed and treated. Existing technologies are complex and rely on expert interpretation, which is costly and inconsistent. Current neonatal seizure detection algorithms lack basis in seizure physiology, rendering them opaque to Neonatal Intensive Care Unit (NICU) physicians. This undermines clinician confidence and limits adoption in clinical practice. NeoRhythm, a novel seizure detection system is proposed. It is the first AI-driven system to integrate electrocardiogram (ECG) data with electroencephalographic (EEG) signals via a multimodal fusion deep-learning (DL) architecture. We hypothesize that integrating physiologically based multi-modal data within an explainable deep-learning framework yields enhanced seizure detection accuracy and transparent clinical insights. We employed four-step engineering methodology that begins with robust data preprocessing (filtering, segmentation, artifact removal), proceeds to deep-learning model training to address class imbalance, integrates explainable AI (XAI) to provide clinically interpretable justifications, and concludes with clinical protocol that centralizes signal acquisition and interpretability. Experimental results demonstrate that incorporation of ECG into EEG-based detection algorithms improves performance across benchmark models. Fusion architecture yields 94.9% sensitivity and 94.2% specificity, outperforming the current standard of care. Explainability features provide clinicians with transparent insights into physiology-based decision-making, building trust. Neurocardiac correlates provide confirmatory markers of seizure, augmenting deep-learning based models. NeoRhythm builds on existing vital-sign monitors and may assist resource-limited NICUs that

lack on-site neurologists. NeoRhythm has the potential to improve neurological outcomes for newborns globally.

Disclosures: R. Amurthur: A. Employment/Salary (full or part-time):; Synovo Inc..

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Presentation Number: NANO025.03

Topic: B.08. Epilepsy

Support: CURE 241473-01

Title: Mapping and manipulating a model seizure network in vivo

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Abstract: Despite the availability of various antiseizure medications, one in three epilepsy patients experiences drug-resistance. Some patients are subsequently eligible for surgical interventions like tissue resection or neurostimulator therapy. However, even when a seizure onset zone is identified and targeted, these treatments rarely result in complete seizure freedom. A major reason for this is the vast interconnectivity of the brain, where distant regions become recruited to form a “seizure network” that can extend far beyond the focal onset zone. To understand the formation of seizure networks in vivo, we developed an experimental paradigm to characterize excitatory and inhibitory activity across a cortical network in awake mice undergoing seizures. We injected 4-Aminopyridine (4AP), a chemoconvulsant, into a site (S1) in the dorsal neocortex and then performed widefield bilateral imaging of Thy1+ excitatory neurons and PV+ inhibitory neurons with simultaneous intracortical electrophysiology. S1 forms an anatomical and functional network with ipsi- and contra-lateral brain regions, primarily secondary motor cortex (iM2, cM2) and contralateral S1 (cS1). Across mice (N=12), we found that seizures (n=41) initiated in S1 will preferentially (>90%) spread across frontal M2 cortical sites rather than directly to cS1. We then applied microstimulation to this same network, finding that excitatory/inhibitory balance is significantly higher in cM2 compared with cS1 ($p<.001$), providing one mechanism for the observed frontal M2 seizure propagation. We next tested different focal neurostimulation frequencies and durations, finding that long, high frequency neurostimulation at any network node results in slow adaptation of excitatory Thy1+ cells, but little to no adaptation of PV+ inhibitory cells (Thy1 vs PV adaptation index, $p<.001$). Meanwhile, 3 Hz stimulation, the dominant frequency during 4AP seizures, induces robust facilitating activity in both Thy1+ and PV+ cell types. Overall, our data suggest that propagating focal onset seizures will spread through distant brain sites by differentially recruiting excitatory and inhibitory cell activity. Our neurostimulation experiments reveal that this recruitment is dependent on the stimulation frequency and suggest that differential excitatory and inhibitory

cell recruitment could be leveraged to disrupt ongoing seizures with electrical stimulation outside of the seizure onset zone, a possibility that will guide subsequent studies of network-oriented neurostimulation. This work could ultimately provide improvements to neurostimulator therapy in human epilepsy patients.

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NIH T32 DK007646

Title: Differences in Cortical Participation During Absence Seizures Revealed by Silicon Probe Recordings and Ca^{2+}-Imaging

Authors: *S. KILIANSKI[1], E. DULKO[1], S. LASHKERI[1], S. HE[2], A. CARNS[1], M. PIKUS[1], S. M. BACA[1], M. P. BEENHAKKER[1];
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Abstract: Absence epilepsy is characterized by unpredictable seizures that begin abruptly and cause a brief, yet typically complete, lapse in conscious awareness, which can greatly impair daily life. During these seizures, high-amplitude, rhythmic electrographic events called spike-wave discharges (SWDs) can be recorded broadly across the cortex. However, studies indicate that the entire cortex does not participate uniformly in these seizures. To better resolve the spatial variability in cortical participation during SWDs, neural activity across the cortex was recorded using both silicon probe electrode arrays and widefield imaging of cortical fluorescence in Thy1-GCaMP x C3H/HeJ mice. Preliminary results from both the silicon probe recordings and widefield GCaMP imaging suggest that the dorsal cortex is not uniformly engaged during these events. Instead, anterior and central cortical regions, such as the somatosensory and motor cortices, exhibit much greater sensitivity to SWDs than posterior regions like the visual cortex. Silicon probe recordings further reveal that population synchrony—that is, the relative timing of neuronal firing—is greatly enhanced in these more sensitive areas. Moreover, SWD termination appears to coincide with a general increase in neural activity, as observed using both electrophysiological and imaging. These results, along with future analyses, will contribute to a deeper understanding of SWDs and why specific brain regions, such as the somatosensory cortex, are more actively engaged during these events than others.

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Title: Aperiodic Activity at Mesoscale Tracks Homeostatic Network Adaptations and E/I dynamics during seizure in a Chronic Epilepsy Mouse Model

Authors: *G. CHAUHAN[1,2], K. KUMAR[2], D. CHUGH[2], S. GANESH[2], A. RAMAKRISHNAN[2];
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Abstract: Epileptic activity is frequently linked to a higher excitation/inhibition (E/I) ratio, although the underlying mechanisms vary across epilepsy and seizure phenotypes. Quantitative assessment of this balance remains particularly challenging in clinical settings due to the limited feasibility of invasive measurement techniques. This study analyzed both aperiodic components and periodic EEG spectral power from bilateral frontal and temporal EEG recordings in C57BL/6 wild-type mice and chronic epileptic Laforin knockout (Epm2a−/−) mice. Specifically, we demonstrate that the 1/f spectral slope representing the scale-free, non-oscillatory component of neural activity was significantly steeper for epileptic mice, suggesting a compensatory increase in global inhibitory tone. Additionally, shifts in the central frequency of periodic oscillations were observed within the delta (0.5-4 Hz) and beta (16-30 Hz) bands, further implicating network-level adaptations in oscillatory dynamics. Notably, administration of the GABAergic anaesthetic Isoflurane induced a pronounced steepening of the aperiodic slope in wild-type mice, an effect that markedly varied in Epm2a−/− mice, potentially reflecting altered GABAergic signalling in KO animals. Importantly, spectral slope and offset values could reliably distinguish dynamic seizure phases (preictal, ictal, postictal) and characterize seizure severity indicating dynamic E/I ratio. Collectively, our findings challenge the notion that epilepsy is uniformly associated with an increased E/I ratio. Instead, we show that this balance varies across seizure phases (preictal, ictal, postictal) and seizure types (absence vs. tonic-clonic), emphasizing the importance of temporally resolved biomarkers in understanding seizure dynamics.

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Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia)
European Joint Program on Rare Disease Project TREAT-SGS

Title: Convergent excitatory and inhibitory dysfunction drives epileptogenesis in Schinzel-Giedion syndrome

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Abstract: Schinzel-Giedion syndrome (SGS) is a rare neurodevelopmental disorder caused by *de novo* mutations in SET binding protein 1 (SETBP1), leading to pathological protein accumulation. SETBP1 is a multifunctional protein involved in gene regulation and chromatin remodeling. Although the most debilitating symptom in SGS is epilepsy, the mechanisms of seizure generation remain unexplored. How does SETBP1 accumulation affect the developing brain and contribute to seizure susceptibility? To explore this, we designed a Cre-dependent conditional mouse model that enables cell type-specific accumulation of mutant human SETBP1. Whole-brain (Nestin Cre) mutants showed spontaneous seizures and increased susceptibility to kainic acid (5mg/kg, n=20/group), along with dentate gyrus (DG) hypoplasia. Immunofluorescence (IF) analysis across DG development revealed disrupted granule cell (GC) migration and ectopic differentiation, assessed at the molecular level by MERSCOPE spatial transcriptomics. While patch clamp recordings revealed GC hyperexcitability due to abnormal short-term facilitation of EPSCs, single-nucleus RNA-sequencing hinted at synaptic abnormalities. Increased excitatory input and immature spine morphology on GC dendrites were then validated by IF and Golgi staining. Electron microscopy revealed vesicle dispersion in presynaptic terminals, implicating disrupted synaptic dynamics. To confirm the contribution of excitatory neurons, we generated an excitatory-specific mutant (Emx1 Cre), which showed DG hypoplasia and GC hyperexcitability but lacked seizure vulnerability (n=10/group). Although the combination of structural and functional defects could create the perfect seizure-prone *milieu*, this seems not to be sufficient to establish the epileptic phenotype, suggesting a beneficial buffering role of the unaffected interneurons. Optogenetics and IF revealed enhanced interneuron-mediated inhibition, supporting a homeostatic plasticity mechanism. Conversely, inhibitory-specific (Gad2 Cre) mutants showed neither structural defects nor seizure vulnerability (n=10/group), indicating interneuron impairment alone is insufficient to trigger the pathology. Our findings show that SGS-related epilepsy results from a “constructive interference” between excitatory and inhibitory dysfunctions within the hippocampal circuitry.

Impairment of either population alone is instead insufficient to overcome brain resilience against seizure initiation. We hypothesize that this concept may extend to other seizure-associated neurodevelopmental disorders caused by genes not classically linked to epilepsy (like SETBP1).

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Topic: B.08. Epilepsy

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Title: Uncovering the mechanism by which Cannabidiol (CBD) decreases seizure burden in a mouse model of viral induced epilepsy

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Abstract: Affecting 70 million people worldwide, epilepsy is characterized by recurrent seizures that can significantly impact quality of life, with 1/3 being resistant to antiseizure medications. Moreover, patients who survive a viral infection of the central nervous system are 16 times more likely to develop epilepsy. Studies have linked neuroinflammation to seizures, which is characterized by infiltration of inflammatory blood macrophages into the brain, presence of reactive glia, and secretion of proinflammatory cytokines. Understanding how these factors affect neuronal excitation is critical for the development of novel treatments. Cannabidiol (CBD), approved by the FDA and commercialized as Epidiolex, effectively reduces seizure frequency for patients with certain genetic epilepsies. Similarly, in a mouse model of viral-induced epilepsy, CBD treatment was effective in reducing seizure burden and incidence. While studies suggest that CBD may attenuate the secretion of inflammatory cytokines such as IL-6 and TNF-alpha, and decrease glial cell activation, the precise anticonvulsant mechanism of CBD is not entirely understood. We hypothesized that CBD reduces seizure burden by modulating neuroinflammation, particularly by decreasing peripheral immune cell infiltration and inflammatory cytokines within the brain. To test this hypothesis, we used a mouse model of viral-induced epilepsy, in which C57BL/6J mice intracranially infected with Theiler's Murine Encephalomyelitis Virus (TMEV) develop acute seizures from 3 to 8 days post-infection (dpi). Male C56BL/6J mice (n=50) were intracortically infected with TMEV and administered either CBD or a vehicle twice daily via intraperitoneal injection (150 mg/kg). Seizures were scored

2x/day (1 hour post CBD treatment, from 3-7 dpi) using the Modified Racine Scale by a researcher blinded to the treatment group. Flow cytometry was conducted on mouse brains at 7 dpi to determine immune cell infiltration and reactive state. Compared to the vehicle group, CBD-treated mice had a significant reduction in seizure burden, inflammatory peripheral cells infiltrating the brain, and expression of the inflammatory marker MHC-II in microglia and macrophages. We are conducting hippocampal cytokine profiling and immunohistochemistry to further elucidate the anti-seizure mechanism of CBD. Furthermore, we plan to investigate oral administration of CBD, which is more aligned with clinical applications. This research advances our understanding of cannabinoid-based therapies; future studies are needed to further investigate the anti-inflammatory properties of CBD and other cannabinoids.

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Presentation Number: NANO025.08

Topic: B.08. Epilepsy

Title: Positive allosteric modulation of adenosine A_{2A}receptors ameliorates pentylenetetrazol-induced seizures

Authors: A. B. YAKUT[1], K. ROY[1], K. E. VOGT[2], T. SAITOH[1], ***M. LAZARUS**[3,4];
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Abstract: In modern society, daily stress and insufficient sleep increase the risk of neurological disorders, including epilepsy. Adenosine, a neuromodulator with anti-excitatory properties, shows promise in regulating seizure activity through A_{2A} receptors in critical brain regions, such as the nucleus accumbens and striatum. We developed an A_{2A}R positive allosteric modulator (PAM) with no peripheral side effects. According to previous reports from our laboratory, A_{2A}R PAMs can promote slow-wave sleep, indicating a potential protective role against pentylenetetrazol (PTZ)-induced seizures. We investigated the effects of PTZ injection with and without A_{2A}R PAM pretreatment by combining video analysis of seizure events with EEG/EMG recordings to monitor epileptic brain activity. Our analysis focused on the first hour post-injection to capture key phenomena. First, we quantified the number of seizure spikes in the EEG during seizures following PTZ injection. PTZ-induced seizure duration significantly decreased with A_{2A}R PAM pretreatment. We then classified seizure episodes into three categories based on duration: short (≤4 seconds), medium (4-12 seconds), and long (>12 seconds) continuous seizures. Mice pretreated with A_{2A}R PAM exhibited a significant reduction in short and total

seizure episodes. Additionally, the EEG power density significantly decreased in the δ power range in $A_{2A}R$ PAM pretreated mice compared to non-pretreated mice. We also examined postictal depressive episodes (PDEs), which are commonly observed after generalized tonic-clonic seizures (GTCS), which are characterized by continuous high-amplitude EEG waves. We observed reduced occurrence of both PDEs and GTCS in $A_{2A}R$ PAM-pretreated mice (Fig. 1).

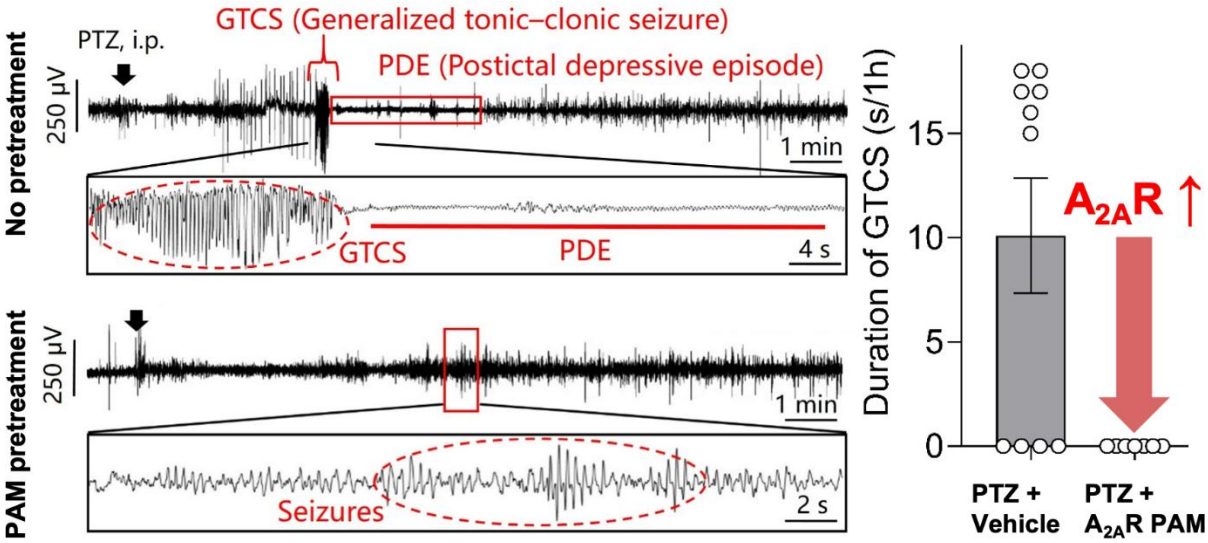


Fig. 1 $A_{2A}R$ PAM suppresses PTZ-induced seizures.

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Topic: B.08. Epilepsy

Title: Kr233: selective kcnq2/3 opener with best-in-class potential for epilepsy

Authors: *Z. TAI, R. WU, L. PENG, N. YAN, S. YAN;
Jiangxi Kvvit Pharmaceut. Co., Ltd., Shanghai, China

Abstract: KR233 is differentiated KCNQ2/3 opener being developed for the treatment of for epilepsy**.** Epilepsy affects approximately 50-65 million people globally. Approximately 30 % of patients with epilepsy resistant to the approved antiseizure medications (ASM). In addition, most of the ASMs associated high withdrawal rate and require careful clinical titration. The KCNQ2/3 (Kv7.2/7.3) channel, a key regulator of the excitatory/inhibitory balance in neurons, has verified

as a therapeutic target for epilepsy treatment but with no drug on market. Ezogabine (retigabine), the first KCNQ opener approved in 2011 for the treatment of focal seizures, was voluntarily withdrawn from the market in 2017 due to chemical issues unrelated to its action on Kv7.2/3. The 2nd generation KCNQ opener XEN-1101 has demonstrated superior efficacy in various preclinical models and also in clinical studies. Despite its benefits, XEN-1101 is associated with notable side effects (e.g. dizziness, balance disorder, dysarthria and gait disturbance), highlighting the need for novel pharmacological therapies that can improve upon its limitations. A key challenge in developing Kv7.2/3 openers is achieving specificity over closely related channels e.g. Kv7.4/7.5. **Here we report KR233, a novel potent KCNQ2/3 opener demonstrating improved subtype selectivity, long-lasting effects, and a superior safety profile compared to XEN-1101.** KR233 shifts the half voltage dependence of activation of Kv7.2/7.3 channels to approximately -49 mV at nanomolar range but with no or minimal effects on Kv7.1 /7.4/7.5 channels at up to 0.2 μM (Better than XEN-1101 which was only 3~4 fold selective for Kv7.2/7.3 over Kv7.4 and Kv7.5 channels), suggesting lower potential for off-target toxicities. In preclinical models, KR233 provided robust protection against seizures in both the pentylenetetrazole (PTZ) and maximal electroshock (MES) -induced seizure models, with a good PK/PD correlation ($EC_{50,plasma}$ <50 ng/ml). Critically, KR233 exhibited no adverse effects on neuronal or motor function at doses up to 60 mg/kg. In contrast, XEN-1101 induced significant impairments at equivalent doses with TD_{50} < 40 mg/kg in the same condition. This highlights the significantly broader safety margin of KR233. In the tox studies, KR233 shows good tolerability (MTD≥1000 mg/kg) and >50-fold therapeutic windows based on a 28-day rat DRF study. Additionally, KR233 exhibited favorable PK profiles across all tested animal species. Overall, these results indicate that KR233 is a promising novel therapeutic candidate for the treatment of epilepsy. IND filling expected in Q3 2025.

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UVA Brain Institute.

Title: Hydroxycarboxylic acid receptor 2 mediates beta-hydroxybutyrate's antiseizure effects

Authors: *S. NADERI;
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Abstract: *The Ketogenic diet (KD), a high-fat, low-carbohydrate regimen, is commonly used to treat drug-resistant seizures.* β-hydroxybutyrate (β-HB), a primary circulating ketone body, is a

key mediator of KD's antiseizure effects. However, its antiseizure mechanism is unknown. Hydrocarboxylic acid receptor 2 (HCAR2) is a Gi-coupled receptor and a receptor for Niacin. β-HB binds to HCAR2 and mediates neuroimmune modulatory effects in neurodegenerative diseases, but its role in epilepsy remains unexplored. We investigated whether HCAR2 mediates the antiseizure effects of β-HB. We used 4- to 8-week-old C57BL/6, HCAR2 knockout ($HCAR2^{-/-}$), wild-type ($HCAR2^{+/+}$), and microglia-deficient mice ($FIRE^{-/-}$). We generated $HCAR2^{-/-}$ using the CRISPR-Cas technique on an S129 mouse background. Two complementary in vivo mouse models—continuous hippocampal stimulation to induce status epilepticus (SE) and kindling—were used to induce seizures. β-HB or saline was administered after SE onset or in fully kindled mice. Whole-cell current-clamp was performed to measure the passive and active membrane properties of dentate gyrus cells (DGCs) in hippocampal slices. The voltage clamp was performed to record synaptic currents. *Calcium imaging was performed using viral delivery to inject a genetically encoded calcium indicator (GCaMP7) into the hippocampus.* The RNAscope in situ hybridization assay was performed to localize HCAR2. Real-time qPCR was used to quantify the HCAR2 expression in the mouse brain. *Systemic β-HB administration reduced the duration of SE and the high-frequency discharges in C57BL/6 mice. β-HB showed no effect on seizures induced by CHS or kindling paradigms in $HCAR2^{-/-}$ mice. However, it diminished both the duration and severity of seizures in SE and in fully kindled $HCAR2^{+/+}$ mice.* β-HB reduced the calcium dynamics in CA1 hippocampal neurons. β-HB hyperpolarized resting membrane potential, raised action potential threshold, and reduced firing frequency of DGCs in C57BL/6 and *$HCAR2^{+/+}$ mice*. Also, β-HB suppressed excitatory synaptic transmission. These effects were nullified in $HCAR2^{-/-}$ mice. RNAscope in situ hybridization assay showed robust HCAR2 expression in the hippocampal DGCs and microglia. HCAR2 was expressed in $HCAR2^{+/+}$ mouse brains but absent in $HCAR2^{-/-}$ mice. The suppressive effect of β-HB was independent of microglia. These findings highlight that HCAR2 mediates β-HB's antiseizure effects. β-HB through neuronal HCAR2 modulates the excitability and synaptic transmission of hippocampal neurons. These effects were observed in mice lacking microglia, underscoring a neuron-intrinsic mechanism for β-HB's action**.**

Disclosures: S. Naderi: None.

Nanosymposium

NANO025: Epilepsy Mechanisms and Interventions

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Time: Monday, November 17, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO025.11

Topic: B.08. Epilepsy

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Title: The fast-dissociating D2 antagonist antipsychotic JNJ-37822681 is a neuronal Kv7 channel opener: potential repurposing for epilepsy treatment

Authors: *F. MICELI[1], L. CAROTENUTO[1], O. KEMINER[2], G. CARLEO[1], A. LEO[3], N. DIRKX[4], M. KAJI[4], S. WECKHUYSEN[4], V. BARRESE[1], N. GUIDA[1], C. OSTACOLO[5], M. TAGLIALATELA[1];
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Abstract: Epilepsy is among the most prevalent chronic neurological disorders, affecting approximately 0.5-1% of the population. Despite the availability of several antiseizure medications (ASMs), about one-third of patients continue to experience pharmacoresistant epilepsy. Extensive research has identified the activation of Kv7 voltage-gated potassium (K^+) channels as a validated mechanism underlying the anticonvulsant effects of novel ASMs. The Kv7 channel family includes five subunits (Kv7.1-Kv7.5), encoded by the KCNQ1-5 genes. Notably, Kv7.2, Kv7.3, and Kv7.5 are predominantly expressed in the nervous system, where they play a key role in modulating neuronal excitability. However, following the market withdrawal of the prototype Kv7 activator, retigabine, no Kv7-targeting treatment is currently available for epilepsy. In the present work we pursued a drug repurposing campaign to identify new Kv7.2/3 channels activators. Using a fluorescence-based high-throughput assay in cells stably expressing Kv7 channels, two repurposing libraries comprising more than 8,000 compounds were screened. Among the hits, JNJ-37822681, a fast-dissociating D2 dopamine receptor antagonist in late-stage clinical development as an antipsychotic, consistently exhibited activity as a neuronal Kv7 channel opener. Electrophysiological whole-cell patch-clamp recordings revealed that JNJ-37822681 had efficacy and potency comparable to those of retigabine in reducing the activation threshold of Kv7.2, Kv7.2/7.3, Kv7.4 and Kv7.5 channels, and no effect on cardiac Kv7.1 channels, when heterologously expressed in CHO cells. In human iPSC-derived cortical glutamatergic neurons, JNJ-37822681 enhanced Kv7-mediated currents, hyperpolarized the resting membrane potential, and reduced spontaneous action potential firing. These effects were blocked by the Kv7 inhibitor XE-991 and were not replicated by the D2 receptor antagonist (-)-sulpiride, confirming that the observed effects of JNJ-37822681 were specifically mediated via Kv7 channel activation. In vivo, JNJ-37822681 significantly reduced the severity and frequency of pentylenetetrazole-induced seizures in C57BL mice and sound-induced seizures in genetically epilepsy-prone DBA/2 mice, with a potency comparable to retigabine. Pretreatment with XE-991 attenuated the antiseizure effects of JNJ-37822681 in both models, further supporting a Kv7-dependent mechanism of action. Given its favorable safety profile in humans and lack of the chemical liabilities associated with retigabine, JNJ-37822681 represents a compelling candidate for further development of novel ASMs.

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Nanosymposium

NANO025: Epilepsy Mechanisms and Interventions

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Presentation Number: NANO025.12

Topic: B.08. Epilepsy

Title: Scn1a gene upregulation mediated by artificial transcription factors as a treatment for Dravet Syndrome

Authors: *S. BAGNASCO[1,2,3], S. G. GIANNELLI[1], M. LUONI[2,1], G. COLASANTE[1], V. BROCCOLI[1,2];
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Abstract: Dravet syndrome (DS) is a severe developmental and epileptic encephalopathy characterized by drug-resistant febrile and afebrile seizures, developmental delay, and behavioral impairments, with 20% of patients dying from Sudden Unexpected Death in Epilepsy (SUDEP). DS is caused by haploinsufficiency of the *SCN1A* gene, which encodes the alpha subunit of the $Na_v1.1$ voltage-gated sodium channel. Current pharmacological treatments are ineffective and new therapies are urgently needed. Since classic gene supplementation methods are unsuitable due to the large *SCN1A* coding sequence, an activating CRISPR/dCas9-based approach has been developed to enhance its expression; however, issues such as the risk of immune response and the necessity to use dual-AAV vectors to deliver it are still present. Here, we exploit Zinc-Finger Proteins (ZFPs) fused to a VP64 transcriptional activator domain, to be used as artificial transcription factors of eukaryotic origin that fit in a single AAV vector. We designed 12 ZFPs targeting *Scn1a* gene promoter and identified two of them able to upregulate *Scn1a* mRNA and $Na_v1.1$ protein levels of about 2 and 1.5-folds respectively, in mouse primary neurons. A subset of ZFPs targeting a regulative region conserved between mouse and human species were tested in human Neural Progenitor Cells (NPCs)-derived neurons, where their ability to upregulate *SCN1A* mRNA level was confirmed. One selected activatory ZFP was V5 tagged and cloned under a constitutive promoter to produce an adeno-associated viral vector (AAV). ZFP-AAV was delivered *in vivo* by intracerebroventricular injection (ICV) in perinatal DS mice. AAV transduction efficiency was assessed by quantification of V5 positive cells over the total NeuN positive, showing that 70% (± 7.5) and 80% (± 2.9) of neurons were transduced in the cortex and hippocampus respectively. DS mice injected with activatory ZFP-AAV displayed an amelioration of SUPED incidence, decreasing from 40% to 20% of mutant injected mice (DS control mice n=13; DS ZFP-AAV injected mice n=13) and a reduced susceptibility to thermal induced seizures, with an increase in the temperature of induction of about 2 C° with respect to control conditions. Additionally, ZFP-AAV-injected DS mice showed an increase of *Scn1a* mRNA of about 3 and 1.5-folds in cortex and hippocampus respectively, and $Na_v1.1$ protein levels (1.2 and 1.4-folds in cortex and hippocampus respectively) compared to DS control mice. Our data suggest the efficacy of activatory ZFPs treatment in boosting *Scn1a* gene transcription both *in vitro* and *in vivo*, together with a good transduction efficiency and an amelioration of DS phenotypic manifestations.

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Nanosymposium

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Topic: B.08. Epilepsy

Support: NIH Grant R35NS132326

Title: The role of T-cells in seizure-induced brain pathology and seizure-associated cognitive impairments

Authors: *D. KLEIDONAS[1,2], Y. WAN[2], L. HARRIS[1], P. PALLEGAR[1,2,3], J. ZHENG[1,2], Y. LIU[1,2], S. ZHAO[1,2,3], L.-J. WU[1,2];
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Abstract: Epilepsy is a neurological condition that is characterized by recurrent seizures which are caused by excessive, hypersynchronous discharge of neurons that leads to transient alterations in behavior. While traditionally considered a neuronal disorder, emerging evidence highlights the neuroimmune axis as a key contributor to seizure-induced pathology. Recent studies have revealed the emergence of T-cells in brain parenchyma following seizures. However, little is known about the functional role of T-cells in the context of epilepsy and their interactions with other brain cells such as microglia, the resident immune cells of the CNS. In addition, the mechanisms through which T-cells migrate to brain parenchyma following seizures remain elusive. To address these questions, we employed the kainic acid (KA) model of seizures on 6-8-week-old mice and multiple experimental approaches such as flow cytometry, immunohistochemistry and behavioral tests. We found an increase in the number of CD4- and CD8-positive cells in the mouse brain 7 days post-seizures. This increase was more prominent in the hippocampal CA3 region, a primary site of KA-induced pathology. Although infiltrated T-cells were found in close proximity to microglia in the CA3, depletion of the latter through a CSF1R inhibitor (PLX3397), did not prevent T-cell recruitment in brain parenchyma. Nevertheless, PLX3397 treatment modified the percentage of activated T-cells in the brain and affected cognitive functions. To directly dissect out the role of T-cells in seizure-induced brain pathology, we used an anti-CD3e antibody to deplete peripheral T-cells. Interestingly, using a CXCR6 knock-in/knock-out mouse line, we observed that CXCR6-CXCL16 axis may participate in the recruitment of T-cells in the brain in response to seizures. Overall, our study provides new insights into the mechanisms that mediate T-cell recruitment in brain parenchyma, their interactions with microglia and their contribution to pathology within the framework of seizures.

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Nanosymposium

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Title: Gaba dysregulation in human and mouse astrocytes is implicated in cortical hyperexcitability

Authors: *V. WAGNER[1], A. KULINICH[2], M. RAIS[3], M. L. CASTRÉN[4], K. A. RAZAK[5], I. M. ETHELL[3];
[2]Div. of Biomed. Sci., [3]Biomed. Sci., [1]Univ. of California, Riverside, Riverside, CA; [4]Medicum, Dept. of Physiol., Helsinki Univ., Helsinki, Finland; [5]Psychology, Univ. California, Riverside, Riverside, CA

Abstract: Fragile X syndrome (FXS) is a leading genetic cause of autism-like symptoms, including sensory hypersensitivity and cortical hyperexcitability, resulting from epigenetic silencing of the Fragile X messenger ribonucleoprotein (Fmr1) gene. Recent observations in humans and Fmr1 knockout (KO) animal models of FXS suggest symptoms are mediated by abnormal GABAergic signaling. As most studies have focused on neuronal mechanisms, the role of astrocytes in mediating defective inhibition in FXS is largely unknown. First, we found that human FXS astrocytes derived from patient-specific induced pluripotent stem cells (iPSCs) show ~7-fold increase in GABA levels compared to their control counterparts using high-performance liquid chromatography (HPLC). Similar to FXS human astrocytes, Fmr1 KO mouse astrocytes showed increased levels of GABA, potentially due to an up-regulation of GABA-synthesizing enzymes GAD65/67 assessed with western blotting and immunostaining. Second, we observed that astrocyte-specific Fmr1 deletion during P14-P28 period reduces inhibitory connections in the cortex, leading to increased locomotor activity and decreased socialization in open field test and social novelty preference test, respectively. Astrocyte-specific Fmr1 conditional KO also had increased resting delta-gamma power coupling and impaired sound-evoked synchronization using EEG recordings. Finally, inhibition of GABA transport in astrocytes normalized cortical responses. Our findings suggest astrocytes play a key role in the development and function of inhibitory circuits, and astrocytes are a valuable target for therapies to relieve FXS-associated phenotypes.

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Nanosymposium

NANO026: Astrocytes in Neuronal Function and Dysfunction

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Time: Monday, November 17, 2025, 1:00 PM - 4:30 PM

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Topic: B.09. Glial Mechanisms

Support: NINDS R01NS116059

Title: Adrenergic signaling regulates the K^+ uptake capacity through modulation of astrocytic calcium-activated small conductance K^+ channel (SK) in the mouse hippocampus.

Authors: *Z. A. LI[1,2], S. TIMSINA[1], X. LIU[1], M. MCNABB[1], X. LI[1], M. ZHOU[1];
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Abstract: The expression of an ohmic, or passive, K^+ conductance enables astrocytes to perform K^+ uptake and release with equal efficiency, a process known as K^+ spatial buffering. However, the long-standing "passive" view implies a static nature of astrocytic K^+ conductance. This study identifies SK2, a calcium-activated small conductance K^+ channel, as highly expressed at the mRNA level in mouse hippocampal astrocytes. SK2 current, isolated using the selective inhibitor apamin (300 nM), increases with development and reaches a mature level by postnatal day 18. SK2 displays weak inward rectification, suggesting a role in both K^+ uptake and release. The open probability of SK2 is regulated entirely by intracellular Ca^{2+} ($[Ca^{2+}]_i$). Indeed, activation (CZH, 100 μM) or inhibition (NPH, 100 μM) of the astrocytic Gq-coupled α1-adrenergic receptor (α1-AR), respectively, enhanced or reduced passive K^+ conductance. This α1-AR-induced potentiation was fully blocked by apamin, confirming SK2 as the underlying dynamic regulator of passive K^+ conductance. To test whether SK2 confers a dynamic capacity for K^+ uptake, high K^+ (10 mM) was puff-applied before and after α1-AR activation. Activation of α1-AR markedly enhanced SK2 activity and significantly increased astrocytic K^+ uptake capacity. These findings identify SK2 as a long-sought K^+ channel contributing to astrocyte passive K^+ conductance. Furthermore, our results demonstrate that astrocyte K^+ conductance is dynamically regulated through SK2-adrenergic signaling interactions, providing a previously unrecognized mechanism for homeostatic K^+ regulation in the brain.

Disclosures: Z.A. Li: None. **S. Timsina:** None. **X. Liu:** None. **M. McNabb:** None. **X. Li:** None. **M. Zhou:** None.

Nanosymposium

NANO026: Astrocytes in Neuronal Function and Dysfunction

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Presentation Number: NANO026.03

Topic: B.09. Glial Mechanisms

Support: Foundation in Republic of Korea (2021R1A2C3005704)
Institute for Basic Science (IBS- R025-A1) (to W.-S.C.)

Title: Elucidating the role of astrocyte-mediated synapse phagocytosis during systems consolidation during sleep

Authors: *C. SHIN[1], W.-S. CHUNG[2];
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Abstract: Episodic memories are initially encoded in the hippocampus and subsequently transferred to the medial prefrontal cortex (mPFC), a process known as systems consolidation. During systems consolidation, synapses in the mPFC undergo robust formation and elimination, facilitating the memory transfer to mPFC circuits. Despite the pivotal role of synapse remodeling in the mPFC during systems consolidation, the mechanisms and functional importance of this process remain largely unknown. Here, we demonstrate a crucial role of astrocytes in the mPFC in precise memory transfer through the elimination of specific synapses. Astrocytes actively phagocytose excitatory post-synapses of mPFC neurons within three to six days following memory encoding via MEGF10, a phagocytic receptor of astrocytes. Moreover, astrocytes in the mPFC preferentially phagocytose post-synapses on engram cells, enabling synapse potentiation during systems consolidation. Among the pre-synaptic inputs to the mPFC, astrocytes selectively eliminate pre-synaptic terminals from the retrosplenial cortex. This process is required for precise memory transfer, as mPFC-specific astrocytic *Megf10* knockout mice showed impaired memory discrimination at a remote time point. Remarkably, we found that the activity of hippocampal engram cells during sleep, but not during wakefulness, is essential for astrocytic phagocytosis in the mPFC and behavior. By taking advantage of chronic sleep wave recordings, we found that theta activity during rapid eye movement (REM) sleep in the hippocampus is transiently increased, showing a similar trend of astrocytic phagocytosis following memory encoding. Taken together, this study reveals a novel phagocytic role of astrocytes in long-term memory and provides evidence that synaptic plasticity mediated by astrocytic phagocytosis is indispensable for sleep-dependent systems consolidation.

Disclosures: C. Shin: None. **W. Chung:** None.

Nanosymposium

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Topic: B.09. Glial Mechanisms

Support: Stanford MCHRI postdoctoral fellowship
Bill and Melinda Gates Foundation

Title: Circadian Clock Modulates Astrocyte Elimination of Synapses via MEGF10

Authors: *L. LI[1], C. SHIN[4], J. CHOI[2], A. VOSS[5], E. GIBSON[3], S. A. SLOAN[6], W.-S. CHUNG[4], A. M. PASCA[2];
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Abstract: The circadian clock homeostasis is achieved through delicate clock gene/protein feedback loop, which regulates various cellular functions in a cell-type specific manner. Astrocytes emerge as key cell type maintaining appropriate circuit function in the brain through eliminating synapses. It remains unknown whether the circadian clock in astrocytes play a role in modulating their function of synapse elimination and circuit function. Here we report that cellular clock in astrocytes fine-tunes their elimination of synapses in both human and mouse species and disrupting cellular clock causes cognitive function deficits. Our studies started from examining the effects of hypoxia on human astrocytes derived from long-term cultured human cortical organoids (hCOs). We first observed decreased synapse elimination in both human and mouse astrocytes exposed to hypoxia and dysregulated circadian rhythm in hypoxic hCOs at molecular and functional levels. Unexpectedly, the synapse elimination defect caused by hypoxia can be rescued by alleviating clock gene dysregulation in human astrocytes. To assess whether circadian clock directly modulates astrocyte phagocytosing synapses, we next demonstrated that manipulating key clock genes, *REV-ERBa* and *PER1*, directly affects the levels of synapse elimination by human astrocytes. We further elucidated that the key phagocytosis receptor MEGF10 mediates the regulatory axis of cellular clock and synapse elimination function in human astrocytes. To substantiate these findings, we leveraged the previously developed *in vivo* fluorescent phagocytosis reporters in mouse hippocampus and demonstrated that align with human astrocyte findings, disrupting clock gene *Rev-erbα* specifically in mouse hippocampal astrocytes reduces their elimination of excitatory synapses along with MEGF10 expression decrease. Importantly, these mice show declined learning and memory functions. Together, our findings provide strong evidence for the conserved regulatory role of intrinsic cellular clock in astrocyte eliminating synapses and circuit homeostasis, and highlight the power of integrating stem-cell derived cellular model with *in vivo* model to systematically uncover the role of circadian clock in brain function at molecular, cellular and behavioral levels.

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Nanosymposium

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Topic: B.09. Glial Mechanisms

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Wings for Life

Title: Transcriptional plasticity of wound repair astrocytes

Authors: *M. V. SOFRONIEW;
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Abstract: Astrocytes contribute to the pathophysiology of all neurological and neuropsychiatric disorders. Disorder-associated astrocyte changes can be primary or secondary, can result in gain or loss of functions, and can be beneficial or detrimental to disorder outcome. Astrocytes are highly adaptable cells that can exhibit substantial context specific changes that influence their interactions with multiple other cell types including neurons, other glia, vascular cells, stromal cells and immune cells; and disorder-associated astrocyte changes can in turn modulate the functions of those cells. Astrocyte changes can vary markedly in different disorders. One pronounced type of astrocyte change is the formation of 'wound repair astrocytes' in response to CNS insults that cause overt loss of tissue due to stroke, trauma, infection or autoimmune disease. In such situations, local astrocytes dedifferentiate, proliferate and form borders around CNS lesions. These wound repair astrocytes interact with stromal and immune cells in lesion cores and form dense neuroprotective borders that isolate inflammatory cells from immediately adjacent functioning neural parenchyma. The generation of proliferative wound repair astrocytes from local G0 astrocytes involves pronounced transcriptional plasticity over a prolonged subacute period of up to 28 days in mice (PMID 38907165). Transcriptional analyses reveal similarities and differences with other border forming astrocytes along meninges. Border formation by astrocytes occurs via interactions with multiple other cell types. Single cell spatial transcriptional analyses are revealing molecular signalling candidates involved in mediating such interactions. Lastly, transcriptional and other analyses are revealing that astrocyte plasticity in disorder contexts can lead to unexpected gene expressions not encountered in healthy astrocytes, in some cases challenging what have traditionally been regarded as "cell-type specific" marker genes for certain cell types based on analyses of healthy tissue or in vitro data.

Disclosures: M.V. Sofroniew: None.

Nanosymposium

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Topic: B.09. Glial Mechanisms

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Title: Distributed Dynamic Changes of Cortical Astrocytes During Motor Learning

Authors: E. KANG[1], T. MA[1], Z. KOU[1], ***X. YU**[2];
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Abstract: The ability to acquire new knowledge and skills and retrieve information is a fundamental feature of the brain that becomes deranged in numerous neurological and psychiatric disorders. Understanding the principles of learning and memory has thus been one of the critical questions in neuroscience. Compared with explicit (declarative) memory for facts and events, less is known about the mechanisms of implicit (procedural) memory for motor skills. Previous studies demonstrated that substantial remodeling of synaptic connections in the mouse primary motor cortex (M1) occurred during motor skill learning, providing a structural basis for enduring motor memory. However, the neurobiological substances modulating this process remain incompletely defined. As abundant non-neuronal cells, astrocytes tile the entire brain and form close contacts with neurons and other glial cells. Increasing evidence has supported the significance of astrocyte calcium signaling in regulating neural circuit function and various aspects of behaviors. In this study, we examined the temporal and spatial dynamics of astrocyte calcium signals in the M1 at different stages of motor skill learning with repetitive two-photon *in vivo* imaging. Using machine learning-based analysis, we further found that astrocyte calcium signals encode information predictive of improved motor performance. These findings suggest that astrocytes play an active role in motor learning and may contribute to the information storage for long-lasting motor memories.

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Nanosymposium

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Topic: B.09. Glial Mechanisms

Support: NIH Grant NS121137
Klingenstein-Simons Fellowship Award in Neuroscience

Title: Developmental plasticity is restricted in a highly regenerative motor circuit

Authors: H. JETTER[1], J. P. BRANDT[2], ***S. D. ACKERMAN**[3];
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Abstract: During development, neural circuits undergo brief windows of heightened activity-dependent structural refinement (*e.g.* plasticity) to establish long-term circuit structure and function. Recent work from our lab and others indicate that these developmental windows, or critical periods, are tightly regulated by peri-synaptic glial cells called astrocytes in both fly and mouse. Here, we extend these studies into zebrafish, a system capable of complete recovery following spinal cord transection in juvenile and adult stages, to ask whether plasticity is developmentally restricted in a highly regenerative model system. Coupling optogenetics with *in vivo* imaging, we determined that acute silencing of zebrafish motor neurons at 3 days post-fertilization (dpf) resulted in dramatic dynamicity of motor dendrites and inputs, along with experience-dependent dendrite growth. Silencing at 4 dpf and beyond had no effect on motor dendrite structure. Interestingly, loss of plasticity correlated with morphological and functional maturation of neighboring astrocytes and oligodendrocytes (the myelinating glia of the central nervous system), suggesting that glia may similarly suppress motor plasticity in the zebrafish spinal cord. Using CRISPR/Cas9 screening and stable mutant lines, our work aims to address how distinct glial cell types, alone and in concert, flexibly suppress plasticity in a highly regenerative motor circuit.

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Title: Astrocytic Ca^{2+} signal related to stress-coping behavior in the ventral hippocampus

Authors: *J. YASHIMA[1,2], K. KASEDA[3], J. NAGAI[3], E. SHIGETOMI[1,2], S. KOIZUMI[1,2]; [1]Yamanashi GLIA Center, Interdisciplinary Grad. Sch. of Medicine, Univ. of Yamanashi, Chuo city / Yamanashi prefecture, Japan; [2]Dept Neuropharmacol, Interdisciplinary Grad Sch. Med, Univ. Yamanashi, Yamanashi, Japan; [3]Lab. for Glia-Neuron Circuit Dynamics, RIKEN Ctr. for Brain Sci., Wako, Saitama, Japan

Abstract: When confronted with a threat or danger, animals exhibit characteristic stress-coping behaviors. Stress-coping behaviors are closely related to health and overall fitness of animals.

Therefore, understanding the brain functions and mechanisms underlying stress-coping behaviors contributes to a deeper understanding of stress-related disorders. Acute stress-coping behaviors are typically classified into two types: active coping, involving struggle and resistance, and passive coping, characterized by immobility and depressive-like responses. In mice, the tail suspension test (TST) is often used to evaluate such behavioral responses to acute stress. Recent findings have revealed that astrocytes actively participate in information processing and behavioral regulation through interactions with neurons in stress-related behaviors. The ventral hippocampus (vHPC) is involved in emotion and stress-related behaviors, and previous studies suggest that astrocytes in the vHPC are associated with anxiety-like and stress-coping behaviors. In this study, we aimed to clarify how astrocytic Ca^{2+} signal in the vHPC of male mice are involved in stress-coping behaviors during the TST. To examine how manipulation of astrocytic Ca^{2+} signal affects behavior, we conducted the TST while selectively activating astrocytes using chemogenetic stimulation (Gq-DREADD). The TST was conducted twice, with a one-week interval between the first and second tests. In the first test, no clear behavioral changes were observed by Gq-DREADD activation. In the second test, immobility time increased significantly in the control group, but this increase was inhibited following four times repeated Gq-DREADD activation, suggesting that astrocytic Ca^{2+} signals may regulate behavioral patterns depending on the timing and frequency of stimulation. To reveal astrocytic Ca^{2+} signals in the vHPC during the TST, we conducted fiber photometry of Ca^{2+} signals in astrocytes expressing GCaMP6f. The preliminary data indicate that astrocytic Ca^{2+} signals markedly increased at the beginning of the behavioral shift from passive to active coping, suggesting that astrocytic Ca^{2+} signals are involved in behavior change. These results indicate that astrocytic Ca^{2+} signal in the vHPC is associated with changes in stress-coping behavior and may modulate such behaviors in a causative manner. Future studies will focus on examining the mechanisms underlying astrocytic Ca^{2+} signals and their impact on local neuronal activity.

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Nanosymposium

NANO026: Astrocytes in Neuronal Function and Dysfunction

Location: SDCC Rm 25A

Time: Monday, November 17, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO026.09

Topic: B.09. Glial Mechanisms

Title: Sex-specific diurnal changes in astrocytes within the mouse substantia nigra pars compacta and its implications for Parkinson's disease

Authors: *D. DAS[1], C. RODRIGUEZ[1], K. LINARES[1], E. BANCROFT[1], R. SRINIVASAN[2]; [1]Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Univ. Hlth. Sci. Center; Texas A&M Inst. for Neurosci. (TAMIN), Bryan, TX

Abstract: Astrocytes are key regulators of neuronal function, influencing neurotransmitter reuptake, calcium signaling, and the release of ATP and cytokines. These processes depend on brain region-specific astrocyte-neuron interactions. We previously showed that in the mouse substantia nigra pars compacta (SNc), S100B labeled astrocytic processes completely envelope the somata of tyrosine hydroxylase (TH) expressing dopaminergic (DA) neurons. In this study, we sought to assess if this unique morphological relationship between astrocytic processes and DA somata in the SNc changes in a diurnal and sex-dependent fashion. 3-4-month-old male and female C57BL/6 mice, exposed to a 12:12 h light-dark cycle, were transcardially perfused at Zeitgeber Time (ZT) 3 and ZT 15. We discovered that when compared to the ZT 3 timepoint, at ZT 15, wrapping of S100B-containing astrocytic processes around SNc DA somata was significantly decreased by ~50 % only in male and not in female mice, and this corresponded with time-dependent changes in S100B astrocytic process density only in male mice. Interestingly, in male mice, we found a correlation between diurnal changes in SNc astrocyte processes with alterations in acetylcholine-evoked DA release at axonal terminals within the dorsolateral striatum (DLS). To elucidate the molecular mechanisms underlying these diurnal structural changes in astrocytes, we focused on the 5-hydroxytryptamine (5-HT)-6 Receptors (5-HT6Rs). We found a ~ 50% higher number of 5-HT6Rs in astrocytes from male mice at ZT 15 than ZT 3. In mouse primary midbrain neuron-astrocyte co-cultures expressing 5-HT6Rs, 5-HT administration changed actin dynamics *in vitro*, which could be inhibited by pre-treatment with the selective 5-HT6R antagonist SB399885. Intraperitoneal injection of SB 399885 12 hours prior to perfusions led to a significant decrease in the extent of astrocytic wrapping and density of astrocytic processes in the field of view at ZT 3 and altered patterns of mean intensity of TH between the two time points compared to vehicle-injected male mice. Together, these data suggest that diurnal changes in astrocyte process density proceed via 5HT6 receptors expressed in SNc astrocytes and may affect dopamine release in the DLS. Taken together, our results have important implications for understanding diurnal and sex-specific differences during the pathogenesis of Parkinson's disease.

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Nanosymposium

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Topic: B.09. Glial Mechanisms

Support: NIH R01NS133047
NS133047

Title: Linking circadian rhythms and synaptic regulation: the role of astrocytic calcium signaling

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Abstract: Astrocytes are fundamental for normal synaptic function, which they modulate through a combination of physical contact and the release of secreted factors such as proteins and gliotransmitters. A major driver of these processes is the release of stored calcium from the endoplasmic reticulum (ER) in response to crosstalk with neurons and other glial cells. Astrocytic calcium fluctuations show circadian rhythmicity, and astrocytes express core clock genes, however their role in rhythmic changes observed in cortical synapses, including alterations in synaptic protein expression, spine density, and excitation/inhibition balance are unknown. Here we investigate the role of astrocytic store-released calcium signaling in synaptic rhythmicity in the mouse visual cortex (VC), which integrates visual input and modulates outputs that feed forward into the circadian system. Leveraging genetic ablation of inositol 1,4,5-trisphosphate receptor 2 (IP_3R2), the ER target of IP_3 in astrocytes, we identify a developmental delay affecting excitatory, but not inhibitory synapses of the VC. Specifically, we observe a deficiency in presynaptic vesicular glutamate transporter protein (VGluT) levels in IP_3R2 KO mice. We show that these disruptions correspond with functional deficits, revealing attenuated immediate early gene activation across multiple visual circuit brain regions, as well as reduced defensive behaviors in response to visually evoked stimuli, and disrupted circadian regulation of free-running motor behavior. To understand the astrocytic factors underlying these changes we analyzed our previously published single nucleus RNA sequencing data and identified differentially expressed genes implied in circadian processes in IP_3R2 KO mice, suggesting a link between molecular clock function, astrocytic calcium activity, and synaptic rhythmicity. Further, through expression of membrane tethered astrocytic eGFP, we provide evidence that astrocyte morphogenesis, which is sensitive to circadian regulation, is disrupted in mice lacking IP_3R2. Collectively, our findings suggest an important role for astrocytic store-released calcium signaling in the modulation of cortical synapse rhythmicity and function. Building on these results, ongoing studies focus on how calcium mediated astrocytic functions vary rhythmically across light/dark transitions.

Disclosures: G. Imrie: None. **I. Farhy-Tselnicker:** None.

Nanosymposium

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Time: Monday, November 17, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO026.11

Topic: B.09. Glial Mechanisms

Title: Maternal immune activation and adolescent cannabis converge on NF-κB-COX-2 signaling in striatal astrocytes to induce hyperdopaminergic behaviors

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Abstract: Chronic use of cannabis during adolescence leads to long-term negative behavioral and health consequences in vulnerable individuals. Genetic mutations relevant to adult psychopathology exacerbate the adverse effects of adolescent cannabis by promoting astrocytic neuroinflammation; similarly, environmental adversities such as maternal immune activation (MIA) can trigger these same pathways. We hypothesized that convergence of MIA and adolescent cannabis on astrocytic neuroinflammatory signaling will produce lasting neurochemical and behavioral abnormalities in adult mice. MIA was induced by a viral mimetic, polyriboinosinic-polyribocytidilic acid [Poly(I:C), 5 mg/kg; IP] to dams (CD1 mice, GD 12.5). After weaning, male and female offspring received delta-9-tetrahydrocannabinol (THC; 8 mg/kg; SC) or vehicle during adolescence (PND 30-51). Three weeks after THC, mice were tested in a series of behavioral tests. A separate cohort was used for *in vivo* brain microdialysis to assess extracellular levels of dopamine (DA) and glutamate (GLU) in the dorsal striatum (DS). *Ex vivo* electrophysiological recordings were performed to assess gliotransmission and GLU synaptic transmission in DS. A separate cohort of mice were sacrificed three weeks after THC treatment for RNAseq of isolated astrocytes. The behavioral tests included 12-18 mice per group, the neurochemical, electrophysiological, and RNAseq experiments included 5 mice per group. MIA × THC interaction synergistically increased exploratory behaviors in the hole board test, open field, elevated plus maze, and elevated amphetamine-induced (2 mg/kg, IP) hyperactivity in mice. MIA × THC interaction increased extracellular levels of DA and GLU in DS. MIA × THC interaction increased the frequency of slow inward currents, suggesting increased astrocytic, but not neuronal, GLU release. Transcriptomic profiling of DS astrocytes showed significant upregulation of dozens of genes related to the immune and inflammatory pathways. Single sample gene set variation analysis identified the NF-κB signaling pathway as highly enriched in astrocytes derived from MIA × THC mice. Consistent with this, immunostaining revealed a significant and synergistic increase in COX-2 expression in DS astrocytes of MIA × THC mice. The hyperdopaminergic behavior was prevented in MIA × THC mice by simultaneous adolescent treatment with the COX-2 inhibitor, NS398. Our data demonstrate that MIA can exaggerate cannabis-produced behavioral impairments via convergent inflammatory signaling in astrocytes, suggesting possible targets for preventing adverse effects of cannabis within susceptible individuals.

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Nanosymposium

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Topic: B.09. Glial Mechanisms

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Belfer Neurodegeneration Consortium

Title: Epigenomic regulation of astrocyte responses to acute inflammation

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Abstract: Astrocyte reactivity is a hallmark of nearly all diseases and injuries of the central nervous system. While classically recognized by morphological changes, recent studies have identified transcriptomic remodeling as a core feature of reactive astrocytes. Single-cell RNA sequencing studies have further demonstrated that astrocyte reactivity is defined not by a single homogenous transcriptomic state, but rather consists of a collection or continuum of many disparate sub-states - a product of stimulus-specific, spatial, and temporal variables. Understanding the effects of astrocyte reactivity in various neurological disorders will require disentangling the molecular mechanisms behind these varied states; however, the gene regulatory mechanisms driving reactive transcriptomic changes remain incompletely defined. Using paired single-nucleus RNA and assay for transposase-accessible chromatin (ATAC) sequencing, we profiled the transcriptomes and epigenomes of over 150,000 astrocytes across the adult mouse brain, both in healthy animals and at multiple time points following an acute inflammatory insult (intraperitoneal lipopolysaccharide injection). Pairing these data with spatial transcriptomics, we find that the predominant molecular diversity present in astrocytes in the healthy brain corresponds to broad anatomical regions. We further identify that the reactive gene expression changes induced in the LPS model of neuroinflammation are broadly consistent across these astrocyte subtypes, with select temporal and regional differences. Notably, we observe complete recovery from reactivity, with no lasting transcriptomic or chromatin accessibility changes two weeks following the inflammatory challenge. Lastly, we apply deep learning DNA sequence models of chromatin accessibility to identify how transcription factors dynamically regulate reactive gene expression changes across the onset of neuroinflammation. This work enhances our understanding of the gene regulatory mechanisms underlying astrocyte reactivity, which may provide new avenues for therapeutic targeting across neurological diseases.

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Nanosymposium

NANO026: Astrocytes in Neuronal Function and Dysfunction

Location: SDCC Rm 25A

Time: Monday, November 17, 2025, 1:00 PM - 4:30 PM

Presentation Number: NANO026.13

Topic: B.09. Glial Mechanisms

Title: Disruption of calcium signals in the mitochondria of astrocytic endfeet within the dorsolateral striatum of mice alters the blood brain barrier

Authors: *G. M. HALL[1], D. AYALA[3], Z. KHAN[5], L. LOPEZ VILLAGRAN[5], A. PAULSON[5], N. NOUNOU[5], S. BAKE[4], R. SRINIVASAN[2];
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Abstract: Calcium signals in astrocytes have emerged as a primary mechanism by which these cells communicate with neurons. Apart from their well-established role in modulating neuronal function and behavior, astrocytes are coupled to the blood brain barrier (BBB) via endfoot processes. However, we do not fully understand the relationship between astrocytic endfoot calcium signals and the BBB. In this study, we assessed the role that astrocytic mitochondrial calcium events play in maintaining BBB integrity. To accomplish this, we specifically altered calcium signals in astrocytic mitochondria within the dorsolateral striatum (DLS) of male and female C57Bl6J mice by using a novel AAV, called Mito-NCS1, which expresses the neuronal calcium buffer, NCS1 only in the mitochondria of astrocytes. Another AAV, called Mito-GCaMP6f was used to visualize calcium events in astrocytic mitochondria. Both AAVs were stereotaxically co-injected into the DLS of mice and astrocytic mitochondrial calcium events were characterized. We found that when compared to control Mito-GCaMP6f injected mice, there were no differences in frequency or amplitude in Mito-NCS1 expressing DLS astrocytes. However, there was a significant decrease in duration of astrocytic mitochondrial calcium events in the DLS following expression of AAV-NCS1. Having found that Mito-NCS1 expression specifically alters calcium signal duration in astrocytic mitochondria within the DLS, we next examined Mito-NCS1-induced changes in mitochondrial morphology. To do this, we co-injected DLS astrocytes with Mito-NCS1 and an AAV specifically expressing GFP in astrocytic mitochondria of the DLS, called Mito-GFP. These experiments revealed that NCS1 expression in DLS astrocytes caused an overall decrease in mitochondrial connectivity. Interestingly, despite an overall decrease in mitochondrial connectivity within the entire astrocyte, astrocytic endfeet showed a paradoxical increase in mitochondrial connectivity following expression of Mito-NCS1. These data demonstrate striking subcellular differences in how mitochondrial calcium signals in astrocytes within the DLS govern mitochondrial dynamics and morphology at a subcellular level. Finally, we assessed the extent to which astrocytic mitochondrial expression of NCS1 in the DLS affects expression of the astrocyte endfoot protein, Aqp4 as well as astrocyte reactivity with GFAP, and microglial reactivity with Iba-1. We found a significant decrease in the expression of Aqp4, with an increase in GFAP and no changes in Iba-1. Together, these

results suggest that calcium signals in astrocytic mitochondria within the DLS play a critical role in BBB biology.

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Nanosymposium

NANO026: Astrocytes in Neuronal Function and Dysfunction

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Topic: B.09. Glial Mechanisms

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Title: Reduction of astrocyte Sema3c improves Rett Syndrome phenotypes

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Abstract: During neurodevelopment, astrocytes secrete proteins that regulate the formation and function of neuronal connections. In neurodevelopmental disorders, including Rett Syndrome, synapse formation and function are altered. Rett Syndrome is an X-linked disorder involving loss-of-function of the *MECP2* gene resulting in regressive motor, visual, and cognitive deficits. While Rett Syndrome research has primarily focused on neurons, astrocytes are an emerging player. The co-culture of wildtype neurons with Rett Syndrome astrocytes stunts dendritic outgrowth, indicating a non-cell autonomous effect of astrocytes on Rett Syndrome pathology, postulated to be through secreted factors. Yet, the identity of these secreted factors and their impact on Rett Syndrome phenotypes is largely unknown. Towards identifying proteins with altered secretion in neurodevelopmental disorders, we previously conducted unbiased proteomic analysis of astrocyte secreted factors in models of Rett, Fragile X, and Down Syndrome. Among the increased astrocyte secreted proteins across disorders is Sema3c, a member of the Class 3 semaphorin family of secreted factors that are involved in nervous system development, including neurite outgrowth, and synapse formation, elimination, and maintenance. Here we show that Sema3c is inhibitory to cortical neuron outgrowth *in vitro*, and identify the specific neuropilin and plexin receptors targeted by Sema3c that mediate this inhibitory effect. Using female Rett Syndrome mouse models, which recapitulate human mosaic *MECP2* levels and neuroanatomical and behavioral phenotypes, we show that genetically reducing the level of Sema3c in astrocytes improves Rett Syndrome phenotypes. Specifically, we find that astrocyte Sema3c reduction can improve neuron morphology, synaptic function, visual acuity, and motor

deficits in Rett Syndrome models. Understanding the role of astrocyte secreted proteins in Rett Syndrome may identify novel avenues for therapeutic targets while also informing on fundamental molecular mechanisms underlying brain function.

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Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

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Presentation Number: NANO027.01

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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Title: Tau Isoform Ratios with a Long-Acting Antisense Oligonucleotide Alleviates 4R-Tauopathy Phenotypes

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Abstract: Tau is a microtubule-associated protein whose dysregulation underlies several tauopathies, including Alzheimer's disease and frontotemporal lobar degeneration (FTLD). It exists as two main isoforms, 3-repeat (3R) and 4-repeat (4R). Excess accumulation of 4R-tau characterizes disorders such as FTLD, progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD). We previously demonstrated that loss of Fused in Sarcoma (FUS) or Splicing Factor Proline/Glutamine-Rich (SFPQ) drives 4R-tau buildup, leading to FTLD-like behavioral deficits and neurodegeneration in mice. Building on this, we designed antisense oligonucleotides (ASOs) incorporating 2'-O,4'-C-ethylene-bridged nucleic acids (ENAs) that selectively lower the 4R-/3R-tau ratio while keeping overall MAPT expression intact. In vitro screening pinpointed NK-18 as the lead candidate. A single intracerebroventricular (ICV) dose of NK-18 normalized 4R/3R-tau splicing in both FUS-knockdown humanized tau mice, alleviating abnormal behaviors, dendritic spine pathology, and neurodegeneration. Pharmacokinetic analysis revealed a brain half-life of roughly six months, with splicing correction sustained for up to two years. Notably, NK-18 outperformed a matched 2'-O-methoxyethyl (MOE) ASO. Collectively, these results position ENA-modified ASOs such as NK-18 as promising, long-acting therapeutics that diminish pathogenic 4R-tau without suppressing total tau, offering a safer strategy for treating 4R-tau-driven tauopathies.

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Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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This abstract does not represent the views of the United States government.

Title: Brd4 regulates microglial activation and synapse elimination in PS19 tauopathy mice

Authors: *D. MA[1,2], E. HOLETS[1], Q. BAI[1,2], V. FIESLER[1,2], D. SUN[1,2], E. A. BURTON[1,2,3]; [1]Pittsburgh Inst. for Neurodegenerative Dis., [2]Neurol., Univ. of Pittsburgh, Pittsburgh, PA; [3]Geriatric Res. Educ. and Clin. Ctr., Pittsburgh VA Healthcare Syst., Pittsburgh, PA

Abstract: Progressive supranuclear palsy (PSP) is a fatal 4-repeat (4R) tauopathy. We recently reported that (+)JQ1, an inhibitor of bromodomain-containing protein 4 (Brd4), rescued neurological deficits in a zebrafish model of PSP by preventing microglial synapse elimination (Bai et al. 2024 *Nature Communications* 15, 8195). The current study was designed to determine whether (+)JQ1 shows similar activity in the PS19 mouse model, which expresses human 4R-Tau in CNS neurons, causing early microglial activation and synapse loss. We tested the hypothesis that: *Brd4-dependent microglial synapse elimination is conserved in a mammalian tauopathy model*. There were three experimental groups: (i) non-transgenic siblings treated with vehicle (n=12); (ii) PS19 mice treated with vehicle (n=12); and (iii) PS19 mice treated with (+)JQ1 (n=13). (+)JQ1 was administered at 50 mg/kg by intraperitoneal injection daily from 3 to 5 months of age. Mice in all groups showed an increase in body weight throughout treatment. Following trans-cardiac perfusion, brains were harvested for histopathological analysis at 5 months of age. Compared with siblings, the abundance of Iba1-labeled microglia in the hippocampus of PS19 mice, and both the number and volume of CD68-immunoreactive puncta within microglia (a marker of microglial activation), were increased robustly. Both abnormalities were prevented by (+)JQ1 treatment. PS19 mice showed a decreased density of PSD95-immunoreactive synaptic puncta, and an increased frequency of intra-microglial PSD95-immunoreactive puncta, indicating microglial synaptic phagocytosis. Both changes were prevented by (+)JQ1 treatment. The proportion of PSD95-immunoreactive synaptic puncta labeled with complement C1q and C3 (early markers of pathological synaptic pruning in

neurodegeneration) was increased in PS19 mice. PS19 mice showed more C1q- and C3-labeled PSD95-immunoreactive puncta within microglia. These changes were prevented by (+)JQ1 treatment. Together, these new data suggest that (+)JQ1 inhibits microglial synaptic phagocytosis in a mammalian tauopathy model, possibly by preventing Brd4-dependent deposition of complement components C1q and C3 at synapses. These findings support the validity of the zebrafish model and are potentially important, since the pharmacological inhibition of Brd4 presents a possible therapeutic strategy to prevent neurological deficits resulting from synapse loss in PSP.

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Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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Tau Consortium

Title: Antisense Oligonucleotide Development for 4R Tau Mutations: Evaluating Phenotypic and Sleep Changes in Tauopathy Models

Authors: *M. HU[1], K. M. SCHOCH[2], J. PARK[1], D. M. HOLTZMAN[3], T. M. MILLER[4]; [1]Washington Univ. in st Louis, St Louis, MO; [2]Neurol., Washington Univ. in St. Louis Dept. of Neurol., Saint Louis, MO; [3]Dept Neurol., Washington Univ., Saint Louis, MO; [4]Neurol., Washington Univ, Sch. Med., Saint Louis, MO

Abstract: Background: Tau mis-splicing, leading to an imbalance of 3R:4R tau isoforms, is a key feature of many primary tauopathies. 3R and 4R tau isoforms selectively deposit in neurons and glia. 4R tau deposition is present in tauopathies like progressive supranuclear palsy (PSP), corticobasal degeneration, and frontotemporal dementia. Many tau mutations alter MAPT splicing, producing more 4R tau. The Miller group and others identified a role for 4R tau in increasing phosphorylation and aggregation, suggesting targeting 4R tau may be beneficial. We developed antisense oligonucleotides (ASOs) to alter MAPT splicing at exon 10, modulating 3R and 4R tau expression to understand their roles in tauopathies. Objective: Our goal is to investigate phenotypic and functional changes following ASO-mediated changes to tau isoform levels in mice. We aimed to characterize the novel N279K MAPT mutant mouse model from MODEL-AD, which recapitulates exon 10 mis-splicing and increases 4R tau expression. We identified behavioral and morphological deficits in the brain, including sleep disturbances, and evaluated whether tau splicing ASOs could rescue these deficits. Methods: Phenotypic profiling of N279K MAPT mice was performed to elucidate the impact of 4R tau overexpression. This

included sensorimotor test, nestlet behavior, and Piezoelectric sleep monitoring. Brain morphology and cell type-specific changes were evaluated using histological and immunohistochemical analyses. Following characterization, mice were treated with 4R to 3R splicing ASOs to assess their ability to rescue these deficits. Results: N279K MAPT mice exhibited elevated 4R tau mRNA and protein levels, consistent with human tauopathies. These mice showed behavioral deficits, including altered sleep patterns and impaired nesting behavior. Morphological analysis revealed brain abnormalities consistent with tauopathy. Treatment with 4R to 3R ASO led to significant behavioral improvements in the nestlet test and increased sleep percent. Western blot and gene expression analyses confirmed a shift from 4R to 3R tau isoform expression. These data demonstrate the efficacy of 4R to 3R ASOs in rescuing 4R-induced phenotypes in N279K mice. Conclusion: Our study supports the broader application of isoform-specific ASO therapy for treating tauopathies caused by MAPT mutations and emphasizes the need for continued development and testing of ASOs in specific mutation contexts. Ongoing studies include evaluating ASOs in other MAPT mutations and exploring their therapeutic potential in iPSC-derived neurons from N279K donors, providing further validation for tau splicing as a targeted treatment strategy.

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Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO027.04

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Xu startup package
Showalter grant
NIH R21NS140907
NIH U01AG088662

Title: Develop a Human Tauopathy Model Using Human-Mouse Neuronal Chimeras

Authors: *Y. JI[1], J.-C. ROCHET[2], F. LIU[4], R. XU[3];
[2]Medicinal Chem. &Molecular Pharmacol, [3]Basic Med. Sci., [1]Purdue Univ., West Lafayette, IN;
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Abstract: Intraneuronal aggregation of tau protein is one of the hallmarks of Alzheimer's disease (AD) and strongly correlates with cognitive decline. Under normal conditions, tau binds to microtubules, regulating axonal transport and microtubule dynamics. Hyperphosphorylation causes tau to detach from microtubules and self-assemble into toxic aggregates, leading to synaptic loss and neuronal death. Conventional tauopathy models, such as hiPSCs-derived *in vitro* models, transgenic rodents, and seed-dependent transgenic mice models, significantly advance our knowledge of disease mechanisms. However, several limitations persist: (1) *in vitro* cultured neurons do not resemble the aged phenotype of AD brains; (2) most rodent models rely on frontal temporal dementia (FTD) mutations, producing tauopathies that diverge from those in AD; and (3) human and rodent tau differ in splice form composition at adulthood. Thus, a more pathophysiologically relevant human model is needed to elucidate AD-specific tau pathology and to facilitate therapeutic discovery. To this end, we generated a tauopathy model by engrafting human neurons into mice brains and stereotaxically injecting AD patient-derived tau seeds into the hippocampus. The transplanted neurons preserved the physiological 1:1 ratio of 3R/4R tau isoforms seen in mature human neurons by 6 months of age. Seed inoculation induced misfolding of endogenous tau, producing pre-tangles and mature neurofibrillary tangles over time. The number of pathology-bearing neurons increased progressively, and lesions disseminated across connected brain regions, mirroring the spreading in AD. Notably, human neurons represented a disproportionate fraction of tangle-positive cells, indicating higher vulnerability to human-derived seeds. Chimeric mice also exhibited progressive memory deficits, linking tau accumulation to functional impairment. In summary, we have established a human-mouse chimera that faithfully models the initiation, propagation, and behavioral consequences of AD-related tauopathy in mature human neurons. This platform offers a powerful tool for mechanistic studies in tau-mediated neurodegeneration and may be adaptable to other seed-related neurological disorders, as well as for therapeutic testing.

Disclosures: Y. Ji: None. **J. Rochet:** None. **F. Liu:** None. **R. Xu:** None.

Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

Location: SDCC Rm 23A

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Presentation Number: NANO027.05

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Ed and Ethel Moore, Florida department of health
NIH/NINDS 1R61NS115178
NIH/NINDS 4R33NS115178

Title: Characterization of a constitutive, monogenic mouse model of tauopathy overexpressing P301L human tau in the brain

Authors: *M. HAMM[1], K. MCNAUGHT JR[3], J. SHUBIN[2], E. GAZAROV[2], Z. STRICKLAND[2], J. HOWARD[1], S. FROMHOLT[2], G. XU[2], D. R. BORCHELT[2], J. M. LEWIS[1];

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Abstract: Objectives: Characterization of a novel model of tauopathy that constitutively overexpresses P301L human tau
Methods: Transgenic founders were generated via DNA pronuclear microinjection of a CamK2-tauP301L construct into murine zygotes. Brain tissue from the lead line, "cTauP301L", was assessed biochemically and histologically for expression and biodistribution of tau. Brains were detergent-fractionated and immunoblotted with a tau antibody panel or sagittally sectioned and stained with 3,3'-diaminobenzidine-based immunohistochemistry and Gallyas silver staining.
Results: Immunoblotting tris-soluble fractions of cTauP301L brains ranging 2-12 months of age revealed a steady level of total human tau expression across ages. Immunoblots with antibodies targeting disease-associated phospho-tau epitopes, including serine 396/404, revealed a significant increase in specific phospho-tau species in 12-month animals compared to 2-month animals. We performed similar immunoblots of detergent-insoluble cTauP301L brain fractions with total and phospho-tau antibodies. These revealed a paucity of insoluble tau in 2 and 4-month brains but a marked presence of insoluble tau in both 8 and 12-month cTauP301L animals. Immunohistochemical staining of cTauP301L brain sections revealed an age-related accumulation of tau protein in the hippocampus, frontal cortex, and olfactory bulb. At 12 months of age, Gallyas silver staining revealed silver-positive neurofibrillary tangles in the same neuronal populations. Additional staining for glial markers GFAP and IBA1 demonstrated an age-related increase in signal suggestive of a gliosis that is spatially and temporally associated with tau pathology in the cTauP301L brain.
Conclusions: Our data indicate the constitutive "cTauP301L" mouse stably expresses P301L human tau in the hippocampus and forebrain, resulting in age-dependent accumulation of tau and accompanying gliosis in these brain regions. This data further indicates that the cTauP301L model exhibits a tau pathology profile similar to that of the commonly used, bigenic, rTg4510 mouse model of tauopathy. As a monogenic, constitutively-expressing model, the cTauP301L represents a straightforward, potentially cheaper alternative for studying tau pathology in vivo.

Disclosures: M. Hamm: None. **J. Shubin:** None. **E. Gazarov:** None. **Z. Strickland:** None. **J. Howard:** None. **S. Fromholt:** None. **G. Xu:** None. **D.R. Borchelt:** None. **J.M. Lewis:** None.

Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

Location: SDCC Rm 23A

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Presentation Number: NANO027.06

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Department of Veterans Affairs IK6BX006467
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Title: Amelioration of tauopathy phenotypes via the tri-snRNP

Authors: *K. R. LEBLANC[1], R. ECK[2], C. S. LATIMER[3], N. LIACHKO[4], R. L. KOW[5], B. C. KRAEMER[6];
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Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative condition characterized pathologically by extracellular beta amyloid deposits and intracellular hyperphosphorylated tau tangles. Interest has grown in investigating pathological tau both due to its correlation with cognitive status in patients with AD, as well as the presence of tau tangles in a number of other neurodegenerative conditions, collectively termed tauopathies. Tauopathies can be modeled using *Caenorhabditis elegans* (*C. elegans*), a small, low cost, rapidly growing, and genetically tractable model organism. Transgenic insertion of human normal or mutant tau recapitulates human disease features including behavioral deficits, neuron loss, accumulation of insoluble hyperphosporylated tau, and shortened lifespan. We have used *C. elegans* forward genetic approaches to identify genes that impact tau toxicity, both to better understand the molecular mechanisms of tauopathies, and to identify new potential targets for therapeutic intervention. Partial loss of function in *dib-1* and *prp-8*, two crucial core splicing factors, ameliorates tau-driven neurodegeneration in *C. elegans*. Both human homologues, TXNL4A and PRPF8, are also depleted in tissue from individuals with AD. This result is surprising given that disruption of splicing has been observed in individuals with Alzheimer's disease and wholesale disruption of splicing would be expected to have severely detrimental effects on cellular function. To characterize splicing related changes in the partial loss of function mutants suppressing tau, we conducted RNAseq on *dib-1* and *prp-8* mutants and observe a spectrum of unusual splicing events. Additionally, disruption of Nonsense Mediated Decay (NMD) via *smg-2* mutations further improves *dib-1* rescue of tauopathy related behavioral phenotypes suggesting NMD may be degrading differentially spliced transcripts containing premature stop codons responsible for tauopathy rescue. To understand the potential shared mechanisms driving tauopathy suppression by *dib-1* and *prp-8* partial loss of function, we set out to uncover if a specific transcript or set of transcripts may act as the drivers. We are currently analyzing RNA sequencing data to uncover shared splicing changes in *prp-8* and *dib-1* partial loss of function strains that result in premature stop codon contain transcripts to identify new candidate partial loss of function tauopathy suppressor genes. This approach will allow us to better probe the molecular pathways of tau toxicity and to identify more specific targets with potential translational relevance for further drug discovery.

Disclosures: K.R. LeBlanc: None. **R. Eck:** None. **C.S. Latimer:** None. **N. Liachko:** None. **R.L. Kow:** None. **B.C. Kraemer:** None.

Nanosymposium

NANO027: Animal Models of Tauopathies and Synucleinopathies

Location: SDCC Rm 23A

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO027.07

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Redox Imbalance and Nrf2/NOX2 Axis Dysregulation in a Gba D409V Mouse Model of Parkinson's Disease

Authors: *E. ESPOSITO[1], A. ARDIZZONE[1], M. LANZA[2], A. CAPRA[3], M. CAMPOLO[4]; [1]Dept. of Chemical, Biological, Pharmaceut. and Envrn. Sciences, Univ. of Messina, [2]CHIBIOFARAM, [3]Dept. of Chemical, Biological, Pharmaceut. and Envrn. Sci., [4]department of biological, chemical, pharmaceutical and environmental science, Univ. of Messina, Messina, Italy

Abstract: Background: GBA1 gene mutations, which cause Gaucher disease, are among the most common genetic risk factors for Parkinson's disease (PD), contributing to lysosomal dysfunction and impaired redox homeostasis. The Nrf2/NOX2 axis has emerged as a critical modulator of neuroinflammation and oxidative stress in neurodegenerative conditions.
Objective: To investigate the temporal progression of redox imbalance and Nrf2 signaling dysregulation in an in vivo Gba D409V knock-in mouse model, and its relevance to GBA1-associated PD pathogenesis.
Methods: Homozygous Gba D409V knock-in mice and C57BL/6J wild-type (WT) controls (8-weeks old) were evaluated longitudinally at 7, 14, 30, 60, and 90 days after starting the experiments. Behavioral assessments were performed alongside molecular analyses of dopaminergic markers (TH, DAT, α-synuclein) and oxidative stress parameters (Nrf2, NOX2, MDA, nitrate/nitrite levels) in midbrain-
Results: At early time points (7-14 days), no significant behavioral or molecular differences were detected between Gba D409V and WT mice. However, beginning at 30 days, and more pronounced at 60 and 90 days, Gba D409V mice exhibited significant upregulation of NOX2, downregulation of Nrf2, and increased oxidative stress markers. These molecular alterations correlated with early dopaminergic dysregulation and behavioral changes.
Conclusions: These findings demonstrate a time-dependent disruption of redox homeostasis in Gba D409V mice, resulting in motor phenotypes. The dysregulated Nrf2/NOX2 axis may represent a key pathogenic mechanism in GBA1-associated PD and a potential target for early intervention.

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Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO028.01

Topic: H.01. Fear and Aversive Learning and Memory

Title: Frontal cortical plasticity mechanisms mediating fear extinction are sex-specific

Authors: *K. GRAHAM[1], G. O'BRIEN[2,1], J. POMEROY-TUCK[2,1], E. BLOSS[1];
[1]The Jackson Lab., Bar Harbor, ME; [2]

Abstract: Past experience is fundamental for adaptive, goal-directed behaviors. For responses to be adaptive in dynamic environments, learned responses must be abandoned once the task contingencies change - a process referred to as extinction. Rodent models leveraging extinction of associative fear memories have shown this process requires recruitment of neurons in the infralimbic (IL) frontal cortex. Here, we present data that test the mechanisms of IL neuron recruitment during extinction learning that support persistent extinction memories. By leveraging viral and genetic strategies, we examined the requirements for neural activity and synaptic plasticity in two specific IL projection neurons: IL neurons targeting the basolateral amygdala (BLA) and IL neurons targeting the nucleus reuniens of the thalamus (RE). We found that both male and female mice require two days of extinction learning to store a persistent extinction memory. Interestingly, silencing of IL-to-BLA neurons in male but not female mice results in impaired extinction memory retrieval, suggesting activity of IL-to-BLA neurons are required for extinction memory in male but not female mice. Both IL-to-BLA and IL-to-RE neurons show robust evidence for structural synaptic plasticity following extinction learning in male mice, but these effects are either significantly weaker or absent in the same projection neurons in female mice. Projection specific genetic deletion from IL-to-BLA projection neurons of the glutamate receptor subunit Grin2b, which has been linked to synaptic plasticity and new spine formation, coordinately blocks learning-related synapse plasticity and the ability of male mice to form and consolidate the extinction memory. This effect is also absent in female mice, suggesting female mice fail to utilize this circuit to form and store extinction memories. Last, we show that pan calcium imaging recordings of IL neurons during extinction learning and retrieval results in differing activity patterns between male and female mice, in which males have more plastic ensemble activity across extinction learning and retrieval while females have more stable ensemble activity across days. Together, our results demonstrate that the cellular plasticity mechanisms of extinction diverge within frontal cortical circuitry between male and female mice. As sex differences are well documented in post-traumatic stress disorder, which can be thought of as a failure of extinction, our work highlights the need for sex-specific approaches to therapeutic modulation of cortical circuits in anxiety disorders.

Disclosures: K. Graham: None. **G. O'Brien:** None. **J. Pomeroy-Tuck:** None. **E. Bloss:** None.

Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO028.02

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01MH123768

Title: The rostral lateral septum orchestrates state-dependent inhibition of cued threat memory across the estrous cycle

Authors: *N. E. BAUMGARTNER[1,2], N. S. DECHACHUTINAN[1], K. H. ADCOCK BINION[2], G. C. BELL[2], A. M. DAHLEN[1], G. NEWBERRY[1], M. PENUMUDI[1], T. RUMBELL[3], J. SIMON[4], E. K. LUCAS[1,2];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Dept. of Mol. Biomed. Sci., North Carolina State Univ., Raleigh, NC; [3]IBM Res., Yorktown Heights, NY; [4]UNC Sch. of Med., Chapel Hill, NC

Abstract: Clinical research implicates cycling reproductive hormones as a primary mechanism driving enhanced female susceptibility to post-traumatic stress disorder (PTSD). Hormone levels at the time of trauma are inversely correlated with subsequent development of PTSD, and the mid-cycle estradiol surge coincides with less severe PTSD symptoms. Here, we tested the hypothesis that cycling reproductive hormones contribute to state-dependent memory, a phenomenon in which memory encoding and recall occur most efficiently in the same physiological state. Using cued threat conditioning, we compared male mice to female mice that underwent conditioning and recall under the same or opposite estrous cycle stage. We targeted high (proestrus, P) and low (diestrus, D) hormone states for a total of 5 groups: male, P→P, P→D, D→D, D→P. Cued recall was consistent between males and P→P females, but all other groups exhibited increased recall. Next, we investigated regional ensembles using c-fos expression as a neural activity marker. Few brain regions exhibited state-dependent recruitment following conditioning. However, only the rostral lateral septum (LS) exhibited state-dependent reengagement following recall, with increased reengagement in P→P females. Using chemogenetic manipulations, we next found that increased LS activity is necessary and sufficient to induce state-dependent inhibition of threat memory in females, but not males. We then sought to identify LS cellular populations involved in this neuronal ensemble. Single nucleus sequencing in naïve and conditioned P females identified 52 distinct LS cellular clusters. Only two neuronal clusters exhibited immediate early gene activation in response to conditioning: one expressing both neurotensin and somatostatin ($LS^{Nts\text{-}Sst}$), and the other expressing corticotropin-releasing hormone receptor 2 (LS^{Crhr2}). Fluorescent in situ hybridization confirmed unique engagement of $LS^{Nts\text{-}Sst}$, but not LS^{Crhr2} neurons, following conditioning in P females. We then measured $LS^{Nts\text{-}Sst}$ calcium dynamics during threat memory processes across hormone states. $LS^{Nts\text{-}Sst}$ neurons were uniquely responsive to cue onset during recall in P→P females, and transients coincided with releases in bouts of freezing in females but not males. Ongoing anterograde tracing will quantify sex differences in strength of $LS^{Nts\text{-}Sst}$ projections to many limbic regions including the nucleus accumbens, the bed nucleus of the stria terminalis, and the medial amygdala. Together, we report a novel female-specific neuronal ensemble that limits the overexpression of behavioral responses to learned threats during high reproductive hormone states.

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Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

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Presentation Number: NANO028.03

Topic: H.01. Fear and Aversive Learning and Memory

Support: NRF Grant 2018R1A3B1052079

Title: Amygdalar dopamine transmission mediates the transition of defensive behavior

Authors: *J. PYO[1], K. PARK[2], S. CHOI[1], S. LEE[1], J.-H. KIM[1];
[1]POSTECH, Pohang-si, Korea, Republic of; [2]postech, Pohang-si, Korea, Republic of

Abstract: As survival strategies, animals confronted with threats respond with passive or active defensive behaviors. While recent reports have disclosed the neural circuits mediating passive freezing behavior and active avoidance behavior, the neural substrate orchestrating the transition from passive to active defensive behavior remains unclear. This study aims to reveal the neural circuits mediating this transition. By adopting an auditory two-way active avoidance paradigm, we found that 77% of the tested mice, classified as Good Performers (GPs), demonstrated high avoidance behavior and low freezing levels after learning. In contrast, the remaining 23% of mice, categorized as Poor Performers (PPs), consistently exhibited low avoidance behavior and high freezing levels. We observed lower dopamine concentration in the BLA of PPs than GPs, suggesting a crucial role in the deficit in active avoidance learning. We utilized fiber photometry, single-cell level calcium imaging, and opto, chemogenetics to study how dopamine contributes to these behavioral changes. Our findings emphasize the critical role of dopaminergic transmission from the ventral tegmental area (VTA) to the posterior basolateral amygdala protein phosphatase 1 regulatory inhibitor 1b (pBLA$^{Ppp1r1b+}$) in the transition of defensive behavior. Furthermore, we demonstrate that the divergent downstream of pBLA$^{Ppp1r1b+}$ neurons toward the nucleus accumbens (NAc) and central amygdala (CeA) have individual roles. Finally, we propose dopaminergic transmission, which potentiates neural circuits of the pBLA $^{Ppp1r1b+}$ as neural substrates mediating the transition of defensive behaviors.

Disclosures: J. Pyo: None. **K. Park:** None. **S. Choi:** None. **S. Lee:** None. **J. Kim:** None.

Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

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Topic: H.01. Fear and Aversive Learning and Memory

Support: 1R21MH131363
1R21EB032609
2P20GM121310

Title: Distinct roles of somatostatin and parvalbumin interneurons in trace eyeblink conditioning

Authors: *Q.-Q. SUN[1], J. DAI[2];
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Abstract: Learning involves evaluating multiple dimensions of information and generating appropriate actions, yet how the brain assigns value to this information remains unclear. In this study, we show that two types of interneurons (INs) in the primary somatosensory cortex—somatostatin-expressing (SST-INs) and parvalbumin-expressing (PV-INs) neurons—differentially contribute to information evaluation during trace eyeblink conditioning (TEC). An air puff (unconditioned stimulus, US) delivered after a whisker stimulus (conditioned stimulus, CS) elicited both reflexive eye closure and stress-related locomotion. However, only self-initiated, anticipatory eye closure during the CS window, measured via electromyography (EMG), was directly relevant to learning performance. We found that SST-IN activity changes aligned with the learning induced changes of the anticipatory eye blinks during the CS period, correlated with the EMG changes across learning. In contrast, PV-IN activity was positively correlated with stress-related locomotion following the US and showed no learning related changes, suggesting a role in processing the emotional or aversive component of the task. Furthermore, cholinergic signaling via nicotinic receptors modulated both SST- and PV-IN activities, in a manner consistent with their distinctive roles, linking these interneurons to the regulation of learning-related actions and emotional responses, respectively. These findings demonstrate that distinct interneuron populations evaluate different dimensions of information—SST-INs for predictive, adaptive actions and PV-INs for stress-related emotional responses—to guide learning and behavior.

Disclosures: Q. Sun: None. **J. Dai:** None.

Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO028.05

Topic: H.01. Fear and Aversive Learning and Memory

Title: Gustatory Thalamic Neurons Mediate Aversive Behaviors

Authors: *F. CAO[1], S. PARK[2], R. D. PALMITER[3];
[1]Psychology, Western Washington Univ., Bellingham, WA; [2]Biochem., Howard Hughes Med. Inst. and Univ. of Washington, Seattle, WA; [3]Univ. of Washington, Seattle, WA

Abstract: The parvicellular part of the ventral posteromedial nucleus (VPMpc) of the thalamus, also known as the gustatory thalamus, receives input from the parabrachial nucleus and relays taste sensation to the gustatory (or insular) cortex. Prior research has focussed on the role of the VPMpc in relaying taste signals. Here we provide evidence showing that VPMpc also mediates aversive behaviors. By recording calcium transients *in vivo* from single neurons in mice, we show that neurons expressing cholecystokinin and the mu-opioid receptor in the VPMpc respond to various noxious stimuli and fear memory. Chemogenetic and optogenetic activation of these neurons enhances the response to aversive stimuli, whereas silencing them attenuates aversive behaviors. The VPMpc neurons directly innervate neurons in the insular cortex and rostral lateral amygdala. This study expands the role of the VPMpc to include mediating aversive and threating signals to the insular cortex and lateral amygdala.

Disclosures: F. Cao: None. **S. Park:** None. **R.D. Palmiter:** None.

Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

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Topic: H.01. Fear and Aversive Learning and Memory

Support: NIMH grant R21MH132052
BBRF YI Award

Title: The Role of Striatal Acetylcholine in Learning Under Social and Non-Social Threats

Authors: *O. DINCKOL[1,2], N. WENGER[2], C. MADDOX[2], T. GOOD[3], M. G. KUTLU[2]; [1]Temple Univ., Philadelphia, PA; [2]Temple Univ. Lewis Katz Sch. of Medicine, Philadelphia, PA, United State, Philadelphia, PA; [3]Cell Biol. and Neurosci., RowanSOM, Lake Hopatcong, NJ

Abstract: Acetylcholine (ACh) is a neurotransmitter with multifaceted roles in cognitive processes, including attention and facilitation of learning. Here, we investigated ACh release in the dorsolateral striatum (DLS), a region critical for cognitive functions and reward processing, to understand its role in threat perception across social and non-social contexts. Using in vivo fiber photometry recordings in male and female C57BL/6J mice, we observed that DLS ACh release increased in response to aversive stimuli and the conditioned stimulus during a fear conditioning paradigm, with this response progressively decreasing as conditioned fear extinguished. Conversely, in an appetitive conditioning paradigm, the DLS ACh response to a reward-predictive cue decreased over time and remained unchanged with learning, suggesting a valence-dependent effect of ACh signaling. In social contexts, we found that DLS ACh release was elevated when animals avoided a same-sex novel conspecific but was unaffected in opposite-sex interactions during the three-chamber sociability test. Similarly, during social competition in a warm spot task and tube test, ACh levels increased as animals approached their opponent or distanced from the desired resource, highlighting ACh's role in modulating

responses to social threats. Notably, these effects disappeared when competitive elements were removed. Optogenetic stimulation of basal forebrain ChAT+ cholinergic terminals induced DLS ACh release reversed social exploration toward novel conspecifics and increased competitiveness in the warm spot and tube tests. Collectively, our findings demonstrate that striatal ACh release signals social and non-social threats, enhances vigilance, and exhibits valence-dependent learning effects.

Disclosures: O. Dinckol: A. Employment/Salary (full or part-time):; Temple University Lewis Katz School of Medicine, Philadelphia, PA, United State. **N. Wenger:** A. Employment/Salary (full or part-time):; Temple University Lewis Katz School of Medicine, Philadelphia, PA, United State. **C. Maddox:** A. Employment/Salary (full or part-time):; Temple University Lewis Katz School of Medicine, Philadelphia, PA, United State. **T. Good:** None. **M.G. Kutlu:** A. Employment/Salary (full or part-time):; Temple University Lewis Katz School of Medicine, Philadelphia, PA, United State.

Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO028.07

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIH 2T32NS041228-21
NIH 5T32NS007224-37
NIH K08MH122733
NIH F31MH138088

Title: Prefrontal neuromodulators perform divergent roles in aversive learning through cyclic AMP

Authors: *A. BASU[1], A. ROSADO[2], A. P. KAYE[3];
[1]Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; [3]Dept. of Psychiatry, [2]Yale Univ., New Haven, CT

Abstract: Individuals must learn to predict levels of threat in environments containing uncertainty to respond with appropriate defensive behaviors. Neuromodulators have been shown to represent components of predictive learning models such as prediction error. However, the intracellular mechanisms by with neuromodulators enact their proposed computational roles have not been well examined, especially during aversive learning. To illuminate the roles of different neuromodulators, we measured release of prefrontal dopamine (DA) and norepinephrine (NE) during trace aversive conditioning, and found that while NE encodes prediction error, DA encodes stimulus salience. Accordingly, increasing NE release during aversive conditioning increases fear recall, while increasing DA release during fear recall serves as a distractor stimulus. We next sought to understand the intracellular consequences of extracellular

neuromodulator signals by fluorometric measurement of cyclic AMP (cAMP). We found sustained cAMP responses to both novel and aversive-conditioned stimuli. Consistent with responses to novel stimuli, threat-evoked cAMP requires D1-dopamine receptor activation. Acutely increasing cAMP during fear recall increases threat-evoked freezing, suggesting a role in threat recall. While norepinephrine has no direct effect on threat cAMP, increasing NE release during learning increases cAMP responses to conditioned cues, in line with NE's role in learning. Finally, increasing NE during learning shifts prefrontal representations of a conditioned cue towards the unconditioned stimulus (shock), revealing an ensemble mechanism of neuromodulator teaching signal function. Future work will determine how cAMP mediates the effects of neuromodulator teaching signals in cortex, as well as how they mediate their behavioral effects. This work will inform the mechanisms of aversive learning and of neuromodulatory teaching signal function.

Disclosures: A. Basu: None. **A. Rosado:** None. **A.P. Kaye:** None.

Nanosymposium

NANO028: Circuit Regulation of Fear and Extinction Learning

Location: SDCC Rm 24A

Time: Monday, November 17, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO028.08

Topic: H.01. Fear and Aversive Learning and Memory

Title: Neural circuit-specific regulation of fear memory by Intersectin-1

Authors: A. FARHAT[1], ***R. LAMPRECHT**[2];
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Abstract: Intersectin-1 (ITSN1) is a multi-domain scaffolding protein that functions as a guanine nucleotide exchange factor (GEF) for Cdc42. To investigate the role of Intersectin-1 in memory formation and maintenance, we used a targeted approach to manipulate its activity with high spatial and temporal precision during and after fear conditioning. We examined the functions of Intersectin-1 in various cell populations and neural pathways. We found that Intersectin-1 modulates long-term memory processes in a neural circuit-specific manner. Ongoing work is exploring how Intersectin-1 influences the synaptic mechanisms underlying memory maintenance. These findings provide new insights into the molecular basis of fear memory in specific brain circuits and suggest that Intersectin-1 may be a potential target for therapeutic intervention in memory disorders.

Disclosures: A. Farhat: None. **R. Lamprecht:** None.

Nanosymposium

NANO029: Ingestive Behavior and Homeostasis in Health and Obesity

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO029.01

Topic: G.08. Food and Water Intake and Energy Balance

Support: DK075168

Title: An amygdalopontine pathway promotes motor programs of ingestion

Authors: D. S. LAFFERTY, **J. S. WOLCOTT**, J. ISAAC, L. RECK, *A. LUTAS; NIH/NIDDK, Bethesda, MD

Abstract: Overconsumption of energy-dense, palatable food leads to obesity. We investigated neural circuit mechanisms that allow for the overconsumption of food despite visceral cues that signal fullness. We examined an inhibitory projection from the central amygdala (CeA) to the pons that targets the parabrachial nucleus (PBN), a well-established meal termination center, as well as the nearby premotor circuits that control orofacial movements. Optogenetic activation of CeA-to-pons axons caused excessive drinking of available liquid, regardless of palatability. When precisely timed to the onset of bouts of licking, photostimulation prolonged ingestive bout duration without affecting the number of lick bouts initiated, suggesting that this pathway modulates consummatory behaviors rather than triggering appetitive responses. Photostimulation-induced overconsumption remained sensitive to visceral satiety cues, as mice eventually halted ingestion. Stimulating CeA-to-pons axons in the absence of food drove ingestion-like orofacial behaviors including licking and chewing as well as food-handling behaviors such as grasping and hand-to-mouth movements in the absence of any target, implicating this pathway in the control of motor programs of ingestion. In a pellet grasping task, photostimulation drove excessive grasping of and overconsumption of food pellets, indicating that photostimulation-induced motor programs are flexible and context-dependent. Leveraging the machine learning toolkits DeepLabCut and Simple Behavioral Analysis (SimBA), we performed facial tracking and behavioral classification during liquid food consumption. We found that while photostimulation increased the frequency of licking, it did not impact the kinematics of the behavior. Taken together, these findings suggest that CeA-to-pons photostimulation promotes a flexible, goal-directed ingestive state by recruiting consummatory motor circuits rather than simply suppressing satiety signals. These findings highlight how forebrain-brainstem interactions can drive feeding behavior beyond homeostatic need.

Disclosures: D.S. Lafferty: None. **J.S. Wolcott:** None. **J. Isaac:** None. **L. Reck:** None. **A. Lutas:** None.

Nanosymposium

NANO029: Ingestive Behavior and Homeostasis in Health and Obesity

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO029.02

Topic: G.08. Food and Water Intake and Energy Balance

Support: NIH 2-R01-DK103808
DOD Grant CP22093P1

Title: Lateral Preoptic Neurotensin Receptor 1 Neurons Promote Weight Loss Behavior

Authors: *B. LEE, C. SCHULTZ, A. ZHAO, R. BUGESCU, G. M. LEINNINGER;
Michigan State Univ., East Lansing, MI

Abstract: The medial preoptic area of the hypothalamus plays a pivotal role in body weight control yet the function of the adjacent lateral preoptic area (LPO) has been largely unexplored. The LPO expresses Neurotensin (Nts) receptor 1 (NtsR1), which has been shown to contribute to energy homeostasis via other brain regions. We hypothesized that NtsR1-expressing neurons in the LPO (LPO^{NtsR1} neurons) modulate ingestive behaviors and physiology that can support weight loss. To test this we injected $NtsR1^{Cre}$ mice in the LPO with AAV-DIO-hM3D-mCherry to express excitatory Designer Receptors Exclusively Activated by Designer Drugs (DREADDq) in LPO^{NtsR1} neurons, permitting their activation after treatment with the DREADD ligand (0.3 mg/kg, ip). Acute DREADDq-mediated activation of LPO^{NtsR1} neurons modestly reduced homeostatic drinking and feeding behavior in normal-weight chow-fed mice compared to vehicle treatment, and the effects were more robust in diet-induced obese (DIO) mice. Sustained activation of LPO^{NtsR1} neurons for 3 consecutive days had no effect on body weight of chow-fed mice but significantly reduced feeding and body weight in DIO mice. However, DREADDq-mediated activation of LPO^{NtsR1} neurons did not alter locomotor activity, indicating that reduced feeding is not an artifact due to malaise or hyperactivity. Intriguingly, analysis in metabolic cages showed that activating LPO^{NtsR1} neurons also lowered respiratory exchange ratio (RER), indicative of biasing energy substrate usage from carbohydrate to fat. Next, we investigated neural mechanisms by which endogenous Nts might engage LPO^{NtsR1} neurons and whether it can support weight loss. Tract tracing revealed that Nts-expressing neurons of the lateral hypothalamic area (LHA^{Nts} neurons) project densely to the LPO and might provide endogenous Nts to activate LPO^{NtsR1} neurons. Optogenetic activation of LHA^{Nts} neurons projecting to LPO (LHA^{Nts} ->LPO neurons) will reveal the contribution of this source of Nts to the LPO, and for LPO^{NtsR}-mediated energy balance. Together, our findings identify a role for LPO^{NtsR1} neurons in modulating feeding, metabolism, and body weight, and suggest that these neurons and the NtsR1 system may be promising targets to support weight loss in obesity.

Disclosures: B. Lee: None. **A. Zhao:** None. **G.M. Leinninger:** None.

Nanosymposium

NANO029: Ingestive Behavior and Homeostasis in Health and Obesity

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO029.03

Topic: G.08. Food and Water Intake and Energy Balance

Support: AHA Grant

Title: Can a BCAA-lowering compound reduce sucrose intake?

Authors: *F. DEHGHANI[1], A. C. SHIN[2];
[1]Nutritional Sci. Dept., [2]Nutritional Sci., Texas Tech. Univ., Lubbock, TX

Abstract: Overconsumption of palatable foods is a key driver of the obesity epidemic. Elevated circulating levels of branched-chain amino acids (BCAAs) are observed in both human and animal models of obesity and type 2 diabetes. BCAA-restricted or low-protein diets stimulate the liver-derived hormone fibroblast growth factor 21 (FGF21), which suppresses sweet intake and preference in mice. BCAA levels are regulated by diet, hormones, and pharmacological agents such as 3,6-dichlorobenzo[b]thiophene-2-carboxylic acid (BT2), an allosteric inhibitor of BCKDK that enhances BCAA catabolism. BT2 lowers circulating BCAA levels and improves metabolic outcomes, including insulin sensitivity, glucose tolerance, and cardiac function in mice. However, the role of BCAAs in food reward behavior remains unclear. This study aims to investigate whether reducing circulating BCAAs alters sucrose intake and the motivation for sweet reward in lean and diet-induced obese mice. Forty-eight male C57BL/6J mice (8 weeks old) were fed either a high-fat diet (60% kcal from fat) or standard chow for 12 weeks. They were assigned to one of four groups and received daily intraperitoneal injections of either BT2 (40 mg/kg) or vehicle for one month: HF + BT2, HF + vehicle, Chow + BT2, and Chow + vehicle. Behavioral tests including the two-bottle choice and progressive ratio tasks were conducted at the end of the treatment period. Obese mice showed significantly lower sucrose intake and motivation to obtain sucrose pellets compared to lean controls. While BT2 treatment did not affect sucrose intake in obese mice, it showed a reduction trend in lean mice. BT2 treatment did not alter sucrose “wanting” in either lean or obese mice, in both ad libitum and fasting states. Our findings suggest that lowering circulating BCAAs may reduce sucrose intake in lean mice, which may serve as a preventative approach for managing obesity. Determining the appropriate dosage of BT2 to reduce sucrose preference is warranted. This study sheds light on the novel role of BCAAs in food reward.

Disclosures: F. Dehghani: None. **A.C. Shin:** None.

Nanosymposium

NANO029: Ingestive Behavior and Homeostasis in Health and Obesity

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Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO029.04

Topic: G.08. Food and Water Intake and Energy Balance

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NIH Grant R01DK075632
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NIH Grant T32GM144636

Title: A spatial and projection-based transcriptomic atlas of paraventricular hypothalamic cell types

Authors: *Y. LI[1], T. BUTLER[1], S. NARDONE[2], A. DOUGLASS[2], J. C. MADARA[2], M. MCDONOUGH[1], J. TAO[2], E. LOWENSTEIN[2], L. WANG[2], J. CAMPBELL[3], J. RESCH[1,4,5]; [1]Univ. of Iowa, Iowa City, IA; [2]Beth Israel Deaconess Med. Ctr., Boston, MA; [3]Biol., Univ. of Virginia, Charlottesville, VA; [4]Iowa Neurosci. Inst., Iowa City, IA; [5]Fraternal Order of Eagles Diabetes Res. Ctr., Iowa City, IA

Abstract: The paraventricular hypothalamus (PVH) controls many behavioral and physiologic processes, including appetite, social behavior, autonomic outflow, and pituitary hormone secretion. However, molecular markers for functionally-specific PVH neuron populations remain largely undefined, and a complete census of PVH cell types has not been established.To address this, we have created a detailed spatially-resolved transcriptomic atlas of the PVH at single-cell resolution. In total, we profiled over 40,000 cells using single-cell/nucleus RNA sequencing. Through additional utilization of publicly available data, this effort resulted in transcriptomic information from more than 16,000 neurons belonging to neuronal subtypes that express the PVH marker *Sim1*. High-sensitivity spatial transcriptomics with multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) further characterized PVH cell types, yielding 26 $Sim1^{+}$ neuron populations, categorized based on their rostral-to-caudal spatial location. Additionally, projection-based profiling identified neuronal subtypes that project to the parabrachial region (PB), helping to identify PVH neuron populations that regulate satiety. Among the PB-projecting PVH neurons, one subtype was enriched for bombesin-like receptor 3 (*Brs3*), an orphan receptor known to regulate feeding behavior and metabolism. Consistent with prior studies, silencing of PVH^{Brs3} neurons resulted in weight gain, while stimulation of PVH^{Brs3} → PB projections was sufficient to reduce food intake. Together, this atlas of PVH cell types provides critical insights into the functional organization of the PVH and serves as a valuable resource for the field of homeostasis.

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Nanosymposium

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Topic: G.08. Food and Water Intake and Energy Balance

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NIH Grant R00HL144923
AHA Grant 935362
F31HL170784

Title: Afferent regulation of hindbrain aldosterone-sensing HSD2 neurons

Authors: *A. KURALAY[1], M. MCDONOUGH[2], T. BUTLER[5], Y. LI[3], J. C. GEERLING[4], J. RESCH[2];
[1]The Univ. Of Iowa - Neurosci. Grad. Program, Iowa City, IA; [2]Neurosci. and Pharmacol., [4]Neurol., [3]Univ. of Iowa, Iowa City, IA; [5]The Univ. Of Iowa Neurosci. Grad. Program, Iowa City, IA

Abstract: Adequate sodium intake is essential for maintaining effective circulatory volume. Aldosterone-sensing neurons in the nucleus of the solitary tract (NTS) that express 11-β hydroxysteroid dehydrogenase type 2 (HSD2) play a key role in driving sodium appetite. Selective ablation of these HSD2 neurons significantly reduces sodium ingestion, whereas their activation drives sodium consumption. Moreover, sodium deficiency and systemic administration of mineralocorticoids both increase c-Fos expression in HSD2 neurons. However, c-Fos expression quickly declines following sodium ingestion, suggesting rapid afferent inhibition of HSD2 neurons independent of circulating hormone levels. To define the afferents regulating HSD2 neuron activity, we combined projection-specific single-nucleus RNA sequencing (snRNA-seq) with optogenetic circuit mapping in *ex vivo* brain slices. We found that GABAergic neurons from the central amygdala (CeA) and local inhibitory neurons within the dorsal vagal complex (DVC) form strong monosynaptic inhibitory connections with HSD2 neurons. Optogenetic stimulation of GABAergic afferents robustly suppressed HSD2 neuron activity in *ex vivo* brain slices from sodium-deprived animals. Interestingly, inhibitory current amplitudes from local DVC inputs were reduced after sodium deprivation, consistent with reduced HSD2 neuron inhibition in this deficiency state. Projection-specific snRNA-seq on CeA neurons that innervate the NTS identified somatostatin (*Sst*)- and neurotensin (*Nts*)-expressing neurons as the primary afferents to HSD2 neurons, whereas other molecularly defined CeA projections to the NTS lacked detectable functional connectivity. These findings identify parallel inhibitory circuits that modulate HSD2 neuron activity and reveal molecularly defined CeA populations that may contribute to salt satiation by suppressing sodium appetite. Future work will address the behavioral relevance of these inhibitory pathways in sodium homeostasis.

Disclosures: A. Kuralay: None. **M. McDonough:** None. **T. Butler:** None. **Y. Li:** None. **J.C. Geerling:** None. **J. Resch:** None.

Nanosymposium

NANO029: Ingestive Behavior and Homeostasis in Health and Obesity

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO029.06

Topic: G.08. Food and Water Intake and Energy Balance

Support: NIH Grant NS099425
NIH Grant NS130038

Title: Parabrachial Foxp2-expressing neurons are necessary for sustaining core body temperature in the cold

Authors: *F. S. GRADY[1], S. GRAFF[2], M. WARNOCK[3], S. GASPARINI[4], M. M. TISH[5], Y. LI[4], G. F. BUCHANAN[6], J. RESCH[7], J. C. GEERLING[6];
[1]Intrnl. Med., Univ. of Washington, Seattle, WA; [2]Fndn. for the Natl. Inst. of Hlth., Bethesda, MD; [3]; [5]Neurosci. Program, [6]Neurol., [7]Neurosci. and Pharmacol., [4]Univ. of Iowa, Iowa City, IA

Abstract: Cold environmental temperatures are a threat to survival. Sustaining core body temperature in the cold requires a dynamic set of adaptive responses known as “cold defense,” but the neural circuitry that senses these threats and orchestrates these responses remains unclear. We identified a cluster of Atoh1-derived, Foxp2-expressing glutamatergic neurons in the lateral parabrachial nucleus (PB) that are activated by exposing mice to cold environmental temperature. We eliminated these neurons in a cell-type-specific manner with a viral vector expressing Caspase 3, an apoptotic protein. Control mice received a vector expressing mCherry. Eliminating all glutamatergic PB neurons as well as their Foxp2-expressing subset caused body temperature to plummet in the cold. The core body temperature of these mice dropped as low as 15° C and exhibited symptoms of cold narcosis. However, mice lacking these neurons had normal wakefulness, movement and appetite at room temperature, and their autonomic cold-defense responses remained intact. However, these mice do not appropriately increase metabolism and locomotor activity in the cold, and thermal discrimination was impaired. Our results indicate that thermosensory information relayed through Foxp2-expressing PB neurons is essential for sensing and surviving a cold environment.

Disclosures: F.S. Grady: None. **S. Graff:** None. **S. Gasparini:** None. **M.M. Tish:** None. **Y. Li:** None. **G.F. Buchanan:** None. **J. Resch:** None. **J.C. Geerling:** None.

Nanosymposium

NANO029: Ingestive Behavior and Homeostasis in Health and Obesity

Location: SDCC Rm 11

Time: Monday, November 17, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO029.07

Topic: G.08. Food and Water Intake and Energy Balance

Title: Tirzepatide improved high fat diet-induced locomotor activity impairment without inducing anxiety-like behaviours in mice.

Authors: M. BURNETT[1], M. BRAHMA[1], E. BILLINGHAM[1], M. CONWAY[1], A. CHAND[1], *D. RIAL[1], J. S. DAVIES[1], J. UNITT[1], N. MIRZA[2], F. MCINTOSH[1], W. PIJACKA[1], S. P.

VICKERS[1];
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Abstract: Obese patients generally have reduced locomotor activity and are at greater risk of anxiety and depression than non-obese individuals. This can be complicated by pharmacological treatments that affect locomotor activity or mood (e.g. fenfluramine, sibutramine and rimonabant). Incretin-based therapies have revolutionised obesity treatment by reducing food intake without the adverse effects of previous treatments. However, adverse effects, in particular anxiety, have been reported in clinical and real-world studies although preclinical data remains scarce. In this study we investigated the impact of Tirzepatide (TZP), a glucose-dependent insulinotropic polypeptide/glucagon-like peptide-1 receptor agonist, on behaviour in a mouse model of diet induced obesity (DIO). Male C57BL/6J mice fed a 45% high fat diet for 31 weeks were randomised to treatment by body weight, food and water intake, lean mass and grip strength. TZP (10 nmol/kg, sc, n=8) or vehicle (n=7) were administered one in three days for 5 weeks. Body weight, food and water intake were measured daily. Locomotor activity and behaviour of DIO mice and age matched controls were measured on Day 33 in a novel environment using the Deep Learning Ethovision software. Body composition was assessed by DEXA at baseline and Day 35. Grip strength was measured at baseline and Day 34. Skeletal muscles (gastrocnemius, tibialis anterior, soleus, and quadriceps), heart and liver were weighed on Day 36. TZP treatment reduced food intake, body weight (23.5%), fat mass (51.9%) and lean mass (5.5%) from baseline to completion. Locomotor activity (cm), average velocity (cm/s), and mobility time (%) were significantly decreased in vehicle treated DIO mice compared to age matched controls. TZP treatment increased locomotor activity (cm), average velocity (cm/s), mobility time (%), sniffing (%) and unsupported rearing (%) compared to vehicle. TZP treatment had no effect on time in central zone (%), grip strength (gF) or skeletal muscle weight, although it did reduce heart and liver weights. As expected, TZP administration resulted in a reduction in food intake, body weight, fat mass and lean mass. Lean mass loss was not associated with a reduction in quantity or function of skeletal muscle. As with clinical studies, locomotor activity was negatively impacted in obese mice. TZP treatment restored locomotor activity to age matched control levels, with less periods of immobility and increased frequency of exploratory behaviours. This effect is likely to be associated with health benefits of weight loss rather than stimulation. TZP treatment did not reduce time in central zone, suggesting a lack of anxiety-like behaviours.

Disclosures: M. Burnett: None. **M. Brahma:** None. **E. Billingham:** None. **M. Conway:** None. **A. Chand:** None. **D. Rial:** None. **J.S. Davies:** None. **J. Unitt:** None. **N. Mirza:** None. **F. mcintosh:** None. **W. Pijacka:** None. **S.P. Vickers:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.01

Topic: A.09. Development and Evolution

Support: NHMRC grant 1194206
NHMRC grant 2019011
NHMRC grant 2041019
National Science and Technology Innovation 2030 Major Program No. 2022ZD0205000
NHMRC grant 2011733

Title: Left is always larger: Highly Consistent Interhemispheric Asymmetry in a Small-Brained Primate, the Marmoset Monkey

Authors: I. CARRIL MUNDIÑANO[1], N. ATAPOUR[1], R. SAMANDRA[1], C. LIU[3], F. ALIZADEH MANSOURI[1], ***M. G. P. ROSA**[2];
[1]Dept. of Physiol. and Biomedicine Discovery Inst., [2]Monash Univ., Clayton, Australia; [3]Chinese Acad. of Sci., Inst. of Neurosci., Shanghai, China

Abstract: Although lateralisation of brain function is found in many animals, this is rarely linked to morphological differences between cerebral hemispheres. For example, many mammal species show functional lateralisation according to criteria such as the preferred use of one of the limbs, but this is rarely accompanied by pronounced anatomical asymmetries. Humans and apes show consistent hemispheric differences including in cortical areas related to the evolution of language, but even this trend is less marked and inconsistent across individuals in macaques and baboons. This has led to the idea that marked anatomical lateralisation of the cortex is linked to the evolution of larger brains. This would impose constraints related to the efficiency of axonal connections, which would lead to the segregation of neural structures into more specialised subnetworks, and ultimately hemispheric asymmetries. We studied high resolution magnetic resonance imaging (MRI) in 302 marmoset monkeys (*Callithrix jacchus*), which are among the smallest primates (brain volume ~8g). The sample included individuals from colonies in 4 countries (Australia, China, Japan and the USA), and both *in vivo* and *ex vivo* scans. Remarkably, we found that adult marmosets (>2 years old, n=210) show consistent anatomical asymmetry, with the left hemisphere being on average 2.34% larger than the right (range 0.22-4.37%). No individual in the sample had a larger right hemisphere. Male and female marmosets did not differ in this respect, and data from the different colonies demonstrated the same trend, despite the individuals therein being genetically isolated for at least 10 generations. Voxel-based morphometry revealed that the differences could be attributed to larger left hemisphere volumes in the higher-order visual, auditory and polysensory areas, ventral temporal areas areas (parahippocampal, perirhinal and entorhinal regions), and the posterior caudate nucleus. The asymmetry develops gradually postnatally, becoming clearly discernible and consistent only in adolescence (> 1 year old). These results argue against the model whereby hemispheric asymmetry derives from constraints associated with connectivity in larger brains. The postnatally developing larger left temporal lobes in marmosets likely represents a case of parallel evolution with humans, species characterised by highly social behaviour underpinned by a rich repertoire of auditory and visual communication.

Disclosures: I. Carril Mundiñano: None. **N. Atapour:** None. **R. Samandra:** None. **C. Liu:** None. **F. Alizadeh Mansouri:** None. **M.G.P. Rosa:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

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Presentation Number: NANO030.02

Topic: A.09. Development and Evolution

Support: Serra-1709-16981

Title: Dolphin brains don't fold like ours:: Quantifying a fundamental difference in multi-scale gyrification between cetaceans and non-cetaceans

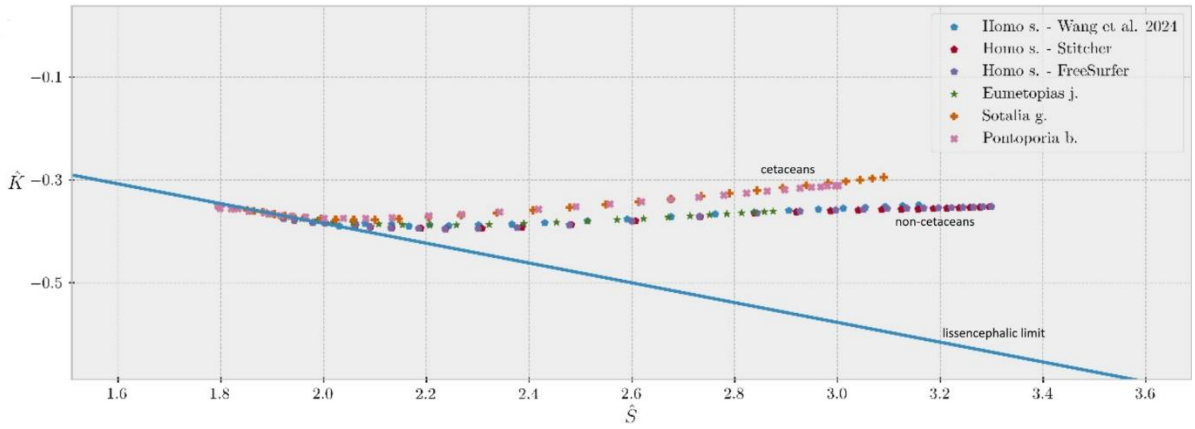
Authors: H. MYNSSEN PINTO LOPES[1], K. A. DE SOUZA[2], N. PATZKE[4], ***B. MOTA**[3]; [1]Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Physics Inst., Univ. Federal Do Rio De Janeiro, Rio De Janeiro, Brazil; [3]Univ. Federal Do Rio De Janeiro, Rio de Janeiro, Brazil; [4]Dept. of Biol. Sci., Hokkaido Univ., Sapporo-Shi, Japan

Abstract: Underneath the large diversity in shapes and sizes of the mammalian cerebral cortex lies a stark regularity: A theory-derived morphometric scaling law for gyrification applies precisely to a broad range of terrestrial mammals. Moreover, primates (and likely other orders) follow a universal self-similar pattern of gyrification, with a universal fractal dimension of 2.5 and a shared fundamental aspect ratio for sulci and gyri. Across species, cortices differ primarily in the range of length scales for which they approximate a fractal of $d_f = 2.5$. However, until now, extending this analysis to cetaceans has remained elusive, due to the difficulty of reconstructing their complex cortices.

As the only fully aquatic mammals, cetaceans cortices have many unique features. Indeed, previous inconclusive evidence indicated they could be systematically more gyrified than those of other mammals. Using a new method of surface reconstruction that integrates anatomical expertise with high-resolution MRI, we conducted a multi-scale analysis in 2 cetaceans and 3 non-cetaceans cortices, abstracting each shape as a trajectory in morphometric space.

These trajectories are starkly distinct for cetaceans and non-cetaceans, with different fractal dimensions yet consistent fundamental aspect ratios. If we imagine cortical surfaces as composed of small triangular tiles of finite thickness, cetaceans would have the same tile shape found in non-cetaceans, but arrange them in a significantly more jagged fashion. Strikingly, the semi-aquatic but non-cetacean sea lion scales identically to humans.

The origins of this distinction are unclear. Does it stem from the mechanical response of brain tissue to aquatic environment—perhaps the greater hydrostatic pressure experienced during diving? Or as an adaptive feature evolved for aquatic life? To address this, we plan to analyze shallow-diving cetaceans like the Amazonian pink dolphin. A mechanical hypothesis predicts its cortex should resemble non-cetaceans', while an evolutionary hypothesis suggests it would scale like its deep-diving relatives.



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Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.03

Topic: A.07. Development of Neural Systems

Support: NIH R21 EY030588

Title: Mapping microstructural gradients of the human putamen from infancy to adulthood

Authors: *V. S. NATU[1], X. YAN[5], C. S. TYAGI[2], E. KUBOTA[2], S. TUNG[2], H. WU[3], A. MEZER[6], E. DRORI[7], N. S. WANG[4], C. LIAO[8], K. SETSOMPOP[4], K. GRILL-SPECTOR[2]; [2]Dept. of Psychology, [3]Ctr. for Cognitive and Neurobiological Imaging, [4]Dept. of Radiology, [1]Stanford Univ., Stanford, CA; [5]Inst. of Sci. and Technol. for Brain-Inspired Intelligence, Fudan Univ., Shanghai, China; [6]Edmond and Lily Safra Ctr. for Brain Sci. (ELSC), [7]The Hebrew Univ. of Jerusalem, Jerusalem, Israel; [8]Dept. of Radiology and Biomed. Imaging, Univ. of California, San Fransisco, San Francisco, CA

Abstract: The putamen, a part of the dorsal striatum, emerges around the 11th week of gestation. It is involved in the integration of information from the cortex to facilitate multiple sensory, motor, and cognitive functions. Recent work has established that the hallmark spatial gradients of the putamen can be characterized in vivo, using quantitative MRI, along its axis in both healthy young and older adults[1]. However, it is unknown if the microstructural gradients exist in infancy or develop across the lifespan. To address this gap, we obtained quantitative MRI (longitudinal relaxation rate (R_1 [s^{-1}]) and diffusion MRI in 20 full-term, healthy infants (ages: 16-479 days) and 20 adults (ages: 19-42 years). In adults, we replicate prior results: we find microstructural gradients of increasing R_1 along the putamen's anterior-posterior axis. However, in newborns' putamen, R_1 is low and does not vary spatially. Nonetheless, this anterior-posterior

R_1 gradient emerges in 6-12-month-olds. Analysis of the putamen's white matter connectivity using diffusion imaging and endpoint density analysis across the brain revealed a topographic arrangement of cortico-striatal white matter connections in adults: anterior segments of the putamen have more connections to frontal regions, but its posterior segments have more connections to sensorimotor and premotor regions. This cortico-striatal connectivity pattern is present in infants but is less robust. We find that from infancy to adulthood, there are increases in connectivity between the anterior putamen to the dorsolateral prefrontal and anterior cingulate cortex, as well as increases in connections between the posterior putamen to somatosensory motor cortex and early visual cortex. Finally, we find a correlation between the development of R_1 and white matter connections: subjects with higher R_1 in the anterior portions of the putamen have more connections from this segment to the dorsolateral prefrontal and anterior cingulate cortex, whereas subjects with higher R_1 in the posterior portions of the putamen have more connections from this segment to sensory-motor and visual areas. Our findings reveal not only the topographic development of microstructure across the putamen but also the relation between the development of microstructure and white matter connections to different functional networks, opening avenues for studying neurodevelopmental disorders related to motor and cognitive control in infants and children.

Reference: 1. Drori, E., Berman, S. & Mezer, A. A. Mapping microstructural gradients of the human striatum in normal aging and Parkinson's disease. Sci. Adv. 8, eabm1971 (2022).

Disclosures: V.S. Natu: None. **X. Yan:** None. **C.S. Tyagi:** None. **E. Kubota:** None. **S. Tung:** None. **H. Wu:** None. **A. Mezer:** None. **E. Drori:** None. **N.S. Wang:** None. **C. Liao:** None. **K. Setsompop:** None. **K. Grill-Spector:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.04

Topic: A.09. Development and Evolution

Support: R01NS095654

Title: New genes enable structural innovation and function in the brain

Authors: *V. LURIA[1], M. W. KIRSCHNER[2], N. SESTAN[3];
[1]Neurosci., Yale Univ., New Haven, CT; [2]Systems Biol., Harvard Med. Sch., Boston, MA; [3]Yale Univ. Sch. of Med., New Haven, CT

Abstract: How genuinely new protein-coding genes originate is a central question in biology. Many new genes arising from non-copying mechanisms such as de novo from intergenic genomic DNA long considered to be "junk", or from long non-coding RNAs, were recently found in Eukaryotes. New genes are taxon-restricted, may encode structurally novel proteins with new protein domains, and illuminate the emergence of protein structure. To understand how

new genes arise, we built a mathematical model based on genomic parameters and dynamic factors including mutation. Combining gene age evaluation and proteogenomics, we identified taxon-restricted genes in >100 eukaryotic genomes from human to paramecium and evaluated their predicted biophysical properties. Compared to ancient proteins, new proteins are shorter, more fragile, disordered and promiscuous, yet less prone to forming toxic aggregates. We experimentally measured structure content and protease resistance of new human proteins and showed new genes function in vivo in zebrafish brains. We showed new human genes have fewer regulatory elements than ancient genes but more than control intergenic open reading frames. Our GTEx RNA expression analysis shows new human genes are expressed in many tissues. Our gnomAD mutational constraint analysis shows some new human genes are functional. Using single-cell RNA-Seq and proteomics, we found new genes are expressed in human brains at multiple ages. Thus, genomic sequence turnover generates many new genes encoding short proteins with distinct structural features and functioning in the brain. Variation in large eukaryotic genomes having large intergenic "dark matter" regions continuously generates new protein structures and new functions.

Disclosures: V. Luria: None. **M.W. Kirschner:** None. **N. Sestan:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.05

Topic: I.08. Learning and Memory

Support: Helen L. Dorris Fellowship
NIH Grant R01NS121223

Title: Micrornas direct purkinje cell development

Authors: *M. GHANEM[1], G. LIPPI[2], N. ZOLBOOT[2], J. X. DU[3], F. ZAMPA[4];
[1]Neurosci., Scripps Res., San Diego, CA; [2]Neurosci., The Scripps Res. Inst., La Jolla, CA; [3]The Scripps Res. Inst., San Diego, CA; [4]Dorris Neurosci. Ctr., Scripps Res. Inst., San Diego, CA

Abstract: Neuronal subtype specification requires precise regulation of gene expression. MicroRNAs (miRNAs), critical regulators of post-transcriptional gene expression in neurons, are essential for brain development, but their temporal and cell-type-specific functions during development are not well understood. Here, we employed a fast and reversible miRNA loss-of-function (LoF) approach to dissect miRNA roles in developing Purkinje cells (PCs), a cerebellar neuron subtype previously thought to differentiate independently of miRNA activity. Acute miRNA LoF revealed that miRNAs are critical for key steps in PC differentiation, including dendritogenesis and climbing fiber synaptogenesis—structural hallmarks of PC identity. Using new mouse models enabling high-resolution mapping of miRNA-target interactions in rare cell types, we identified a PC-specific regulatory network involving miR-206 and its target proteins

Shank3, Prag1, En2, and Vash1 to be essential for shaping PC specification. Functional manipulation demonstrated that miR-206 repression of these targets is necessary for proper dendritic and synaptic development, with miR-206 knockdown or target overexpression partially recapitulating the miRNA LoF phenotype. These findings establish a critical role for temporally coordinated miRNA activity in shaping neuronal subtype identity through post-transcriptional control of structural differentiation programs.

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

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.06

Topic: A.09. Development and Evolution

Support: RiLo-18/20/21/22 from the University of Turin

Title: Structural Basis and Functional Roles of FOXP2 Liquid-Liquid Phase Separation

Authors: *M. DELLOCA, S. BOGGIO BOZZO, F. GENTILE, S. VAGLIETTI, F. FIUMARA;
'Rita Levi Montalcini' Dept. of Neurosci., Univ. degli Studi di Torino, Torino, Italy

Abstract: The transcription factor FOXP2 is a key regulator of vocalization-, speech-, and language-related phenotypes in human and non-human species. The protein harbors several domains, including a DNA-binding forkhead domain (FHD), a zinc finger (ZnF), a leucine zipper (LZ), and an N-terminal low-complexity region (LCR) containing long polyglutamine (polyQ) repeats. We recently discovered that FOXP2 polyQ tracts form coiled-coil structures promoting protein condensation and fibrillization via liquid-liquid phase separation (LLPS), regulating FOXP2 transcriptional activity. Moreover, their evolutionary length variation correlates quantitatively with vocalization- and hearing-related parameters. These observations established a previously unrecognized form of polyQ length-dependent, LLPS-related molecular encoding of vocalization frequency in mammals. Based on these findings, we further investigated the molecular basis of FOXP2 LLPS, exploring the possible interplay of the N-terminal polyQ LCR with other domains of the protein in shaping its overall LLPS behavior. These analyses revealed that none of the domains individually can recapitulate the condensation properties of the full-length protein, indicating that the interplay of multiple protein regions defines the overall LLPS behavior of FOXP2 and the architecture of its condensates. Furthermore, we are currently exploring functional roles of FOXP2 LLPS in neuronal cells beyond its role in transcriptional regulation. These findings provide new mechanistic insight into FOXP2 LLPS behavior and its functional implications in neural cell biology.

Disclosures: M. DellOca: None. **S. Boggio Bozzo:** None. **F. Gentile:** None. **S. Vaglietti:** None. **F. Fiumara:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.07

Topic: A.09. Development and Evolution

Support: RiLo2022/2023 grants from the University of Turin

Title: Neurodevelopmental disease-related truncations impair CDKL5 phase separation and catalytic activity

Authors: *S. BOGGIO BOZZO, M. DELLOCA, S. VAGLIETTI, A. GURGONE, V. CARDINALE, M. GHIRARDI, M. GIUSTETTO, F. FIUMARA;
'Rita Levi Montalcini' Dept. of Neurosci., Univ. degli Studi di Torino, Torino, Italy

Abstract: Cyclin-dependent kinase-like 5 (CDKL5) is a serine/threonine protein kinase with a crucial role in the regulation of fundamental cellular processes, including DNA damage response, neuronal development, synaptogenesis, and synaptic function. Mutations in the *CDKL5* gene give rise to *CDKL5 deficiency disorder* (CDD), an X-linked epileptic encephalopathy with severe neurodevelopmental impact. CDD is clinically characterized by early-onset seizures, neurocognitive impairment, and complex sensorimotor dysfunction. CDKL5 comprises an N-terminal catalytic domain (NTD) and a longer, less characterized C-terminal domain (CTD). Importantly, pathogenic mutations target not only the kinase domain but also the CTD, leading to the cellular production of truncated CDKL5 variants. These disease-related mutant proteins retain an intact NTD and variably shortened portions of the CTD, indicating the importance of this distal region of the protein for the physiological regulation of CDKL5 catalytic activity. We have found that the CTD has a key role in mediating CDKL5 liquid-liquid phase separation (LLPS), a fundamental biophysical process regulating intracellular protein spatial organization and function. This behavior results in the formation of dynamic, membraneless CDKL5 condensates within both neuronal and non-neuronal cells. We demonstrate that LLPS is predominantly driven by serine-rich, evolutionarily conserved CTD low-complexity regions (LCRs). A critical CTD internal fragment (CTIF) acts as a pivotal driver of CDKL5 phase separation. Disease-related truncating mutations (S726X and R781X) which elide portions of the CTIF, both impair CDKL5 condensate formation and reduce its ability to phosphorylate EB2, one of its known targets. These findings corroborate the crucial role of the CTIF and distal CTD regions in promoting LLPS and maintaining CDKL5 functional integrity. Collectively, our results identify LLPS as a novel regulatory mechanism for CDKL5 driven by a CTD region elided by most truncating mutations. Its loss--through the impairment of CDKL5 LLPS and functional activity--may represent a central molecular event in the pathogenesis of CDD.

Disclosures: S. Boggio Bozzo: None. **M. DellOca:** None. **S. Vaglietti:** None. **A. Gurgone:** None. **V. Cardinale:** None. **M. Ghirardi:** None. **M. Giustetto:** None. **F. Fiumara:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.08

Topic: A.07. Development of Neural Systems

Support: Alfred P. Sloan Foundation

Title: Axonal branch points orchestrate mitochondrial biogenesis

Authors: *T. WAINGANKAR[1], C. ZURITA[3], C. VUONG[4], D. BAUTISTA[5], K. DRERUP[6], S. C. LEWIS[2];
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Abstract: In the peripheral nervous system, mitochondria are trafficked along sensory axons that are several centimeters to a meter in length, facilitating local energy conversion and biosynthesis away from the soma. Mitochondrial biogenesis and function require genes encoded in both the nuclear and mitochondrial genomes, yet how the expression of these two genomes is coordinated far from the cell body remains unclear. In the present study, we leveraged metabolic labeling and high-resolution 4D fluorescence microscopy to identify a subset of mitochondria positioned at axonal branch points that undergo cycles of biogenesis. We found that, in mouse neurons and live zebrafish larvae, branch point-anchored mitochondria preferentially perform mtDNA synthesis and transcription, serve as hubs for local translation of mitochondrial Complex I mRNA encoded in the nucleus, and undergo a specialized asymmetric division, resulting in two distinct daughter classes. Our results showed that daughters exiting the branch point have decreased mitochondrial nucleoid content, inner membrane potential, and ATP compared to daughters that remain at the branch point, creating a spatially constrained pattern of mitochondrial biogenesis that generates heterogeneity and shapes the bioenergetic landscape of peripheral axons.

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Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.09

Topic: A.07. Development of Neural Systems

Support: R01GM141280
R21EY034706

Title: Protocadherin-19 canalises the development of neural dynamics in the zebrafish optic tectum

Authors: S. BISWAS, M. R. EMOND, G. PHILIP, ***J. D. JONTES**;
Ohio State Univ., Columbus, OH

Abstract: Protocadherin-19 (Pcdh19) is a homophilic cell adhesion molecule. In humans, mutations in *PCDH19* are linked to schizophrenia and autism, and cause a female-limited developmental epileptic encephalopathy. We previously showed that *pcdh19* is expressed in neuronal columns within the zebrafish optic tectum and that larvae lacking *pcdh19* exhibit defects in visually-guided behaviors. To explore the effects of Pcdh19 loss on the function of visual circuits, we used *in vivo* 2-photon calcium imaging to record neural activity in the optic tectum, while providing visual stimulation. Using principal component analysis to perform linear dimensionality reduction, we find that the first 5 dimensions capture ~90% of the variance in our data. Each dimension constitutes a "neural mode" defined by a major pattern of neuronal covariation. The population responses are remarkably consistent among individual wild type larvae, with the variation among individuals being comparable to the variation among different trials within the same fish. To assess the effects of *pcdh19* loss on tectal function, we generated a promoterless *pcdh19* allele, $pcdh19^{\Delta prom}$. This allele eliminates the possibility of genetic compensation that can be activated by mutations that result in frameshifts and premature stop codons. The neuronal population responses of $pcdh19^{\Delta prom}$ mutants to visual stimulation deviated from the wild type, as the individual neural modes were less well correlated to the wild type average. Importantly, the deviations from wild type dynamics varied stochastically among the mutants; the within-group variation of the mutant larvae was greater than for the wild type larvae. The increased variation in neural responses among the mutants, indicates stochastic changes in the underlying network organization. We imaged individual larvae on consecutive days, starting at day 3, when the tectum first becomes visually responsive. While individual larvae exhibit increased variance and less stereotyped population responses at day 3, most exhibit mature neural modes by day 4. We find that the developmental trajectories are tightly clustered in wild type larvae, while those of the mutants are more divergent. Our data are consistent with the idea that Pcdh19 contributes to the canalization of the development of the optic tectum, providing a constraint that contributes to the assembly of networks responsible for highly stereotyped neural dynamics. Loss of Pcdh19 leads to developmental trajectories and outcomes that vary stochastically. These results could be relevant to the variable penetrance and phenotypic variability associated with most genes linked to neurodevelopmental disorders.

Disclosures: S. Biswas: None. **M.R. Emond:** None. **G. Philip:** None. **J.D. Jontes:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.10

Topic: A.07. Development of Neural Systems

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Title: Retinal calcium waves coordinate uniform tissue patterning of the Drosophila eye.

Authors: *B. J. CHOI[1], Y.-C. CHEN[2], C. DESPLAN[1];
[1]New York Univ., New York, NY; [2]New York Univ., New York, NY.

Abstract: Optimal neural processing relies on precise tissue patterning across diverse cell types. Here, we show that spontaneous calcium waves arise among non-neuronal support cells in the developing *Drosophila* eye to drive retinal morphogenesis. Waves are initiated by Cad96Ca receptor tyrosine kinase signaling, triggering PLCγ-mediated calcium release from the endoplasmic reticulum. A cell-type-specific 'Innexin-code' coordinates wave propagation through a defined gap junction network among non-neuronal retinal cells, excluding photoreceptors. Wave intensity scales with ommatidial size, triggering stronger Myosin II-driven apical contractions at interommatidial boundaries in larger ommatidia. This size-dependent mechanism compensates for early boundary irregularities, ensuring uniform ommatidial packing critical for precise optical architecture. Our findings reveal how synchronized calcium signaling among non-neuronal cells orchestrates tissue patterning in the developing nervous system.

Disclosures: B.J. Choi: None. **Y. Chen:** None. **C. Desplan:** None.

Nanosymposium

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Presentation Number: NANO030.11

Topic: A.07. Development of Neural Systems

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u Tsai NeurosciencesInstitute of Stanford University
Fondation IRCM

Title: Teneurin-3 and Latrophilin-2 function in somatotopic map patterning

Authors: *A. KANIA[1], X. ZHANG[2,3], D. DEL TORO, Sr.[4], C. SARANTOPOULOS[3,2], S. MAHASENAN[5], D. PEDERICK[6], R. B. ROOME[7], E. SEIRADAKE[8], L. LUO[6];
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Abstract: Despite first being described nearly a century ago, the mechanisms governing the formation of somatotopic (ST) maps, such as the cortical homunculus of Penfield, remain largely unknown. The mediolateral (ML) axis of the dorsal horn (DH) of the spinal cord is organised as an ST map of the proximodistal (PD) axis of the body surface. We show that this map correlates with complementary ML gradients of Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2) cell surface receptors. A corresponding expression pattern is also evident in the sensory neurons of the dorsal root ganglia (DRG). Since Ten3-Lphn2 mediated repulsion in conjunction with homophilic Ten3-Ten3 mediated attraction control the wiring of hippocampal circuits, we studied the function of these proteins in DRG-DH connectivity. First, we demonstrated that these proteins are capable of guiding DRG axons *in vitro*. Next, we generated conditional knockouts (cKOs) of Ten3/Lphn2 in the DH and DRG, which revealed a range of ML miswiring phenotypes depending on the ablated gene and location, with the Ten3 DH cKO being the most severe. Furthermore, Ten3 DH cKOs also showed an abnormal distribution of Fos protein, a proxy of neuronal activity, induced by a noxious stimulus. Importantly, such mutants display an impaired ability to accurately attend to a noxious stimulus along the PD axis of the limb. We thus provide the first evidence that these molecules act in complementary gradients, are required for ST map formation, and disruption of DH ST map development is accompanied by deficits in directed somatosensory behaviours.

Disclosures: A. Kania: None. **X. Zhang:** None. **D. Del Toro:** None. **C. Sarantopoulos:** None. **S. Mahasenan:** None. **D. Pederick:** None. **R.B. Roome:** None. **E. Seiradake:** None. **L. Luo:** None.

Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

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Topic: A.07. Development of Neural Systems

Support: NIH Grant R01 EY025670
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Title: Early face experience is critical for normal adult face processing

Authors: *S. SHARMA, M. S. LIVINGSTONE;
Harvard Med. Sch., Boston, MA

Abstract: Faces convey rich social information, including identity and expression, and face-selective neurons in primate inferotemporal (IT) cortex play a key role in their processing. Prior work has shown that monkeys restricted from seeing faces in early life instead develop behavioral preferences and IT selectivity for the experienced stimuli (hands). However, it remains unclear whether such face restriction disrupts higher-level face processing and if these functions can recover following later re-exposure to faces. Here, we combined behavioral and electrophysiological experiments in three face-restricted monkeys (tested 1-8 years after re-exposure to faces) and three typically raised control monkeys. To assess behavioral preferences, we conducted two experiments: (1) monkeys freely viewed images of faces, hands, and objects to examine recovery of face detection; and (2) they viewed paired images of threat and neutral expressions to probe facial expression processing. To characterize neural selectivity and tuning, we conducted three passive viewing experiments: (1) to assess category selectivity, we presented images of faces, hands, and objects; (2) to evaluate expression and identity tuning, we presented eighteen monkey identities across two expressions (threat/neutral); and (3) to assess identity and viewpoint tuning, we presented seven monkey/human identities across five viewpoints. We found that, behaviorally, face-restricted monkeys recovered from their early hand bias and showed increased attention to faces. However, though their overall facial expression preferences resembled control monkeys, they differed in gaze patterns, looking longer at the lower half of the face, whereas controls focused on the upper half. Moreover, IT neurons of all three face-restricted monkeys never developed normal face selectivity, instead showing mixed face and hand selectivity. Finally, none of the face-restricted monkeys showed lower decoding of facial expression, identity, or viewpoint information from their IT neural activity compared to controls. These findings indicate that early face experience is critical for the normal development of higher-order neural mechanisms of face processing, underscoring the importance of early experience in shaping the social brain.

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Nanosymposium

NANO030: Building a Brain: From Molecules to Experience

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO030.13

Topic: A.07. Development of Neural Systems

Title: Socioeconomic Origins of Salience Detection: Evidence from Auditory Mismatch Negativity

Authors: *Y. HAO[1], L. HU[2];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Grad. Sch. of Educ., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Early life experiences shaped by socioeconomic status (SES) may leave lasting imprints on brain function. One key aspect of neural processing is the ability to detect salient changes in the environment while maintaining focus on a task. This study investigates whether childhood subjective social status is associated with neural responses to irrelevant auditory changes in adulthood. Across two experiments (total n = 58), we recorded frontocentral event-related potentials while participants engaged in visual tasks and were simultaneously presented with streams of auditory tones, including frequent standard tones (80%) and infrequent deviants (20%). In both studies, individuals with lower childhood SES consistently showed enhanced mismatch negativity (MMN, study 1, standard beta = 0.47, p = 0.008; study 2, standard beta = 0.39, p = 0.001), indicating strong automatic change detection in the brain. This effect was robust across variations in auditory features (e.g., pitch manipulated in both studies), task type (e.g., passive viewing in study 1 vs. active detection in study 2), and emotional content (manipulated in study 2: neutral vs. negative). Despite heightened MMN responses, lower-SES participants did not differ from their higher-SES counterparts in task performance or in the P3a component, which indexes involuntary attentional orienting to deviant sounds. These findings suggest that lower childhood SES is associated with increased neural sensitivity to environmental changes, possibly reflecting an adaptive response to unpredictable early environments.

Disclosures: Y. Hao: None. **L. Hu:** None.

Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

Location: SDCC Rm 1

Time: Tuesday, November 18, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO031.01

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

Support: NIA K01-AG-081484-01
NINDSR01-NS109260-01A
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Title: Selectively vulnerable myeloarchitecture in behavioral variant frontotemporal dementia

Authors: *D. T. OHM[1], N. CAPP[1], P. SABATINI[1], K. A. COUSINS[1], J. S. PHILLIPS[1], K. RASCOVSKY[1], D. A. WOLK[1], E. B. LEE[2], C. T. MCMILLAN[1], D. J. IRWIN[1];
[1]Neurol., [2]Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Select cortical circuits degenerate in neurodegenerative disorders, but they remain poorly characterized in behavioral variant frontotemporal dementia (bvFTD), the most common young-onset dementia primarily caused by tau (bvFTD-tau) or TDP-43 (bvFTD-TDP) pathology. Healthy long-range projection neurons have well-myelinated axons that traverse gray matter in distinct—often vertical—orientations, but myeloarchitectonic changes in disease are understudied. Our recent work found that frontal cytoarchitecture enriched for projection neurons degenerates more in bvFTD-tau compared to bvFTD-TDP. Thus, we hypothesized greater myelin loss and disorganization in bvFTD-tau versus bvFTD-TDP. In bvFTD-tau (n=27), bvFTD-TDP (n=47), and healthy controls (HC; n=32) for reference, we immunostained for myelin basic protein (MBP, SMI94), tau (AT8), and TDP-43 (1D3) pathology in semi-adjacent sections of clinically relevant anterior cingulate, orbitofrontal, and mid-frontal cortices examined previously for neurodegeneration. We digitally measured areas occupied by total MBP immunoreactivity and by distinct vertical (V) and non-vertical (NV)-MBP bundles classified using gradient structure tensors. We tested MBP metrics in linear mixed-effects and linear models adjusted for demographic and biologic variables to compare groups and test associations with pathology within groups. In HC, we validated our MBP metrics by finding distributions expected for canonical myeloarchitecture, including a positive association between V-MBP and projection neurons (β=0.003, $p<0.001$). Compared to HC, total MBP was higher in bvFTD-TDP (β=0.002, p=0.001) and bvFTD-tau (β=0.003, $p<0.001$), and higher in bvFTD-tau versus bvFTD-TDP (β=0.002, p=0.001). While V-MBP was unexpectedly similar between groups, we found more NV-MBP in bvFTD-tau versus bvFTD-TDP (β=0.005, p=0.004) and HC (β=0.006, $p<0.001$), suggesting select axons express higher MBP in bvFTD. Consistent with elevated NV-MBP being a pathologic response, we found NV-MBP positively correlated with TDP-43 (β=0.002, p=0.006) and tau (β=0.004, p=0.004) pathology, whereas V-MBP did not. Our findings provide evidence of disorganized myeloarchitecture in a subset of cortical circuits that may be mediated by local pathology and is more pronounced in bvFTD-tau versus bvFTD-TDP. Thus, tau may preferentially disrupt myelin processes, especially along axons parallel to cortical layers in bvFTD. Future myelo- and cytoarchitectonic studies using multiplex staining will help elucidate which neurons and glia relate to axonal changes, thereby finding the cellular pathways most and least vulnerable in bvFTD.

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Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

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Presentation Number: NANO031.02

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

Support: NIH Grant RF1-AG029577
UCSF Schwab DCDC Innovation Fund

Title: Voxelwise encoding models reveal altered perceptual and semantic representations during narrative movie-watching in frontotemporal dementia

Authors: *M. VISCONTI DI OLEGGIO CASTELLO[1], B. MCEACHEN[2], K. RANKIN[2], J. L. GALLANT[1];
[1]UC Berkeley, Berkeley, CA; [2]UC San Francisco, San Francisco, CA

Abstract: Frontotemporal dementia (FTD) is characterized by complex behavioral symptoms such as reduced sensitivity to socioemotional information or semantic impairments. These symptoms present most severely in everyday activities requiring multimodal cognitive processing. However, the functional representations underlying these complex processes are not probed in most fMRI studies of FTD that rely on resting state or simple tasks. To better understand how FTD impacts the functional representations underlying complex tasks, we designed an fMRI movie-watching paradigm for clinical research. Ten patients with FTD (aged 52-81, 5 females) and 12 healthy controls (aged 50-77, 7 females) watched short movies with speech. Participant-specific voxelwise encoding models were used to predict brain activity simultaneously from feature spaces quantifying low-level auditory, low-level visual, lexical-semantic, and visual-semantic information in the movies. Separate train and test sets were used for each participant to ensure generalizability. Because FTD affects complex cognitive functions, we hypothesized that patients would show altered functional representations manifesting as differential recruitment of semantic processing areas compared to controls. To test this hypothesis, participant-specific prediction accuracy maps were projected to the template surface fsaverage and averaged across participants in each group. Inspection of these maps revealed that, in both groups, low-level features predicted brain activity in early auditory and visual regions and semantic features predicted brain activity in temporal, parietal, and frontal cortex. However, visual-semantic maps appeared sparser in patients than in controls, suggesting that visual-semantic representations were impaired in FTD patients. To evaluate these differences systematically, we quantified the fraction of voxels best predicted by each feature space. Fewer voxels were predicted by the visual-semantic features in FTD patients compared to controls (difference of -5.4%, 95% CI [-9.9%, -0.1%], p = 0.062 permutation test) and more voxels were predicted by the low-level auditory features (difference of 3.7% [0.8%, 7.1%], p = 0.037). No differences were found for the low-level visual and lexical-semantic features. These findings suggest that during complex multimodal processing, FTD may cause a reduction in visual-semantic representations, which in turn leads to an increase in low-level auditory representations. This may explain why FTD patients struggle with complex everyday activities that require multimodal integration of semantic and perceptual information.

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Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

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Parkwood Institute Research Cross-Theme Collaboration Studentship

Title: Trajectories of neurodevelopmental and neurodegenerative outcomes in genetic frontotemporal degeneration

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Abstract: Converging evidence hints at neurodevelopmental effects in genetic frontotemporal degeneration (FTD). For some genes, young adult FTD mutation carriers show cross-sectional differences in brain volumes and cognition compared to familial non-mutation carriers. However, longitudinal trajectories can more sensitively capture FTD-related neurodevelopmental vs. neurodegenerative changes than cross-sectional approaches. This study thus aimed to examine longitudinal trajectories of brain volumes, executive function, and plasma biomarkers in young adult carriers compared to familial non-mutation carriers, as measures of neurodevelopmental and neurodegenerative outcomes of FTD-causing mutations. This longitudinal cohort study comprised participants, aged 18-30 years, from the FTD Prevention Initiative across Europe, Canada, and the USA. Genetic groups included *C9orf72* (50%), *MAPT* (37%), and *GRN* (13%). Linear mixed-effects models were computed to assess longitudinal outcomes between groups, controlling for baseline age, sex, scanner (for brain volumes), and education (for executive function); random effects accounted for between-subject variability nested within family membership. Mutation carriers (n=113) and familial non-carriers (n=102) did not differ in age (mean±SD, 25.8±3.2), sex (56% female), or number of visits (2.2±1.6). Compared to non-carriers, average volumes were smaller in *C9orf72* repeat expansion carriers in the thalami (b= 2286.2, SE=538.3, p=0.0038), insula (b=1174.8, SE=396.6, p=0.018), and medial orbitofrontal cortices (b=1000.3, SE=359.9, p=0.032), without significant group x time interaction. *MAPT* carriers had larger medial orbitofrontal cortices on average (b=-1735.2, SE=611.3, p=0.047) than non-carriers, without significant group x time interaction. For *GRN*, there were no differences in brain volumes in regions of interest between groups or over time. No longitudinal changes were observed in executive function, or plasma NfL or GFAP between groups per genetic group. In conclusion, FTD-causing mutations are linked to changes in brain volumes, but not executive function or plasma biomarkers of neurodegeneration, suggesting potential compensatory mechanisms during young adult years. Consistent with prior research, findings of larger regional brain volumes support potential neurodevelopmental effects in *MAPT*-

associated FTD. Future longitudinal study of youth mutation carriers and non-carriers is necessary to disentangle neurodevelopmental vs. early neurodegenerative changes in FTD.

Disclosures: I. So: None. **E. Finger:** None.

Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

Location: SDCC Rm 1

Time: Tuesday, November 18, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO031.04

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

Title: Pharmacological modulation of UBE2K by molecular glues: A new approach to targeting proteinopathies in Huntington's disease

Authors: *D. B. OLIVER-JOLICOEUR[1], G. MAOR[1], J. LAWRENCE[1], J. RANJAN[1], N. MAHAVEER CHAND[1], G. M. MILLER[1], M. A. KIEBISH[1], N. R. NARAIN[1], K. M. ROSEN[1], D. CHIMMANAMADA[2], V. K. VISHNUDAS[1], S. GESTA[1];
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Abstract: Proteinopathies, disorders characterized by the pathological accumulation of misfolded or aggregation-prone proteins, are recognized as central to the onset and progression of multiple neurodegenerative disorders, including Alzheimer's, Parkinson's and Huntington's diseases (HD). Despite advances in understanding disease mechanisms, therapeutic strategies capable of directly modulating proteostasis remain limited. In an effort to identify previously unrecognized regulatory nodes within neurodegenerative disease biology, we used Bayesian artificial intelligence and systems-level modeling (NAi Platform), to construct causal network maps of molecular drivers in preclinical models. This hypothesis-free, data-driven analysis pinpointed UBE2K, an E2 ubiquitin-conjugating enzyme highly expressed in brain, as a central hub across multiple disease-relevant models. UBE2K is structurally unique among E2 enzymes due to its C-terminal ubiquitin-associated (UBA) domain, which enables binding to polyubiquitin chains and contributes to its high processivity during substrate modification. In a cellular model of HD using SH-SY5Y neuroblastoma cells expressing mutant Huntingtin (mHtt), stable knockdown of UBE2K via shRNA significantly increased lysosomal enzyme activity and led to a pronounced reduction in the accumulation of aggregated mHtt. To evaluate UBE2K as a pharmacological target, we conducted fragment-based screening, surface plasmon resonance (SPR), and in vitro discharge assays, yielding tool compounds that modulate its ubiquitin-transfer activity. X-ray crystallography studies identified a novel binding pocket at the interface between UBE2K and thioester linked ubiquitin, where these compounds acted as molecular glues to prevent ubiquitin discharge. Additional predictive and cell-based assays identified compounds with favorable permeability and other biophysical properties. Using differentiated SH-SY5Y neuroblastoma cells expressing mHtt, these tool compounds demonstrated their ability to increase the abundance of the thiol-sensitive form of ubiquitin-charged UBE2K, consistent with a mechanism of reduction of ubiquitin transfer. These preliminary, potential findings not only

elucidate a novel mechanism by which UBE2K may modulate mHtt aggregation but also demonstrate the feasibility of altering this pathway pharmacologically. Our results nominate UBE2K as a structurally and functionally distinctive target in the ubiquitin-proteasome system and support its potential as a therapeutic lever in HD and related neurodegenerative disorders.

Disclosures: D.B. Oliver-Jolicoeur: A. Employment/Salary (full or part-time):; BPGbio. **G. Maor:** A. Employment/Salary (full or part-time):; BPGbio. **J. Lawrence:** A. Employment/Salary (full or part-time):; BPGbio. **J. Ranjan:** A. Employment/Salary (full or part-time):; BPGbio. **N. Mahaveer Chand:** A. Employment/Salary (full or part-time):; BPGbio. **G.M. Miller:** A. Employment/Salary (full or part-time):; BPGbio. **M.A. Kiebish:** A. Employment/Salary (full or part-time):; BPGbio. **N.R. Narain:** A. Employment/Salary (full or part-time):; BPGbio. **K.M. Rosen:** A. Employment/Salary (full or part-time):; BPGbio. **D. Chimmanamada:** A. Employment/Salary (full or part-time):; Coorg Biosciences. **V.K. Vishnudas:** A. Employment/Salary (full or part-time):; BPGbio. **S. Gesta:** A. Employment/Salary (full or part-time):; BPGbio.

Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

Location: SDCC Rm 1

Time: Tuesday, November 18, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO031.05

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

Support: NIH Grant 1R36AG088283-01

Title: Tmem106b c-terminal fragments induce neuronal damage, nuclear disruption, and tdp-43 mislocalization

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Abstract: Genetic variations in the highly glycosylated lysosomal transmembrane protein 106B (TMEM106B) are linked to increased risk for several neurodegenerative disorders, particularly frontotemporal lobar degeneration (FTLD) with TDP-43 pathology. However, the molecular mechanisms driving this association remain unclear.
Recent advancements in cryo-electron microscopy (cryo-EM) have revealed homotypic assemblies of the C-terminal domain of TMEM106B (CTF) as a novel cytosolic proteinopathy. These CTF assemblies have been identified across multiple neurodegenerative diseases, including FTLD and Parkinson’s disease (PD), suggesting a potential pathogenic role for TMEM106B in neurodegeneration.
To assess the pathogenic potential of TMEM106B-CTF, we expressed the human CTF fragment corresponding to the fibrillar core identified in FTLD cases in both cell lines and primary neurons. One construct was expressed ubiquitously, and the other was routed through the endoplasmic reticulum (ER) and Golgi for glycosylation and lysosomal targeting. Both

constructs formed fibril-like structures within 48 hours in U2OS cells and induced nuclear abnormalities, including nuclear denting, altered lamin B1 distribution, and RanGAP mislocalization. In neurons, longitudinal microscopy revealed that both CTF constructs induced significant neurotoxicity. Given the link between TMEM106B and FTLD, we also observed TDP-43 mislocalization from the nucleus to the cytoplasm. Neurons co-transduced with CTF constructs also showed mislocalization of GFP fused to a nuclear localization signal (NLS), indicating compromised nuclear integrity.
To further understand the underlying mechanisms, we are using proteomics-based approaches to identify proteins that interact with TMEM106B-CTF. These findings will guide future in vivo studies aimed at clarifying the neurotoxic mechanisms of TMEM106B-CTF and its role in disease pathogenesis.

Disclosures: K. Tilahun: None. **J. Jiang:** None.

Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

Location: SDCC Rm 1

Time: Tuesday, November 18, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO031.06

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

Support: NINDS Grant NS085770
NIA ADRC Grant AG072977

Title: Synaptic loss and stability of synaptic proteins in a conditional transgenic mouse model of human TDP-43 proteinopathy

Authors: *Y. ANTEZANA[1], E. ADEBANJO[6], T. HILL[2], I. A. AYALA[2], A. BAHRAMI[7], K. R. SADLEIR[3], H. DONG[4], R. J. VASSAR[8], M.-M. MESULAM[5], C. GEULA[9];
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Abstract: Frontotemporal lobar degeneration (FTLD) is a common early-onset dementia marked by tauopathy or TAR DNA-binding protein 43 (TDP-43, FTLD-TDP) proteinopathy. We utilized a biallelic mouse model of FTLD on a mixed FVB/129SVE background overexpressing wild-type human TDP-43 (hTDP-43) under a tetracycline transactivator (tTA) system (TET off). Cortical intraneuronal punctate phosphorylated TDP-43 positive inclusions appeared at 14 days expression, peaked at 8 weeks and were absent by 24 weeks with concurrent neuronal loss and cortical thinning. Cortical synaptic loss has emerged as a consistent finding in neurodegenerative dementias, particularly in Alzheimer’s disease. In human participants with FTLD-TDP, we have shown reductions in cortical levels of the dendritic spine protein spinophilin and the presynaptic

protein synaptophysin, and a slight increase in the postsynaptic density protein of 95 KD (PSD-95), likely due to reactive upregulation. This study investigated whether similar synaptic changes occur in conditional hTDP-43 transgenic mice. Western blot analysis was conducted using homogenized frontal and temporal cortex from 5 transgenic and 5 wild type mice following 14 days, 8 weeks, and 24 weeks of TDP-43 expression. Antibodies against spinophilin (Cell Signaling Technology; mAb; 1:1000), synaptophysin (Millipore Sigma; mAb; 1:10000), and PSD-95 (UC Davis/NIH Neuromab Facility; mAb, 1:3000) were used. Electron microscopy (EM) was performed on 11 transgenic and 9 wild type mice to quantify frontal cortical synapses. Western blot analysis showed no consistent reductions in synaptic protein levels in transgenics. In some instances, levels were elevated; notably, PSD-95 was significantly increased in temporal cortex at 24 weeks of expression ($p = 0.0005$). However, quantitative analysis of EM images revealed significantly lower numbers of synapses in the frontal cortex of transgenic mice after 24 weeks. These findings suggest that hTDP-43 mice display synaptic loss despite stable or increased synaptic protein levels, likely due to compensatory upregulation. This mirrors observations in human FTLD-TDP and Alzheimer's disease and indicates that synaptic protein levels alone may not accurately reflect true synapse loss.

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Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

Location: SDCC Rm 1

Time: Tuesday, November 18, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO031.07

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

Support: R01NS104437, Pershing Square Foundation
R01 NS104437

Title: Transcriptomic signatures of neuron types selectively vulnerable to FTD and MND

Authors: *A. BREEVOORT[1], D. IVANOV[1], S. C. VATSAVAYAI[3], A. NANA[2], A. TUDORAS MIRAVET[6], F. PEREIRA[7], R. SALONER[2], W. W. SEELEY[4], A. A. POLLEN[5]; [1]Neurol., [2]Memory and Aging Ctr., Univ. of California, San Francisco, San Francisco, CA; [3]Neurol., Univ. of California San Francisco, San Francisco, CA; [4]Neurol., Univ. of California San Francisco, San Mateo, CA; [5]Univ. of California San Francisco, San Francisco, CA; [6]Memory and Aging Center, Univ. of California, San Francisco, San Francisco, CA; [7]Mayo Clin., Jacksonville, FL

Abstract: Frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) are united by shared etiopathogenesis and the selective vulnerability of distinct Later 5 extratelencephalic (L5ET) neuron subtypes: von Economo neurons (VENs) in FTD and Betz cells in ALS.

Although these neurons have been studied extensively using histological approaches, transcriptomic analysis has been challenging due to low abundance. Here, we performed single nucleus RNA sequencing of the frontoinsular cortex in 40 individuals spanning the FTD-ALS clinical spectrum linked to underlying TDP-43 Type B pathology, with and without a pathogenic C9orf72 expansion. By pooling samples into a single experiment and enriching for large neurons, we limited batch effects and enriched for L5ET neurons capturing 7,258 neurons in this cluster from a total of 224,793 cells. Further analysis of this cluster revealed novel, molecularly distinct subtypes. Remarkably, cluster-free differential abundance analysis revealed convergent depletion of one L5ET subtype, as well as one subtype of L2/3 excitatory neurons, in sporadic and *C9orf72* FTD-ALS. Finally, we identified candidate transcriptional changes associated with FTD-ALS and link these to CSF proteomic biomarker results. Together, our findings further illuminate the molecular taxonomy underlying the selective vulnerability of L5ET and L2/3 excitatory neurons in FTD-ALS, highlight candidate biomarkers, and nominate potential therapeutic targets.

Disclosures: A. Breevoort: None. **D. Ivanov:** None. **S.C. Vatsavayai:** None. **A. Nana:** None. **A. Tudoras Miravet:** None. **F. Pereira:** None. **R. Saloner:** None. **W.W. Seeley:** None. **A.A. Pollen:** None.

Nanosymposium

NANO031: Other Dementias, Proteinopathies, and Pathologies

Location: SDCC Rm 1

Time: Tuesday, November 18, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO031.08

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

Support: NIH/NINDS Grant NS127211

Title: Functional and multi-omic approaches to define the brain cell type-specific effect of CSPα mutants on proteostasis

Authors: *M. J. ROSENE[1,3], A. LOPEZ CADAVID[2], M.-Y. J. LAI[4], B. A. BENITEZ[5]; [1]Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Neurol., Beth Israel Deaconess Med. Ctr., Cambridge, MA; [3]Neurol., [4]BIDMC/Harvard Med. Sch., Boston, MA; [5]Neurol., Harvard Med. Sch. - BIDMC, Boston, MA

Abstract: Objective: Combine computational multi-omic approaches with biochemical experiments in *in vitro* and *in vivo* models to investigate the role of CSPα in multiple brain cell types. **Background:** *DNAJC5* encodes for the neuroprotective cochaperone cysteine string protein-α (CSPα). CSPα mutants cause adult neuronal ceroid lipofuscinosis (ANCL), characterized by endolysosomal dysfunction, microgliosis, synaptic loss, and protein aggregation. Single-nuclei RNAseq has shown elevated expression of CSPα in neurons and, interestingly, in microglia. We hypothesized that CSPα regulates brain proteostasis on a cell type-specific basis. **Methods:** We analyzed whole-transcriptome/proteome data from human

neuronal, astrocyte, and microglial lines stably overexpressing wild-type (WT) and mutant CSPα, along with proteomic data from ANCL patients (N=4), healthy controls (N=5). Biochemical analyses were performed on cell lysate/conditioned media from cells treated with ALP modulators. We validated ANCL brain features in ANCL patient-derived iPSC-derived cortical neurons. **Results:** Human cell lines expressing mutant CSPα exhibit high molecular weight aggregates, but to differing extents among neuronal, microglial, and astrocytic cells. Aggregates are also observed to be secreted into the conditioned medium of neuronal cells. Neuronal and microglial lines expressing mutant CSPα showed increased depalmitoylated CSPα and impaired autophagy. Autophagy activators decreased monomeric and aggregated CSPα, while lysosomal inhibitors increased them. iPSC-derived ANCL neurons replicate the phenotype observed in brain tissue. Post mortem brain tissue and cell line proteomics revealed altered ALP protein expression. Secretomes from the cultured cell lines showed altered levels of cochaperones and proteins associated with inflammation in microglial cells. **Conclusion:** Our study reveals cell-type-specific CSPα aggregation phenotypes and altered proteostatic pathways. Ongoing multi-omics analysis will determine cell type-specific CSPα-associated neurodegenerative pathways. Future functional assays will elucidate how CSPα and its related aggregates are processed in each brain cell type.

Disclosures: M.J. Rosene: None. **A. Lopez Cadavid:** None. **M.J. Lai:** None. **B.A. Benitez:** None.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.01

Topic: F.09. Motor Neurons and Muscle

Support: NIH/NCI K01CA190235
The Kavli Foundation
The Hellman Foundation Fellowship
The Natural Sciences and Engineering Council of Canada Fellowship

Title: Spinal motor neuron development, metabolism, and neuromuscular junction formation are transcriptionally regulated by Nuclear Factor IA

Authors: *J. GAUBERG, S. MUNOZ, S. GLASGOW;
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Abstract: The neuromuscular junction (NMJ) is a key site of vulnerability in aging and neuromuscular disease, yet the transcriptional programs that ensure its proper formation during development are not fully understood. Here, we identify **Nuclear Factor-IA (NFIA)** as a critical regulator of both development and metabolism in spinal motor neurons. Using conditional knockout mice, transcriptomic/genomic profiling, biochemical assays, and morphological

analyses, we show that NFIA is required for motor neuron positioning, axonal arborization, and NMJ formation. Loss of NFIA leads to disorganized motor axon arbors and impaired NMJ connectivity, revealing its essential role in motor circuit assembly. Unexpectedly, we also find that NFIA supports mitochondrial function and ATP production, revealing a transcriptional mechanism that links energy metabolism to neuronal maturation. These findings position NFIA as a central coordinator of motor neuron identity, connectivity, and bioenergetics, providing a developmental framework to better understand NMJ vulnerability in aging and disease.

Disclosures: J. Gauberg: None. **S. Munoz:** None. **S. Glasgow:** None.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.02

Topic: F.09. Motor Neurons and Muscle

Title: Netrin-1 participates to motor units' selective vulnerability to ALS

Authors: *X. KOLICI[1,2], V. VALSECCHI[1], G. LAUDATI[1], G. PIGNATARO[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that leads to relentless loss of almost all motor units. Interestingly, extraocular muscles (EOMs) remain relatively spared until the final stages of the disease. Several lines of evidence have suggested a tight link between ALS pathophysiology and axon guidance pathways. Among them, Netrin-1 emerged as one of the most significant. Indeed, Netrin-1 can act as both an attractant and repellent axon guidance signal, influencing axonal projections and pathways during nervous system development and regeneration. It can bind to receptors like DCC and Neogenin to attract axons, or to UNC5 receptors to repel them. Our work aimed to investigate and characterize the possible role of Netrin-1's pathway in neuromuscular junction's differential susceptibility to ALS. To this purpose, the expression of Netrin-1 and its receptors UNC5A and DCC was evaluated in EOMs, gastrocnemius and soleus muscles of G93A-SOD1 mice by means of qRT-PCR and Western Blot analysis, in pre- and late-symptomatic stages of the disease. Our data showed an up-regulation of Netrin-1 in EOMs of ALS mice in a presymptomatic stage. Additionally, while we didn't observe variations in UNC5A receptor's levels, DCC was exclusively expressed by EOMs and not by vulnerable limb muscles. Ongoing experiments are being carried out in the attempt to assess the hypothesis of Netrin-1's implication in muscle responsiveness to disease. To this aim, Netrin-1 has been silenced or overexpressed in the C2C12 myoblast cell line and in the NSC-34 motor neuron-like hybrid cell line exposed to L-BMAA, a reported ALS-like stimulus. Incucyte system, MTT and LDH analysis will be used to evaluate cell proliferation and cell viability in the different experimental conditions. Our results may pave

the way for the pathophysiological characterization of Netrin-1's pathway in ALS skeletal muscles and its influence on muscle-motor neurons communication.

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Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.03

Topic: F.09. Motor Neurons and Muscle

Support: Hightech-Agenda Bavaria
UKRI Turing AI Fellowship (EP/V025449/1)

Title: Motor unit tracking across force paradigms and subject-independent finger force prediction from spike trains

Authors: *R. MIO ZALDIVAR[1,2], J. BODENSCHLÄGEL[1], A. A. FAISAL[2,1];
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Abstract: Unlocking multiple control channels from the spinal cord for advanced neural interfaces requires robust motor unit (MU) tracking during real-world force production and characterisation of the neural drive-to-force relationship in a generalisable, subject- and task-independent manner. This study lays the groundwork by addressing two key aspects: (1) tracking individual MUs across constrained and unconstrained force control tasks, and (2) developing a pipeline for regression models that decode force from MU spike trains across subjects and tasks. We recorded high-density surface electromyography (2 grids of 64 channels) from the forearm flexors of healthy participants (N = 25, age 28.8 ± 7.7 years, 15 female) during isometric thumb, index, and middle finger flexion. Participants performed a total of 30 constrained trials (trapezium force trace with a 60-second plateau at 10% and 20% of maximum finger force tracked by a single digit) and 5 unconstrained trials (30-second multi-digit variable force tasks). MU spike trains were decomposed using convolutive kernel compensation. For our first goal, we tracked each subject's MUs across tasks using the 2D cross-correlation between MU activation maps and MU action potential waveforms at each electrode channel. Only MUs with a cross-correlation above 0.9 were considered matches. Crucially, MUs could be reliably tracked from constrained into unconstrained tasks. On average per subject, 10.6 ± 12.1 MUs (16.3% of the total MU pool for that condition) decoded from thumb flexion, 19.2 ± 19.0 MUs (21.8%) from index finger flexion, and 56.2 ± 80.3 MUs (53.6%) from middle finger flexion were tracked. For our second goal, we focused on the ramp-up phase of the trapezium force trace where MU recruitment occurs. We developed and tested a pipeline to predict varying finger force using the smoothed discharge rates of 10 representative MUs (5 early-, 5 late-recruited) as input features. Unlike prior subject-specific approaches, our regression models (Linear, Ridge, GAM, GLM,

XGBoost, RNN, GRU, LSTM) were trained on data pooled across all subjects and task conditions. After hyperparameter tuning, the LSTM model with a 250 ms window achieved the best prediction accuracy ($R^2 = 0.925$) and showed robust generalisation across subjects and target digit conditions, a critical requirement for user-independent interfaces. Our findings show that specific MUs are identifiable within individuals across constrained and unconstrained conditions. Moreover, our machine learning pipeline, built on a set of representative MUs, supports subject-independent neural interfaces and enables data pooling across subjects, tasks, and datasets.

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Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

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Presentation Number: NANO032.04

Topic: F.09. Motor Neurons and Muscle

Support: Nemours Foundation
Delaware CTR ACCEL Program (U54GM104941)
Moseley Foundation - CP Cell Therapy

Title: A Single Cell RNA-Seq Study of Satellite Cell Derived Myoblast Gene Expression in Spastic Cerebral Palsy

Authors: K. ROBINSON[1], J. HICKS[2], M. BATISH[3], ***R. E. AKINS**[4];
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Abstract: Cerebral palsy (CP) is a static encephalopathy caused by damage to the developing brain that results in neuromotor dysfunction characterized by muscle spasticity, poor coordination and weakness. CP is a leading cause of physical disability in children. Individuals with spastic CP have impaired longitudinal skeletal muscle growth and contractures that may contribute to impaired motor control, muscle atrophy, and increased need for clinical care or surgery. Muscle stem cells (satellite cells) are responsible for skeletal muscle growth and repair, and studies have demonstrated that there is a decreased number of satellite cells in CP and that these cells exhibit inhibited myotube formation in vitro; however, it is unclear if SC heterogeneity contributes to this effect or dysfunction in CP. We investigated differences in SC subpopulations in cells isolated from participants with spastic CP and controls using single cell RNA-sequencing (scRNA-seq). Surgical explants of skeletal muscle were obtained from subjects undergoing orthopedic surgery at the Nemours Children’s Hospital, Delaware, following IRB approved informed consent/assent. Seven subjects with spastic CP and 7 control subjects were enrolled in the study. Satellite cells were isolated by double immunomagnetic selection for CXCR4 and NCAM1. SC phenotype was confirmed by MYF5 immunofluorescence. Single cells were isolated from proliferating satellite cell-derived myoblasts (SC-MBs) and lysed to capture

RNA molecules. Barcoded next-generation sequencing (NGS) cDNA libraries were prepared and sequenced on a NextSeq 2000. scRNA-seq profiles from CP and control cohorts were compared using the R package Seurat. As expected, both cohorts displayed the same overall profile of SC subpopulations with cells separating into 14 clusters. Clusters were identified as representing different phases of the cell cycle and different stages of myogenesis. There were fewer differentiating cells in the CP cohort compared to controls and in the male cohort compared to females. Differentially expressed genes identified within each subpopulation may account for CP-associated differences seen in skeletal muscle satellite cells.

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Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.05

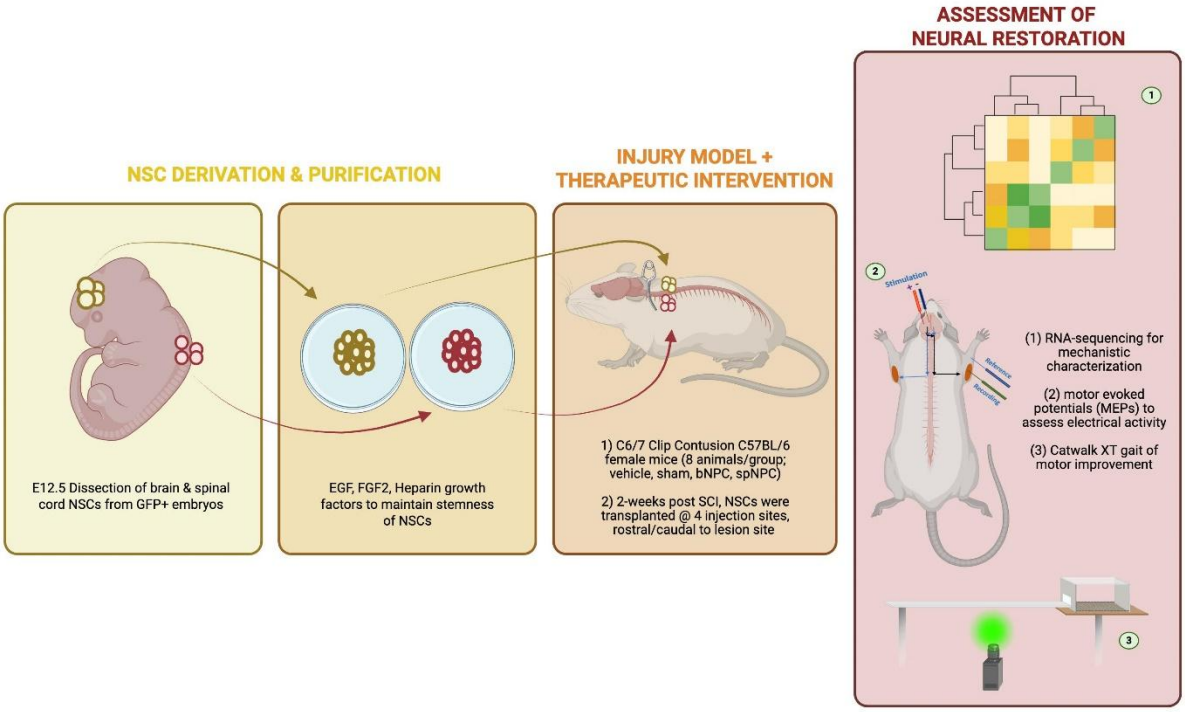
Topic: D.05. Spinal Cord Injury and Plasticity

Title: Regional Spinal Cord Identity of Neural Stem Cells Promote Optimal Synaptic Function and Motor Recovery Following Traumatic Spinal Cord Injury

Authors: *W. MCINTYRE[1], S. KOUHZAEI[3], A. ASGARIHAFSHEJANI[3], M. KHAZAEI[4], M. G. FEHLINGS[2];
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Abstract: Neural stem cells (NSCs) are a viable transplant therapeutic for sparing and replenishing the cellular niche following traumatic spinal cord injury (tSCI). However, optimal cell type and mechanisms of recovery remain poorly characterized. Particularly, it is unclear whether NSCs, or NSCs that are regionalized to the spinal cord, can promote more optimal recovery of motor function following tSCI. Thus, this study aims to directly compare NSCs, derived from the brain (b) and spinal cord (sp), to assess whether a regionally matching graft can promote an optimal therapeutic for tSCI. bNSCs and spNSCs were dissected from E12.5 GFP+ mice and transplanted into the cervically injured spinal cord (N=8/group). Assessment of neural restoration was evaluated through CatWalk XT gait analysis system, electrophysiology, and RNA-sequencing (figure attached). RNA-seq revealed drastic transcriptomic changes in both neuron and astrocytes between regional sources, outlining differences in axonal guidance (GO:0007411) and astrocytic-specific neurotransmitter transport (GO:0006836). in vivo, transplanted spNSCs enhanced recovery of motor forelimb function, specifically swing speed (**sp**: 51.1cm/s$\pm$5.0; **b**: 42.6cm/s$\pm$4.2; **p<0.05**) and stride length (**sp**: 5.4cm$\pm$0.6; **b**: 4.4cm$\pm$0.5; **p<0.05**), outlining improved speed and stability of movement. Motor evoked potentials, which electrically stimulate the motor cortex to relay a signal to the bicep/forelimb, showed spNSC-transplanted animals exhibited a lower electrical threshold to elicit a response (**sp**: 5.1mV$\pm$0.6;

b: 10.0mV±0.9; **p<0.01**). Enhanced motor recovery, alongside a lower threshold of electrical response, indicates enhanced electrophysiological integrity across the lesion, suggesting greater restoration of neural circuitry. This study confirms genetic differences in the mature cells derived from regional NSCs, which can explain their ability to differentially promote recovery after tSCI. Thus, regional identity of grafted NSCs should be a priority consideration when developing future cell therapeutics for human tSCI.



Disclosures: W. McIntyre: None. **S. Kouhzaei:** None. **A. Asgarihafshejani:** None. **M. Khazaei:** None. **M.G. Fehlings:** None.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.06

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Canadian Institutes for Health Research (CIHR)
Wings for Life
International Spinal Research Trust

Title: Rehabilitative Training Modifies Synaptic and Neurogenic Gene Expression in Grafted Neural Progenitor Cells Following Spinal Cord Injury

Authors: *M. KHAZAEI[1], M. M. ZAVVARIAN[2], C. S. AHUJA[2], J. HONG[3], S. KOUHZAEI[2], A. ASGARIHAFSHEJANI[2], D. A. DESKA-GAUTHIER[2], G. BALBINOT[2], K. K. FENRICH[4], K. FOUAD[5], M. G. FEHLINGS[6];
[2]Genet. and Develop., [1]Krembil Res. Institute, Univ. Hlth. Netw, Toronto, ON, Canada; [3]Genet. and Develop., Krembil Res. Inst., Toronto, ON, Canada; [4]Fac. of Rehabil. Med., [5]Physical Therapy, Univ. of Alberta, Edmonton, AB, Canada; [6]Div. Neurosurg., Univ. of Toronto, Toronto, ON, Canada

Abstract: Spinal cord injury (SCI) is a devastating neurological condition with limited treatment options, particularly for cervical SCIs, which severely impair upper limb function. Despite significant progress in preclinical therapeutic strategies, their clinical translation remains challenging. Among promising interventions, neural progenitor cell (NPC) transplantation combined with task-specific rehabilitation capitalizes on the synergistic effects of cellular regeneration and activity-dependent plasticity to enhance functional recovery. However, the underlying molecular mechanisms governing this process remain incompletely understood.Here, we investigated the transcriptomic landscape of human NPCs transplanted into a rat model of cervical SCI subjected to task-specific forelimb rehabilitation. Using a clip compression injury model, GFP-labeled NPCs were engrafted into the lesion site, and their gene expression profiles were analyzed to delineate rehabilitation-induced transcriptional changes. Pathway enrichment analysis revealed significant upregulation of genes associated with neuronal differentiation, synaptogenesis, neurotransmitter signaling, and anti-inflammatory responses. Key transcriptional regulators, including BDNF, NEUROD1, and SOX11, were strongly implicated in synaptic integration and NPC survival. Rehabilitation-induced activity was found to promote synaptic plasticity while modulating the local inflammatory milieu at the lesion site. Functional validation using tetanus toxin light chain (TeTxLC)-mediated synaptic blockade confirmed that neuronal activity is essential for driving these molecular adaptations.Our findings underscore the pivotal role of rehabilitation-driven transcriptional programs in shaping NPC integration and functional recovery after SCI. This study highlights the power of transcriptomic profiling and systems biology in unveiling the molecular underpinnings of rehabilitation-enhanced NPC therapy, offering novel insights for optimizing stem cell-based interventions in SCI.

Disclosures: M. Khazaei: None. **M.M. Zavvarian:** None. **C.S. Ahuja:** None. **J. Hong:** None. **S. Kouhzaei:** None. **A. Asgarihafshejani:** None. **D.A. Deska-Gauthier:** None. **G. Balbinot:** None. **K.K. Fenrich:** None. **K. Fouad:** None. **M.G. Fehlings:** A. Employment/Salary (full or part-time):; Krembil Research Institute.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.07

Topic: D.05. Spinal Cord Injury and Plasticity

Title: Resting-state Functional Connectivity Dynamics Associated with Motor Recovery After Spinal Cord Injury in the Marmoset Brain

Authors: *A. TOGA[1], N. NAGOSHI[1], T. KONDO[1], T. OKUNO[2], J. HATA[2], T. SHIMIZU[1], Y. SATO[1], H. OKANO[3], M. NAKAMURA[1];
[1]Dept. of Orthopaedic Surgery, Keio Univ. Sch. of Med., Tokyo, Japan; [2]Grad. Sch. of Human Hlth. Sci., Tokyo Metropolitan Univ., Tokyo, Japan; [3]Keio Univ. Regenerative Med. Res. Ctr., Kanagawa, Japan

Abstract: Introduction: Spinal cord injury (SCI) disrupts neural pathways, inducing significant neural plasticity in the central nervous system. Clarifying the neural changes after SCI is essential to understand functional recovery mechanisms. Previous studies focused primarily on localized brain regions; however, whole-brain adaptations remain unclear. Resting-state functional magnetic resonance imaging (rs-fMRI) is a powerful tool for examining whole-brain functional connectivity (FC). This study longitudinally characterized FC changes following cervical spinal cord hemisection in common marmosets at early (3 weeks) and late (7 weeks) recovery stages.
Methods: Five common marmosets underwent cervical spinal cord hemisection at C4/5. Motor functions were quantitatively assessed weekly using an open field scoring system. Animals were habituated to the MRI scanning environment prior to rs-fMRI recording. rs-fMRI was conducted at 9.4T across 12 sessions per animal in awake conditions, using head-post fixation to minimize motion artifacts. FC was analyzed across 327 brain regions by calculating correlation matrices. Additionally, average blood-oxygen-level-dependent (BOLD) signal intensities were calculated. Structural connectivity was validated using neuroanatomical tracer data from the Brain/MINDs database.
Results: Motor function scores improved progressively each week following injury. The number of significant FC pairs decreased markedly from 291 pairs at 3 weeks to 93 pairs at 7 weeks post-injury. At 3 weeks post-injury, widespread increases in FC relative to pre-injury were observed, particularly involving motor-related and motivational brain regions, including the anterior cingulate cortex, premotor cortex, and prefrontal cortical areas. Corresponding significant increases in regional BOLD signal intensities were detected in the primary motor cortex, premotor cortex, and anterior cingulate cortex. By 7 weeks post-injury, FC changes became more localized, predominantly involving higher-order cognitive and motivational regions, including the anterior cingulate cortex, orbitofrontal cortex, and nucleus accumbens. Conclusion: The present study longitudinally characterized brain FC changes following cervical SCI in the common marmoset. Early widespread FC alterations evolved into more localized connectivity patterns centered on frontal motor and motivational regions. These temporal FC dynamics and associated BOLD signal changes enhance understanding of neural adaptations underlying motor recovery after SCI, highlighting potential targets for brain-based therapeutic interventions.

Disclosures: A. Toga: None. **N. Nagoshi:** None. **T. Kondo:** None. **T. Okuno:** None. **J. Hata:** None. **T. Shimizu:** None. **Y. Sato:** None. **H. Okano:** None. **M. Nakamura:** None.

Nanosymposium

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Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.08

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH Grant F31HL174079
NIH Grant T32HL134621
NIH Grant R01HL153102
Bryan Robinson Grant for the Neurosciences

Title: Inhibitory circuits contribute to epidural stimulation induced respiratory motor output patterning after spinal cord injury

Authors: *A. MICKLE, C. BRENNAN, J. PENALOZA APONTE, E. A. DALE;
Univ. of Florida, Gainesville, FL

Abstract: Our lab has shown epidural electrical stimulation at C4 can restore some diaphragm EMG in an acute, anesthetized rat model of spinal cord injury (Mickle et al. 2024). Additionally, we have shown stimulation can support endogenous respiratory patterning of the ipsilesional hemidiaphragm, with diaphragm EMG activity concentrated during inspiration in response to constant stimulation across the respiratory cycle. However, the local spinal circuitry that contribute to the ability of the ipsilesional diaphragm to generate appropriate pattern in response to unpatterned stimulus remains unknown. First, to determine what local spinal circuits respond to electrical stimulation at C4, rats were urethane anesthetized, cycle triggered mechanically ventilated, and implanted with diaphragm EMG recording and C4 stimulating electrodes. Rats were then left spinally intact and underwent stimulation (n=8), or a sham period (n=5), or were C2-hemisected and stimulated (n=8) or sham (n=6). An hour post stimulation, rats were perfused and C4 spinal tissue harvested and prepared for RNAScope to stain for FOS (a marker of increased neuronal firing) mRNA. While excitatory neurons did not make up a larger proportion of FOS expressing neurons after stimulation (stim vs. sham %VGLUT 41.3 vs 44.4, $p = 0.08$) stimulation specifically increased the proportion of FOS in inhibitory cells (stim vs. sham %VIAAT 21.7 vs 8.3, $p = 0.0001$), particularly in spinal laminae 3 and 4. To identify if this increase in inhibitory firing contributes to the ability of epidural stimulation to elicit patterned motor output, we next gave C2-hemisected rats stimulation as detailed previously, administered 10 μl of 50 mM picrotoxin (PTX) intrathecally at C4 to block inhibitory firing, and repeated stimulation (n=4). As previously described (Cregg et al. 2017), administration of PTX resulted in spontaneous diaphragm bursts of longer duration and at lower frequency than eupneic bursting in 3/4 rats. These bursts were also commonly elicited at the very onset of stimulation. PTX administration potentiated EMG response magnitude (mean EMG mV 0.022 vs 0.055, $p = 0.029$); however, in the absence of inhibitory inputs, appropriate EMG patterning on the ipsilesional side of the diaphragm in response to constant stimulation was blunted (expiratory/inspiratory ratio pre vs. post PTX 0.76 vs 0.97). These data indicate that while inhibitory neurons play a role in negative feedback loops dampening diaphragm response to electrical stimulation, they also contribute to appropriate ipsilesional diaphragm function by ensuring that EMG response to stimulus is not tonic.

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Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.09

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NSFC 82350002

Title: Engineered clMagR-MSCs with Magnetic Stimulation Enhance Iron Detoxification and Mitochondrial Transfer for Spinal Cord Injury Repair

Authors: *L.-J. ZONG[1], H. WANG[2];
[1]Southeast Univ., Nanjing, China; [2]Zhongda Hosp. Southeast Univ., Nanjing, China

Abstract: Mesenchymal stem cells (MSCs) have demonstrated significant potential in the treatment of spinal cord injury (SCI). However, their efficacy is hindered by the challenge of maintaining viability within the iron-overloaded acute injury microenvironment, where excess iron has been observed to induce neuronal ferroptosis. To address this issue, we genetically engineered mesenchymal stem cells (MSCs) to overexpress an iron-sensing protein from pigeon (*Columba livia*), magnetoreceptor (clMagR). Previous studies have demonstrated that this enables free iron to be converted into iron oxide nanoparticles, thereby potentially reducing iron toxicity. Furthermore, we implemented magnetic stimulation to augment mitochondrial biogenesis and intercellular transfer via tunneling nanotubes (TNTs), a well-established neuroprotective pathway. In female rats with SCI, the combination of clMagR-MSCs and magnetic stimulation resulted in a substantial enhancement of locomotor function, as evidenced by Basso, Beattie, and Brody (BBB) scores and gait analysis. Fluorescence imaging revealed prolonged graft retention, with retention times reaching up to 21 days. In vitro, under conditions of ferric ammonium citrate (FAC)-induced iron overload, clMagR-MSCs exhibited superior viability and neuroprotective efficacy in comparison to unmodified cells. When co-cultured with PC12 neurons, the cells restored mitochondrial membrane potential, reduced reactive oxygen species (ROS), and enhanced neuron survival. The transfer of mitochondria through TNTs was confirmed by microscopy and inhibited by pathway-specific blockers. Transcriptomic analysis of magnetically stimulated clMagR-MSCs revealed upregulation of mitochondrial biogenesis pathways, particularly PGC1α. The results of this study suggest that clMagR-MSCs, through iron detoxification and enhanced mitochondrial delivery, improve cell survival and neurological recovery after SCI. This dual strategy offers a streamlined approach to overcoming acute microenvironmental barriers and advancing stem cell therapies in neuroregeneration.

Disclosures: L. Zong: None. **H. Wang:** None.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.10

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation grant #999331 to DJN

Title: Respiratory rate conditioning for pain control in spinal cord injured mice

Authors: *D. J. NOBLE, M. A. SAWCHUK, S. HOCHMAN;
Cell Biol., Emory Univ., Atlanta, GA

Abstract: Neuropathic pain after spinal cord injury (SCI) is associated with changes in breathing, which could signal acute cardiorespiratory distress and predict long-term outcomes. In particular, respiratory rate (RR) and its variability may predict clinical outcomes in pain research. Our lab previously showed that operantly conditioning slowed RR (sRR) in rats alleviated mechanical hypersensitivity in an inflammatory pain model, suggesting that breath control has untapped potential as a non-pharmacological therapeutic intervention. However, the training paradigm and therapeutic effects have yet to be replicated in mice, precluding a clearer understanding of the neurobiological mechanisms underlying pain relief. This study sought to validate operant conditioning procedures in mice and assess the effects of sRR training on SCI-induced pain. Breathing was recorded over five 30-minute sessions using remote biofield sensors affixed to small acrylic chambers containing individual animals. Mice were operantly conditioned using sensory reinforcement, with half trained to slow their breathing and half to speed it up (fast RR, or fRR). A customized LabVIEW interface turned off an aversive strobe light (negative reinforcement) or released a pleasurable odorant (positive reinforcement) whenever mice breathed below (sRR) or above (fRR) their baseline RR. Resting RR and its variability were quantified using dedicated analysis software. The mice diverged in RR over five training sessions (significant group x session interaction, 2-way ANOVA), with sRR training being more effective in the mice receiving odorant reinforcement and fRR training more effective in those receiving strobe reinforcement. In both cases, RR variability, the standard deviation of resting RR, significantly increased in fRR vs. sRR mice. Preliminary assessments of pain-related behavior revealed altered thermal preferences and a trend toward reduced mechanical hypersensitivity in sRR-trained mice. Our findings validate operant conditioning procedures to control RR in mice. Ongoing studies are aimed at elucidating the neural changes associated with training, and investigating a variety of relaxing and reinforcing odorants for their ability to impact clinically relevant SCI outcomes.

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Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.11

Topic: F.09. Motor Neurons and Muscle

Title: Neurotransmitter receptor change in the motor cortex after peripheral nerve injury

Authors: E. OROZCO[1], ***S. B. SHAH**[2];
[1]Biol. Sci., UC San Diego, La Jolla, CA; [2]UCSD, La Jolla, CA

Abstract: Peripheral nerve injuries result in sensorimotor loss, pain, and loss of independence. Surgical reconstruction of nerves restores some function, but recovery is often incomplete. The central nervous system (CNS) is a critical, yet poorly understood and understudied contributor to improved neuromuscular functional recovery. Electrical mapping in the motor cortex reveals cortical remodeling after nerve injury. In the immediate phase, regions of cortical maps representing the injured nerve shrink or disappear, and pre-existing inputs from neighboring regions rapidly enlarge their representations in these regions ("unmasking"), due to the absence of a competing dominant input. This enlarged representation then strengthens over time. Molecular pathways proposed to underlie unmasking and subsequent strengthening in the motor cortex after peripheral nerve injury have not been formally tested. We hypothesized that this remodeling reflects reduced inhibition by GABAergic neurons and increased activity of excitatory glutamatergic neurons. To test this hypothesis, sciatic nerves of male and female C57/Bl6 mice 8-10 weeks of age were injured unilaterally, and brains were harvested at time points up to 4 months after injury. Motor cortical regions corresponding to the sciatic nerve and adjacent forelimb domains were evaluated bilaterally. GABA, GluR1, GluR2/3, and NMDA receptor transcript levels were measured using quantitative real-time PCR and protein expression of these receptors was measured using capillary electrophoresis (JESS) and immunofluorescence (IF). At time points as early as 3 days after injury, consistent with our hypothesis, reductions in GABA receptor and increases in GluR1 and GluR2/3 were observed in the forelimb/hindlimb transition region of the motor cortex compared to contralateral and sham injury controls. These changes were also observed at 14 days after injury, with higher variability at 7 days after injury. Receptor changes were concurrent with changes in dendritic morphology in motor cortical layers 2-3, as observed by IF. Collectively, our data demonstrate rapid structural and biological changes in the motor cortex after peripheral nerve injury, which may underlie the unmasking phenomenon. We are currently evaluating reversibility of these changes and implications for intervening in receptor plasticity pathways on neuromuscular recovery after nerve injury. The CNS represents a promising new target for therapy after peripheral nerve injury, to enhance functional recovery.

Disclosures: E. Orozco: None. **S.B. Shah:** None.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.12

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NRF grant RS-2022-NR072349
NRF grant RS-2024-00341308
NRF grant RS-2023-00225555

Title: Investigation of NPTX2-associated neural plasticity during vagus nerve chemogenetic stimulation-induced locomotor recovery after spinal cord injury

Authors: *S. RYU[1], S.-E. ROH[2];
[1]Eulji University, Sch. of medicine, Ellicott City, Korea, Republic of; [2]Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Spinal cord injury (SCI) leads to severe and often irreversible motor deficits due to the limited regenerative capacity of the central nervous system. Recent studies suggest that neuromodulatory strategies, such as vagus nerve stimulation (VNS), may enhance functional recovery by promoting circuit-level plasticity. In this study, we employed chemogenetic stimulation of the vagus nerve to investigate its role in facilitating locomotor recovery and plasticity mechanisms after SCI in rats. Adult rats received a thoracic spinal cord contusion injury and were subsequently treated with excitatory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) targeting the vagus nerve. Clozapine (CLZ) was administered to activate the DREADD-expressing vagal circuits. Locomotor recovery was longitudinally assessed over eight weeks using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale. Notably, animals that received chemogenetic stimulation (CS) exhibited progressive improvement in BBB scores compared to SCI-only and control virus groups, suggesting a functional benefit of vagal neuromodulation. To elucidate the molecular and circuit-level mechanisms underlying this recovery, we examined the expression and distribution of NPTX2, an activity-regulated immediate early gene known to mediate excitatory synapse formation and plasticity. NPTX2-related changes were assessed in sensory, motor, and prefrontal cortical regions, given their relevance to injury response and neurorehabilitation. Additionally, in light of the growing recognition of diaschisis—remote cortical and subcortical dysfunction following focal CNS injury—we explored whether NPTX2 expression changes may reflect network-level reorganization beyond the lesion site. Our current investigation focuses on how vagus nerve stimulation influences neuroplasticity across functionally interconnected regions via NPTX2-associated pathways. These findings may provide a foundation for novel rehabilitation strategies leveraging endogenous plasticity mechanisms and clarify the contribution of NPTX2 in the context of CS-driven motor recovery, particularly in the modulation of sensory-motor integration, higher-order cortical remodeling, and resolution of diaschisis-related dysfunction following SCI.

Disclosures: S. ryu: None. **S. Roh:** None.

Nanosymposium

NANO032: Spinal Cord: Development, Injury, and Disease

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO032.13

Topic: F.09. Motor Neurons and Muscle

Title: Individuals with multiple sclerosis exhibit lower force steadiness and intermuscular coherence than healthy controls during isometric plantar flexion

Authors: *M. HENRY[1], T. CROMPTON[1,2], R. M. ENOKA[1];
[1]Integrative Physiol., Univ. of Colorado Boulder, Boulder, CO; [2]Cardiff Univ., Cardiff, United Kingdom

Abstract: Multiple sclerosis (MS) impairs central nervous system function, resulting in significant mobility deficits. Force steadiness and intermuscular coherence provide insight into the neural drive to muscles and may reveal adaptations underlying motor impairment in MS [1,2]. However, intermuscular coherence has not been studied in people with MS and only a few studies have examined limb differences in force steadiness, despite well-documented asymmetry in MS-related motor deficits. This study addressed these gaps by comparing force steadiness and intermuscular coherence across limbs between people with MS and healthy controls. Fifteen people with MS (44 ± 9 years, PDDS score 0-6) and 11 healthy controls (42 ± 13 years) performed five 30-s isometric plantar-flexion contractions at 15% of maximal strength with each leg. Electromyographic (EMG) signals were recorded from the soleus and gastrocnemius medialis (agonists), and the tibialis anterior (antagonist). Force steadiness was quantified as the coefficient of variation (CV) for force. Intermuscular coherence was calculated from the rectified EMG signals between muscle pairs and characterized with the peak amplitude and area under the curve in the alpha (6-15 Hz) and beta (16-30 Hz) bands—respectively linked to afferent feedback and corticospinal control [3]. The CV for force was greater in people with MS than controls ($p = 0.004$), and in the non-dominant/more affected leg ($p = 0.044$). Both coherence amplitude and area under the curve in the beta band were lower in people with MS across all muscle pairs ($p \leq 0.024$). Similarly, coherence in the alpha band was reduced in the MS group only in the soleus-tibialis anterior pair ($p \leq 0.004$). Coherence outcomes did not differ between legs ($p \geq 0.35$). The greater CV for force in people with MS likely reflects increased variability in neural drive [1]. Lower beta-band coherence suggests reduced shared cortical input [2], consistent with impaired corticospinal transmission due to MS lesions [3]. Lower alpha-band coherence in the soleus-tibialis anterior pair may indicate disrupted afferent feedback or alterations in reciprocal inhibition. Leg asymmetry in force steadiness—but not in coherence—suggests that neural drive variability differs between limbs, whereas shared synaptic input does not. These findings provide the first evidence of altered intermuscular coherence in people with MS and introduce new neural markers for characterizing motor dysfunction in MS. 1. Enoka & Farina. 2021. Physiology (Bethesda) 36(2): 114-130 2. Grosse et al. 2002. Clin Neurophysiol 113(10): 1523-1531 3. Pawlitzki et al. 2017. Neurol Neuroimmunol & Neuroinflamm, 4(6): e399

Disclosures: M. Henry: None. **T. Crompton:** None. **R.M. Enoka:** None.

Nanosymposium

NANO033: Neuroprosthetics: Control of Real and Artificial Arm, Hand, and Other Grasping Devices

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO033.01

Topic: F.05. Brain-Machine Interface

Support: CDMRP W81XWH-22-1-1119

Title: A streamlined closed-loop wearable functional electrical stimulation neuroprosthetic for upper limb rehabilitation

Authors: ***N. TACCA**[1], C. DUNLAP[1], S. COLACHIS, IV[1], B. SCHLINK[1], M. HEIMANN[1], P. PUTNAM[1], S. CADY[1], L. WENGERD[2], D. FRIEDENBERG[1];
[1]Battelle Mem. Inst., Columbus, OH; [2]Ohio State Univ., Columbus, OH

Abstract: Injuries to the central nervous system, such as spinal cord injury (SCI) and stroke, can severely limit upper limb function, reducing independence and quality of life. Physical rehabilitation, often supplemented by functional electrical stimulation (FES), remains the standard of care to improve motor outcomes. Recent advances suggest that pairing FES with a user's volitional intent can further enhance recovery by engaging neuroplastic mechanisms. Electromyography (EMG) offers promise as a natural, non-invasive interface for decoding motor intent to activate FES. However, the lengthy and complex calibration procedures required by current EMG-based neuroprosthetic systems present a significant barrier to their practical use in both clinical and home settings. To address this challenge, we evaluated strategies to streamline the setup of a wearable forearm sleeve system that integrates high-density EMG and FES for closed-loop hand function restoration. Our focus was on reducing or eliminating the calibration time required before each session, thereby making the system more accessible and user-friendly. We leveraged data from prior sessions to build pretrained decoders for individuals with chronic SCI and stroke. This approach enabled rapid setup and quick transition to functional task practice, often eliminating the need for calibration entirely or reducing it to under five minutes. In able-bodied participants, we demonstrated that a generic decoder trained across users could achieve high decoding accuracy in a zero-shot setting, with further improvements possible by incorporating a small amount of new user data. Both supervised and unsupervised decoder update methods were explored, with unsupervised approaches showing potential to support operator-free adaptation in the future. These results show that calibration time for EMG-based neuroprosthetic systems can be substantially reduced, supporting more efficient rehabilitation and broader clinical use.

Disclosures: **N. Tacca:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **C. Dunlap:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **S. Colachis:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **B. Schlink:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **M. Heimann:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **P. Putnam:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **S. Cady:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **L. Wengerd:** A. Employment/Salary (full or part-time):; The Ohio State University. **D. Friedenberg:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.

Nanosymposium

NANO033: Neuroprosthetics: Control of Real and Artificial Arm, Hand, and Other Grasping Devices

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO033.02

Topic: F.05. Brain-Machine Interface

Support: National Science Foundation (NSF) under Grants CNS-2340997
National Science Foundation (NSF) under Grants CNS-2349771

Title: Leveraging EEG-based neural signals for user recognition

Authors: S. SUNDAR[1], P. GYREYIRI[2], *D. SHUKLA[2];
[1]Purdue Univ., West Lafayette, IN; [2]Univ. of Wyoming, Laramie, WY

Abstract: Leveraging EEG-based Neural Signals for User Recognition

Shruthika Sundar[1], Paul Sansah Gyreyiri[2], and Diksha Shukla[2]
[1]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, USA
[2]Department of Electrical Engineering and Computer Science, University of Wyoming, Laramie, USA

Abstract:
Reliable user authentication remains a critical challenge in digital security. Unlike traditional methods that can be spoofed or forgotten, neural signals offer a promising path to user identification. This study introduces an EEG-based approach that leverages brain responses to personalized visual stimuli as stable, identity-specific neural signatures.
In this study, thirty-four (34) volunteer participants (ages 18-40 years; Mean = 25.2 ± 6.64) each created a personalized *visual password* by selecting a familiar face (e.g., a public figure they admired/liked) and placing it within a symmetrical two-dimensional grid. These individualized passwords served as visual authentication cues. Each participant completed two EEG recording sessions using a DSI-24 wireless EEG headset, spaced 5-7 days apart. During each session, the participants were shown 60 visual password stimuli (30 personalized, 30 unfamiliar) in a random order presented on a computer screen, with a 1.5-second inter-stimulus interval. EEG data were preprocessed using standard techniques, segmented into 1-second epochs, and analyzed across canonical frequency bands (Delta to Gamma). For each epoch, 935 features were extracted per trial, including spectral power, wavelet coefficients, and inter-hemispheric asymmetries, enabling detailed characterization of individual neural responses.
Personalized stimuli elicited significantly stronger P300 responses at frontal and parietal electrodes (F3, F4, Fz, C3, P3), reflecting attentional engagement and potential for brain-based user recognition. While some variability was observed, the neural signatures showed a reasonable degree of consistency across trials and two sessions. A machine learning framework, such as a convolutional neural network (CNN), can be employed to model EEG responses to personalized versus random stimuli, enabling the development of neural identity tokens as a potential extension of our initial findings.

By tapping into recognition memory and attention processes, the findings in this work are a step toward bridging cognitive neuroscience and real-world biometric security, paving the way for neuroadaptive authentication systems grounded in stable neural signatures.
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Nanosymposium

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Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO033.03

Topic: F.05. Brain-Machine Interface

Support: CDMRP W81XWH-22-1-1119

Title: Evaluation of a High-Density EMG-FES Forearm Sleeve For Use in Neurorehabilitation

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Abstract: Individuals with spinal cord injury (SCI) and stroke experience severe motor and sensory impairments that alters their daily functioning. Loss of hand function significantly impacts the ability to perform activities of daily living, affecting quality of life and autonomy. To address these challenges, functional electrical stimulation (FES) is a widely used non-invasive rehabilitation technique for SCI that delivers electrical pulses to muscles, thereby inducing targeted contraction in paretic muscles. Recent advances in FES have incorporated electromyography (EMG) signals into a closed-loop feedback system. EMG serves as an indicator of voluntary motor intent, and when integrated with FES, allows for modulation of muscle activity in real-time. We hypothesize this EMG-controlled FES closed loop approach could improve outcomes in SCI and stroke rehabilitation by promoting more natural and coordinated muscle activation patterns. We are conducting preliminary studies to evaluate the feasibility of using a novel high-density electrode forearm sleeve that integrates EMG and FES in a closed loop system as a rehabilitative tool in individuals with chronic SCI and stroke. Participants who have completed the study have reported improvements in their ability to perform tasks independently after using the system including increased hand strength, coordination, and flexibility in their paretic hand. Furthermore, analysis of the high-density EMG data provides a unique window into potential changes in muscular activation patterns and neuromuscular connectivity. Our findings highlight the potential of our high-density EMG-FES

system to decode residual motor activity from the paretic arm, leverage these neural signals to facilitate functional motor recovery, and drive neuroplasticity following neurological injury.

Disclosures: **M.K. Heimann:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **B. Schlink:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **N. Tacca:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **C. Dunlap:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **S. Colachis:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **P. Putnam:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **L. Wengerd:** A. Employment/Salary (full or part-time):; The Ohio State University. **D. Friedenberg:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.

Nanosymposium

NANO033: Neuroprosthetics: Control of Real and Artificial Arm, Hand, and Other Grasping Devices

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO033.04

Topic: F.05. Brain-Machine Interface

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SNSF Ambizone grant 223402

Title: Pre-movement alpha oscillations modulate the sense of agency by gating sensorimotor binding

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Abstract: The sense of agency (SoA) is the subjective, pre-reflexive feeling of causing and controlling our actions, and the consequent events in the external world. It is thought to play a key role in motor control and self-awareness. SoA arises from the comparison between efferent motor commands and afferent sensory feedback. In order to perform such comparison, the brain must integrate sensory and motor information at a large scale, gating the information flow between different functional areas. The dynamics of such large-scale process is to the present day largely unknown. In three experiments, we leveraged invasive and non-invasive brain machine interfaces to decouple motor commands and sensory feedback, allowing to study the brain dynamics of sensorimotor comparisons. Experiments 1 and 2 were conducted in a tetraplegic proficient user of an intracranial brain machine interface (BMI) for hand control. Motor commands are decoded from a primary motor cortex (M1) implant and translated into functional hand movements through neuromuscular electrical stimulation (NMES). In

Experiment 1, we manipulated the congruency of sensory feedback with motor commands, and assessed the participant's sense of agency via explicit judgements. In Experiment 2, we developed an adaptation of Libet's task to implicitly measure agency. We hypothesized that slow neural oscillations, due to their role in modulating long-range connectivity, would affect the integration of afferent and efferent information and the subsequent sense of agency. We found that the phase of pre-movement 8 Hz oscillations consistently predicted sense of agency both for explicit judgements and implicit measures. In Experiment 3, we extended our investigation to whole brain dynamics by conceptually replicating Experiment 1 in an EEG setup. We confirmed that the phase of pre-movement alpha-band oscillations in both M1 and SMA modulated participants' agency ratings. Importantly, we found that movements starting in the optimal SMA phase for agency were associated with an increase of post-movement functional connectivity between SMA and the contralateral frontal, temporal and parietal lobes. Our results suggest that the pre-movement M1-SMA phase may gate the information exchange involved in sensorimotor comparisons, modulating the amount of binding between intentions and actions and therefore the SoA. This is in line with evidence about the mechanistic framework linking slow neural oscillations and brain-wide communication. Our findings show a path towards the application of such framework to functional and phenomenological aspects of sensorimotor integration.

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Nanosymposium

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Presentation Number: NANO033.05

Topic: F.05. Brain-Machine Interface

Support: EIC-2021-Pathfinderchallenges-01-02 NEMO-BMI (101070891
EIC 2021-TransitionChallenges-01-01 ReverseParalysis (101057450),
Carnot Leti Institute

Title: Advancing brain signal decoder for autonomous use of the WIMAGINE Bain-Computer-Interface technology

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Abstract: After remarkable proofs of concept in the lab and in clinical settings, Brain-Computer Interfaces (BCIs) are entering a new era. While numerous commercial entities enter the field, the primary goal remains to enable individuals with chronic severe motor handicaps to use motor compensation solutions outside the lab, particularly at home and autonomously. The project team has already developed and clinically validated, using the WIMAGINE ECoG device, a BCI allowing a quadriplegic to control an exoskeleton using motor intents [1] and a Brain Spine Interface allowing a person with paraplegia to walk naturally in ambulatory settings [2]. This communication deals with two major BCI bottlenecks addressed by the team in the NEMO-BMI and REVERSE-PARALYSIS projects: (1) the need for qualified supervision to set up the brain signals decoder model [3], and (2) the requirement for powerful and bulky hardware for real-time decoding to drive effectors. **First Achievement: To reduce the need for supervision during model updates, we extracted from cortical recordings specific signals associated with the match between the motor intent and the action enabled by the effector. By using this new information termed 'SATISFACTION' to label correct predictions during model creation, we demonstrated that unsupervised training can achieve decoding performance comparable to supervised training. **Second Achievement: We first accelerated the decoding algorithms by translating them to optimized C++ code to enable the brain signal decoding on a Raspberry Pi, achieving a significant miniaturization and a 90% power consumption reduction compared to a laptop for both inference and model updates. Since then, we started the design of an integrated circuit dedicated to ECoG-based BCI decoding, that paves the way for a more ergonomic system with increased autonomy, enabling easier use of BCI controlled effectors. [1] Benabid et al, Lancet Neurol. (2019), 10.1016/S1474-4422(19)30321-7 [2] Lorach et al, Nature (2023), 10.1038/s41586-023-06094-5 [3] Moly et al, Journal of Neural Engineering (2022), 10.1088/1741-2552/ac59a0 Acknowledgements: Horizon-EIC-2021-Pathfinderchallenges-01-02 NEMO-BMI (101070891); Horizon-EIC 2021-TransitionChallenges-01-01 ReverseParalysis 101057450, and Carnot Leti Institute.

Disclosures: F. Sauter-starace: None. **S. Karakas:** None. **R. Souriau:** None. **L. Struber:** None. **F. Martel:** None. **V. Spagnolo:** None. **T. Collin:** None. **H. Lorach:** None. **V. Delattre:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ONWARD Medical. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ONWARD Medical. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ONWARD Medical. **G. Charvet:** None. **T. Aksenova:** None.

Nanosymposium

NANO033: Neuroprosthetics: Control of Real and Artificial Arm, Hand, and Other Grasping Devices

Location: SDCC Rm 25A

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Presentation Number: NANO033.06

Topic: F.05. Brain-Machine Interface

Support: DoD Grant W81XWH-20-PRMRP-IIRA

Title: Chronic viability and functional performance of the muscle cuff regenerative peripheral nerve interface for exoskeleton control

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Abstract: Restoring motor control in individuals with limb paresis remains a critical challenge, with exoskeletons offering a promising avenue for functional assistance. However, current exoskeleton control interfaces are limited by instability and unreliable quality of the detected motor control signals. To address this limitation, the Muscle Cuff Regenerative Peripheral Nerve Interface (MC-RPNI) was developed as a novel biologic solution designed to amplify efferent motor signals from peripheral nerves, thereby providing improved control signals to drive exoskeletons. While prior MC-RPNI studies have demonstrated signal amplification (10 to 20-fold) 3 months post-surgery in anesthetized rats *in situ*, the long-term utility of the MC-RPNI construct and its physiologic signaling *in vivo* during volitional movements have not been investigated. In this study, we evaluated the long-term viability (12 months post-surgery) *in situ* and functional signal amplification *in vivo* (6 months post-surgery) of the MC-RPNI in a rat model. For both aims, MC-RPNIs were created on the common peroneal (CP) nerve in rats (n=12). To assess the construct's longevity, we performed histological and electrophysiological analysis at 12 months post-surgery. Immunohistochemistry staining revealed sustained muscle reinnervation, and hematoxylin and eosin (H&E) staining revealed preserved muscle morphology and no signs of pathological degeneration. Electrophysiological recordings *in situ* from 12-month MC-RPNIs following proximal CP nerve stimulation confirmed persistent, high-amplitude evoked muscle signals (4.08 mV on average). These findings indicate lasting structural and functional integrity of the construct. To assess real-time physiologic signaling *in vivo* in free-moving rats, treadmill-trained animals were implanted with recording electrodes (MicroProbes) in the proximal CP nerve and the MC-RPNI at 6 months post-surgery, secured via headstage implants. Simultaneous recordings of these volitional signals and the rat's gait (via Optitrack) demonstrated that the MC-RPNI amplified volitional CP nerve signals—by over 10-fold—with a signal-to-noise ratio of 20 dB. Notably, MC-RPNI signals were predominantly observed during the dorsiflexion phase of gait (from toe-off to mid-swing), suggesting that the MC-RPNI effectively transduced CP nerve signals responsible for transmitting motor commands associated with foot dorsiflexion. In summary, the MC-RPNI demonstrates chronic viability and reliable amplification of physiologic, volitional motor signals. These findings support its potential as a robust biologic interface for advanced exoskeleton control.

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Nanosymposium

NANO033: Neuroprosthetics: Control of Real and Artificial Arm, Hand, and Other Grasping Devices

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO033.07

Topic: F.05. Brain-Machine Interface

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Title: Encoding of object weight in human motor cortex during object transport

Authors: *Z. CHEN, A. R. SOBINOV, C. RAMAN, N. G. HATSOPOULOS;
The Univ. of Chicago, Chicago, IL

Abstract: Millions of people in the U.S. live with spinal cord injury or limb loss, limiting their autonomy. Brain-computer interfaces (BCIs) offer a path to restore function by decoding neural signals to control prosthetic limbs. While most BCI work targets limb kinematics, grasp force - essential for stable object manipulation - remains understudied. Although it has been shown before that force can be decoded from neural activity in the motor cortex (MC), recent evidence suggests that these representations of force weaken during object transport, impairing the performance. We hypothesized that this reflects the lack of somatosensory feedback during control of BCIs in paralyzed participants.

To investigate this, we worked with a unique BCI participant possessing residual grasping ability through finger flexor contractures and coordinated wrist motions, as well as partial hand and arm sensation. The participant was previously implanted with Utah arrays in the motor and sensory cortices targeting hand and arm representations as a part of a clinical trial. They were instructed to grasp and transport a cylindrical object between three target locations on a table in front of them. On each trial, the weight of the object, its starting and target locations were randomized. We used three levels of object weight, 0.5-1.2 kg, which naturally elicited different grasp forces. Real-time force was recorded from four force sensors mounted on the perimeter of the cylinder, and one bottom-facing sensor for detecting the moment the cylinder was lifted. We found that object weight could be classified above chance from the activity in the motor cortex using a linear decoder. The classification accuracy remained similar throughout the trial - from the initial grasp to release. These findings suggest that somatosensory feedback may assist with maintaining the grasp force representation in MC, highlighting its importance for improving BCI-based prosthetic control.

Disclosures: **Z. Chen:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH NINDS R35NS122333, NIH NINDS R01NS125270, The University of Chicago. **A.R. Sobinov:** A. Employment/Salary (full or part-time):; The University of Chicago. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH NINDS R35NS122333, NIH NINDS R01NS125270. **C. Raman:** None. **N.G. Hatsopoulos:** A. Employment/Salary (full or part-time):; The University of Chicago. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Blackrock Neurotech, Inc..

Nanosymposium

NANO034: Opioids: Models, Treatment, and Behavioral Pharmacology

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO034.01

Topic: H.08. Drugs of Abuse and Addiction

Support: Grantseeker 12802B

Title: Development of a novel mouse model of oral oxycodone self-administration and conditioned place preference

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Abstract: Opioid analgesics are among the leading causes of drug overdose deaths in the United States. Oxycodone, a widely prescribed semi-synthetic opioid, is used to treat moderate to severe pain. However, its chronic use leads to tolerance, dependence, and addiction. Here, we report the development of a more translational mouse model of reward following oral self-administration in mice in which we assessed if oral oxycodone self-administration was associated with reward and if this response was mediated via the mu opioid receptor. We used the place conditioning paradigm, which is widely used as an animal model of reward, to achieve our goals. Mice were first confined to one of the conditioning chambers while had access to a 4% sucrose solution in each chamber once daily for one hour. Mice had access to drink from the sucrose solution while conditioned each day. Mice were confined to the opposite chamber for one hour on the following day. The volume of the solution consumed by each mouse was measured at the end of each session. This alternate-day conditioning continued for four days each week for two consecutive weeks. Following the last conditioning on week 2, mice were tested for baseline place preference. This initial phase was to allow animals to habituate to the chambers and obtain a stable intake pattern. On weeks 3 and 4, one of the sucrose solutions was replaced with oxycodone in a 4% sucrose solution and conditioning started with one day with oxycodone

solution and the other day with the sugar solution for a total of 4 days each week. Mice were tested for place preference toward the conditioning chambers 24 h after the last conditioning each week. Mice were then exposed to extinction training for two weeks, in which mice had access to only sucrose solution in the conditioning chambers during each session. Mice were tested for preference 24 h after the last conditioning each week. Mice were then tested for the reinstatement of CPP following a challenge dose of oxycodone (5mg/kg, i.p.). On each test day, mice were placed in the central chamber and allowed to explore the conditioning chambers for 15 min. The amount of time that mice spent in each chamber was recorded. Our results showed that oral oxycodone self-administration induced a robust CPP which was extinguished and reinstated following the challenge dose of oxycodone. Subsequent studies in mice lacking mu opioid receptors and their wildtype littermates showed that the CPP response was blunted in the absence of mu opioid receptors. Together, these results suggest that oral oxycodone self-administration is associated with reward and this response is mediated via mu opioid receptors.

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Nanosymposium

NANO034: Opioids: Models, Treatment, and Behavioral Pharmacology

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Presentation Number: NANO034.02

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant R21DA062164

Title: Xylazine-induced enhancement of the discriminative stimulus effects of fentanyl in rats

Authors: C. A. GALLAGHER[1], *D. MARTINEZ[2], D. F. MANVICH[1];
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Abstract: The highly-potent synthetic opioid fentanyl has remained the top contributor to US overdose fatalities for nearly a decade. However, the expanding detection of xylazine within the US illicit fentanyl supply has threatened to further exacerbate this already dire public health threat. Typically used as a veterinary sedative and anesthetic, xylazine induces central nervous system depression via activation of noradrenergic alpha-2 receptors and has been shown in preclinical studies to potentiate fentanyl-induced respiratory depression, brain hypoxia, and lethality, while also decreasing the efficacy of the opioid receptor antagonist naloxone for overdose reversal. Despite these risks, xylazine is considered a desirable adulterant among some drug users, with anecdotal reports indicating that it may prolong and/or enhance fentanyl's effects. Here we investigate whether xylazine modulates the interoceptive stimulus effects of fentanyl using a two-lever drug discrimination procedure in rats. Male (n=5) and female (n=6) adult Sprague Dawley rats were first trained to discriminate fentanyl (0.032 mg/kg IP) vs. saline under a fixed-ratio 10 schedule of food reinforcement. Upon satisfaction of training criteria, test

sessions were conducted 1-2 times per week, with training sessions performed on days between tests. As expected, fentanyl (0.001-0.032 mg/kg) dose-dependently produced fentanyl-appropriate responding and rate suppression. Xylazine alone (0.1-1.0 mg/kg IP) did not substitute for fentanyl up to doses that produced rate suppression, however it dose-dependently enhanced the discriminative stimulus and rate-suppressant effects of low-dose fentanyl. Subsequent time course studies revealed that 0.032 mg/kg fentanyl failed to produce fentanyl-appropriate lever responding at a pretreatment time of 120 min, however 1.0 mg/kg xylazine administered in combination with 0.032 mg/kg fentanyl produced full substitution at this same time point. Together, our results demonstrate that xylazine enhances the rate-suppressing and discriminative stimulus effects of fentanyl and prolongs fentanyl's duration of action. These findings corroborate clinical evidence that xylazine potentiates the subjective effects of fentanyl in humans.

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Nanosymposium

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Topic: H.08. Drugs of Abuse and Addiction

Support: Discovery Grant from Natural Sciences and Engineering Research Council of Canada

Title: Differential Modulation of Memory Consolidation by Opiates

Authors: B. GINSON, *F. LERI;
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Abstract: It has been proposed that reinforcing drugs promote the development of addiction by enhancing the consolidation of memories formed during drug use. From a cognitive-behavioural perspective, this hypothesis predicts drug-induced modulation of memory consolidation across different memory systems implicated in addiction.Focusing on opiates, our laboratory has consistently demonstrated that heroin facilitates the consolidation of episodic memories. In the current studies, we investigated the effects of post-training heroin administration in male Sprague-Dawley rats on stimulus-affect learning, as well as the consolidation of stimulus-response learning.
Study 1 employed place conditioning with 1 mg/kg heroin (n = 48; Study 1A) or 20 mg/kg cocaine (n = 30; Study 1B), and tested the effects of post-conditioning injections of vehicle, 1 mg/kg heroin, or 1 mg/kg heroin + 3 mg/kg naloxone. Animals were tested after one pairing (Test 1) and four pairings (Test 2). In both studies, post-training heroin impaired the development of a place preference, and this heroin-induced consolidation deficit was prevented by naloxone.

Study 2 examined the effects of heroin on the consolidation of stimulus-response learning using a modified Barnes maze task assessing the acquisition of a habitual response. Rats (n = 60) were trained over ten sessions to find a fixed escape box and received vehicle, 1 or 2 mg/kg heroin post-training. At the end of training, animals were tested in a probe trial in which the escape hole was relocated. The 1 mg/kg heroin dose increased escape latency, target investigation, and locomotion within the training quadrant during the probe trial, suggesting enhanced perseveration of the trained response. In contrast, 2 mg/kg heroin reduced target hole exploration during the probe, likely reflecting impaired motivation to escape.These findings suggest that the opiate heroin does not exert uniform effects on the consolidation of memory traces mediated by different memory systems. Moreover, pharmacological variables such as drug dose may influence motivational aspects of memory during consolidation, rather than trace stability itself.

Disclosures: B. Ginson: None. **F. Leri:** None.

Nanosymposium

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Presentation Number: NANO034.04

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH NIDA R00DA052624

Title: Nucleus Accumbens Parvalbumin Interneurons Regulate Fentanyl-evoked Behavior

Authors: *K. M. JOHNSON[1], E. A. GAUTHIER[4,2], S. E. KING[5], P. E. ROTHWELL[3], E. LEFEVRE[5];
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Abstract: Opioid use disorder is a chronic relapsing disorder characterized by compulsive drug seeking behavior and escalation in drug taking, despite attempts to resist. Opioid use disorder has a high burden of disease, affecting millions of people worldwide. A better understanding of how opioids affect the intrinsic reward circuitry is crucial to generate therapeutic interventions. The nucleus accumbens (NAc) is central within reward circuitry, and interneurons expressing parvalbumin (PV; also known as fast-spiking interneurons) form a prominent source of synaptic inhibition within the NAc. Increasing evidence suggests NAc PV interneuron circuitry is altered in motivation and drug addiction, leading us to investigate whether these interneurons are involved in opioid addiction. First, we demonstrated that opioids can directly target PV interneurons through the mu opioid receptor. Using in-situ hybridization, NAc PV interneurons were shown to express mRNA mu opioid receptors. Subsequent ex-vivo slice electrophysiology experiments confirmed functional activity, whereby application of DAMGO, a mu opioid receptor agonist, decreased the intrinsic firing rate of PV interneurons, as well as their inhibitory

postsynaptic currents to medium spiny neurons. Given this functional connection between opioids and PV interneurons, we next investigated whether chemogenetic (DREADD) inhibition of PV's could modulate fentanyl-evoked behavior. Male and female PV-2A-Cre mice underwent stereotaxic surgery to inject either the Cre-dependent inhibitory (hM4Di) DREADD or control virus, into the NAc shell. Subsequently, animals underwent fentanyl psychomotor sensitization for 5 days, with a daily injection of the DREADD ligand clozapine-N-oxide (CNO) prior to each fentanyl injection. DREADD inhibition of PV interneurons significantly attenuated the induction of fentanyl-evoked locomotor sensitization in females but not males ($p<0.05$). Based on these findings with non-contingent fentanyl, a multi-stage intravenous self-administration paradigm was used to test whether DREADD-mediated inhibition of PV interneurons attenuates progressive ratio responding and cue-induced reinstatement, assessing motivation and relapse, respectively. However, in these contingent self-administration experiments, DREADD inhibition of PV interneurons trended towards increased progressive ratio breakpoint in males but not females, with no effect on reinstatement. Together, these results are indicative of a sex-dependent role for PV interneurons in non-contingent versus contingent fentanyl-evoked behavior.

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Nanosymposium

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Topic: H.08. Drugs of Abuse and Addiction

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TL1 TR002380

Title: High frequency stimulation of the ventral tegmental area rescues respiratory depression in rats

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Abstract: Fentanyl induced opioid use disorder constitutes a significant health crisis in the United States, contributing to high rates of opioid overdose-related deaths. Early in opioid addiction, abnormal increases in extracellular dopamine in the nucleus accumbens (NAc) reinforce excessive drug-seeking behaviors which can lead to fatal respiratory depression. Given

this mechanism, we investigated whether high frequency stimulation (HFS) of the ventral tegmental area (VTA) could block NAc dopamine increase and respiratory depression following an acute lethal dose of fentanyl. We hypothesized that VTA HFS would mitigate these dopaminergic responses and prevent fentanyl-induced respiratory failure. Multiple cyclic square wave voltammetry (M-CSWV), was applied via a carbon fiber microelectrode to measure absolute dopamine concentration in the NAc of urethane-anesthetized male Sprague-Dawley rats (n=6). Dopamine levels were recorded at baseline and following acute fentanyl administration (30 µg/kg, i.v.). VTA HSF (130 Hz frequency, 200 µsec pulse width, and 0.2 mA amplitude) was administered to the VTA before and during fentanyl exposure. Acute fentanyl administration resulted in a 178.2% increase in NAc dopamine levels from baseline, accompanied by a decline in respiratory rates to critically low levels (45 breaths per minute vs. 102), resulting in 100% mortality. VTA HFS did not significantly alter baseline tonic dopamine levels or prevent fentanyl-induced dopamine increase in the NAc but was able to fully rescue fentanyl-induced respiratory failure. Overall, these results provide preliminary evidence for the potential of HFS as a therapeutic strategy for fentanyl-induced respiratory depression.

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Nanosymposium

NANO034: Opioids: Models, Treatment, and Behavioral Pharmacology

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 8:00 AM - 9:45 AM

Presentation Number: NANO034.06

Topic: H.08. Drugs of Abuse and Addiction

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Title: Single-cell transcriptional characterization of rat cortical and striatal cell types after escalated heroin intake and protracted withdrawal

Authors: *C. T. BURKE[1], D. OTTENHEIMER[1], S. FERGUSON[2], G. D. STUBER[1];
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Abstract: The precise molecular mechanisms underlying individual differences in susceptibility to maladaptive opioid use are largely unknown, and likely reflect dysregulated neural activity in multiple brain regions associated with reward and motivation. One central node in opioid reward circuitry is the nucleus accumbens (NAc), which integrates glutamatergic input from diverse brain regions with dopaminergic input from the ventral tegmental area, and projects to the

ventral pallidum (VP). To characterize the transcriptional state of neurons throughout this circuit, we utilized a rodent model of IV heroin self-administration in which rats display polarized heroin intake and can subsequently be classified into high and low addiction risk groups, and conducted snRNAseq in prelimbic cortex (PL), NAc, and VP after self-administration and protracted withdrawal. We hypothesized that subsets of NAc medium spiny neurons (MSNs) would be significantly perturbed specifically in rats with high addiction risk behaviors. Nuclei isolation and library prep were conducted using 10X Genomics Next GEM pipeline, and data analysis was completed using the R package Seurat. Data was integrated with mouse scRNAseq datasets to facilitate identification of shared and distinct neuronal subtypes for each brain region, producing an atlas of known and novel cell types. Analysis of each neuronal subtype revealed significant transcriptional alterations in multiple NAc D1 and D2 expressing MSN sub-clusters, as well as L2/3 and L4/5 IT neurons in PL, including a conserved response in genes associated with postsynaptic densities across these distinct cell types. In contrast, VP neurons were largely unchanged by heroin exposure, despite high expression of the μ-opioid receptor in VP. WGCNA analysis revealed neuronal subtype-specific modules of gene expression with differential expression by heroin addiction risk group. Follow up experiments using spatial transcriptomics and *in situ* hybridization are in progress to elucidate the spatial distribution of novel MSN subtypes and genes differentially expressed between high and low addiction risk rats. To assess their potential contribution to drug-seeking behavior, candidate genes of interest will be manipulated during and/or after heroin self-admin using intersectional CRISPR/Cas9 viral strategies to target specific neuronal subpopulations.

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Society of Neurological Surgeons (SNS) Neurosurgeon-Scientist Training Program (NSTP)

Title: Neurochemical and Electrophysiologic Correlates of Fentanyl Administration in a Swine Model

Authors: K. SCHEITLER[1], J. M. ROJAS CABRERA[2], S. VETTLESON-TRUTZA[1], C. D. BLAHA[1], *H. SHIN[1], Y. OH[1], K. H. LEE[1];
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Abstract: Dysregulation of mesolimbic dopamine contributes to opioid addiction. While tonic dopamine concentrations are not currently measured during deep brain stimulation (DBS) surgery, electrophysiology is recorded. Finding electrophysiologic correlates of dopamine dynamics in response to fentanyl could help clarify the mechanisms of addiction and support development of closed-loop DBS therapies. We applied the Multifunctional Apparatus for Voltammetry, Electrophysiology, and Neuromodulation (MAVEN) in an anesthetized swine model of frame-based DBS. A carbon fiber microelectrode (CFM) was stereotactically implanted in the swine nucleus accumbens (NAc) to record tonic dopamine levels. Another DBS lead was implanted in the contralateral NAc to record LFPs. Tonic dopamine concentrations were measured using multiple cyclic square wave voltammetry (MCSWV) with the following parameters: initial potential -0.2 V, staircase increment +25 mV, square wave amplitude ±0.4 V, pulse duration 1.0 ms, five cyclic square waves per scan, and scan rate 0.1 Hz. Baseline and post-fentanyl recordings were performed. Fentanyl administration resulted in increases in tonic dopamine concentrations in the NAc and increased power in lower-frequency LFP bands. Dopamine sensitivity was validated in vivo using a selective dopamine agonist and stimulation-evoked release. This study demonstrates the feasibility of simultaneous dopamine and electrophysiologic recording during DBS in a large-animal model of opioid administration. MAVEN enables integrated, real-time monitoring of neurochemical and electrophysiologic signals within existing clinical stereotactic workflows. These results support the potential for neurotransmitters to serve as biomarkers in the development of closed-loop neuromodulation systems for opioid addiction and other neuropsychiatric disorders.

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Nanosymposium

NANO035: Neural Circuit Mechanisms of Social Behavior and Cognition

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Presentation Number: NANO035.01

Topic: I.06. Social Cognition

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Title: Neural basis of sick pup approach by mouse dams

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Abstract: Background: Maternal care is critical for offspring well-being and must be directed and flexible depending on the perceived conditions and needs of offspring. It remains unclear which sensory systems and neural mechanisms are necessary for mothers to sense and respond to the needs of their children. Sickness behaviors may serve as socially-useful signals that solicit comfort or caregiving, and the context of maternal care may reinforce caregiving. Here, I use spontaneous and trial-based behavioral tests, intersectional chemogenetics, and optically-tagged *in vivo* electrophysiology to test how the mouse oxytocin system contributes to approach toward sick pups. **Methods**: Dams were tested in a three-chambered social preference assay to quantify preference toward post-natal day 14 pups injected with lipopolysaccharides (LPS, 1 mg/kg) or saline. To determine if vision, olfaction, or somatosensation might be important for sick pup approach, dams were also tested in the dark, with olfactory cues alone, or with trimmed whiskers. We also characterized and compared maternal behaviors of a mouse dam toward sick or healthy pups over the course of 7 hours using a multi-camera longitudinal behavioral monitoring system developed in the lab. To determine the contribution of oxytocin neuron subtypes to sick pup approach, we generated intersectional transgenic mouse lines to genetically target two major subclasses of oxytocin neurons, magnocellular and parvocellular. Dams were injected with Cre- and Flp-dependent hM4D(Gi) in the paraventricular nucleus of the hypothalamus (PVN), a major site of oxytocin synthesis, and tested in the social preference test. Finally, using silicon probe recordings coupled with a fiber for blue light delivery in an Oxt-Flp female mouse with Flp-dependent ChR2, we further test how oxytocin and PVN population dynamics change in response to sick vs. healthy pups. **Results**: Dams spend more time in the chamber with LPS-injected offspring than saline-injected offspring, but not non-offspring. Dams tended to avoid sick olfactory cues alone. Dams also display slightly increased huddling behavior toward LPS-injected pups vs. saline controls within the first 3 hours after sickness induction. Inhibition of parvocellular, not magnocellular, oxytocin neurons prevents sick pup preference. Our *in vivo* PVN recordings indicate highly coordinated activity across the PVN population when interacting with sick pups relative to healthy. Taken together, these data suggest that increased approach toward sick pups is familiarity-dependent, involves integration of multiple sensory cues, and requires parvocellular oxytocin neuron activity.

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Topic: I.06. Social Cognition

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Title: Compositionality of social gaze in the prefrontal-amygdala circuits

Authors: *G. QI[1], O. DAL MONTE[1,3], S. FAN[1,4], S. W. CHANG[1,5,6,2];
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Abstract: Each social gaze can be deconstructed into fundamental components, including social state, gaze content, and gaze duration. To reduce dimensionality and facilitate generalization, the brain needs to represent such primitive components in an abstract format. Here we examined the compositional nature of social gaze primitives in the brain as pairs of macaques engaged in social gaze interaction. Behaviorally, a generalized linear mixed-effects model showed that a partner monkey's likelihood of looking back at the recorded animal's face emerged from a compositional code: social state and gaze content each exerted strong main effects, but their joint influence was sub-additive. Gazing at the partner's face was more predictive of partner's social gaze during low compared to high states of the recorded monkeys. Moreover, the predicted probability of partner's social gaze was also higher when the recorded monkeys gazed at the object during high compared low states. On the other hand, gaze duration contributed little unless paired with specific content. Thus, downstream social gaze behavior is determined by how primitive components are *combined*, rather than by their independent sums, providing evidence for behavioral compositionality. At neural level, neural populations in the basolateral amygdala (BLA) and the gyrus of the anterior cingulate cortex (ACCg) represented state and content in an abstract format and orthogonally to one another, whereas the dorsomedial prefrontal cortex (dmPFC) and orbitofrontal cortex (OFC) showed limited generalization. Notably, linear mixed-selective neurons encoding both state and content in the ACCg and BLA, but not in the dmPFC or OFC, mediated the abstraction underlying the generalization. Moreover, the state and content information exhibited distinct functional connectivity among the four neural populations, with social state information flowing from BLA to dmPFC and ACCg, and gaze content information flowing from dmPFC to OFC and BLA. Our findings provide the neural grammar supporting the compositionality of social gaze in the prefrontal-amygdala circuits.

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Nanosymposium

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Title: Oscillatory dynamics during pup retrieval behavior in mPFC-Amygdala-A1 network

Authors: *D. JUNG[1], H.-B. HAN[2], Y. KIM[1], S. JANG[1], G.-H. LEE[1], R. C. FROEMKE[3], J. CHOI[4];
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Abstract: Pup retrieval is a complex process requiring the seamless integration of sensory cues, emotional evaluation, and motor execution. While hormonal and experience-dependent factors are known to influence this behavior, the fast-timescale circuit dynamics that support it remain unclear. Here, we used CBRAIN, a wireless real-time neural recording system that detects and visualizes oscillatory activity in freely moving animals via onboard computation and LED-based feedback, to record from the auditory cortex (A1), basolateral amygdala (BLA), and medial prefrontal cortex (mPFC) during spontaneous pup retrieval in virgin female mice exploring large-scale arenas. To resolve moment-by-moment transitions, we applied fine-grained behavioral segmentation at tens of millisecond resolution. We observed that distinct beta sub-bands tended to align with specific behavioral phases: low beta (~20 Hz) bursts in the mPFC emerged during the inspection phase, when the mouse approached and examined the pup just before lifting, whereas high beta (~30 Hz) activity increased during pup lifting and the return to the nest. These transitions were circuit-specific and phase-locked to micro-behavioral events. Our findings suggest that retrieval behavior relies on transition beta oscillations at their distinct frequency bands to flexibly coordinate perception, salience attribution, and action selection across distributed networks. The CBRAIN platform enabled the high-resolution mapping of this proess in real time, offering new insights into the neural architecture of social behavior and its modulation by internal states and neuromodulators such as oxytocin.

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Nanosymposium

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Title: Diverse forms of plasticity supporting maternal aggression in female mice

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Abstract: To protect the helpless young, females dramatically increase aggression towards intruders during lactation, known as maternal aggression. However, attack is costly and risky. When pups no longer exist, maternal aggression loses its purpose and rapidly declines. Our study reveals the essential plasticity in the pathway from estrogen receptor alpha-expressing (PA^{Esr1}) cells in the posterior amygdala to neuropeptide Y receptor Y2 ($VMHvl^{Npy2r}$) cells in the ventrolateral part of the ventromedial hypothalamus to regulate maternal aggression. Functional manipulations and photometry recordings demonstrate VMHvl-projecting PA^{Esr1} ($PA^{Esr1\text{-}VMHvl}$) cells are naturally active and required for maternal aggression. *In vitro* slice recordings showed that PA-$VMHvl^{Npy2r}$ connection strengthens and $VMHvl^{Npy2r}$ excitability increases to enhance $VMHvl^{Npy2r}$ responses to intruders and drive attack in lactating dams. Furthermore, we found oxytocin as a critical mediator to link pups' needs to the aggression circuit output. Interestingly, PA, not VMHvl, is the key site for oxytocin to boost the aggression circuit output. The abundant expression of oxytocin receptor (OXTR) in PA^{Esr1} cells enables oxytocin to increase the input-output relationship of the aggression circuit by increasing the input resistance of the PA^{Esr1} cells. The decreased maternal aggression by the oxytocin level drops after pup separation can be restored by optogenetic stimulation of oxytocin neurons in the paraventricular hypothalamic nucleus. This recovered maternal aggression can be canceled by blocking PA OXTR signaling. Thus, diverse forms of plasticity occur at the PA^{Esr1}-$VMHvl^{Npy2r}$ circuit to support maternal aggression, while oxytocin signals the need of the young, enabling the female to rapidly adjust its aggression.

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Nanosymposium

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Topic: I.06. Social Cognition

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Title: Cortical contribution of Neurokinin-3 Receptors to aggression

Authors: L. KRONHEIM[1], R. GATLIN[1], S. PARK[1], H. WALKER[1], N. A. FROST[2], M. ZELIKOWSKY[1];
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Abstract: Social isolation induces an internal state characterized by alterations to social and aggressive behaviors. Previous work from our lab has established a role for the medial prefrontal

cortex (mPFC) Tachykinin-2 (Tac2) neuropeptide system in the top-down control of isolation-induced aggression. We have found that release of the neuropeptide, neurokinin B (NkB), from mPFC Tac2 neurons controls social behaviors that represent an animal's escalation towards attack, however we have not yet examined how manipulating the activity of the cells expressing the receptor for the neuropeptide NkB, the neurokinin-3 receptor (Nk3R), contributes to aggression escalation and attack. Using multiplex fluorescent in situ hybridization we found that Nk3R-expressing cells co-express cFos, a marker of neural activity, in cortical layers 1-3, yet significantly fewer deep-layer (layer 6) Nk3R-expressing cells co-express cFos in mPFC of isolated (SI) compared to group housed (GH) mice. Interestingly, through single nucleus RNA-sequencing, we found that isolation increases the expression of Tacr3, the gene encoding Nk3R, in L2/3 (early layer) excitatory neurons and decreases Tacr3 expression in several populations of layer 6 neurons. As Nk3R's are proposed to depolarize cells, these data suggest a link between Tacr3 expression in distinct layers and their activity - higher Tacr3 expression correlates with more active Tacr3+ cells. Using complementary loss-of-function approaches, we have identified that inhibition of Tacr3+ neurons and CRISPR-mediated mutagenesis of Tacr3 in mPFC of isolated mice differentially impacts aggression escalation (body investigation, anogenital investigation) and attack. These data reveal the complex, layer-specific contribution that local Tac2/NkB signaling makes towards isolation-induced aggression.

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Nanosymposium

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:30 AM

Presentation Number: NANO035.06

Topic: I.06. Social Cognition

Title: Hippocampal CA2 neuronal activity encodes social dominance hierarchy

Authors: *R. NGUYEN[1], S. A. SIEGELBAUM[2];
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Abstract: Social dominance hierarchies are highly adaptive social structures that influence group conflict, resource allocation, and reproductive success. Context-appropriate social behaviors may depend on memory of past experiences with conspecifics. The hippocampal CA2 subregion is essential for social memory, encoding individual identity and social valence. However, the neural circuit mechanisms underlying the formation and representation of social groups are poorly understood. Here, we investigated CA2 neuronal activity during the establishment of dominance hierarchies in mice. Using chemogenetics, we inhibited CA2 pyramidal neurons in groups of unfamiliar mice during their initial social encounter. We then evaluated the hierarchy over 7 days using the dominance tube test assay. Inhibition of CA2

during social familiarization disrupted the consistency of social ranks across days. While in well-established hierarchies, CA2 inhibition during the tube test did not alter match outcomes. To examine the neural representations supporting social hierarchy, we performed in vivo calcium imaging with miniature microscopes. We identified a subset of CA2 neurons whose activity scaled with the social rank of the interacting partner. Moreover, CA2 population activity exhibited a linear axis corresponding to social rank, suggesting a monotonic representation of hierarchy. Our findings underscore a crucial role for the CA2 in forming stable hierarchies and provide insight into hippocampal neural coding of social structures.

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Wu Tsai Institute at Yale University

Title: Neural dynamics of social evidence accumulation in cooperative interactions of freely moving marmosets

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Abstract: Social interactions are inherently complex, requiring outcome evaluations, behavioral adjustments, and coordinated communication. In nonhuman primates, social gaze is a key means through which individuals gather information about others' intentions and actions. To uncover the neural mechanisms underlying the use of social gaze in cooperation, we studied dyadic cooperative pulling behavior in freely moving common marmosets (*Callithrix jacchus*) using an automated 3D behavior tracking system to define and quantify social gaze events. Previous findings revealed that marmosets flexibly adapt both gaze-independent and gaze-dependent strategies to support successful cooperation. Building on decision neuroscience frameworks—where saccadic fixations have been shown to accumulate value information leading to decision outcomes—we hypothesized that social gaze serves as a mechanism for accumulating social evidence prior to cooperative actions. We further predicted that the dorsomedial prefrontal cortex (dmPFC), a region implicated in various social cognitive functions, supports this accumulation process. To test this, we developed a multi-channel wireless system that allowed us to record neural activity in this naturalistic setting (n=460 neurons in animal 1; n=450 in animal 2).

Behaviorally, we observed enhanced social gaze prior to pull actions, particularly in successful trials. At the single-neuron level, dmPFC activity ramped toward pull actions, with slope magnitude negatively correlated with gaze accumulation — shallower slopes for higher levels of gaze accumulation and steeper slopes for lower levels —mirroring slope modulations in traditional evidence accumulation studies. At the population level, neural trajectories derived from dmPFC activity exhibited distinct dynamics as a function of gaze accumulation, with longer trajectories when gaze accumulation levels were higher. Notably, both the gaze accumulation level and its modulation on neural activity were positively correlated with the movement speed of the partner—a behavioral feature reflecting variability in their motion. This suggests that animals may be using social gaze to accumulate information about the partner's behaviors, including movement dynamics. More gaze accumulation may be required to support successful coordination, likely because higher variability introduces greater uncertainty in reading the partner's intentions. Together, our results reveal that the dmPFC encodes accumulated social evidence both at the level of individual neurons and through the neural dynamics at the population level.

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Keck Foundation Junior Faculty Award

Title: Neural representation of prosocial behaviors in the anterior cingulate cortex

Authors: *M. ZHANG, X. ZHANG, E. WU, W. HONG;
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Abstract: Animals show a range of prosocial behaviors—voluntary actions that benefit others—toward distressed conspecifics. Understanding other individual's negative states is essential for providing appropriate prosocial responses to their specific needs. Previous studies have shown that many species, including rodents, avians, and primates, provide affiliative touch to comfort stressed partners. Our recent work found that mice not only display comforting behavior but also

display targeted helping responses specifically toward others in pain. This targeted helping behavior, characterized by allolicking of the injury site, reduced the self-licking of the partners in pain. Activation or suppression of the anterior cingulate cortex (ACC) bidirectionally regulate both types of prosocial behaviors. Using microendoscopic imaging, we found the ACC differentially encodes these two forms of prosocial behaviors in both single-neuron and population level. Furthermore, we performed electrophysiological recording while animals interacted with partners experiencing stress or pain and found that these behavioral actions could be decoded not only in the ACC and but also in the medial amygdala (MeA).

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Topic: I.06. Social Cognition

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Title: Competition Between Prefrontal and Auditory Cortex Blocks Learning to Use Sound to Search for a Social Reward

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Abstract: Research on neural mechanisms of motivated behaviors in animals has largely relied on operant training in artificial behavioral paradigms. In contrast, animals in their natural environments often use default behavioral strategies to solve ethological problems, such as those encountered during social communication, but they can also adapt their behavior based on new sensory information to reach their goal more efficiently. We have been exploiting a search behavior in mice to uncover how the brain learns to recognize sound cues and use them to guide their search for a social reward. Adult female mice are naturally motivated to search for and retrieve mouse pups. We train females to locate pups in a T-maze by approaching a novel synthetic sound that has been reinforced with the delivery of a pup to retrieve. Animals initially start with a default win-stay strategy of returning to search for pups where they last found one. Their improvement in using the novel sound reflects a competition between the default and the new auditory strategy. Recordings in the auditory cortex (ACx) during learning show a prognostic modulation of neural responses as animals progressively use the sound more, while recording in the medial prefrontal cortex (mPFC) reflects the decaying usage of the win-stay strategy. Critically, chemogenetic inhibition of ACx and mPFC reveals surprising opposite roles of the two regions in learning: silencing ACx impairs learning of the sound cue, while silencing mPFC accelerates it. Importantly, when mPFC function is restored after animals have learned the

task under mPFC inhibition, their sound-guided search performance declines, and win-stay behavior re-emerges. A model of direct neural competition explains trial-by-trial behavior accurately. Thus, our findings show how a new modality for informative cues can come to prevail in decision-making and demonstrate opposing roles for prefrontal and auditory cortex in the competition between strategies in a naturalistic search behavior. The work also demonstrates how paradigms dissecting predispositions and plasticity in ethological social behaviors can uncover neural mechanisms and behavioral dynamics that have been overlooked in conventional operant tasks.

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Title: Fatherhood increases auditory sensitivity to pup calls in the biparental California mouse, Peromyscus californicus

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Abstract: In mammalian mothers, sensory systems that detect and process stimuli from pups undergo structural and functional changes at the onset of parenthood. However, the extent to which fathers undergo sensory plasticity is not as well understood. We investigated auditory cortical responses in both anesthetized and awake male California mice (*Peromyscus californicus*), one of only ~5-10% of mammalian species that show biparental care. Virgin males in this species are aggressive or avoidant while fathers are highly nurturant towards pups. We presented virgin males and fathers with neutral auditory stimuli (clicks or noise bursts) or a pup-call sequence while we recorded local field potentials down the depth of their cortical column. Field potentials were transformed into current source density profiles for a spatial-temporal cortical population map of activity. Under anesthesia, fathers (n=8) showed higher baseline spectral power and higher gain in response to quiet pup calls and noise bursts in their supragranular layer, compared to virgins (n=6). Fathers also had higher response peak amplitudes and greater sensitivity specifically to pup calls, in granular and deeper layers. This was borne out in stronger peak amplitudes relative to virgins during quiet (~20 dB) calls compared to loud (~45 dB) calls and higher inter-trial phase coherence to only quiet pup calls compared to virgins. We propose that sensory plasticity in parenthood facilitates improved detection and representation of weaker stimuli, such as those that may arise from a distance or in

noisy environments. Following up in awake, head-fixed, virgins (n=5) and fathers (n=5), we presented a pup call sequences over different levels of noise masking. Awake experimentation is ongoing with further recordings and analysis. As of now, awake fathers have increased sensitivity to pup calls nearer to noise thresholds compared to virgins, further supporting our hypothesis that fatherhood induces a partially specific pup-stimulus sensitivity gain.

Disclosures: K.E. Deane: None. **W. Saltzman:** None. **K.A. Razak:** None.

Nanosymposium

NANO035: Neural Circuit Mechanisms of Social Behavior and Cognition

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Topic: I.06. Social Cognition

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Scan | Design
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Title: Decoding pain mediated shifts in social behavior following neuropathic injury in mice.

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Abstract: While the peripheral detection of pain is well-characterized, the brain mechanisms that transform nociception into affective pain states—and their influence on social behavior—remain unclear. To investigate this interaction, we used an operant social self-administration paradigm in male and female mice paired with the spared nerve injury (SNI) model. Following 8 days of operant training, where lever pressing was rewarded with the introduction of an affiliative social partner, mice received a spared nerve injury (SNI) or sham injury and were reintroduced to the task either 1 day (immediate) or 5 days (delayed) after surgery. One-photon endomicroscopy was conducted in the nucleus accumbens of a subset of experimental mice during baseline and post-injury social testing to determine how neuronal ensembles encoding positive social interactions shift across pain development. In a second subset of experimental mice, single-cell RNA sequencing of the nucleus accumbens was performed to examine transcriptional changes resulting from acute SNI alone and from social intervention for SNI. We found that access to social interaction significantly shaped behavioral and nociceptive outcomes. Mice with immediate post-injury social access exhibited reduced mechanical allodynia (von Frey: $p < 0.05$ compared to groups without social operant access) and higher operant social responding compared to those given delayed access. In contrast, delayed-access mice displayed increased social withdrawal and enhanced allodynia. These effects were sex-dependent, with

females showing stronger behavioral sensitivity to both pain and social disruption. Transcriptomic analysis revealed broad gene expression shifts, including differential regulation of endogenous opioid-related genes, specifically in pair-housed SNI mice who were not given access to the social operant task. These findings identify a critical recovery window in which social interaction modulates both behavioral and molecular signatures of pain, revealing a sex-dependent, time-sensitive neurobiological mechanism that may inform therapeutic strategies targeting affective components of chronic pain.

Disclosures: C.A. Toddes: None. **S.A. Golden:** None. **M. Heshmati:** None.

Nanosymposium

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Title: The intermediate CA1 and CA3 differentially encode social recognition

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Abstract: Mice naturally prefer to investigate novel conspecifics over familiar conspecifics, suggesting that they can recognize and remember previously encountered individuals. Prior research has found that chemogenetically inhibiting the intermediate and ventral hippocampus disrupts social novelty preference. The **intermediate hippocampus** consists of two primary pyramidal layers: the **intermediate CA1 (iCA1)** and the **intermediate CA3 (iCA3)**. Previous studies that have recorded endogenous activity in the iCA1 have revealed that individual neurons preferentially fire for novel and familiar conspecifics, providing evidence that the iCA1 encodes social recognition information. However, no published studies have recorded endogenous neural activity in the iCA3, and thus it remains unknown whether this region also encodes social recognition information. To answer this question, one-photon cellular-resolution calcium imaging was performed to record endogenous neural activity in the iCA1 and iCA3 in two separate cohorts of male mice. We first ran the imaging mice on a social discrimination test in which the imaging mice could investigate two conspecifics housed on opposite ends of a behavioral arena. We found that both the iCA1 and iCA3 conjunctively encode social and spatial information with many neurons only firing for one conspecific in a specific location. To further explore social recognition representations within the iCA1 and iCA3, we built a novel behavioral paradigm called the linear recognition assay, in which the imaging mice could investigate multiple conspecifics at the same spatial location. Permutation tests revealed that a greater

proportion of iCA3 neurons preferentially fired for novel and familiar conspecifics compared to iCA1 neurons. Additionally, support vector machines trained on neural activity decoded novel and familiar conspecifics in the iCA3 with higher accuracy compared to the iCA1. Additionally, neurons were longitudinally tracked across multiple recording sessions to test the stability of social recognition representations across days. We found that a different subset of neurons preferentially fired for novel and familiar conspecifics each day which suggests that there is high social recognition remapping. Together, these findings provide evidence that the iCA3 does encode social novelty information and also suggests that there are functional differences in social recognition encoding between the iCA1 and iCA3.

Disclosures: B. Dykstra: None. **G. Berman:** None. **M. Murugan:** None.

Nanosymposium

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Topic: I.06. Social Cognition

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F32HD106666

Title: An Endocannabinoid-Regulated Social-Active Ensemble Encodes Negative Social Outcomes in the Nucleus Accumbens of Shank3$^{e4\text{-}22}$KO Mice

Authors: *O. FOLKES[1], M. DONAHUE[1], P. N. NEGRON-MORENO[2], Y.-H. JIANG[3]; [2]Neurosci., [1]Yale Univ., New Haven, CT; [3]Dept. of Pediatrics and Neurobio., Sch. of Med., New Haven, CT

Abstract: Social behavior deficits are a common symptom of neuropsychiatric disorders, but there are limited pharmacological treatments for these symptoms. Understanding how neurons encode social information will give insight toward identifying novel pharmacological targets. *SHANK3* encodes a postsynaptic scaffold protein and is a common risk gene for several neuropsychiatric disorders characterized by social deficits, including autism. Our lab developed the *Shank3*$^{\Delta e4\text{-}22}$ mouse model, which shows a loss of social preference and alteration in the connectivity of the nucleus accumbens (NAc), a critical region for social behaviors. Therefore, we tested the hypothesis that *Shank3* deletion alters NAc neural response to social investigation.Using single-cell calcium imaging, we found that the first social investigation bout triggers hyperactivity of NAc cells in *Shank3*$^{\Delta e4\text{-}22}$ mice compared to WT mice, indicating a potential maladaptive encoding in NAc social ensembles. To test this, we next used Targeted Recombination in Active Populations (TRAP) to manipulate social-active ensembles in WT and *Shank3*$^{\Delta e4\text{-}22}$ mice. We found that *Shank3*$^{\Delta e4\text{-}22}$ mice prefer to spend time in a chamber paired with optogenetic inhibition of social-active ensembles. Also, inhibiting social-active ensembles diminishes social preference in WT mice but restores social preference in *Shank3*$^{\Delta e4\text{-}22}$ mice.

These data show that *Shank3*$^{\Delta e4\text{-}22}$ deletion may shift the encoding of social cues from a neutral to a negative valence to induce social deficits. Finally, our data demonstrate that pharmacologically augmenting endocannabinoids (eCBs) restores social preference deficits in *Shank3*$^{\Delta e4\text{-}22}$ mice but causes social preference deficits in WTs. eCB augmentation also normalizes NAc cellular activity in *Shank3*$^{\Delta e4\text{-}22}$ mice during the first social investigation bout, but causes hyperactivity during this bout in WTs. These studies show that initial social investigations may be a critical time point for neural encoding of social interaction. Our future work will focus on understanding how pharmacological and optogenetic inhibition of the encoding of initial social investigations alters subsequent social behaviors.

Disclosures: O. Folkes: None. **M. Donahue:** None. **P.N. Negron-Moreno:** None. **Y. Jiang:** Other; Couragene, Inc, Taysha Gene Therapies.

Nanosymposium

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Topic: I.06. Social Cognition

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HHMI Freeman Hrabowski

Title: Whole-brain activity enhancing auditory-guided maternal behavior

Authors: *B. R. MCRAE[1], D.-L. K. D. FERGUSON[2], H. IBARRA AVILA[2], L. HAMMOND[4], B. J. MARLIN[3];
[1]Neurosci., [2]Zuckerman Inst., [3]Neurosci., Psych., Zuckerman Inst., HHMI, Columbia Univ., New York, NY; [4]Neurol., Ohio State Univ., Columbus, OH

Abstract: From the moment of giving birth, a mother must quickly adapt to the new demands of motherhood, attending to signals from her offspring and using them to inform her behavior. Research in rodents has begun to disentangle innate and learned features of the neural circuitry underlying maternal responses to infant cues. For example, distress vocalizations emitted by isolated mouse pups, termed pup calls, elicit maternal behavior and time-locked neural activity in the left primary auditory cortex (A1) in mothers and virgin females with maternal care experience, termed experienced virgins, but not naïve virgin females. Given the complexity of pup call-evoked behaviors, we hypothesize that pup calls engage different neural circuitry across the whole brain in animals of varying maternal experience, particularly in regions involved in stress, emotion, motivation, and decision-making. We also hypothesize that when looking beyond A1, nuanced differences exist between mothers and experienced virgins, who have acquired maternal behavior through innate and learned mechanisms, respectively. We conducted an auditory preference assay and revealed that mothers, but neither naïve nor experienced

virgins, exhibit a preference for pup calls. We then used whole-brain activity mapping via c-Fos-iDISCO+ to characterize the representation of pup calls throughout the brain, revealing different activation patterns across groups. Our preliminary data suggest that pup calls evoke distinct behavioral responses and brain-wide neural activity in mothers, naive virgins, and experienced virgins. Altogether, this work takes advantage of behavioral tracking and whole-brain imaging methods to uncover how multiple mechanisms, whether they be innate or learned, can support adaptive offspring care.

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Nanosymposium

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Presentation Number: NANO036.01

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Novel spatial tagging of single nuclei spatializes single cell ATACseq and V(D)J sequencing with gene expression for glioma cellular ecosystem profiling

Authors: *W. WANG, J. WILHELMY, D. KOOL, C. CHANG, C. FAN;
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Abstract: Advances in single-cell multiomic technologies such as RNAseq, ATACseq, or V(D)J sequencing have enriched our understanding of gene expression, chromatin accessibility, and immune receptor diversity, but they fall short of capturing the spatial context of cells. This spatial architecture is especially unique in glioma, exhibiting both disorganized arrangements and structured regions of malignant cells surrounded by diverse nonmalignant neighbors. Existing spatial methods, whether microscopy- or sequencing-based, primarily focus on gene or protein expression. Moreover, the resulting data often lacks true single-cell nature, requiring computationally complex cell segmentation or deconvolution, and delivers a limited number of molecules. To address these shortcomings, Trekker™ technology provides a new class of spatial solutions, by transforming single-cell sequencing assays into spatially resolved single-cell data. Based on Slide-tags, the Trekker kit spatially tags individual cells in a tissue section by releasing spatially indexed DNA barcodes from a bead array surface into nearby nuclei. Tagged nuclei are then dissociated from the array and processed by conventional single-cell sequencing methods. This technique expands spatial measurement beyond gene expression, enabling spatial multiomic analyses such as chromatin and V(D)J profiling. Also, given the measurements are true single-cell in nature, the datasets are easier to analyze and interpret. We applied Trekker single-cell mapping kit to existing single-nuclei (sn) ATACseq and V(D)J assays combined with gene expression to perform multiomic spatial profiling of fresh frozen glioma tissue. Copy number aberration inference and joint clustering on snRNAseq and snATACseq data identified spatially defined malignant, glial, neural, immune, and vascular cells by genetic, transcriptomic, and

epigenetic signatures. Malignant cells were categorized into the four canonical substates, and correlation between chromatin accessibility and gene expression identified gene-linked regulatory elements for each substate. Spatial-association analyses between malignant substates and immune cell types across multiple spatial scales, including spatially aware ligand-receptor analysis, revealed the organization and interactions of each substate within its native microenvironment. V(D)J analysis uncovered the spatial distribution of different T cell clones. Our work demonstrates how Trekker technology can advance understanding of glioma architecture, aid treatment research, and overcome the limitations of past spatial options.

Disclosures: W. Wang: A. Employment/Salary (full or part-time):; Takara Bio USA. **J. Wilhelmy:** A. Employment/Salary (full or part-time):; Takara Bio USA. **D. Kool:** A. Employment/Salary (full or part-time):; Takara Bio USA. **C. Chang:** A. Employment/Salary (full or part-time):; Takara Bio USA. **C. Fan:** A. Employment/Salary (full or part-time):; Takara Bio USA.

Nanosymposium

NANO036: Multi-omics

Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO036.02

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatial mapping of FFPE mouse brain: Integrating single-nucleus RNA-seq with spatial transcriptomics to study complex tissue organization

Authors: *C. R. UYTINGCO, J. SAKKOS, B. NGO, D. KOOL, J. WILHELMY, A. BARRETT, W. WANG, C. CHANG, C. FAN;
Takara Bio USA, San Jose, CA

Abstract: Spatial transcriptomics has transformed our understanding of tissue organization and heterogeneity by linking gene expression to spatial context. However, current single-cell technologies rely on complex workflows, proprietary protocols, and computationally intensive segmentation or deconvolution steps to resolve individual cells. These limitations are particularly evident in the analysis of intricate systems, including developing and adult nervous systems, where precise cell-type classification, anatomical mapping, and single-cell gene-level sensitivity are essential. To overcome these challenges, we developed Trekker™ spatial mapping kit, a novel single-cell spatial transcriptomics platform that combines the sensitivity of snRNA-seq with a simple, robust spatial tagging approach. Inspired by Slide-tags technology (Russel et al. 2023), Trekker technology spatially tags nuclei within intact tissue sections, even in FFPE biospecimens, and leverages established snRNA-seq workflows. To demonstrate the technology's capabilities in FFPE samples, we aligned 25-30 µm thick sections from adult mouse brain and E16 mouse embryo onto a spatially indexed tile for tagging. The isolated, spatially tagged nuclei were processed through a standard single-cell sequencing workflow to generate high-sensitivity single-nuclei expression and spatial data. Leveraging existing

bioinformatics tools for clustering, trajectory inference, and spatial analysis, we obtained a detailed characterization of the mouse brain and embryo. This enabled marker-guided regional selection and differential gene-expression analysis based on cell type and spatial location. Combined with same-section hematoxylin and eosin (H&E) staining, the approach allowed for comparative analysis with histological context and the cross-validation of spatial data. Trekker technology represents a major advancement in spatial transcriptomics by offering seamless integration into standard workflows and high spatial fidelity, achieving true single-cell resolution. By bridging the gap between spatial context and transcriptomic depth, this technology opens the door for broader adoption of spatial transcriptomics to deepen our understanding of neuroscience, enabling comprehensive exploration of cellular organization without compromising gene sensitivity, scalability, and interpretability.

Disclosures: C.R. Uytingco: A. Employment/Salary (full or part-time):; Takara Bio USA. **J. Sakkos:** A. Employment/Salary (full or part-time):; Takara Bio USA. **B. Ngo:** A. Employment/Salary (full or part-time):; Takara Bio USA. **D. Kool:** A. Employment/Salary (full or part-time):; Takara Bio USA. **J. Wilhelmy:** A. Employment/Salary (full or part-time):; Takara Bio USA. **A. Barrett:** A. Employment/Salary (full or part-time):; Takara Bio USA. **W. Wang:** A. Employment/Salary (full or part-time):; Takara Bio USA. **C. Chang:** A. Employment/Salary (full or part-time):; Takara Bio USA. **C. Fan:** A. Employment/Salary (full or part-time):; Takara Bio USA.

Nanosymposium

NANO036: Multi-omics

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Presentation Number: NANO036.03

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: MIRA Grant
R21 Grant

Title: Acrylock merfish: embedding acrylate-modified encoding probes for enhanced signal quality in spatial transcriptomics

Authors: *Q. FENG;
Neurosci. Program, Univ. of Illinois Urbana-Champaign Neurosci. Grad. Program, Champaign, IL

Abstract: Multiplexed error-robust fluorescence in situ hybridization (MERFISH) enables high-throughput spatial mapping of transcripts but can suffer from probe dissociation and background noise. To address these challenges, we developed “AcryLock MERFISH,” which employs 5′ acrylate-modified encoding probes that covalently co-polymerize into the polyacrylate gel matrix, decoupling signal retention from mRNA integrity and hybridization kinetics to preserve spot contrast even in cell-dense or RNA-degraded tissue. Encoding probes with a 5′ acrylate

moiety (“acEP”) were co-polymerized into the MERFISH embedding gel with normal probes as control (“nEP”). Three independent honey bee brain sections (∼12,000 cells each) were imaged per condition. Signal-to-noise ratios (SNR) of 130 targeted genes in five cell-dense mushroom body regions were calculated by labeling pixels matching each gene’s decoded barcode as “signal” and using a concentric ring 2-4 pixels away as “background.” Within each Z-plane, the background median and scaled median absolute deviation (MAD) were used to calculate each signal pixel’s SNR as (intensity − background median)/(3 × MAD). To validate spatial fidelity, two dopamine receptor transcripts (DopR2, Dop3) were mapped and the Pearson correlation between nEP and acEP expression profiles was calculated. AcryLock MERFISH increased weighted SNR for 116 of 130 genes (89.2%), with 69 genes (53.1%) showing statistically significant gains by Welch’s t-test; only 14 genes (10.8%) exhibited decreased SNR, and just one was significant. Mean SNR rose for 89.2% of genes, and over half (53.1%) of targets exhibited significant gains compared to the unmodified protocol. These results demonstrate that AcryLock MERFISH substantially improves spot contrast and detection sensitivity in challenging, high-density tissue at minimal cost, enabling more accurate transcript decoding under stringent imaging conditions. Future work will leverage acrylate-modified probes to perform multiple high-formamide stripping cycles to reduce signal loss after each iamging rounds, and extend this approach to diverse tissue types, including human breast cancer cell lines.

Disclosures: Q. Feng: None.

Nanosymposium

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Presentation Number: NANO036.04

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Using a spatial RNA-protein co-detection assay to investigate post-mortem microvascular changes in former American football players

Authors: D. WAKHLOO[1], K. BABCOCK[2], K. SBROCCO[3,4], *A. DIKSHIT[5], J. CHERRY[6]; [1]ACD, a Bio-techne brand, Newark, CA; [2]Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA; [3]Boston Univ. Sch. of Med., Cambridge, MA; [4]Boston Univ. Sch. of Med., Boston, MA; [5]Advanced Cell Diagnostics, Newark, CA; [6]Boston Univ., Boston, MA

Abstract: Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy associated with exposure to repetitive head impacts (RHI) most commonly seen in contact sport athletes such as American football players. CTE is characterized by the presence of neurofibrillary tangles in neurons and glia around small blood vessels at the depths of neocortical sulci that can ultimately progress to deeper brain structures over time. CTE can only be definitively diagnosed after death, thus a better understanding of the cellular and molecular changes in CTE-afflicted brain tissue may lead to the identification of mechanisms that could be useful for novel biomarkers, monitoring progression, or therapeutic development. Assessing CTE in postmortem

human brain tissue requires a multiomic approach to identify unique trauma-related markers and their associated biological processes. Using a [KB1] flagship single-cell spatial RNAscope technology, target gene and protein expression can be visualized to characterize resident cell types in a neuropathological region of interest. Here, we demonstrate a novel method for the simultaneous detection of RNA and protein using a modified co-detection assay to investigate trauma-related microvascular changes. With this novel TSA amplification-based co-detection assay, we visualized RNA and protein marker panels in human postmortem samples taken from the dorsolateral frontal cortex. Hypoxia was detected using RNA probe *HIF1a* to target hypoxic cells. The vascular basement membrane, collagen-4, was targeted using RNA probe *Coll4*. Morphological antibodies were used to mark resident microglia (IBA1), astrocytes (GFAP), and endothelial cells (CD31), along with a marker for pathological tau (AT8). Image analysis was performed using the HALO® analysis from Indica labs. Co-expression of hypoxic and cell type-specific markers in RHI-exposed and -unexposed brain samples enabled us to assess the effect of head trauma on brain microvasculature, including alterations in cell-specific hypoxic changes and basement membrane alterations. Single cell spatial RNAscope technology is a valuable tool for multiomic analysis and interrogation of complex tissues to obtain insight into novel prognostic and therapeutic biomarkers.

Disclosures: D. Wakhloo: A. Employment/Salary (full or part-time):; ACD, a Bio-Techne brand. **K. Babcock:** None. **K. Sbrocco:** None. **A. Dikshit:** A. Employment/Salary (full or part-time):; ACD, a Bio-Techne brand. **J. Cherry:** None.

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Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: 5U19MH114830-05
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Title: A spatial transcriptomics atlas of the adult mouse brain

Authors: *M. KUNST[1], R. MATHIEU[1], L. CHING[1], J. QUON[1], D. MCMILLEN[1], J. CAMPOS[2], N. MARTIN[1], S. DANIEL[2], P. OLSEN[2], N. VALERA CUEVAS[1], A. RUIZ[3], J. ARIZA TORRES[4], M. HEWITT[2], S. C. SEEMAN[2], C. M. PAGAN[6], S. SUNKIN[2], L. A. ESPOSITO[5], R. ABBASI-ASL[7], L. NG[2], J. WATERS[9], H. ZENG[2], A. LEE[8];
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Abstract: Understanding the spatial organization of cell types in the brain is essential for deciphering its intricate functional and anatomical architecture. Spatial transcriptomics offers a method to correlate transcriptomic cell types with their anatomical locations. Recently, we published a whole-brain multiplexed error-robust fluorescence in situ hybridization (MERFISH) dataset (Yao et al., 2023), which includes 4.3 million cells, complementing our single-cell RNA sequencing (scRNAseq) taxonomy of 5322 clusters. This spatial dataset allowed us to examine the distribution of cell types within the mouse brain.Following this achievement, we expanded our datasets. Our study encompasses six adult datasets, comprising four P56 and two P28 samples, all sex-matched to facilitate robust comparative analyses. A novel segmentation and processing pipeline was employed, resulting in a dataset containing approximately 33 million cells, registered to the Common Coordinate Framework (CCFv3) for uniform spatial alignment and comparative studies. This comprehensive dataset provides insights into brain region relationships based on cell type composition and enables analyses of cell type distributions across sexes with enhanced statistical power.Additionally, employing the transformer-based spatial domain detection tool, CellTransformer, we generated computationally derived brain parcellations for detailed spatial mapping. These datasets represent a valuable resource for advancing the understanding of cell type composition and spatial organization within the mammalian brain.

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Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: R01 DK120667
P50 DA037844

Title: Identification of novel neuronal regulators for diet-induced obesity in outbred heterogeneous stock rats

Authors: T. LE[1], O. SESHIE[2], T. BUI[2], O. POLESSKAYA[3], A. A. PALMER[4], W. VALDAR[5], R. MOTT[1], ***L. SOLBERG WOODS**[2];
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Abstract: Obesity is caused by genetics, the environment (e.g., diet) and their interactions. Identifying genes that interact with diet to influence obesity risk, however, has proven challenging. The brain regulates both peripheral metabolism and satiety signaling and thus plays an important role in development of obesity. High fat diets are known to dysregulate the transcriptome of the hypothalamus, but most studies use inbred strains and do not account for genetic variation. Our laboratory uses outbred heterogeneous stock rats for genetic fine-mapping of obesity and related traits. In the current study, we measured 36 metabolic traits in 2,000 HS rats, split equally by sex, where half the rats were placed on a low-fat diet (LFD) and the other half were put on a high-fat diet (HFD) for 12 weeks. Traits measured included fat and lean mass before and after starting the diet, fasting glucose, insulin and lipid levels, glucose tolerance, food intake, activity levels, fatty liver score, serum biochemistries and multiple tissue weights. All rats were genotyped using imputation from low coverage whole genome sequence. RNAseq analysis of hypothalamus was conducted in a subset of 400 HS rats split equally by sex and diet. We used linear mixed models to detect physiological and expression quantitative trait loci (pQTLs and eQTLs, respectively) in the full dataset as well as separately by diet. Genes with cis-eQTLs that overlapped pQTLs were assessed as candidate causal genes through mediation analysis. We identified 37 pQTLs for 34 traits where eight QTLs mapped multiple traits and 17 QTLs were diet-specific (e.g., found only in the LFD or HFD condition). Using mediation analysis, we identified 14 candidate causal genes for eight QTLs in the full dataset, including hypothalamic *Pcare*, a ciliary gene within a pQTL for fat mass change and *Tnsfs9,* a cytokine in the tumor necrosis factor (TNF) family within a pQTL for omental fat pad weight. Four candidate genes were identified for three diet-specific pQTL including *Fpr1* for food intake when animals were on a HFD, but not LFD and *Ccdc77* for activity levels when animals were on a LFD, but not HFD. Future studies are needed to validate the role of these novel candidate genes. This work demonstrates the importance of accounting for diet in genetic studies for obesity and identifies novel neural regulators of diet-induced obesity and related traits.

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Nanosymposium

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Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH/NIDA Grant P30DA060810

Title: Reanalyzing Legacy Behavioral Phenotypes in Outbred Heterogeneous Stock Rats Using Modern Genomic Tools

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Abstract: We used modern genomic methods to reanalyze a large legacy dataset, which included 195 behavioral and physiological phenotypes measured in 1,407 Heterogeneous Stock (HS) rats, using recently developed genotyping and analysis pipelines for the HS rats population by the Center for Genetics, Genomics, and Epigenetics of Substance Use Disorders.We re-genotyped the original cohort using low-cost, high-throughput, low-coverage whole genome sequencing (lcWGS), followed by genotype imputation with STITCH, leveraging reference data from more than 20,000 HS rats. This method identified more than 7 million SNPs across the genome. Thus, we enhanced genetic resolution and improved power and precision for the genetic analysis of the original traits.We estimated heritability and performed a genome-wide association study (GWAS). We also computed genetic correlations between the legacy phenotypes and newly collected behavioral and physiological traits from the Center and performed phenome-wide association studies (PheWAS) across more than 300 traits, revealing novel pleiotropic associations and shared genetic architecture.Our analysis demonstrates how application of advanced genomic tools can reintegrate and extend the relevance of historical phenotypic data, enhancing their value for current genetic and behavioral research.

Disclosures: O. Polesskaya: None. **A. Baud:** None. **Y. Xin:** None. **B. Johnson:** None. **T. Missfeldt Sanches:** None. **M.K. Lara:** None. **E. Keung:** None. **R. Avila:** None. **N. Suzuki:** None. **A.A. Palmer:** None.

Nanosymposium

NANO036: Multi-omics

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO036.08

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH P30DA060810
NIH U01DA051234
NIH F31DA063333
NIH P30DA060810

Title: Complex genetic variants associated with gene expression across brain regions in Heterogeneous Stock rats

Authors: *D. CHEN[1], K. COHEN[2], D. MUNRO[2], M. MORTAZAVI[2], J. GUEVARA[2], O. POLESSKAYA[2], J. SEBAT[2,3,4], M. GYMREK[5,6], A. A. PALMER[2,3];
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Abstract: The Heterogeneous Stock (HS) rats outbred population, derived from eight inbred strains, has been used in experimental and genetic studies to unravel biological insights for

addiction-related behaviors for years. However, determining the causal genes remains challenging. Most genetic studies focus primarily on single nucleotide polymorphisms (SNPs) and overlook complex variants such as insertions/deletions (INDELs), tandem repeats (TRs) and structural variants (SVs). To begin to address this gap, we generated a comprehensive catalog of SNPs, INDELs, TRs and SVs in the outbred HS rats population using short and long read sequencing technologies, and performed expression quantitative trait loci (eQTL) mapping across five brain regions. Our analysis discovered over two million non-SNP genetic variants. While some of them are tagged by SNPs, a significant proportion are not tagged by SNPs and thus have been overlooked by current approaches. We identified over twenty thousand cis-QTLs associated with expression across all five brain regions. More than half of the top associations are with non-SNP genetic variants. Ultimately, our goal is to integrate complex genetic variation into all HS rats genetic studies to uncover novel mechanisms underlying addiction-related behaviors.

Disclosures: D. Chen: None. **K. Cohen:** None. **D. Munro:** None. **M. Mortazavi:** None. **J. Guevara:** None. **O. Polesskaya:** None. **J. Sebat:** None. **M. Gymrek:** None. **A.A. Palmer:** None.

Nanosymposium

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

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Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIDA U01DA043098
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Hope for Depression Research Foundation
Pritzker Neuropsychiatric Disorders Research Consortium

Title: Using spatial transcriptomics to characterize the effects of cocaine self-administration on brain gene expression in a selectively bred rat model of individual vulnerability to psychiatric disorders and drug addiction

Authors: *M. H. HAGENAUER[1], E. K. HEBDA-BAUER[2], M. A. EMERY[5], D. M. KROLEWSKI[2], P. M. MARAS[1], B. LUMA[1], F. LI[2], S. KOONSE[1], M. WASELUS[3], S. B. FLAGEL[2], J. B. BECKER[4], S. J. WATSON[6], J. LI[1], A. A. PALMER[7], H. AKIL[2];
[2]Michigan Neurosci. Inst., [3]MNI, [4]Psychology, [1]Univ. of Michigan, Ann Arbor, MI; [5]Neurosci., Michigan Neurosci. Institute, Univ. of Michigan, Ann Arbor, MI; [6]MNI, Michigan Neurosci. Inst., Ann Arbor, MI; [7]Psychiatry, UCSD, La Jolla, CA

Abstract: Background: To better understand individual vulnerability to psychiatric disorders and drug addiction, we have selectively bred rats for many generations to produce two lines with highly divergent behavioral temperaments: bred Low Responders (bLR) are highly inhibited and

anxious in a novel environment, whereas bred High Responders (bHR) are highly exploratory, sensation-seeking, and prone to drug-seeking behavior. In our current study we used spatial transcriptomics to characterize the differential impact of cocaine self-administration (SA) on brain gene expression in bHR versus bLR rats and relate these findings to known genetic and genomic differences between the two lines.
Methods: bHR/bLR rats (generation F81, N=24: n=3/line/group/sex) were given access to 3 weeks of cocaine SA (0.3 mg/kg/infusion, 2hrs/day for 5 days/week), and were then sacrificed 48-72 hrs following SA with matched unhandled controls. One coronal slice per subject was collected for spatial RNA-sequencing (A/P: ~ -3.5 mm, includes hippocampus) using the Visium platform (10x Genomics; 28x151bp sequencing: Illumina NovaSeqXPlus). Data was preprocessed using SpaceRanger (v.3.0.1, mRatBN7.2), and analyzed using Seurat (v.5.2.1).
Results: Spatial barcodes ("spots") cleanly clustered, mapping to known anatomical regions, including hippocampal subregions. Initial analyses revealed a large number of genes that were differentially expressed between the two lines (1,542 genes with FDR<0.05 in at least one cluster), especially in the hippocampal molecular layer. The differentially expressed genes replicated previous findings related to bioenergetics (*Ist1*, *Ucp2*), microglial function (*C1qc, Ucp2, Fcrl2*), reactive oxygen species (*Nqo2, Ucp2*), and growth and development (*Bmp4*). There were fewer effects of cocaine SA vs controls (33 genes with FDR<0.05 in at least one cluster), but a high percentage of these genes were also differentially expressed in association with bred line in at least one cluster (12/33 or 36%), including large effects for a gene essential to the electron transport chain*,* apoptosis, oxidative damage response, and pruning *(Cycs).*
Conclusion: Multiple levels of evidence now implicate a key set of genes in behavioral temperament, potentially modulating vulnerability to psychostimulant use. By characterizing their expression and regulation by cocaine in specific regions and circuits, we lay the groundwork for mechanistic studies and pharmacological targeting. Next, we will obtain finer-grained results by integrating our current data with previously collected bHR/bLR spatial transcriptomics data and single nuclei RNA-Seq data.

Disclosures: M.H. Hagenauer: None. **E.K. Hebda-Bauer:** None. **M.A. Emery:** None. **D.M. Krolewski:** None. **P.M. Maras:** None. **B. Luma:** None. **F. Li:** None. **S. Koonse:** None. **M. Waselus:** None. **S.B. Flagel:** None. **J.B. Becker:** None. **S.J. Watson:** None. **J. Li:** None. **A.A. Palmer:** None. **H. Akil:** None.

Nanosymposium

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Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO036.10

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Arrayed genome-wide CRISPR screening for regulators of human neuronal health and degeneration enabled by automation and generative AI

Authors: *A. WESTERHAUS, Y.-D. KWAK, R. IHRY;
Novartis, Cambridge, MA

Abstract: The global rise in neurodegenerative diseases underscores the urgent need to identify novel genes critical to neuronal health and degeneration for the development of therapeutic strategies. Arrayed screens offer superior ability to explore complex phenotypes in human neurons derived from induced pluripotent stem cells (iPSCs). However, the high technical complexity and costs have limited arrayed screens for functional genomics in complex human models. To address this, we leveraged standardized iPSC neuronal culture, automation, brightfield microscopy, and in silico labeling (ISL) to quantify neurite length at scale. These technical advances enabled a high-performance genome-wide arrayed CRISPR screen in human neurons from iPSCs. The screen identified both neuron-specific essential genes and suppressors of a neurotoxin that induces axon degeneration. This research represents a significant step toward understanding the complex neuronal processes underlying neuronal health and paves the way to increase our understanding of the genetic factors either preventing or contributing to neurodegenerative disease in humans.

Disclosures: A. Westerhaus: A. Employment/Salary (full or part-time):; Novartis. **Y. Kwak:** A. Employment/Salary (full or part-time):; Novartis. **R. Ihry:** A. Employment/Salary (full or part-time):; Novartis.

Nanosymposium

NANO036: Multi-omics

Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO036.11

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant P30DA060810

Title: Deep transcriptomic analysis identifies potential molecular mediators of substance use disorders in outbred rats

Authors: *D. MUNRO[1,2], H. CHEN[3], R. J. HITZEMANN[4], T. C. JHOU[5], S. H. MITCHELL[6], L. SOLBERG WOODS[7], F. TELESE[8], A. G. GUSEV[9], P. MOHAMMADI[2,10], A. A. PALMER[1];
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Abstract: Behavioral studies in outbred rats that include a genome-wide association study (GWAS) component can reveal genetic contributions to variation in complex traits. However, they can only identify chromosomal regions, rather than specific genes. The integration of transcriptomic data, such as through transcriptome-wide association study (TWAS), can identify

genes that may mediate the genetic associations, enhancing their biological interpretation and their translation to human biology. While TWAS originally used transcriptomic data in the form of gene expression, methods such as Pantry have increasingly used additional modalities of variation, such as alternative splicing and alternative transcription start and end sites, extracted from the same RNA-seq data, to detect more routes by which a gene can mediate a genetic association. We processed 2,737 RNA-seq samples from nine brain regions and three non-brain tissues collected in the RatGTEx project (www.RatGTEx.org), quantifying six RNA modalities and building transcriptomic prediction models. Using the TWAS approach, we applied these to GWAS summary data for 281 behavioral and physiological traits collected from 13 outbred rat studies and found over 2,000 gene-trait associations. We further analyzed associations specific to each brain region for traits in categories including conditioned reinforcement, delay discounting, cocaine, nicotine, oxycodone and heroin self-administration, identifying genes known to be involved in related behavior as well as genes with no previously reported role. These findings provide evidence for the involvement of certain genes, specified by brain region and modality of RNA variation, in complex traits. Further experiments can use this evidence to confirm these molecular mediators and target them for the treatment of substance use disorders. To facilitate use of these results, we provide an interactive TWAS web portal as part of the RatGTEx resource (twas.RatGTEx.org).

Disclosures: D. Munro: None. **H. Chen:** None. **R.J. Hitzemann:** None. **T.C. Jhou:** None. **S.H. Mitchell:** None. **L. Solberg Woods:** None. **F. Telese:** None. **A.G. Gusev:** None. **P. Mohammadi:** None. **A.A. Palmer:** None.

Nanosymposium

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO036.12

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: A highly efficient AAV capsid engineering platform produced a panel of novel capsids targeting various tissues

Authors: *C. FERNANDES, V. YUAN, S. STORRIE, T. SONG, C. RICH, J. HONG, G. YU, B. LAHN;
VectorBuilder Inc., Chicago, IL

Abstract: We have developed a highly efficient AAV capsid engineering platform capable of identifying novel AAV capsids with dramatically enhanced specificity for various target tissues in mice and non-human primates (NHP). This platform has several key advantages, including 1) the ability to search through very large sequence space, 2) high reproducibility in screening results, 3) the ability to target very specific cell types such as motor neurons in the spinal cord, and 4) low cost.
Employing this platform, we've identified a panel of novel AAV capsids capable of targeting a

variety of therapeutically relevant tissues with far more efficiency than existing serotypes, including neurons by systemic or intrathecal injection, muscle by systemic injection, and retina by intravitreal injection. One novel capsid was demonstrated to efficiently target spinal cord motor neurons. Its utility was further demonstrated by the enhanced ability of this capsid in treating spinal muscular atrophy (SMA) in mice, as compared to other capsids..

Disclosures: C. Fernandes: None. **V. Yuan:** None. **S. Storrie:** None. **T. Song:** None. **C. Rich:** None. **J. Hong:** None. **G. Yu:** None. **B. Lahn:** None.

Nanosymposium

NANO036: Multi-omics

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Time: Tuesday, November 18, 2025, 8:00 AM - 11:15 AM

Presentation Number: NANO036.13

Topic: J.02. Systems Biology and Bioinformatics

Title: A Cross-Species Single-Cell Atlas of Microglia Reveals Conserved and Divergent Subtypes and States in Mouse and Human Brain

Authors: *M. R. MOUSSA[1], A. L. BURGHARD[2];
[1]Sch. of Computer Sci. and Stephenson Sch. of Biomed. Engin., Univ. of Oklahoma, Norman, OK; [2]Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Microglia are resident immune cells in the central nervous system and a heterogenous population with notable heterogeneity across development phases, regions, and disease states. While a few 'microglia' single cell atlases have recently emerged to profile large microglia datasets, a comprehensive, single cell/nuclei-resolution map of microglial subtypes and functional or activation states across different species remains lacking, especially one that performs an integrative secondary analysis of existing data. Here, we present a cross-species single-cell transcriptomic atlas of microglia from mouse and human brains, spanning multiple regions, sexes and ages. We use integrated analysis of single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics datasets and tackle computational challenges like batch effect and modality integration. Our dataset encompasses over 200,000 microglial cells from adult human postmortem brains and sex as well as age-matched murine counterparts. We benchmark several integration approaches using anchor-based and deep manifold alignment methods. Our secondary analysis reveals a core set of conserved microglial subtypes, including homeostatic microglia, interferon-responsive microglia, and various activated states. Notably, we identify a 'lipid-associated microglia' (LAM) state present in both species, potentially enriched in aging and neurodegenerative conditions. Our dataset collection, which is available via a web-interactive atlas tool, provides a valuable resource for understanding microglial diversity across species and lays the foundation for translating findings from mouse models to human.

Disclosures: M.R. Moussa: None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

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Presentation Number: NANO037.01

Topic: A.09. Development and Evolution

Support: Horizon 2020 Innovative Training Network EpiSyStem (GT), H2020 Marie Skłodowska-Curie Actions, Grant/ Award Number: 765966
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A.V. was supported by Telethon Research Grant GGP19226 (GT) and RE-MEND (101057604).

Title: Regulatory logic of human cortex evolution by combinatorial perturbations

Authors: *A. VITRIOLO;
Human Technopole Fndn., Milano, Italy

Abstract: Comparative genomic studies between contemporary and extinct hominins revealed key evolutionary modifications, but their number has hampered a system level investigation of their combined roles in scaffolding modern traits. Through multi-layered integration we selected 15 genes carrying nearly fixed *sapiens*-specific protein-coding mutations and developed a scalable design of combinatorial CRISPR-Cas9 bidirectional perturbations to uncover their regulatory hierarchy in cortical brain organoids. Interrogating the effects of overexpression and downregulation for all gene pairs in all possible combinations, we defined their impact on transcription and differentiation and reconstructed their regulatory architecture. We uncovered marked cell type-specific effects, including the promotion of alternative fates and the emergence of interneuron populations, alongside a core subnetwork comprising *KIF15*, *NOVA1*, *RB1CC1* and *SPAG5* acting as central regulator across cortical cell types.

Disclosures: A. Vitriolo: None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.02

Topic: A.09. Development and Evolution

Support: NHMRC Grant 2023/GR001373

Title: Evaluating Non-CpG Methylation as a Marker of Neuronal Maturity in Human In Vitro Models

Authors: *D. POPPE[1,2];
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Abstract: Non-CpG methylation (mCH, where H = A, T, or C) is an epigenetic feature largely restricted to postmitotic neurons in the mammalian brain. It accumulates during early postnatal development—after birth in humans and following eye opening in mice—and is closely associated with synaptogenesis and neuronal activity. This developmental specificity makes mCH a promising candidate for assessing neuronal maturity. However, its reliability as a marker in human neuronal cell culture systems remains uncertain. In mouse models, neurons derived from pluripotent stem cells acquire global mCH levels in vitro on a timeline comparable to in vivo development. In contrast, human stem cell-derived neurons typically exhibit minimal mCH accumulation, even after prolonged differentiation. This discrepancy raises key questions: Does the absence of mCH in human cultures reflect true developmental immaturity? Are current protocols insufficient to support mCH accumulation? Or does mCH require in vivo-like activity patterns or cellular environments that standard cultures fail to provide? Recent culture strategies—such as extended differentiation periods, three-dimensional organoids, co-culture with additional cell types, and epigenetic modulation—have shown promise in promoting the expression of late-onset neuronal gene programs. Emerging methylome data suggest that under these conditions, global mCH levels may begin to rise. Yet, the distribution, reproducibility, and functional consequences of this methylation remain poorly understood. Here, we explore the hypothesis that non-CpG methylation could serve as a molecular readout of neuronal maturity in vitro. Drawing from its developmental trajectory in vivo and initial findings from human neuronal cultures, we outline experimental approaches to assess mCH as a functional maturity marker. Does mCH reflect intrinsic neuronal identity—or does its emergence depend on environmental factors that are difficult to replicate outside the brain?

Disclosures: D. Poppe: None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.03

Topic: A.02. Stem Cells and Reprogramming

Title: Multiple MAPT Mutations Introduced by a Novel Genome Editing Platform in hiPSCs Reveals Defects in Neuronal Development and Function

Authors: *B. ZAHERI[1], T. ZHANG[1], A. YAN[2], F. CORRALES[1], A. MATSUDA[1], F. LI[1], G. LI[1];
[1]ALSTEM, Richmond, CA; [2]Crystal Springs Uplands Sch., Hillsborough, CA

Abstract: Mutations or SNPs in the MAPT gene can alter tau protein structure or expression, leading to its pathological aggregation and contributing to neurodegenerative disorders such as frontotemporal dementia and Alzheimer's diseases. Studying the role of these genetic alterations in disease progression often requires the creation of cell lines carrying multiple mutations within both intronic and exonic regions of the MAPT gene. However, introducing such complex modifications efficiently remains a significant challenge. In this study, we developed a highly effective platform that combines a CRISPR/Cas9-based genome editing approach with the PiggyBac transposon system to precisely introduce six mutations across exon 10 and its flanking introns in MAPT gene in human induced pluripotent stem cells (hiPSCs). This model was then used to investigate the effects of these mutations on neuronal development and function. To introduce these mutations into the MAPT gene, a pair of gRNAs was used to remove the entire exon 10 and adjacent intronic regions, followed by insertion of a donor vector carrying the desired mutations, flanked by PiggyBac elements with both positive and negative selection markers. Single-cell cloning enabled isolation of homozygous clones. The selection cassette was later excised using PiggyBac transposase, and the cells without selection cassette excision were eliminated by ganciclovir treatment as they expressed negative selection marker, HSV-TK gene. Final clones were validated by PCR and Sanger sequencing, confirming precise and footprint-free integration of the mutations. Furthermore, we successfully integrated an inducible Neurogenin-2 (NGN2) expression cassette into the AAVS1 safe harbor locus of the mutant MAPT hiPSCs to enable neuronal differentiation and functional assessment. The MAPT mutant hiPSCs successfully differentiated into neurons with typical morphology and baseline electrophysiological properties, however, the neuronal function was impaired compared to wild-type NGN2-induced neurons. We developed a precise and efficient platform to introduce multiple MAPT mutations in hiPSCs and demonstrated that these mutations impair neuronal function following NGN2-induced differentiation, providing a valuable model for studying tau-related neurodegeneration.

Disclosures: B. Zaheri: A. Employment/Salary (full or part-time):; ALSTEM. **T. Zhang:** A. Employment/Salary (full or part-time):; ALSTEM. **A. Yan:** A. Employment/Salary (full or part-time):; ALSTEM. **F. Corrales:** A. Employment/Salary (full or part-time):; ALSTEM. **A. Matsuda:** A. Employment/Salary (full or part-time):; ALSTEM. **F. Li:** A. Employment/Salary (full or part-time):; ALSTEM. **G. Li:** A. Employment/Salary (full or part-time):; ALSTEM.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.04

Topic: A.02. Stem Cells and Reprogramming

Support: NIH grant U01DA054170
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Title: Apoe4 impacts cortical neurodevelopment and alters network formation in human brain organoids

Authors: *K. K. MEYER-ACOSTA[1], N.-E. VANESA[3], E. DIAZ GUERRA[2], P. VARMA[4], J. HSIEH[5];
[1]Neuroscience, Developmental, and Regenerative Biol., [2]Neuroscience, Developmental and Regenerative Biol., Univ. of Texas at San Antonio, San Antonio, TX; [3]Univ. of Texas At San Antonio, San Antonio, TX; [4]Univ. of Texas San Antonio, San Antonio, TX; [5]Neuroscience, Developmental, and Regenerative Biol., The Univ. of Texas At San Antonio, San Antonio, TX

Abstract: *APOE4* is the leading genetic risk factor for Alzheimer's Disease (AD). While most studies examine the role of *APOE4* in aging, *APOE4* causes persistent changes in brain structure as early as infancy and is associated with altered functional connectivity that extends beyond adolescence. We used human iPSC derived cortical (COs) and ganglionic eminence organoids (GEOs) to examine *APOE4*'s influence on the development of cortical excitatory and inhibitory neurons, respectively. We show that *APOE4* reduces cortical neurons and increases glia by promoting gliogenic transcriptional programs in COs. In contrast, GEOs exhibited distinct yet convergent phenotypic and transcriptional changes with *APOE4*, marked by an early increase in GABAergic progenitors and a persistent increase in neurons, indicative of accelerated differentiation. Additionally, we found a dysregulation of genes related to GABA's inhibitory action in COs. Multi-electrode array recordings in assembloids, generated by fusing COs and GEOs, revealed that *APOE4* disrupts network function resulting in heightened synchronicity and altered GABA responsiveness. Recordings in mixed genotype assembloids revealed that changes in *APOE4* COs were sufficient to alter GABA function and heighten network synchrony. Together, our findings provide evidence that neurodevelopmental changes driven by *APOE4* can shape early neural networks, potentially contributing to structural alterations observed in infant *APOE4* carriers. This research raises the possibility that early changes in brain structure and function may have implications on AD vulnerability later in life. To our knowledge, this is the first study to explore the neurodevelopmental impact of *APOE4*, underscoring the need for more research exploring the significance of early brain changes on AD susceptibility.

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Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

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Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.05

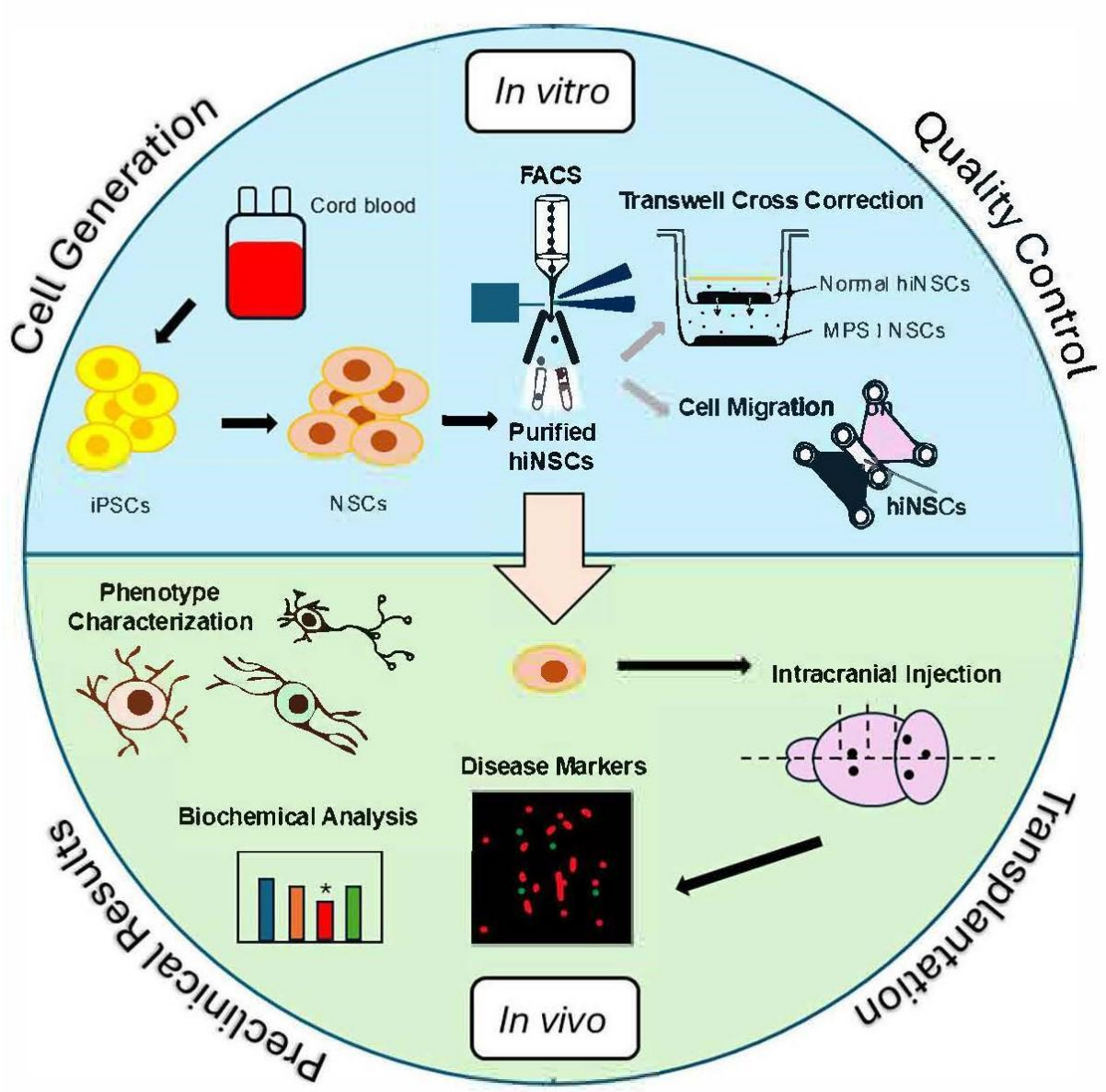
Topic: A.02. Stem Cells and Reprogramming

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Title: CNS-targeted cell therapy for MPS I: assessing iPSC-derived neural stem cell transplants

Authors: S.-H. KAN[1], C. CALHOUN[1], A. STOVER[1], J. HARB[1], R. WANG[1], E. S. MONUKI[2], P. H. SCHWARTZ[1];
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Abstract: Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by deficiencies of α-l-iduronidase (IDUA), leading to glycosaminoglycan (GAG) accumulation and progressive multi-organ damage, including neurodegeneration. Current treatments, such as enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT), show limited efficacy in the central nervous system due to poor blood-brain barrier (BBB) penetration. To address this unmet need, we generated human neural stem cells (NSCs) by differentiating induced pluripotent stem cells (iPSCs) derived from cord blood. Purified NSCs were transplanted in the brains of neonatal MPS I (*Idua*$^{-/-}$) mice on an immunodeficient NSG background. Eight months post-transplantation, donor NSCs were detected throughout the brain, showing widespread migration and partial restoration of IDUA activity. Importantly, this was accompanied by a significant reduction in β-hexosaminidase, an indicator of lysosomal burden. Histological analyses using human-specific STEM121 antibody staining revealed extensive engraftment and integration of donor cells (human NSCs) throughtout the brains of *Idua*$^{-/-}$ mice. Co-localization with GFAP and Olig2 indicated differentiation into astrocytes and oligodendrocytes. Notably, brains of treated mice exhibited decreased levels of CD68 and LAMP1, markers associated with inflammation and lysosomal dysfunction. These results demonstrate that iPSC-derived NSCs can migrate, engraft, differentiate, and deliver therapeutic benefit in the MPS I brain. This approach holds promise for treating neurological manifestations of MPS I and potentially other lysosomal storage diseases.



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Nanosymposium

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Topic: A.02. Stem Cells and Reprogramming

Support: NINDS 2T32NS007473-21A1
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Title: Characterization of Oligodendrocytes in an iPSC-Derived Model of Tuberous Sclerosis Complex

Authors: *M. R. GLASS[1], M. SAHIN[2];
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Abstract: Tuberous sclerosis complex (TSC) is caused by heterozygous mutations in either TSC1 or TSC2 which lead to hyperactive mTOR signaling. Patients present with benign tumors, epilepsy, and with TSC-associated autism spectrum disorder (ASD). TSC patients with an ASD diagnosis had decreased fractional anisotropy in white matter tracts relative to TSC patients without a diagnosis, suggesting that hypomyelination may contribute to ASD in TSC. Mouse models of the disorder have identified cell-autonomous and non-cell autonomous mechanisms that could lead to hypomyelination. 1) mTOR hyperactivation in oligodendrocytes (OLs) leads to increased cell death during maturation and inhibits the development of fully functional myelinating OLs. 2) TSC1-deficient neurons secrete connective tissue growth factor (CTGF), a known myelination inhibitor that reduces white matter and myelin basic protein production in wildtype OLs. How these cell-autonomous and non-cell autonomous mechanisms interact to contribute to hypomyelination in TSC remains unknown. We used forebrain assembloids differentiated from patient derived induced pluripotent stem cells (iPSC), their isogenic controls and biallelic knockdown of TSC2 to study cellular and molecular mechanisms of OL dysfunction in TSC. Forebrain assembloids model OL development in a sophisticated cellular environment that includes cell-autonomous and non-cell autonomous effects, and our preliminary data suggest OL development and MBP production are altered in TSC2 -/- cultures. After 20 weeks of differentiation, TSC2 -/- assembloids secrete 4-fold more CTGF than TSC2 +/+ assembloids (N=2). Electron microscopy confirmed compact myelination in TSC2 +/+ and TSC2 +/- assembloids, but no remaining compact myelin was identified in TSC2 -/- (N=1). Orthogonal measurements using immunofluorescence confirmed that TSC2 -/- assembloids produce less MBP than TSC2 +/+ assembloids and GALC+ oligodendrocytes were more compact (N=3). CTGF application (10ng/mL for 6 days) to TSC2 +/- and TSC2 +/+ forebrain assembloids reduced MBP without altering GALC+ morphology of OLs (N=1). Future work will examine earlier timepoints to test for increased apoptosis in maturing TSC2-deficient OLs and test if domains of CTGF are necessary or sufficient in MBP reduction.

Disclosures: M.R. Glass: None. **M. Sahin:** None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.07

Topic: A.02. Stem Cells and Reprogramming

Support: NIH #R21NS08847-01
NIH 1R01NS113314-01A1

Title: Tsc2 regulates RhoA signaling in induced pluripotent stem cells and neurons independent of mTOR

Authors: *A. PIER[1], T. S. CATLETT[2], N. NAMRU[3], S. RAFFERTY[3], T. M. GOMEZ[2]; [1]Neurosci., Univ. of Wisconsin - Madison Neurosci. Training Program, Madison, WI; [2]Dept. of Neurosci., [3]Univ. of Wisconsin - Madison, Madison, WI

Abstract: Tuberous Sclerosis Complex (TSC) is an autosomal dominant neurodevelopmental disorder caused by a monogenic mutation to either *TSC1* or *TSC2*. Nearly one-half of TSC patients have mild to profound intellectual disabilities and autism, with the majority developing seizures. The TSC1 and TSC2 proteins are known to interact and form a protein complex (TSC1-TSC2), which negatively regulates mTORC1-mediated protein synthesis and activates mTORC2-mediated cytoskeletal rearrangements. Regulation of local protein synthesis and the cytoskeleton are vital for proper development, axon guidance, and neural network formation. In a previous study, we found that human forebrain neurons differentiated from $TSC2^{+/-}$ induced pluripotent stem cells (iPSCs) exhibited dramatic defects in axon outgrowth and sensitivity toward several canonical axon guidance cues. Surprisingly, these defects were found to be *independent* of both mTORC pathways, while basal and cue-activated RhoA signaling was diminished. Next, we wanted to determine if differences in RhoA activity could be seen at earlier stages of development. We began by examining our corrected, $TSC2^{+/+}$, and null, $TSC2^{-/-}$, patient-derived iPSCs. Interestingly, we discovered significant differences in RhoA activity in $TSC2^{-/-}$ iPSCs, which exhibit decreased phosphorylation levels of myosin light chain compared to $TSC2^{+/+}$ iPSCs. Following up on our previous work in neurons and our current research into stem cells, we are continuing to characterize RhoA defects in $TSC2^{-/-}$ iPSCs and neurons. To this end, we have identified several exciting new phenotypes in $TSC2^{-/-}$ neurons, including increased neurite branching and a higher frequency of actin waves. These new phenotypes will be useful for conducting rescue experiments using a lentivirus (LV) engineered to express a Tet-inducible, Halo-tagged TSC2. This LV allows us to acutely restore the normal expression levels of TSC2 in $TSC2^{+/-}$ and $TSC2^{-/-}$ iPSCs and neurons. We have confirmed the efficacy of our Halo-TSC2 construct by demonstrating its ability and to reduce elevated mTOR signaling in $TSC2^{-/-}$ iPSCs and neurons. Additional experiments will investigate TSC2 activity and localization within live stem cells, and growth cones during stimulation with guidance cues and after plating on inhibitory substrates. These experiments will also assess whether this LV restores RhoA activity in both cell types and guidance sensitivity in neurons. Future modifications to the construct will enable us to further analyze the relevant domains of TSC2 that are necessary for regulating RhoA activity, as well as for regulating axon extension and cue responses.

Disclosures: A. Pier: None. **T.S. Catlett:** None. **N. Namru:** None. **S. Rafferty:** None. **T.M. Gomez:** None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.08

Topic: A.02. Stem Cells and Reprogramming

Support: Institutional Funds

Title: Fighting The Fire: HIKESHI-associated Leukodystrophy

Authors: *S. RATHAN KUMAR[1], G. VATINE[2], K. ESS[3];
[1]Vanderbilt Univ., Nashville, TN; [2]Ben-Gurion Univ. of the Negev, Beer Sheva, Israel; [3]Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: HIKESHI-associated leukodystrophy (HAL) is a lethal genetic condition with most patients dying in childhood. Patients have hypomyelination associated with developmental delay, paraparesis, and high risk of death following febrile illnesses and infections. HAL is caused by homozygous mutations in the *HIKESHI* gene leading to loss of protein. HIKESHI serves as a nuclear import carrier to the chaperone HSP70 during heat shock response (HSR). When exposed to physiological stressors, cellular proteins are prone to misfold resulting in dysfunction. In response, cells initiate HSR with chaperone proteins refolding damaged proteins to maintain homeostasis. As the primary symptoms in HAL are related to white matter, we hypothesized that lack of HIKESHI impairs oligodendrocyte HSR resulting in abnormal neural development and function. Skin samples were collected from affected homozygous patients, non-symptomatic heterozygous relatives, and healthy individuals. iPSCs were reprogrammed from primary fibroblast cultures. After validation, iPSCs were differentiated into neurons and oligodendrocytes and exposed to stressors, followed by immunofluorescent staining and immunoassays for various proteins. Oligodendrocytes were also cultured on nanofiber plates to assess myelination and development. Cells were also treated with proteosome inhibitors to attempt rescue of HIKESHI protein and cellular function. Immunofluorescent staining indicates HSP70, and other proteins displayed altered nuclear localization in *HIKESHI* homozygous mutant cells. We also noted that homozygous mutant cells had fewer number of Olig2+ cells, aberrant myelination, and enlarged and less circular nuclei. *HIKESHI* homozygous mutant neurons appeared to develop normally but displayed altered electrical firing upon heat shock exposure. Proteosome inhibitor treatment restored some HIKESHI protein and upon heat shock, cells displayed nuclear localization of HSP70 indicating that proteosome inhibitor treatment can restore some physiological function. Bulk RNA sequencing was done and revealed changes in gene expression during oligodendrocyte development. The effect of proteosome inhibitors on oligodendrocyte development and physiology is being further analyzed. We conclude that loss of HIKESHI results in heat shock pathway alterations and potential dysfunction of neurons and oligodendrocytes. Due to the urgent clinical need, greater understanding would facilitate improved therapeutics to improve the quality and duration of patients with HAL.

Disclosures: S. Rathan Kumar: None. **G. Vatine:** None. **K. Ess:** None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.09

Topic: A.02. Stem Cells and Reprogramming

Title: Targeting hyperexcitability of YWHAG R132C iPSC-derived neurons as a potential therapeutic intervention for rare genetic epilepsies

Authors: *A. M. SCHREIBER[1], R. D'SOUZA[1], A. THOMPSON[1], D. BHATTARAI[2], L. RIZZARDI[2], J. D. PEREIRA[1];
[1]Neurol., [2]Biochem. and Mol. Genet., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Mutations in the *YWHAG* gene cause rare childhood epilepsy associated with neurodevelopmental delays, with no therapeutic intervention available. *YWHAG* encodes the 14-3-3γ protein, which interacts with various targets and modulates their downstream activity. Despite evidence of an essential role in various brain disorders, little is known regarding the molecular consequences of *YWHAG* mutations and their effect on neuronal function and survival. Here, we profiled an induced pluripotent stem cell (iPSC)- derived $YWHAG^{R132C}$ disease model. The control iP11NK iPSC line was CRISPR-engineered to introduce the $YWHAG^{R132C}$ mutation and a doxycycline-inducible Neurogenin 2 (NGN2) transgene, inserted into a safe harbor locus, facilitating robust differentiation of iPSCs into cortical-like neurons by expression of NGN2. Using live cell imaging combined with immunocytochemistry and calcium imaging, we performed a detailed characterization of the functional activity of YWHAG R132C neurons compared to isogenic controls across different time points and multiple independent differentiation batches. Additionally, we utilized RNA sequencing (RNAseq) to assess global gene expression in control and mutation neurons to identify genes dysregulated with the $YWHAG^{R132C}$ mutation. Early bulk RNAseq analysis of NGN2-derived neurons shows 2,000 misregulated transcripts, including 5-fold upregulation of genes involved in regulating neuronal differentiation and organization and 2-fold downregulated expression of gene activity of various ion channels. We detected differences in neural morphology and growth in $YWHAG^{R132C}$ mutation cells, including early changes in neuronal organization. YWHAG R132C neurons have a higher calcium baseline, displaying distinct firing patterns consistent over time (n=3), and correlate with neuronal hyperexcitability and an epileptic phenotype. YWHAG protein levels were rescued by 1-hour treatment with 10 μM Lovastatin, as was the increased calcium baseline, implying a potential therapeutic intervention targeting hyperexcitability. Together, those findings suggest that the *YWHAG* gene appears to be a master regulator of neuronal differentiation, organization, and activity, that the R132C mutation leads to downregulation of YWHAG protein levels, and that treatment with Lovastatin can rescue YWHAG protein levels, higher calcium baseline, and hyperexcitability in our *in vitro* disease model.

Disclosures: A.M. Schreiber: None. **R. D'Souza:** None. **A. Thompson:** None. **D. Bhattarai:** None. **L. Rizzardi:** None. **J.D. Pereira:** None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.10

Topic: A.02. Stem Cells and Reprogramming

Title: Neural rosette development in a dorsal forebrain spheroid model of Down syndrome

Authors: *A. AYOUB[1], T. F. HAYDAR[2];
[1]Children's Natl. Hosp., Washington, DC; [2]Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Early events in fetal corticogenesis are preferentially affected in Down syndrome (DS) and may contribute to the cerebral hypocellularity, microcephaly, and altered cortical lamination characteristic of the condition. Three-dimensional culture models of trisomy 21 have emerged as experimentally tractable systems for investigating early cortical development. Neural rosettes - proliferative, morphogenic centers within organoids - develop from the self-organization of differentiating neural precursor cells, recapitulating the neural tube primordium. Direct comprehensive characterization of neural rosette morphogenesis and maturation during early development in DS has not been done. Here we present an exploratory analysis of these structures during the first 30 days in vitro (DIV). We differentiated one isogenic line of induced pluripotent stem cells into human cortical spheroids with dorsal forebrain characteristics in three technical replicates. In entire optically cleared spheroids, the number, size and maturation state of neural rosettes were quantified from high-resolution 3D reconstructions generated by multiphoton imaging and compared by genotype. Mitotic index measurements and EdU pulse-labeling assays were performed to assess cell cycle progression within rosettes at multiple stages of growth. We find that spheroid growth is consistent between genotypes until DIV20, after which trisomic spheroids diverge from controls in growth rate and size. Notably, both genotypes show a bimodal size distribution, with trisomic spheroids skewed toward the smaller peak relative to euploid. This growth disturbance is correlated with altered neural precursor proliferation and dysmaturation of the neural rosettes. Specifically, at DIV16, euploid spheroids exhibit a higher density of medium to large rosettes, based on luminal area, whereas trisomic spheroids display fewer and less coherent rosettes, reflecting immature development. Large, disorganized rosettes are observed at DIV 23 and 30 in trisomic spheroids, suggesting disordered compensatory remodeling. Conversely, euploid spheroids display smaller, contracted rosettes consistent with expected late-stage maturation. Additionally, we observed a concomitant decrease in PH3+ mitotic cells in trisomic spheroids as early as DIV16. We expect EdU analyses to reflect increased total cell-cycle and/or S-phase length, indicating delayed cell cycle progression in trisomic spheroids. By characterizing differences in rosette formation and maturation, we begin to elucidate the effects of early neural development on growth dynamics in the brains of individuals with DS.

Disclosures: A. Ayoub: None. **T.F. Haydar:** None.

Nanosymposium

NANO037: Using Human iPSCs to Model Neuronal Function and Dysfunction

Location: SDCC Rm 30

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO037.11

Topic: A.02. Stem Cells and Reprogramming

Title: Exploring sex influences of aneurysmal subarachnoid hemorrhage early brain injury on neural tissue

Authors: *K. GEMENES[1], A. EBERLE[3], S. AFEWORK[3], R. JAVELOSA[3], A. KUMAR[3], E. MCNALLY[3], M. MILLER[3], R. M. JHA[3], M. ANDREWS[2];
[2]Sch. of Biol. & Hlth. Systems Engin., [1]Arizona State Univ., Tempe, AZ; [3]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Aneurysmal subarachnoid hemorrhage (aSAH) is a type of stroke that occurs when an aneurysm spontaneously ruptures and bleeds into the subarachnoid space. Early brain injury (EBI) occurs during the first 72 hours after the aneurysm rupture and is a key contributor to poor outcomes after an aSAH. During EBI, a secondary injury occurs contributing to negative neurological outcomes, yet the molecular drivers of this neurological injury are unknown. Of particular concern, biological females experience worse outcomes as a consequence of aSAH, compared to biological males. The mechanistic causes driving sex-dependent subarachnoid hemorrhage outcomes have not been studied in humans, despite impaired female patient outcomes.
Identifying vulnerable cell types in the brain during EBI by using human stem-cell-derived neural models is crucial to advancing therapy. We will evaluate how neural injury is mediated after an aSAH stroke by exposing COs to patient-derived cerebrospinal fluid (CSF). We are evaluating the impact of injury response on human neural cell types by using 3D cortical organoids (COs), derived from human pluripotent stem cells (PSCs), that recapitulate some properties of the human brain, including neural cell populations that self-organize into a cortex-like cytoarchitecture. COs are highly tractable models that facilitate the temporal study of EBI to assess damage-mediated changes in human cell types, like neurons and astrocytes. We have begun to explore changes using male and female stem cell lines differentiated into COs treated with male and female patient-derived CSF, after a variety of clinical outcomes and observe changes to neuronal and glial health. We are investigating molecular drivers of cellular vulnerability using gene expression and morphological profiling. We hypothesize that female patient aSAH CSF, collected during EBI, impacts neuronal health and astrocyte reactivity more than male aSAH CSF, which influences clinical outcomes. This study will increase our understanding of molecular drivers of injury response after aSAH and identify potential differences in outcomes based on sex.

Disclosures: K. Gemenes: None. **A. Eberle:** None. **S. Afework:** None. **R. Javelosa:** None. **A. Kumar:** None. **E. McNally:** None. **M. Miller:** None. **R.M. Jha:** None. **M. Andrews:** None.

Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO038.01

Topic: B.09. Glial Mechanisms

Title: Uncovering oligodendrocyte diversity in the rat brain using high-resolution spatial transcriptomics

Authors: T. CAO[1], L. GUO[1], L. WANG[1], T. NGUYEN[2], T. PHAM[2], S. PHAM[2], L. CHEN[3], J. ZHOU[6], ***Y. WANG**[4,3], W. LIAO[7], S. MAHAJANI[5];
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Abstract: Understanding the spatial diversity of resident brain cell populations is essential for deciphering region-specific functions and disease mechanisms. Recent advancements in spatial transcriptomics technologies facilitate unprecedented resolution and scale, enabling the study of region-specific disruptions in cellular composition and gene expression within anatomically complex tissues, such as the brain. In this study, we utilized a sequencing-based spatial transcriptomics workflow that combined Stereo-seq, DNBSEQ-T7 sequencing, and the SpatialX analysis platform to profile over 1.5 million cells in sagittal sections of the adult rat brain. Using nanometer-scale resolution and a large field-of-view (2 × 3 cm) chip, we identified 15 transcriptionally distinct clusters corresponding to anatomical brain regions. These spatial clusters revealed region-specific gene expression signatures, enabling the identification of different cell types, including neurons, astrocytes, oligodendrocytes, and microglial cells. The spatial co-expression and differential gene expression analyses revealed two oligodendrocyte subpopulations, distinguished by their expression of key myelination markers including *Mbp*, *Plp1*, and *Mobp*. One of those clusters demonstrated comparative enrichment of genes associated with active myelin synthesis, such as *Ermn* and *Fa2h*, indicating functional heterogeneity within oligodendrocyte lineages. SpatialX enabled robust annotation and visualization of these populations, validating their identity through deep-learning-based cell type prediction utilizing reference annotation datasets. This study demonstrated the power of combining the Stereo-seq spatial transcriptomics platform with efficient DNBSEQ sequencing and cloud-native analytics to resolve cellular complexity in the brain, providing an unbiased and scalable framework for future investigations in neurodevelopment and demyelinating disorders.

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Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO038.02

Topic: E.06. Vision

Support: IHU FOReSIGHT [ANR-18-IAHU-0001]

Title: Myelination eliminates the need for neurovascular coupling in the optic nerve

Authors: *S. CHARPAK[1,2];
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Abstract: In the brain gray matter, neurovascular coupling (NVC) maintains brain metabolism homeostasis by modulating blood flow according to neuronal activity. In the white matter, the energy cost of information transmission along myelinated axons is reduced and the need for NVC is unknown. Here, we used two-photon imaging at extreme depth and high-field BOLD fMRI (17.2T) to investigate NVC along the along the entire length of the rodent optic nerve, a unique model of myelinated axonal tract. We found that flickering light and drifting grating stimulations increased blood flow in the retina, the unmyelinated optic nerve head, and at the level of the nerve synaptic terminals. However, it did not affect blood flow and oxygenation in the myelinated part of the optic nerve, i.e. the intracranial optic nerve and the optic tract. We conclude that during natural visual stimulation the metabolic cost of action potential propagation along myelinated axons does not require NVC.

Disclosures: S. Charpak: None.

Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

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Topic: B.09. Glial Mechanisms

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ARCS Foundation
Dr. Miriam and Sheldon G. Adelson Research Foundation

Title: Targeted lentiviral knockout of Chst10 in oligodendrocyte progenitor cells promotes maturation after white matter stroke

Authors: *N. SHIH[1], D. J. DITULLIO[1], S. CARMICHAEL[2];
[1]UCLA, Los Angeles, CA; [2]Neurol., UCLA Sch. Med., Los Angeles, CA

Abstract: White matter stroke is a progressive vascular disease that leads to neurological deficits and can cause dementia. It produces an area of cell death and axonal disruption (the “infarct”) and in 70% of clinical studies, white matter stroke infarcts expand from preexisting lesions into adjacent white matter (the “peri-infarct” region) further damaging and disrupting neuronal connections, summating in lesion size to cause vascular dementia, and producing causing substantial disability. The death of oligodendrocytes, one of the major constituents in white matter, leads to the demyelination of axons in the peri-infarct region. This region is of clinical interest compared to the infarct itself as a target for potential repair. Whereas the axons are lost in the infarct, axons in the peri-infarct region are intact but are chronically demyelinated. We investigate the role of oligodendrocyte progenitor cells (OPCs) in white matter stroke repair, using a mouse model that replicates advanced-stage WMS pathology. We characterized OPC responses to injury and identified a critical window of early proliferation followed by restricted differentiation. Using a stroke-specific OPC transcriptome and identification of candidate pro-differentiation genes, Csrp2 and Chst10 were selected. Distinct gene delivery strategies were developed to enable selective loss-of-function analysis in OPCs. We developed lentiviral tools incorporating a modified PDGFRα promoter and miR124-based neuronal off-target suppression. Using this system, we conducted targeted gene manipulation studies in the corpus callosum following white matter stroke. Knockout of Chst10 in OPCs not only increased the number of virally labeled cells at later time points but also promoted oligodendrocyte maturation. Csrp2 manipulation had limited impact on OPC lineage progression. Together, this work establishes a robust viral platform for selectively targeting white matter OPCs and highlights Chst10 as a key regulator of OPC fate and differentiation in the context of white matter stroke. Ultimately, this contributes to a deeper understanding of remyelination failure in WMS and identifies potential molecular targets for therapeutic intervention.

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Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO038.04

Topic: B.09. Glial Mechanisms

Title: Clinical sphingosine 1-phosphate receptor agonists protect oligodendrocytes in the cuprizone demyelination model

Authors: *H. SONG[1], J. D. TEO[2], J. KHOR[2], J. LEE[2], A. S. DON[2];
[1]The Univ. of Sydney, Sydney, Australia; [2]Sch. of Med. Sciences, Fac. of Med. and Health, and Charles Perkins Centre, The Univ. of Sydney, Camperdown, NSW, Australia

Abstract: Therapeutics that promote oligodendrocyte survival and remyelination are needed to restore neurological function in multiple sclerosis (MS). Dual sphingosine 1-phosphate receptor 1/5 (S1PR1/5) agonist siponimod is one of few drugs approved for secondary progressive MS,

acting through sequestration of lymphocytes in secondary lymphoid organs. Limited evidence also suggests that siponimod has direct myelin-protective effects in the CNS, however its precise mechanism of action in the CNS and direct effects on oligodendrocytes are unclear. 10-week-old C57BL/6 mice were fed a diet containing 0.2% cuprizone for 5 weeks to induce demyelination, together with daily administration of vehicle control, siponimod (0.04 - 1 mg/kg/day) or ozanimod (1 mg/kg/day), which is an S1PR1-selective agonist in mice. We found that siponimod but not ozanimod protected against demyelination and mature oligodendrocyte loss with 5 weeks of cuprizone administration, in both male and female mice. Interestingly, neither drug protected against acute loss of oligodendrocytes following one week of cuprizone feeding. Tracing newly-proliferated cells via 5-ethynyl-2-deoxyuridine (EdU) administration during the last 10 days of a five week cuprizone diet revealed an increased density of EdU-labelled mature oligodendrocytes but not oligodendrocyte progenitors in siponimod- compared to vehicle-treated mice, indicating that siponimod promotes oligodendrocyte maturation but not progenitor cell proliferation. Proteomic analysis of corpus callosum tissue provided evidence that both siponimod and ozanimod activate G-protein signalling in the CNS, whereas only siponimod showed neuroprotective effects, preserving not only myelin, but also synaptic and axonal proteins. These neuroprotective effects were supported with immunofluorescence staining. Both S1PR1- and S1PR5-selective agonists protected mature oligodendrocytes against cytokine-induced cell death in an *in vitro* oligodendrocyte model, suggesting that the mechanisms of protection *in vivo* are more complex.In conclusion, siponimod has direct neuroprotective actions in the CNS. As siponimod but not ozanimod protected against oligodendrocyte loss, it is likely that the protective effects of siponimod *in vivo* are mediated at least partially through S1PR5.

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Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

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Topic: B.09. Glial Mechanisms

Support: Children's Glaucoma Foundation
Vision for Tomorrow
Fight for Sight student summer fellowship

Title: Spontaneous remyelination and a novel role for Pax6 in ocular nerve remodeling

Authors: *S. MOHAN[1], J. D. LAUDERDALE[2];
[2]Cell. Biol., [1]Univ. of Georgia Interdisciplinary Neurosci. Program, Athens, GA

Abstract: Myelination is essential for the rapid and efficient conduction of nerve impulses, and its disruption is a hallmark of several neurological disorders, including multiple sclerosis. These conditions highlight the importance of understanding the molecular mechanisms that govern both

myelination and demyelination. In the eye, the ophthalmic branch of the trigeminal nerve innervates several ocular tissues, including the cornea and limbus. While limbal nerve fibers remain myelinated, corneal nerves are typically unmyelinated—a feature believed to be critical for preserving corneal transparency. Interestingly, our findings reveal that corneal nerves are not always unmyelinated. Using a mouse model and two distinct myelination markers, we show that corneal nerves are initially myelinated during early postnatal development but undergo a natural demyelination as they mature. This developmental demyelination appears to be a specialized adaptation that supports corneal clarity, suggesting that, unlike in pathological contexts, demyelination in the cornea may serve a beneficial and tightly regulated role. In contrast to the cornea, myelination in the limbal region remains stable throughout development. Notably, in a heterozygous Pax6 loss-of-function mutant, this developmental demyelination in the cornea is delayed. In adult Pax6 mutants, we observe remyelination of select corneal nerve fibers, confirmed through antibody labeling and electron microscopy, which correlates with alterations to the ocular surface. These findings uncover a previously unrecognized instance of spontaneous corneal nerve remyelination and reveal unexpected plasticity in corneal nerve architecture. Moreover, we identify a novel role for Pax6 in regulating nerve fiber remodeling, suggesting it may facilitate spontaneous remyelination and offering new insights into mechanisms of nerve maintenance and repair in the eye.

Disclosures: S. Mohan: None. **J.D. Lauderdale:** None.

Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

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Presentation Number: NANO038.06

Topic: B.09. Glial Mechanisms

Support: DBT grant BT/PR21413/MED/122/40

Title: Elucidating the mechanistic role of the JAK-STAT cell signaling pathway in neonatal hypoxic brain injury in vitro

Authors: *S. SEN[1], S. TYAGI[2], D. DEY[2], V. SHRIVASTAVA[2], S. RANI[2], M. FAHAD[3], A. ETHAYATHULLA[3], J. SHARMA[4], P. SETH[5], J. PALANICHAMY[2];
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Abstract: In normal fetal brain development, oligodendrocytes (OL) pass through multiple stages of maturation. Premyelinating OL (pre-OL) are prevalent in preterm neonatal brain (24-32 weeks of gestation), and are especially vulnerable to hypoxic injury. White matter injury in preterm neonates can result in neurological deficits like cerebral palsy (CP). To elucidate mechanisms underlying the etiopathogenesis of CP, human fetal neural stem cells (hFNSCs)

were differentiated into pre-OL (day 14) and mature OL (day 28), as indicated by their respective markers, followed by bulk RNA sequencing. Analysis of transcriptional datasets demonstrated the involvement of the JAK-STAT pathway in OL maturation. To validate our findings, we used the MO3.13 cell line, resembling pre-OL (day 0), that could be differentiated into mature OL (day 7) using PMA. Elevated expression of IL6 and STAT3, along with pSTAT3 (Y705) using qPCR, ELISA, and Western blotting, indicated the involvement of the JAK-STAT pathway in OL maturation. This was confirmed by demonstrating reduced MBP expression in mature MO3.13 after using a pSTAT3-specific inhibitor. To elucidate the role of pSTAT3 in myelination, we also analyzed the MBP promoter and identified putative pSTAT3 binding sites in it, which were further validated by molecular docking simulations with the crystal structure of STAT3 and the MBP promoter. To further study the dynamics of this pathway during hypoxic injury, pre-OLs (hFNSC-derived primary pre-OL as well as MO3.13) were exposed to hypoxia (0.2% oxygen, 48 hours). Elevated HIF-1α (Western blotting) and CA9 (qPCR), confirmed hypoxia exposure. Bulk RNA sequencing demonstrated downregulation of the JAK-STAT pathway in hypoxia-exposed pre-OL. This was validated by reduced expression of IL-6 family of ligands, pSTAT3 levels and increased expression of PIAS3 (negative regulator of pSTAT3), in pre-OL exposed to hypoxia, indicating a link between downregulation of the JAK-STAT pathway and pre-OL undergoing a maturation arrest after hypoxic injury. These novel findings highlight the involvement of the JAK-STAT pathway in OL maturation and hypoxic brain injury, and indicate a neurotherapeutic potential of targeting this pathway in treating cerebral palsy.

Disclosures: S. Sen: None. **S. Tyagi:** None. **D. Dey:** None. **V. Shrivastava:** None. **S. Rani:** None. **M. Fahad:** None. **A. Ethayathulla:** None. **J. Sharma:** None. **P. Seth:** None. **J. Palanichamy:** None.

Nanosymposium

NANO038: Mechanisms of Myelination, Demyelination, and Remyelination

Location: SDCC Rm 23A

Time: Tuesday, November 18, 2025, 1:00 PM - 2:45 PM

Presentation Number: NANO038.07

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

Support: HHMI Emerging Pathogens Initiative

Title: Early life influenza infection results in multicellular neural dysregulation and disrupted myelin development

Authors: *K. E. MALACON[1], K. SHAMARDANI[2], S. ARTANDI[3], N. ZERNICKA-GLOVER[4], M. MONJE[1];
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Abstract: Children, with their actively developing brains, are particularly vulnerable to neuroimmune challenges during early life. Millions of children world-wide are currently living

with long COVID, and among them, up to 44% experience symptoms of cognitive impairment, often described as “brain fog.” Similarly, millions of children contract influenza each year, with thousands requiring hospitalization. To investigate the neuroimmune and cognitive effects of respiratory immune challenges during early postnatal life, we exposed juvenile mice to a mild respiratory influenza (H1N1) infection at postnatal day 14 (P14) via intratracheal administration. One week after infection, we observed white-matter-specific microglial reactivity, accompanied by a loss of oligodendrocytes. Behavioral testing revealed that mild H1N1 infection resulted in cognitive deficits, as evidenced by impaired performance in the Novel Object Recognition Task (NORT) and T-maze tests of attention and memory, conducted 4 weeks post-infection (at 6 weeks of age). By 7 weeks post-infection, oligodendrocyte numbers and reactive microglia levels in white matter had normalized to control levels, but deficits in myelin ultrastructure, including decreased myelinated axon density and decreased myelin sheath thickness in the frontal subcortical white matter, were evident by transmission electron microscopy (TEM). Concordant with these myelin deficits, behavioral testing at 8 weeks post-infection (10 weeks of age) revealed persistent deficits in attention and memory, which persisted 6 months post-infection. As respiratory influenza infection increased blood and CSF chemokine levels, we tested whether targeting chemokine receptors could mitigate the neurobiological effects of H1N1 infection. We administered a small-molecule CCR3 inhibitor that is blood-brain barrier (BBB)-permeable from day 7 to day 28 after infection. Inhibiting the multi-chemokine receptor CCR3 reduced reactive microglia and increased oligodendrocyte numbers in the subcortical white matter after H1N1 infection. Concordantly, cognitive performance in the novel object recognition test was rescued in CCR3i-treated animals after H1N1 infection. Taken together, these findings underscore the neuroimmune response to mild respiratory influenza during critical developmental stages, shedding light on potential glial mechanisms underlying cognitive impairment and suggesting avenues for targeted interventions to mitigate long-term neurocognitive effects following respiratory immune challenge in pediatric populations.

Disclosures: K.E. Malacon: None. **K. Shamardani:** None. **S. Artandi:** None. **N. Zernicka-Glover:** None. **M. Monje:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); M.M. holds equity in MapLight and CARGO Therapeutics..

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.01

Topic: C.06. Neuromuscular Diseases

Support: Guangdong Basic and Applied Basic Research Foundation 2023B1515020109
Major Program of Shenzhen Bay Laboratory S241101003

Title: Trpv1 promotes stress granule assembly and neurodegeneration in als/ftd

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Abstract: Stress granules (SGs) are membrane-less, cytoplasmic RNA/protein condensates assembled in cells upon diverse cellular stressors. Aberrant SGs can trigger the aggregation of SG proteins, such as TDP-43, FUS, etc. As the aggregation of these proteins is a pathological hallmark of ALS/FTD, two related neurodegenerative diseases, it is believed that SGs play a critical role in ALS/FTD pathogenesis. Consistent with this idea, mitigating SG assembly suppresses neurodegeneration in cellular and animal models of ALS/FTD, suggesting SGs as a potential therapeutic target. However, current SG inhibitors often have detrimental side effects in preclinical studies, highlighting the need to identify novel approaches to target SG assembly. To identify novel ways to target SGs, we performed RNAi screens on ~300 SG-related genes in *Drosophila* models of ALS/FTD and then neurons derived from patient-induced pluripotent stem cells (iPSCs). We identified ~10 novel genes as potential therapeutic targets, among which a top candidate is transient receptor potential vanilloid 1 (TRPV1), a non-selective calcium channel. To study how TRPV1 promotes SG assembly and neurodegeneration in ALS/FTD, we first showed that stress upregulated cytoplasmic calcium, suggesting a role of calcium in SG assembly. Consistent with this notion, BAPTA, a calcium chelator, suppressed SG assembly by disrupting the interactions among SG proteins. Secondly, we showed that the cytoplasmic calcium is upregulated in iPSC-derived neurons (iPSNs) from ALS/FTD patients, compared to the control, and is further upregulated upon excitation. Agreeing with these data, BAPTA suppressed glutamate-mediated excitotoxicity in these iPSNs, suggesting that calcium promotes neurodegeneration by sensitizing neurons to excitotoxicity. Furthermore, antisense oligonucleotides (ASOs) or antagonists against TRPV1 suppressed cytoplasmic calcium levels, SG assembly, and excitotoxicity. Combined, our findings identified a novel mechanism by which calcium promotes SG assembly, excitotoxicity, and neurodegeneration and suggested TRPV1 as a potential therapeutic target for ALS/FTD.

Disclosures: C. Li: None. **S. He:** None. **K. lu:** None. **K. Zhang:** None.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.02

Topic: C.06. Neuromuscular Diseases

Support: NIA T32AG057468
Patrick Grange Foundation
Mitsubishi Tanabe Pharma America, Inc.

Title: Focal neuroinflammation in upper motor neuron axons and injury-induced inflammation in lower motor neurons could explain clinical symptom progression in ALS

Authors: *H. C. CROPPER[1,2], F. ESLAMI[2], K. KOPECKY[2], M. RAMIZUDDIN[2], V. SRIVASTAVA[2], F. MIR[2], J. LIU[2], E. MOCANU[2], T. VALYI-NAGY[3], C. ABRAMS[2], F. SONG[2], J. A. LOEB[2];
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Abstract: Background: While genetics plays a strong role in amyotrophic lateral sclerosis (ALS) susceptibility, environmental factors may better explain clinical heterogeneity in disease presentation (limb- vs bulbar-onset). Interestingly, there is a higher prevalence of ALS in athletes and veterans, two groups that have higher rates of nerve injuries. Using the SOD1 rat model, we have shown sciatic nerve injury triggers disease onset in the injured hindlimb. Here, we examined the effect of brachial plexus nerve injury in SOD1 rats and the role of peripheral nerve injury in ALS onset in patients through clinical and postmortem examination of upper (UMN) and lower motor (LMN) neuron systems. Methods: We analyzed charts from 66 ALS patients for rates of nerve injury. In a subset of 10 patients, we performed rapid autopsies and collected tissue for immunohistochemistry (microglia, axon loss, pTDP43) and bulk RNA-sequencing. In SOD1/WT rats, we performed left brachial plexus crushes and collected tissue post-injury (n=6-7 at 1wk, 2wk, 4wk, end stage) to quantify functional recovery and histopathology (microglia, SOD1, neuronal loss). Results: 83% of arm onset ALS patients had clinical or MRI evidence of a cervical nerve injury and 67% of leg onset patients had evidence of a lumbar nerve injury. This suggests that a previous nerve injury may increase susceptibility of that location to disease onset. Histologically, LMN loss was greatest at the site of onset, but UMN axons show all or nothing degeneration and neuroinflammation of the corticospinal tract in the spinal cord through the medulla ($p<0.05$). Surprisingly, the corticospinal tract was intact in the midbrain and motor cortex, suggesting Wallerian degeneration of UMN axons. RNA-sequencing in the same tissues shows enrichment of inflammatory pathways at the sites of disease onset, implicating neuroinflammation in disease spread. Following a left brachial plexus crush in SOD1 rats, they never regained full function ($p<0.01$). While SOD1 rats typically have hindlimb onset, 4/7 brachial plexus injury SOD1 rats had left forelimb onset. Injured animals had ipsilateral neuroinflammation greater in SOD1 vs WT rats and disease onset showed more pathology in the cervical and lumbar enlargements ($p<0.001$). Conclusion: These data suggest that the location of ALS disease onset could be mediated by focal nerve injuries, which are also sufficient to produce focal onset in SOD1 rats. While nerve injury induced inflammation may drive ALS focality for LMN loss, in contrast we found a lack of neuronal degeneration in the motor cortex. Instead, there was focal loss of axons distal to the lower brainstem associated with neuroinflammation.

Disclosures: H.C. Cropper: None. **F. Eslami:** None. **K. Kopecky:** None. **M. Ramizuddin:** None. **V. Srivastava:** None. **F. Mir:** None. **J. liu:** None. **E. Mocanu:** None. **T. Valyi-Nagy:** None. **C. Abrams:** F. Consulting Fees (e.g., advisory boards); Guidepoint Global, Atheneum Partners, NIH. **F. Song:** None. **J.A. Loeb:** None.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.03

Topic: C.06. Neuromuscular Diseases

Support: FightMND Foundation, Australia
FightMND Drug Development Grant Program

Title: Validation of the clinical stage therapeutic agent RRx-001 as a novel disease-modifying treatment for Amyotrophic Lateral Sclerosis

Authors: *R. GORDON[1], M. KUZNETSOVA[2], K. BHATT[3], B. ORONSKY[4], S. CAROEN[4]; [1]Med., Univ. of Queensland - TRI, Brisbane, Australia; [2]Queensland Univ. of Technol., Brisbane, Australia; [3]Translation Res. institute-QUT, Brisbane, ; [4]EpicentRx, Inc., La Jolla, CA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a terminal adult-onset neurodegenerative disorder characterized by a progressive loss of motor neurons in the brain and spinal cord. Current treatments only provide minimal benefits and do not effectively slow or stop disease progression. There is still an urgent need to develop more effective disease-modifying therapeutics for ALS. Failure of recent clinical trials with therapeutic agents targeting single mechanisms, such as immune activation or oxidative stress, indicates that multiple mechanisms may converge to drive motor neuron death and disease pathology. RRx-001 (nibrozetone) is a clinical-stage CNS permeable dual NLRP3 inflammasome inhibitor and NRF2 activator currently in clinical-stage development as a radio and chemo-protective agent for cancer supportive care indications. In this study, we evaluated the therapeutic potential of RRx-001 for neuroprotection using a combination of pre-clinical animal models and mechanistic studies in isolated immune and neuronal cell cultures. In the SOD1 G93A mouse model of ALS, RRx-001 therapy reduced multiple markers of inflammasome activation, both in the muscle and spinal cord. RRx-001 therapy also increased protective NRF2 pathways in the muscle, spinal cord and motor cortex. Once weekly dosing with RRx-001 at 2 to 5 mg/kg also improved exercise intolerance and multiple markers of skeletal muscle pathology and mitochondrial . Crucially, RRx-001 therapy improved motor deficits and increased median survival in SOD1 G93A ALS model. Our studies in primary cultures identified that low nanomolar doses of RRx-001 were effective at reducing markers of inflammasome activation and inflammatory neuropathology triggered by neurotoxic C9ORF72 dipeptide aggregates (poly-GA) in immune cells. Similarly, in pure neuronal cultures, we found that that RRx-001 improved mitochondrial function and protective NRF2 activation markers. Together, our results suggest that RRx-001 could be effective in slowing disease progression by blocking both pathological inflammasome activation, and independently, by increasing protective NRF2 in skeletal muscle, blood, and the central nervous system. Given that RRx-001 has been tested in over 300 patients to date, with no dose limiting toxicities or related serious adverse events, our results highlight the prospect of RRx-001 as a new disease-modifying therapeutic for ALS with the potential for rapid clinical translation.

Disclosures: R. Gordon: A. Employment/Salary (full or part-time):; Translational Research Institute, QUT, University of Queensland. 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.; EpicentRx Inc.. **M. Kuznetsova:** None. **K. Bhatt:** None. **B. Oronsky:** A. Employment/Salary (full or part-time):; EpicentRx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpicentRx. **S. Caroen:** A. Employment/Salary (full or part-time):; EpicentRx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpicentRx.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.04

Topic: C.06. Neuromuscular Diseases

Title: LINE-1 reverse transcriptase inhibition is neuroprotective in a sporadic model of ALS

Authors: *S. KNUTSON[1], S. PULLEY[1], M. AHLIJANIAN[2], K. DEDUCK[3], L. BREUILLAUD[3], B. BEDELL[3], K. LONG[1], H. KEILHACK[1];
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Abstract: Chronic neurodegenerative disorders involve progressive neuronal loss and hallmarks such as genomic instability and neuroinflammation. The endogenous transposable element Long Interspersed Nuclear Element-1 (LINE-1) and its reverse transcriptase (RT) activity increase with aging, epigenetic changes, and cellular stress, contributing to neurodegeneration. LINE-RT can be inhibited by nucleoside reverse transcriptase inhibitors (NRTIs), a class of anti-retroviral drugs originally developed for HIV. In ALS, increased LINE-1 expression is seen in human cases and mouse models with TAR DNA Binding Protein 43 (TDP-43) pathology, and there is clinical precedent for NRTI use in ALS. We hypothesize that LINE-1 RT plays a central role in ALS via two mechanisms: (1) in the cytosol, LINE-1 RT generates DNA that activates neuroinflammatory nucleic acid sensing pathways like cGAS-STING; (2) in the nucleus, LINE-1 RT integrates into new genomic loci, driving genomic instability, DNA damage, and neuronal death. To test our hypothesis, we utilized the TDP-43ΔNLS doxycycline (dox) regulated mouse model, which results in inducible cytoplasmic TDP-43 aggregation in motor neurons under low-dox conditions. These mice were treated for 8 weeks with potent, orally bioavailable, and brain penetrant LINE-1 RT inhibitors, RPT-A and RPT-B. Over the course of the study, we observed an improvement in composite clinical scores, significantly driven by improvement in hindlimb clasping, with RPT-A. This improvement correlated with a significant decrease in Neurofilament Light (NfL) in the CSF at the terminal endpoint. Histologically, we observed lower astrocyte activation and a decrease in phosphorylated TDP-43 in the motor cortex with RPT-A. To correlate efficacy with exposure, we confirmed concentrations of the parent molecules RPT-A and -B in plasma and brain. We observed comparable exposure in plasma, however, the brain exposure of RPT-A was significantly higher than RPT-B. Free fraction brain exposure of RPT-A

but not RPT-B suggested substantial target coverage, thereby providing a quantitative pharmacological basis for the benefits observed with treatment. In summary, neuronal protection, decreases in inflammatory response, and improvement in clinical scores were observed in mice treated with RPT-A in a model of cytoplasmic TDP-43 pathology. These effects corresponded with brain exposure, strongly suggesting that inhibition of CNS LINE-1 RT underlies these observations. Translation of these in vivo mouse findings to human mechanistic models and patient samples will enable the advancement of LINE-1 RT inhibitors for therapeutic and clinical development in ALS.

Disclosures: **S. Knutson:** A. Employment/Salary (full or part-time):; ROME Therapeutics. **S. Pulley:** A. Employment/Salary (full or part-time):; ROME Therapeutics. **M. Ahlijanian:** F. Consulting Fees (e.g., advisory boards); ANI Consulting, LLC. **K. DeDuck:** A. Employment/Salary (full or part-time):; Biospective Inc. **L. breuillaud:** A. Employment/Salary (full or part-time):; Biospective Inc. **B. Bedell:** A. Employment/Salary (full or part-time):; Biospective Inc. **K. Long:** A. Employment/Salary (full or part-time):; ROME Therapeutics. **H. Keilhack:** A. Employment/Salary (full or part-time):; ROME Therapeutics.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.05

Topic: C.06. Neuromuscular Diseases

Support: 1R21AG075814-01A1

Title: The role of Valosin Containing Protein (VCP) in nuclear TDP-43 aggregate pathology.

Authors: ***S. BALASUBRAMANIYAN**[1,2], C. BERGMANN[3], A. JAIN[1], K. Z. WANG[4], J. K. KOFLER[6], C. J. DONNELLY[5], C. T. CHU[7];
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Abstract: Protein accumulation and aggregation are pathological hallmarks of several neurodegenerative disorders. Cytoplasmic aggregation of TAR-DNA binding protein 43 (TDP-43) is common in Amyotrophic Lateral Sclerosis (ALS) - Frontotemporal Dementia (FTD) spectrum, with nuclear TDP-43 aggregation observed in the subtype that is associated with mutations in the molecular chaperone Valosin Containing Protein (VCP). VCP is a ubiquitously expressed member of the AAA+ ATPase protein family with myriad functions, including protein degradation and aggregate handling. While the mechanisms of TDP-43 aggregation remain unknown, stressors such as mutations, protein or RNA concentrations, and environmental factors may contribute to TDP-43 aggregation in various subcellular compartments. Furthermore, the

failure of the proteostasis machinery within the cell may also lead to a buildup of toxic species over time. Using an RNA-binding deficient recombinant construct, we successfully modeled nuclear TDP-43 aggregation in mouse primary cortical neurons. Following transfection, we observed mobile TDP-43 droplets and/or immobile, discrete structures in neuronal nuclei. Using Fluorescence Recovery After Photobleaching (FRAP), we demonstrated that the discrete structures show reduced solubility consistent with aggregates. We discovered that the presence of aggregate-like structures in the nucleus was associated with loss of dendritic arbors as quantified by Sholl analysis. Using a novel real-time TDP-43 loss-of-function sensor (CUTS), we showed that the different TDP-43 expression patterns in the nucleus are associated with a varying degree of TDP-43 loss of splicing function, with aggregates in the nucleus having the most severe loss-of-function effect. We observed that endogenous VCP co-localizes with discrete aggregates in the nucleus. Further, the expression of catalytically inactive VCP exacerbated TDP-43 pathology in the nucleus in neurons, suggesting a potential role for VCP in clearing nuclear TDP-43 aggregates. Our preliminary data suggests a partial loss of function caused by a familial FTD mutation in VCP relative to overexpressed wild-type VCP. Future experiments will investigate VCP's involvement in modulating TDP-43 nucleocytoplasmic shuttling and aggregate clearance. TDP-43 aggregation is a common pathology observed in multiple neurodegenerative diseases. VCP regulates different cellular pathways to help maintain protein homeostasis. Understanding VCP's role in TDP-43 aggregate clearance could shed light on how different upstream processes culminate in a common pathology across various neurodegenerative diseases.

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Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

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Presentation Number: NANO039.06

Topic: C.06. Neuromuscular Diseases

Support: European Regional Development Funds No. CZ.02.1.01/0.0/0.0/16_019/0000868 ENOCH.

Title: Multiple motor proteins regulate diverse ALS-linked TDP-43 anterograde axonal transport

Authors: *M. FEOLE[1], V. M. POZO DEVOTO[3], N. DRAGISIC[4], A. SANSA[2], M. CARNA[5], D. LALL[2], G. FORTE[6], R. SMITH[7], G. B. STOKIN[8], C. N. SVENDSEN[2];
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London, United Kingdom; [7]Director, Ctr. For Neurologic Study, La Jolla, CA; [8]Inst. of Mol. and Translational Medicine, Fac. of Med. and Dentistry, Palace Univers, Olomouc, Czech Republic

Abstract: TDP-43 is an RNA-binding protein (RBP) essential for RNA metabolism. Under physiological conditions, it predominantly resides in the nucleus, where it regulates transcription and splicing, but it is also detected in the cytoplasm, where it participates in mRNA trafficking and local translation. In nearly 97% of Amyotrophic Lateral Sclerosis (ALS) cases, this nucleocytoplasmic balance is disrupted, leading to TDP-43 nuclear depletion and cytoplasmic aggregation. While its nuclear functions have been well characterized, less is known specifically about its axonal roles, despite early axonal degeneration being a hallmark of ALS pathology. This raises the critical question of whether TDP-43's involvement in ALS arises from a nuclear loss of function or a toxic cytoplasmic/axonal gain of function.To address this, we investigated TDP-43 localization and dynamics in axons, aiming to understand how its transport and interactions with motor proteins might regulate mRNA availability during motor neuron (MN) degeneration. Fluorescently tagged TDP-43 and three canonical axonal cargoes, Rab5, Synaptophysin, and APP, were transfected into H9-derived human neurons, and their trafficking was analyzed using semi-automated tracking. TDP-43 transport differed from that of the other cargoes, appearing to engage multiple kinesins with distinct dynamics, suggesting that RBPs may use flexible transport strategies to ensure widespread mRNA distribution. We further examined protein-protein interactions via biochemical assays and Proximity Ligation Assays, confirming that TDP-43 associates with various components of the anterograde transport machinery. In iPSC-derived MNs from healthy donors and sporadic ALS (sALS) patients, we observed accumulation of TDP-43 in axons, with increased granule density and size in sALS, consistent with pathological aggregation. These findings support a model in which impaired axonal trafficking of TDP-43 may contribute to mRNA dysregulation and neuronal vulnerability. Our work highlights the critical role of axonal transport in maintaining neuronal health and identifies axonal TDP-43 transport mechanisms and dysfunction during disease as key aspects in understanding ALS pathogenesis.

Disclosures: M. Feole: None. **V.M. Pozo Devoto:** A. Employment/Salary (full or part-time):; Faculty of Medicine Masaryk University Brno. **N. Dragisic:** None. **A. Sansa:** None. **M. Carna:** None. **D. Lall:** None. **G. Forte:** None. **R. Smith:** None. **G.B. Stokin:** A. Employment/Salary (full or part-time):; Department of Neurology, Gloucestershire Royal Hospital, Gloucester, UK. **C.N. Svendsen:** None.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

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Presentation Number: NANO039.07

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01GM114564-09
NIH Grant T32GM136536

Title: Nedd4 ubiquitinates tdp-43 and regulates its escrt-dependent endolysosomal clearance

Authors: *L. J. MARMORALE, R. BUCHAN;
Mol. and Cell. Biol., Univ. of Arizona, Tucson, AZ

Abstract: Cytoplasmic mislocalization and aggregation of the nuclear RNA-binding protein, TDP-43 is a common pathological hallmark of neurodegenerative diseases including >95% of all ALS cases as well as some cases of frontotemporal dementia and Alzheimer's disease. Driving clearance of cytoplasmic TDP-43 reduces toxicity in various disease models, though how TDP-43 clearance is regulated in physiological contexts remains controversial. Understanding TDP-43 proteostasis is key for understanding toxicity associated with TDP-43 proteinopathy, and upregulating clearance of toxic TDP-43 species could be an avenue of therapeutic potential. We are interested in studying endolysosomal clearance of TDP-43, a pathway we discovered that functions independent of macroautophagy and the proteasome. Using both unbiased and targeted approaches we identified genes related to the E3 ubiquitin ligase, NEDD4(human)/Rsp5(budding yeast) and the Endosomal Sorting Complex Required for Transport (ESCRT) as key effectors of TDP-43 endolysosomal clearance. Inactivation of Rsp5/NEDD4 function increased TDP-43 protein stability in yeast, HEK293A, and iPSC-derived neurons (iNeurons) models. NEDD4 overexpression also increased K63-specific ubiquitination of endogenous TDP-43. ESCRT mutants in yeast and HEK293A cells exhibited increased TDP-43 protein stability, cytoplasmic mislocalization, and aggregation. Moreover, NEDD4 and the ESCRT-associated protein VPS4A co-immunoprecipitated with endogenous TDP-43 in HEK293A cells. NEDD4 KD weakened the interaction between TDP-43 and VPS4A, and impaired targeting of TDP-43 within multi-vesicular body (MVB)-like structures. Therefore, we hypothesize NEDD4 ubiquitination facilitates ESCRT dependent internalization of TDP-43 into MVBs. Future studies will focus on understanding TDP-43 endolysosomal degradation in greater mechanistic detail as well as uncovering how TDP-43 levels, localization, and physical state determine its degradation pathway. We are also interested in cytoplasmic clearance of disease-associated TDP-43 isoforms. Broader impacts of our study include potential identification of novel therapeutic targets for neurodegenerative disease, and a greater understanding of proteostasis regulation.

Disclosures: L.J. Marmorale: A. Employment/Salary (full or part-time):; University of Arizona. **R. Buchan:** A. Employment/Salary (full or part-time):; University of Arizona.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.08

Topic: C.06. Neuromuscular Diseases

Title: A Human iPSC-Based Platform for Functional and Molecular Evaluation of ALS Therapeutics Targeting TDP-43 Pathology

Authors: S. JAIN, B. SAMSON-COUTERIE, C. VAN BERKEL, K.-C. PITSA, *C. LEACH; Ncardia, Leiden, Netherlands

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder primarily affecting motor neurons, with most cases exhibiting pathological aggregation and mislocalization of the RNA-binding protein TDP-43. In ~97% of patients, TDP-43 pathology is associated with loss of nuclear function and splicing dysregulation, including downregulation of *STMN2*, a microtubule regulator critical for axonal maintenance and repair. The lack of predictive, disease-relevant human models has hindered drug discovery, with most preclinical systems failing to recapitulate key molecular and functional ALS hallmarks. To address these limitations, Ncardia developed a scalable, human iPSC-based ALS platform using motor neuron-enriched cultures that robustly reproduce patient-relevant phenotypes. Upon TDP-43 stress induction, these iPSC-derived motor neurons display prominent aggregation and nuclear clearance of TDP-43, accompanied by significant reduction in full-length *STMN2* mRNA and protein and increased expression of aberrantly spliced truncated *STMN2*. Functional impairment is evidenced by altered network activity, including reduced firing, burst frequency, and synaptic connectivity. This model was used to evaluate a lead small molecule therapeutic from Neurizon, a Biotechnology company dedicated to revolutionizing the treatment of neurodegenerative diseases.. The compound, NUZ-001, is designed to restore proteostasis via autophagy activation and has demonstrated promising results in both preclinical and clinical studies for ALS, with the trial reporting a 39% slowdown in motor function decline, as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R). Treatment of ALS iPSC-derived motor neurons for 5-7 days led to a significant decrease in TDP-43 aggregation, with a 50-65% reduction in total TDP-43 levels as measured by HTRF ($p < 0.001$). Electrophysiological function, assessed via multi-electrode array (MEA), showed a significant improvement in burst and network burst metrics ($p < 0.001$). Additionally, spontaneous calcium imaging confirmed normalization of neuronal network activity, including restoration of peak rate and peak-to-peak intervals to wild-type levels. Collectively, these results underscore the predictive power of Ncardia's iPSC-based ALS platform for evaluating therapeutic compounds targeting TDP-43. The integration of molecular, cellular, and functional endpoints in a human-relevant system enhances translational value and provides a powerful tool for advancing ALS drug discovery.

Disclosures: S. Jain: None. **B. Samson-Couterie:** None. **C. van Berkel:** None. **K. Pitsa:** None. **C. Leach:** None.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

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Topic: C.06. Neuromuscular Diseases

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Title: Oral treatment with Fasudil diminishes the capacity of ALS patient-derived exosomes to induce TDP-43 neuropathology in vitro

Authors: *D. A. LINSEMAN[1], C. C. H. BARKER[1], A. N. GROSSBERG[2], K. M. REYNOLDS CAICEDO[1];
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Abstract: Background: TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA-binding protein that undergoes nuclear-to-cytoplasmic translocation and aggregation in motor neurons during the progression of ALS. Therefore, targeting TDP-43 neuropathology is a major goal of ALS research. Rho-associated protein kinase (ROCK) is a pathogenic player in ALS; however, its role in modulating TDP-43 neuropathology has not been previously investigated. **Objective:** We examined whether TDP-43 neuropathology induced *in vitro* in motor neuronal cells by incubation with ALS patient-derived exosomes could be reduced by oral treatment with the ROCK inhibitor Fasudil. **Methods:** Neuron-derived exosomes (NDEs) were isolated from plasma of two healthy control subjects and six ALS patients at baseline and after 6 months of treatment with oral Fasudil at a dose of 180 mg/day. The presence of TDP-43 in NDEs was confirmed by ELISA. Differentiated NSC34 motor neuronal cells were transfected with plasmid expressing wild-type human TDP-43 fused to Td-tomato fluorescent protein, then incubated with NDEs for 24 hours and examined by confocal microscopy to observe changes in TDP-43 subcellular localization and aggregation. **Results:** NSC34 cells treated with NDEs from healthy control subjects showed a normal TDP-43 distribution, with predominantly nuclear localization and very few aggregates. Incubation of NSC34 cells with NDEs from ALS patients at baseline (before Fasudil treatment) induced a dramatic redistribution of TDP-43 from the nucleus to the cytoplasm along with marked aggregation. In contrast, NDEs from ALS patients treated with Fasudil did not cause TDP-43 to redistribute to the cytoplasm and aggregation was markedly reduced compared to baseline, essentially to levels comparable to healthy controls. Proteomic analysis of NSC34 cells following treatment with control versus baseline ALS patient NDEs revealed marked differences in expression of proteins associated with neurodegeneration, stress granules, and RNA-binding dysfunction, further supporting the functional impact of exosome cargo. **Conclusions:** Our findings suggests that NDEs from ALS patients harbor pathogenic cargo capable of inducing TDP-43 redistribution and aggregation in motor neuron-like cells *in vitro*, and that this activity is attenuated following oral ROCK inhibition with Fasudil. Ongoing work will investigate changes in exosome proteomic signatures and alterations in TDP-43 structure or post-translational modifications that may contribute to the pathogenic potential of ALS patient-derived NDEs.

Disclosures: D.A. Linseman: F. Consulting Fees (e.g., advisory boards); Woolsey Pharmaceuticals, New York, NY, USA, Biotech. **C.C.H. Barker:** None. **A.N. Grossberg:** None. **K.M. Reynolds Caicedo:** None.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.10

Topic: C.06. Neuromuscular Diseases

Title: Uncovering the functional impact of novel ALS-specific fusion genes

Authors: ***A. BOUDI**[1], T. PETROZZIELLO[2], H. XU[3], J. LEMANSKI[3], M. KESAVAN[3], A. CASTILLO-TORRES[2], R. B. MONSANTO[2], C. DE ESCH[3], M. CUDKOWICZ[2], R. MOURO PINTO[3], D. GAO[3], G. SADRI-VAKILI[2];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease, typically resulting in death in 2-5 years from diagnosis. The underlying pathogenic mechanisms remain largely elusive, particularly in its sporadic form which accounts for approximately 90% of cases. While numerous genetic factors have been associated with the onset and progression of ALS, we recently identified a new class of genetic alterations enriched in ALS tissues, fusion genes, by leveraging large scale RNA-Seq datasets from the Target ALS Foundation and the New York Genome Center ALS Consortium. Historically, fusion genes have been associated with cancers, and little is known about their implications in neurodegenerative diseases. We confirmed and refined our findings by performing RNA-Seq on additional post-mortem ALS and control tissues from Massachusetts General Hospital. Our analysis identified over 400 gene fusion events unique to post-mortem ALS tissues, with an odds ratio of 3.24, suggesting a strong association between fusion genes and ALS. We then manually curated potential fusion genes predicted to generate in-frame chimeric proteins. Among these, the FAM69A-EVI5 fusion emerged as a top candidate based on its ALS specificity, predicted chimeric protein and biological relevance. FAM69A encodes for a putative calcium-regulated kinase localized in the endoplasmic reticulum, while EVI5 is a Rab11 GTPase-activating protein implicated in recycling endosomes. We hypothesized that the FAM69A-EVI5 ALS-specific fusion disrupts their respective cellular roles, contributing to ALS pathogenesis.
To test this, we employed a multi-tiered approach by transfecting SH-SY5Y neuroblastoma cells line with the fusion gene and: (1) assessed cell metabolism, proliferation and death, (2) characterized the subcellular localization and functional impact of the fusion proteins, and (3) examined structural and functional anomalies in differentiated SH-SY5Y neuron-like cells. Preliminary results suggest that expression of the fusion gene alters cellular proliferation, indicative of fusion-driven dysfunctions.
Ongoing studies will validate these findings in induced pluripotent stem cell (iPSC)-derived motor neurons and develop ELISA-based assay for detecting the fusion proteins.
This work represents the first functional analysis of ALS-specific fusion genes, with potential implications for understanding disease mechanisms, identifying novel biomarkers and therapeutic targets in ALS.

Disclosures: A. Boudi: None. **T. Petrozziello:** None. **H. Xu:** None. **J. Lemanski:** None. **M. Kesavan:** None. **A. Castillo-Torres:** None. **R.B. Monsanto:** None. **C. de Esch:** None. **M. Cudkowicz:** None. **R. Mouro Pinto:** None. **D. Gao:** None. **G. Sadri-Vakili:** None.

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Presentation Number: NANO039.11

Topic: C.06. Neuromuscular Diseases

Title: Assessing tau and pTau-T181 as biomarkers for amyotrophic lateral sclerosis

Authors: *T. PETROZZIELLO[1], E. MIZERAK[4], A. CASTILLO-TORRES[2], R. B. MONSANTO[3], B. HAMMERSCHLAG[2], B. FILLINGHAM[1], P. KIVISÄKK[2], K. FOX[4], S. E. ARNOLD[2], J. B. COHEN[4], M. CUDKOWICZ[5], G. SADRI-VAKILI[6];
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Abstract: To date, the diagnosis of amyotrophic lateral sclerosis (ALS), on average, could take over a year, highlighting an urgent and unmet need for reliable diagnostic biomarker testing. Recent studies have demonstrated changes in the levels of the microtubule-associated protein tau and its phosphorylated form at T181 (pTau-T181) in cerebrospinal fluid (CSF) derived from individuals with ALS. Specifically, we and others reported a significant decrease in the ratio between pTau-T181 and total tau levels in ALS CSF compared to healthy control (HC). Additionally, we also demonstrated a significant increase in total tau levels in CSF derived from individuals diagnosed with bulbar-onset ALS compared to HC. Lastly, we demonstrated that increases in CSF total tau levels positively correlated with faster ALS progression. Building on these findings, here we assessed total tau and pTau-T181 in plasma samples from participants with ALS and HC using two methods - Quanterix Simoa and Meso Scale Discovery (MSD) assays. Both assays revealed an increase in pTau-T181 and pTau-T181:tau ratio in ALS compared to HC. Additionally, increases in both pTau-T181 and pTau-T181:tau ratio were independent of region of onset and observed in plasma derived from both bulbar- and limb-onset ALS. Importantly, longitudinal analysis demonstrated that while increases in plasma pTau-T181:tau levels were not associated with the ALS functional rating scale revised (ALSFRS-R), pTau-T181 levels were associated with faster decline in ALSFRS-R over time and, therefore, with faster disease progression. Total tau levels, instead, were increased in ALS plasma compared to HC on the MSD assay and decreased on Quanterix Simoa assay. These changes in plasma total tau levels were independent of region of ALS onset. Lastly, longitudinal analysis demonstrated a positive, yet non-significant association between plasma total tau levels and the ALSFRS-R. Collectively, our results confirm recently published findings demonstrating an increase in pTau-T181 levels in ALS plasma and expand to reveal an increase in pTau-T181:tau ratio, suggesting that plasma pTau-T181 could serve as a biomarker for ALS. Ongoing studies aim at clarifying tau's role as a diagnostic or prognostic biomarker for ALS.

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Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

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Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.12

Topic: C.06. Neuromuscular Diseases

Title: Axon-specific transcriptome alterations including PLK1 activation and RIG-I-mediated type I IFN signaling define motoneuron-specific vulnerability and biomarker-driven therapeutic opportunities in FUS-ALS

Authors: *V. ZIMYANIN[1], A. HERMANN[2];
[1]Univ. of Virginia, Charlottesville, VA; [2]Dresden Univ. of Technology, Dept. of Neurol., Dresden, Germany

Abstract: Mutations in FUSED IN SARCOMA (FUS) cause juvenile-onset amyotrophic lateral sclerosis (ALS). Early pathogenesis of FUS-ALS involves impaired transcription and splicing, DNA damage response, and axonal degeneration. However, the molecular pathophysiology and the link between somatic and axonal phenotypes are still poorly understood. We evaluated whether compartment-specific transcriptome differences could distinguish and drive early axonal degeneration. We used iPSC-derived cortical and spinal motor neurons (MNs) coupled with microfluidic approaches to generate RNA-sequencing profiles from axonal and somatodendritic compartments. In FUS-ALS, spinal motor neurons show greater vulnerability than cortical neurons due to impaired DNA damage response, disrupted stress granule dynamics, and activation of cell cycle pathways involving PLK1, which are not seen in cortical neurons. We demonstrate that the axonal transcriptome in spinal motoneurons is unique and distinct, with RNA metabolism, extracellular secretion, and matrix disassembly pathways especially enriched in all distal axonal compartments. FUS mutation leads to changes in distinct pathways that were clustered in only a few distinct protein-protein interaction (PPI) networks. Somatodendritic changes upon FUS mutation include WNT signaling, mitochondrial, ECM-, and synapse-related functions. In contrast, analysis of the axonal transcriptome in mutant motoneurons centers on the PLK1 pathway, mitochondrial gene expression, and regulation of inflammation. We have validated PLK1 upregulation in FUSP525L motoneurons. PLK1 upregulation did not activate cell-cycle re-entry but contributed to mutant motoneuron survival, and its inhibition increased neuronal cell death. We propose that upregulation of PLK1 represents an early event in the pathogenesis of ALS and could act in response to DNA damage, mitochondrial damage, and immune response activation in the affected cells. In addition our research highlights RIG-I-mediated innate immune activation and mitochondrial-derived dsRNA accumulation as key contributors to axonal degeneration in FUS-ALS, suggesting JAK-STAT inhibition as a potential therapeutic strategy. Overall we provide a novel valuable resource of the potential targets and affected processes changed in the specific compartments of disease FUSP525L motoneurons.

Disclosures: V. Zimyanin: None. **A. Hermann:** None.

Nanosymposium

NANO039: ALS: Human Genetics, Cellular Mechanisms, and Potential Treatments

Location: SDCC Rm 11

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO039.13

Topic: C.06. Neuromuscular Diseases

Support: Les Turner ALS Foundation

Title: Life extension, motoneuronal excitability, and extrasynaptic glutamate: the role of system x_c^- in amyotrophic lateral sclerosis

Authors: *B. HEIT, M. JIANG, C. HECKMAN;
Neurosci., Northwestern Univ., Chicago, IL

Abstract: In amyotrophic lateral sclerosis (ALS), neural networks exhibit elevated glutamate concentrations coupled with hyperexcitability which ultimately beget excitotoxic death. Not surprisingly, the contribution of *synaptic* glutamate has been heavily scrutinized in ALS etiology; the influence of ambient, *extrasynaptic* glutamate, however, has not been appraised. In the CNS, ambient glutamate is regulated by the cystine/glutamate antiporter, system x_c^-, with protein subunit xCT. Importantly, the antiporter is markedly upregulated in animal ALS models and human patients. We therefore examined the role of system x_c^- in ALS pathogenesis by comparing three mouse genotypes: 1) $xCT^{-/-}$ mice, a transgenic model lacking system x_c^-, 2) $SOD1^{G93A}$ mice, a transgenic ALS model, and 3) wild-type (WT) controls. Specifically, we employed an adult *in vitro* spinal cord preparation to electrophysiologically measure motoneuronal excitability, mass spectrometry to quantify cerebral spinal fluid (CSF) glutamate concentrations, and Western blot analyses to evaluate xCT protein expression. Experiments were performed on male and female mice during the early- and late-life stages. Our results revealed decreased motoneuronal excitability in $xCT^{-/-}$ mice, as evidenced by enhanced short-term depression (STD), when compared to WT and $SOD1^{G93A}$ counterparts. Contrarily, $SOD1^{G93A}$ mice exhibited attenuated STD as compared to $xCT^{-/-}$ and WT mice, thus revealing increased motoneuronal excitability. Moreover, the dampened excitability in $xCT^{-/-}$ mice was concomitant to a robust reduction in CSF glutamate levels and an absence of xCT protein expression, whereas the increased excitability in $SOD1^{G93A}$ mice was attendant to heightened glutamate levels and elevated xCT protein expression. All differences between genotypes remained consistent or became exacerbated with the aging process. Remarkably, $xCT^{-/-}$ mice exhibited a ~17% extension in lifespan compared to WT mice, whereas $SOD1^{G93A}$ mice displayed severely truncated lifespan. Moreover, our preliminary data shows extended lifespan of $SOD1^{G93A}$ mice with genetic deletion of xCT. Collectively, these findings suggest that the ALS-induced upregulation of system x_c^- increases ambient, extrasynaptic glutamate concentrations which elicits motoneuronal hyperexcitability thereby expediting degeneration. Due to the availability of FDA-approved system x_c^- inhibitors, our results portend high translational potential for ameliorating ALS pathology in humans. Furthermore, these studies warrant future investigations

comparing and/or combining system x_c^- antagonism with current therapies, such as Riluzole treatment.

Disclosures: B. Heit: None. **M. Jiang:** None. **C. Heckman:** None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.01

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Rita Allen Foundation

Title: Calcium dynamics in choroid plexus endothelial cells are regionalized and activity-dependent

Authors: *N. DANI;
Cell and Developmental Biol., Vanderbilt Univ., Nashville, TN

Abstract: Cerebral vascular barriers regulate the entry of beneficial factors into the brain while blocking toxins and pathogens. Endothelial cells are crucial for these functions and influence the physical, metabolic, and transport properties of these brain barriers including the choroid plexus. Transcriptional and spatial analyses have identified conserved and barrier-specific endothelial programs. For example, choroid plexus endothelial cells exhibit gene expression parenchymal blood vessels, including transcription factors, receptors, and transporters; however, they possess distinct features like fenestrae and engage with unique stromal and non-vascular cell types. Recent single-cell transcriptional studies have shown changes in choroid plexus endothelial gene expression linked to development, aging, and disease. However, tools to visualize choroid plexus endothelial cell properties in real time are limited. This study adapts an explant model to visualize calcium activity in intact choroid plexus tissue from embryonic and adult mice. By using transgenic mouse lines, we drive the expression of a genetically encoded calcium indicator (GCaMP) specifically in endothelial cells of the lateral ventricle choroid plexus, enabling the visualization of intracellular calcium dynamics, which serves as a surrogate for cell activity. Preliminary findings show distinct region-specific patterns of endothelial cell activity in explanted tissue. These cells can also sense and respond to mechanosensory cues, neurotransmitters, and activation of G protein-coupled receptors. While the lack of continuous blood and cerebrospinal fluid perfusion is a limitation of the explant model, it allows for rapid screening of activity-dependent responses in specific endothelial cell subtypes across the choroid plexus. Understanding these properties may enhance in vivo studies focused on improving drug delivery to the central nervous system and deepen our knowledge of choroid plexus remodeling in disease states.

Disclosures: N. Dani: None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.02

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Wellcome Trust 225950/Z/22/Z

Title: Building and Breaking the Blood-CSF Barrier: Human choroid plexus organoids reveal injury-repair dynamics

Authors: *L. PELLEGRINI;
King's Col. London, Cambridge, United Kingdom

Abstract: The choroid plexus (ChP) is a vital tissue located in the brain ventricular system. This tissue displays a number of important functions such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF). To explore the development and function of the human ChP, we recently established a protocol to generate human ChP organoids using a combination of signalling molecules that are physiologically present during the stages of development of this tissue. These organoids recapitulate fundamental functions of ChP such as CSF secretion and formation of a tight epithelial barrier selectively permeable to small molecules. To characterise the development of ChP cell populations over time, we have performed a longitudinal scRNA sequencing analysis of the organoids. We found that, similarly to ChP tissue *in vivo*, organoids stop proliferating *in vitro* and develop features of mature tissue, comparable to adult human ChP. Next, we used this model to investigate the epithelial response to mechanical injury and we discovered that ChP epithelial cells secretes cytokines and chemokines in response to injury and undergo a transitional state involving tissue remodelling leading to repair. Finally, we are using this model as a platform to study mechanisms of peripheral regulation in the ChP in response to environmental stimuli, CNS-targeting drugs and inflammatory agents. In conclusion, ChP organoids have been proven useful in multiple applications and represent a powerful tool to study not only developmental diseases but also tissue repair response.

Disclosures: L. Pellegrini: None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.03

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

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The New York Stem Cell Foundation
Cure Alzheimer's Fund
Human Frontier Science Program (HFSP) research program grant #RGP0063/2018
NIH R01 NS088566
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Title: The roles and functions of the choroid plexus in brain inflammation and neurodegeneration

Authors: *H. XU[1], M. LEHTINEN[2];
[1]Harvard Med. School/Boston Children's Hosp., Boston, MA; [2]Pathology, Boston Children's Hosp., Boston, MA

Abstract: Excessive neuroinflammation exacerbates neurodegeneration. The choroid plexus (ChP) is emerging as a key regulator of brain inflammation, but the cellular mechanisms are poorly understood. The ChP forms the main blood-CSF barrier, receives and integrates signals from both the brain and the periphery, controls access of peripheral molecules and immune cells to the brain, and through its secretory epithelia influences CSF composition and thereby brain cell activities. Our recent work provides a broad and detailed overview of important immune cell types, their origins, and the mechanisms that govern their blood-ChP-CSF movements during brain inflammation. The work discovered extensive interaction between a specialized population of epithelial cells and immune cells using longitudinal multi-photon imaging in awake mice combined with single-cell transcriptomics and lineage tracing. The epithelial cells coordinate stepwise immune cell recruitment, infiltration, differentiation, and adhesion to the ChP upon acute brain inflammation. In turn, the immune cells modulate the structural integrity of the epithelial barrier. Our on-going work identified consistent signatures of ChP inflammation and barrier disruption in patient specimens and mouse models of Alzheimer's disease. Collectively, our work provides critical first steps towards a mechanistic understanding of the complex epithelial-immune synergy that governs ChP inflammation. The findings guide our future investigation into ChP inflammation and barrier dysfunction in brain aging and Alzheimer's disease and possible new therapies to control neuroinflammation.

Disclosures: H. Xu: None. **M. Lehtinen:** None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

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Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

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The Shurl and Kay Curci Foundation

Title: Adeno-associated virus (AAV) as a tool for advancing CSF biology: safety implications for the developing ventricular system

Authors: *L. LIU, A. NOGUERA, R. M. FAME;
Neurosurg., Stanford Univ., Stanford, CA

Abstract: Cell-type-specific expression of well-characterized or novel (synthetic/exogenous) proteins and genetic sequences has significantly advanced our understanding of the central nervous system (CNS). In cerebrospinal fluid (CSF) biology, AAV-mediated transfection of target cells enables sustained delivery of therapeutic secretory proteins into the CSF, offering promising avenues for both CNS therapy and mechanistic studies. However, despite AAV's advantages of tropism-based cellular selectivity and transgene delivery, both preclinical studies and clinical trials report short- and long-term adverse effects, including excessive immune responses. Especially relevant for CSF biology, CNS immune insults raise the risk of CSF dysregulation, including hydrocephalus. These risks may be exacerbated in pediatric populations, where ongoing CNS development—along with immature meninges, choroid plexus, and skull structures—may impair the ability to compensate for CSF dysregulation. To systematically address these risks and provide guidelines for minimizing CNS immune insults after delivering AAV vectors, we test the effects of intracerebroventricular (i.c.v.) injections of increasing concentrations of 3 AAV serotypes (AAV2/5, AAV2/4, and AAV.PHP.eB) to neonatal (P0/P1) CD1 mouse pups (n=4). We find that i.c.v. injections of all 3 AAV serotypes induced ventriculomegaly by postnatal day 7 (P7) when administered at higher doses. CSF ELISA assays detected elevated CSF levels of the pro-inflammatory cytokine CCL2 in AAV-injected P7 pups, indicating immune activation. These proinflammatory effects were independent of AAV tropism. Immunohistochemical analysis verified AAV2/5 tropism limited to choroid plexus epithelial cells, whereas AAV2/4 and AAV.PHP.eB transfected choroid plexus epithelial cells in addition to also transfecting ependymal and parenchymal regions, respectively. Taken together, these results emphasize critical safety considerations for using AAV to study CSF biology, particularly in developing systems. Further, these results suggest an optimal dosage for perinatal i.c.v. AAV delivery and provide a suite of methods to analyze immune insult after i.c.v. AAV delivery. This study enables a range of future directions, including further elucidating the mechanisms underlying AAV-induced ventriculomegaly—whether via increased CSF production, impaired clearance, or both—and assessing associated glial/immune reactivity.

Disclosures: L. Liu: None. **A. Noguera:** None. **R.M. Fame:** None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

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Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.05

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Hydrocephalus Association

Title: Adeno-associated virus serotype comparison for human choroid plexus transduction using ex vivo tissue specimens

Authors: O. CHECHNEVA[1], A. HOCHSTETLER[2], B. J. CORD[1], M. LEHTINEN[3], ***C. SADEGH**[1], A. MOSKALIK[1], T. BACHA[1], Y. REN[4], J. FAN[1], M. MCMAHON[1], A. PANIGRAHI[1], D. WARNER[1], V. VIJAYAKARTIK[5];
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Abstract: Adeno-associated virus (AAV)-based gene therapy holds significant promise for treating neurological disorders through targeted delivery to the central nervous system. Multiple studies have examined the transduction characteristics of different AAV serotypes delivered into the cerebrospinal fluid (CSF) in rodent and large-animal models, revealing species-specific tropism patterns in the choroid plexus (ChP). For example, while AAV5 efficiently transduces rodent ChP epithelial cells, the same virus demonstrates poor transduction efficiency in feline ChP. However, transduction characteristics in human ChP models remain unreported, representing a critical translational gap. We developed an *ex vivo* assay to evaluate the specificity of AAV-mediated gene therapy using surgically derived tissue specimens. All protocols approved by the Institutional Review Board. Human tissue specimens were cultured in human cerebrospinal fluid for 48 hours with selected AAV serotypes (AAV1, AAV4, AAV5, AAV6, and AAV9) encoding a green fluorescent protein (GFP) reporter gene. Transduction efficiency (percentage of GFP-positive cells) at different viral titers was quantified via immunofluorescence microscopy.For each AAV serotype, we identified the overall transduction efficiency and the specificity of epithelial targeting within the ChP, as compared to specimens of pia and neocortex. Dose-dependence was also characterized for each AAV serotype. These descriptive findings provide a foundation for delivering proteins of interest for the restoration of the CSF composition in conditions such as hydrocephalus, chemotherapy-related cognitive impairment, and other disorders of CSF homeostasis.

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Nanosymposium

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Presentation Number: NANO040.06

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Human choroid plexus development, pathologies, and implications for the developing and aging human brain

Authors: *E. S. MONUKI[1], B. A. JOHNSON[2], H. MASTERS[3], M. NEEL[4], V. ESPERICUETA[5];
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Abstract: As the interface between blood and the cerebrospinal fluid (CSF) that it produces, the choroid plexus (ChP) is a crucial support for the developing and adult brain. The ChP has also been neglected historically, particularly in humans, but has gained momentum as a tissue worthy of scientific inquiry. Our inquiries have focused on stem cell derivations of human ChP cells and defining their common pathologies to model in the stem cell systems. We recently improved and simplified the derivation of human ChP epithelial cells (CPECs) from human stem cells, including induced pluripotent stem cells (iPSCs). Using single-cell RNA sequencing (scRNA-seq) and human tissues, we found an unexpectedly complex CPEC lineage tree in prenatal development that has important implications for CPEC roles in human brain development. After establishing a human ChP biorepository, we identified a number of common aging-associated ChP pathologies, some of which have not been described in other species. One distinctive pathology is an intracellular CPEC amyloid known as the Biondi body (BB). Using manual and machine learning approaches, we found a marked and abrupt increase in BB prevalence during human midlife as well as statistical evidence for its clustering and prion-like spread. Importantly, BB accumulation precedes the accumulation of other amyloids and neuropathologies associated with brain aging and neurodegeneration, such as amyloid-beta implicated in Alzheimer's disease (AD). Stem cell-derived CPECs readily take up exogenous amyloid-beta, and at the cellular level, BBs have significant impacts on multiple functional proteins expressed by CPECs. Collectively, these findings suggest ChP contributions to brain aging and neurodegeneration, particularly during midlife, but potentially lifelong. To begin interrogating these contributions, we acquired and generated human iPSCs that are isogenic for the three human-specific isoforms of APOE, the amyloid-beta chaperone and strongest genetic risk factor for sporadic AD. Initial scRNA-seq studies identify significant differences between iPSC-derived CPECs with different APOE isoforms and their ability to take up amyloid-beta, raising the possibility of early-life differences in amyloid-beta clearance. To further improve our human ChP models, we also describe the development of iPSC-derived CPEC organoids and vascularized assembloids, which have the added value of being potential delivery vehicles for neurotherapeutics that cannot cross the blood-brain barrier.

Disclosures: E.S. Monuki: None. **B.A. Johnson:** None. **H. Masters:** None. **M. Neel:** None. **V. Espericueta:** None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.07

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS110665
NSF Grant 2033800
NSF Grant 2328276
NIH Grant NS129735

Title: Exploring the impact cell-cell signaling and fluid flow has on the assembly and maturation of the human choroid plexus

Authors: *H. MASTERS;
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Abstract: As the interface between the blood and cerebrospinal fluid (CSF), the choroid plexus (ChP) conditions and produced CSF and mediates body-brain homeostasis, largely via their epithelial cells (CPEC) which act as the functional unit of the tissue. The expression of amyloid beta production and clearance paired with the high expression of AD risk genes (particularly APOE) in human CPECs suggests the cells and tissue are involved in the pathogenesis of age-related diseases such as Alzheimer disease (AD). Despite this, remarkably little is known about the human ChP or its CPECs, how they change in disease, and how they can be manipulated for therapeutic benefit. This is in part due to the lack of robust model CPEC and ChP systems available for in-depth mechanistic studies. To address this gap, my initial studies involved developing an efficient protocol for CPEC differentiation from human pluripotent stem cells (hPSCs), that unfortunately exhibited immature properties, a signature that hinders studies on age-related diseases such as AD. While the protocol mimics the early developmental *in vivo* environment of CPECs, it lacks vascular and stromal cells (basal interacting factors) and fluid flow (apical interacting factors) that are acquired later. Transcriptome mining of the dCPECs revealed the cells to be a source of vascular and stromal promoting factors, such as VEGF and FGFs, and various mechanosensory tools. These findings suggest the immature CPECs are primed to interact with factors that developmentally are acquired later in development. As such, I hypothesis that immature CPECs rely on interactions with vascular and stromal cells along with exposure to fluid flow to fully reach maturity. To test this, I leveraged innovative genetic engineering techniques and microfluidic devices not previously applied to ChP models, to recreate the choroid plexus microenvironment in a dish. This system involves co-culturing dCPECs with derived endothelial and stromal cells while perfusing the system to mimic the flowing CSF. Preliminary studies showed a subtle increase in age-related markers and decrease in progenitor-related markers. Such a representative model will inform mechanisms of human ChP development, assembly, and role of the ChP in aging and AD.

Disclosures: H. Masters: None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.08

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: A role for mechanosensation in choroid plexus biology

Authors: *C. P. PROFACI[1], A. DEY[3], R. LUO[2], S. H. YUAN[4], A. PATAPOUTIAN[3];
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Abstract: The choroid plexus, a villous structure that projects into each of the brain's ventricles, is widely considered to be the source of cerebrospinal fluid (CSF). Studying this floating structure within the brain has long presented a technical challenge, and many fundamental questions about choroid plexus biology and physiology remain unanswered. One important outstanding question is whether the choroid plexus is able to sense CSF flow, hydrostatic pressure, or CSF osmolarity, and how it might respond to these signals. This work explores a physiological role for mechanosensation at the choroid plexus.

Disclosures: C.P. Profaci: None. **A. Dey:** None. **R. Luo:** None. **S.H. Yuan:** None. **A. Patapoutian:** None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.09

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R01NS125074
NIH , R01AG083164

Title: Loss of ChP/CSF increases plaque load and accelerates plaque pathology progression in mouse AD models

Authors: A. TARANOV, ***A. LUO**;
Univ. of Cincinnati, Cincinnati, OH

Abstract: Correlative evidence from human studies suggests that impaired function the choroid plexus (ChP), which produces cerebrospinal fluid (CSF) may contribute to pathology seen in

normal aging and Alzheimer's disease (AD), in the latter case it is hypothesized to be driven by reduced clearance of amyloid beta (Aβ) from the brain though the CSF, although these hypotheses have not been directly tested in rodent models. To this end, we utilized the novel inducible non-invasive ChP ablation model developed in the lab (ROSA26iDTR mice), and our data show a moderate and stage-dependent acceleration of plaque pathology progression in two mouse models of Alzheimer's disease (5xFAD and Denali hAβ-SAA mice), demonstrating the direct causal link between ChP/CSF and amyloid plaque pathology. Furthermore, we identified a potential behavioral phenotype of impaired novelty-induced responses, including reduced exploration and increased freezing in novel contexts, and impaired responses to social novelty. This possible shared phenotype between ChP ablation and plaque pathology suggests potential common mechanisms of pathogenesis in these distinct aging and AD-associated pathological states. Additionally, CSF proteomics analysis revealed CSF-borne factors that are differentially regulated by ChP ablation and SAA genotype, and that warrant investigation in future studies to determine whether they may be causative of the observed phenotypes.

Disclosures: A. Taranov: None. **A. Luo:** None.

Nanosymposium

NANO040: Choroid Plexus and CSF Biology

Location: SDCC Rm 24A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO040.10

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Neurological Foundation of New Zealand Postdoctoral Fellowship

Title: Age and amyloid-beta dependent circadian changes in the rodent choroid plexus

Authors: *D. J. JANSSON[1], R. O'BOYLE[4], R. J. VERED[2], T. PEDERSEN[5], E. GINO[6], M. SEVAO[7], K. L. SUCHLAND[3], S. KEIL[8], M. BRAUN[9], J. J. ILIFF[2];
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Abstract: The choroid plexus (CP) is a highly vascularized stromal tissue located in each of the four brain ventricles. Encased by ciliated epithelial cells, the CP is responsible for the majority of cerebrospinal fluid (CSF) production in the brain. The CP also delivers trophic factors to surrounding brain cells, forms the brain-CSF barrier, and is important in brain immune cell surveillance.Still, relatively little is known about what factors regulate the CP under healthy conditions. CSF production in humans is diurnally regulated, and evidence in animal models demonstrate CP circadian-dependent transcriptional regulation. Moreover, CSF movement and

clearance is regulated by both sleep and circadian rhythms.However, CP function and CSF dynamics are altered with age and in the setting of Alzheimer's disease (AD), where sleep and circadian disruption often co-occur. We hypothesized that the CP is functionally regulated by sleep and circadian rhythms, and that this regulation would be disrupted with old age and with AD.To test this, we collected CP tissue from wild-type mice at 3 months (young), 12-14 months (aged), and from 12-14-month-old J20 (B6.Cg-*Zbtb20*$^{Tg(PDGFB-APPSwInd)20Lms}$/2Mmjax) mice as a model of amyloid pathology (AD mice). Tissue was collected at opposite circadian times to coincide with the middle of light and dark phases of the 24 hour clock. We performed bulk RNA sequencing on the tissue to examine the transcriptional profile across the day in the three conditions (young, old, AD).We observed that in young healthy mice, circadian rhythms are the main governing factors driving changes in gene expression in the CP. Circadian-dependent gene changes are enriched for protein stability, metabolism and cellular respiration in young mice, but in old mice shift to prioritizing membrane transport, vesicular function and ion homeostasis. Alternatively, in mice with amyloid accumulation CP circadian regulation is lost. Our data suggest age-related circadian shifts in the choroid plexus may drive disease processes. Future work aims to disentangle the effects of circadian-dependent dysregulation at the choroid plexus with age-related impairment of glymphatic function.

Disclosures: D.J. Jansson: None. **R. O'Boyle:** None. **R.J. Vered:** None. **T. Pedersen:** None. **E. Gino:** None. **M. Sevao:** None. **K.L. Suchland:** None. **S. Keil:** None. **M. Braun:** None. **J.J. Iliff:** F. Consulting Fees (e.g., advisory boards); Applied Cognition.

Nanosymposium

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Presentation Number: NANO040.11

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

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Brain Research Foundation Seed Grant
NIH R01 MH136258
NIH T32 HL110852
OFD/BTREC/CTREC Faculty Development Fellowship Award
NIH R01 NS088566
NIH RF1 DA048790

Title: Diurnal cycles in the choroid plexus regulate CSF

Authors: *R. M. FAME;
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Abstract: Cerebrospinal fluid (CSF) exchanges with the central nervous system's immediate environment and interfaces with systemic circulation at the blood-CSF barrier. CSF composition

reflects brain states, contributes to brain health and disease, is modulated by circadian rhythms and behaviors, and turns over multiple times per day, enabling rapid signal relay. Mechanisms of how CSF elements change over circadian time and influence function can be harnessed for diagnostic biomarkers and therapeutic intervention.
Transmission and secretion of signals via the choroid plexus brain barrier can modulate brain states via regulation of CSF composition. We analyzed diurnal variations in mouse choroid plexus and CSF of male adults. Ribosome profiling of choroid plexus epithelial cells revealed diurnal translatome differences in metabolic machinery, secreted proteins, and barrier components. Using choroid plexus and CSF metabolomics and blood-CSF barrier analyses, we observed diurnal changes in metabolites and cellular junctions.
Transthyretin (TTR), a thyroid hormone chaperone secreted by the choroid plexus is diurnally regulated. Diurnal variation in choroid plexus TTR depended on Bmal1 clock gene expression. We achieved real-time tracking of CSF-TTR in awake $Ttr^{mNeonGreen}$ mice via multi-day intracerebroventricular fiber photometry. Diurnal changes in choroid plexus and CSF TTR levels correlated with CSF thyroid hormone levels, for which we developed a new mass spectrometry based detection method. All data were rigorously analyzed following power calculations based on the variance. Together, these findings highlight integrated diurnal control of brain states by the choroid plexus and CSF.

Disclosures: R.M. Fame: None.

Nanosymposium

NANO041: Sleep Mechanisms Contributing to Cognitive Function

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO041.01

Topic: G.07. Biological Rhythms and Sleep

Support: CIHR 254572

Title: Auditory Cued-Based Targeted Memory Reactivation Timed to Sleep Spindles Enhances Declarative Memory and Reorganizes Hippocampal-Cortical Networks

Authors: *V. MUTREJA[1], P. GUPTA[2], O. LUNGU[3], L. LAZZOUNI[3], E. GABITOV[4], G. ALBOUY[5], B. R. KING[6], A. BOUTIN[7], J. CARRIER[8], J. DOYON[9];
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Abstract: Sleep spindles - brief bursts of 11-16 Hz activity during non-rapid eye movement (NREM) sleep - are known to support memory consolidation by reactivating memory traces endogenously and facilitating the reorganization of associated neural networks. It remains,

however, unclear whether this process can be manipulated directly, via targeted stimulation during sleep. In response to this gap, we tested whether closed-loop targeted stimulation delivered at spindle onset improves memory retention and alters post-sleep brain activity. Participants (n=25) learned locations of two types of images (animals and clothing) in a square grid that were each paired with a specific sound. The memory task was performed while fMRI data were acquired using a 3T MRI scanner, followed by an overnight sleep session in the lab. During NREM sleep, only one of the two sounds was then replayed at spindle onset using a closed-loop targeted stimulation that detected spindles in real time, such targeted memory reactivation (TMR) technique being designed to boost the reactivation of memory traces specific to the learned material associated with a given sound at encoding. The next morning, participants underwent a retest and recognition session while being scanned again, enabling a direct assessment of post-sleep retrieval. Behavioral results showed a significant improvement in memory performance and hit rate in the recognition test for the TMR-cued items compared to the non-TMR ones ($p<0.01$). These findings suggest that auditory cueing during spindles selectively stabilizes memory traces. Post-sleep fMRI analyses revealed reduced hippocampal activation during retrieval of TMR-cued items relative to non-TMR items, accompanied with increased activity in parietal and sensorimotor regions, including the postcentral gyrus, and superior parietal lobule. ROI-to-ROI connectivity analyses showed that, during TMR-cued retrieval, connectivity between the hippocampus (left & right) and posterior temporal fusiform cortex (right) correlated positively with memory performance. These findings thus indicate that TMR promotes a reconfiguration of retrieval pathways, reducing reliance on medial temporal lobe circuitry and engaging a more distributed cortical network, particularly involving perceptual and associative cortices. Together, they also imply that spindle-timed auditory stimulation enhances declarative memory and reshapes post-sleep retrieval dynamics by modulating hippocampal-cortical communication, hence supporting a systems-level shift towards cortical-based memory retrieval and offering a mechanistic pathway for precision memory enhancement during sleep.

Disclosures: V. Mutreja: None. **P. Gupta:** None. **O. Lungu:** None. **L. Lazzouni:** None. **E. Gabitov:** None. **G. Albouy:** None. **B.R. King:** None. **A. Boutin:** None. **J. Carrier:** None. **J. Doyon:** None.

Nanosymposium

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Presentation Number: NANO041.02

Topic: G.07. Biological Rhythms and Sleep

Support: ERC Grant Sleep Balance
Stipend - Max Planck School for Cognition

Title: Spindle-slow oscillation coupling facilitates schema memory formation in humans

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Abstract: Memory schemas are thought to be formed through the abstraction of regularities from multiple experiences, while episodic details of specific experiences often fade from memory. We hypothesized that sleep plays an active role in the abstraction of schema memories, beyond the consolidation of episodic details. We designed an ecologically relevant task in virtual reality inspired by the object-place recognition task commonly used in rodent studies. Our task consists of a schema build-up phase, during which participants (N = 60) learn spatial distributions (i.e. a spatial schema) of two object categories via object-location-associations. Subsequently, the implicitly acquired schema is retrieved, either immediately or following a period of sleep or sleep deprivation. When controlling for episodic memory, participants who slept after schema acquisition show evidence for superior schema memory compared to the immediate retrieval and the sleep deprivation groups. Furthermore, spindle-slow oscillation coupling during non-REM (NREM) sleep was associated with a better schema memory. These findings validate our paradigm to study schema memory in virtual reality and highlight the role of NREM sleep in the abstraction of spatial regularities in humans.

Disclosures: L. Bastian: None. **H. Hamann:** None. **K. Rauss:** None. **J. Born:** None.

Nanosymposium

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Presentation Number: NANO041.03

Topic: G.07. Biological Rhythms and Sleep

Support: ERC AdG 883098 SleepBalance

Title: Spatial schema memory formation in rats is dependent on post-encoding sleep

Authors: *M. HARKOTTE[1,2,3], F. HEIMEL[1], S. DIMITROV[1], D. GRAMLING[1,2,3], M. INOSTROZA[1], J. BORN[1,4,5];
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Tuebingen (IDM), Tuebingen, Germany; [5]Ctr. for Integrative Neuroscience, Univ. of Tuebingen, Tuebingen, Germany

Abstract: Generalizing from past experiences supports flexible behavior in novel but related situations. In this study, we investigated whether post-encoding sleep facilitates the generalization of a spatial rule across multiple experiences (schema memory). Rats were trained on an elaborated version of the object-place recognition (OPR) task, which allowed for abstraction of a spatial rule across eight encoding episodes spaced 20 minutes apart. During each episode, animals freely explored two objects in an open-field arena. Following the encoding phase, animals either slept or were sleep-deprived for two hours, after which they remained undisturbed for 22 hours. Schema memory was assessed the next day. Only animals allowed to sleep during the two-hour post-encoding window demonstrated evidence of schema memory. Furthermore, histological analysis of c-Fos expression during retrieval indicated that successful schema recall was associated with higher inter-regional coactivation, whereas total c-Fos expression was elevated in non-performing animals. These findings highlight the critical role of immediate post-encoding sleep in schema memory formation and demonstrate the utility of an adapted OPR task for probing sleep-dependent memory generalization in rodents.

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Nanosymposium

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Title: Dual memory processing during sleep with consolidation of the past and preparation for the future

Authors: *K. GHANDOUR[1,3], T. HAGA[4], N. OHKAWA[5], C. A. FUNG[6], M. NOMOTO[2,3], M. FAYED[1,3], H. ASAI[7], M. SATO[8], T. FUKAI[9], K. INOKUCHI[1,3];
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Communications Technol., Osaka, Japan; [5]Dokkyo Med. Univ., Mibu Machi, Shimotsuga-Gun, Japan; [6]Dept. of Neurosci., RIKEN BSI, Hong Kong SAR, Hong Kong; [7]Univ. of Tokyo, Tokyo, Japan; [8]Neuropharm., Hokkaido Univ., Sapporo, Hokkaido, Japan; [9]Okinawa Inst. of Sci. and Technol., Onna-son, Japan

Abstract: Memories are stored in specific engram cells, but how distinct engram populations are chosen for current versus future learning episodes remains unclear. In this study, we show that hippocampal CA1 neurons exhibit organized, synchronous activity during prelearning home cage sleep. This activity, present only in engram cells, aligns with future learning patterns and is referred to as *preconfigured ensembles*. After learning, a subset of non-engram cells begins to display coordinated activity, which is shaped during postlearning offline periods, and we termed these cells engram-to-be. These cells later re-emerge to represent new experiences. Our computational model suggests that synaptic depression and scaling contribute to the reorganization of non-engram cell activity. These findings point to two parallel processes occurring during offline periods: the stabilization of past memories through reactivation and the preparation for future ones via synaptic plasticity mechanisms.

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Nanosymposium

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Title: Closed-loop disruption of cortico-cortical communication in NREM impairs motor cortex manifold consolidation

Authors: *J. KIM[1,2,3], L. HE[2,3], K. GANGULY[2,3];
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Abstract: Systems consolidation refers to the gradual integration of newly encoded experiences from the hippocampus into cortical networks for long-term memory storage. Recent studies suggest that the prefrontal cortex (PFC) plays an important role in the later stages of this process. However, prior investigations have typically relied on short-duration exposure paradigms,

limiting insight into the full temporal dynamics of systems consolidation. In this study, we tracked daily changes in motor skill performance, neural representational dynamics in motor cortex (M1), and PFC-M1 slow oscillation (SO) coupling during NREM sleep over a 20-day period in rats trained on a reach-to-grasp task. Employing optogenetic closed-loop stimulation, we selectively disrupted PFC-M1 SO coupling during sleep to test its causal role in long-term motor memory consolidation. Disruption of PFC activity during PFC-M1-coupled SOs delayed the emergence of coordinated PFC-M1 activity and impaired the stabilization of motor performance, particularly in the refinement of reach trajectories. This perturbation also impaired manifold consolidation in M1-there was not the expected decrease in neural trajectory variance-and diminished clustered spindle activity, elucidating how sleep contributes to motor memory stabilization. Together, our findings provide causal evidence that the long-term changes in PFC-M1 communication during NREM sleep over the course of skill learning are essential for the consolidation of neural manifolds and reductions in motor variability, underscoring the dynamic long-term cortico-cortical interactions that support long-term memory stabilization.

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Nanosymposium

NANO041: Sleep Mechanisms Contributing to Cognitive Function

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Presentation Number: NANO041.06

Topic: G.07. Biological Rhythms and Sleep

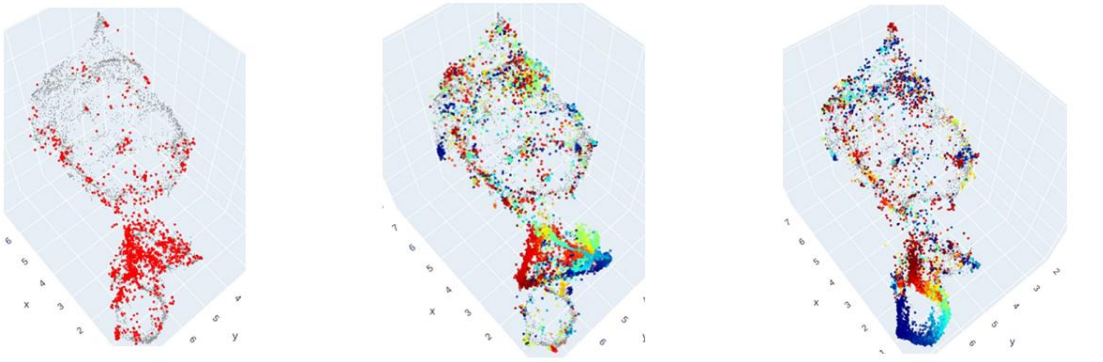
Support: Nos. 12271429
Nos. 12090021
Nos. 12226007

Title: Rem and nrem reactivate separate experiences differentially

Authors: *Y. LIU[1], W. YANG[2], R. HUSZAR[3], M. VOROSLAKOS[4], C. QIAO[1], G. BUZSAKI[5];
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Abstract: Hippocampal activity during sleep is critical for memory consolidation and selection. While sharp-wave ripples (SWRs) in non-rapid eye movement sleep (NREM) are known to support the replay and strengthening of recent experiences, the computational role of rapid eye movement (REM) sleep remains less understood. Sigmund Freud famously suggested that "dreams prefer the impressions of the day before the previous day," raising the possibility that REM may not prioritize memories differently from NREM. To test this hypothesis, we recorded hippocampal population activity in rodents across multiple wake-sleep cycles in different environments. Using the geometry of neural representation during NREM sharp wave ripple

events as a reference manifold, we projected population activity from both wake and REM periods. We observed that REM activity consistently mapped to regions of the manifold associated with earlier wake episodes (i.e., the one prior to the most recent), rather than the most recent waking period. These preliminary results suggest a temporal division in memory reactivation: NREM ripples support consolidation of recent experiences, whereas REM may revisit and restructure older memory traces. Assuming that further experiments and analysis will support these observations, the distinct embedding patterns also imply that REM may help to separate overlapping memory episodes, serving a functional role akin to partitioning memory streams. Importantly, our data may challenge the view that sharp wave ripple activity during NREM is dominated by noise or arbitrary reactivation, instead suggesting a structured process tied to different experiences. These findings may help advance our understanding of how different sleep stages coordinate to preserve memory integrity over time and inform the design of biologically inspired learning systems that are resilient to catastrophic forgetting.



REM data (epoch5) embed on SWR manifold (epoch5)

Recent behavior data (epoch2) embed on SWR manifold, colored by behavior position (epoch5)

Brand-new behavior data (epoch4) embed on SWR manifold, colored by behavior position (epoch5)

Disclosures: Y. Liu: None. **W. Yang:** None. **R. Huszar:** None. **C. Qiao:** None. **G. Buzsaki:** None.

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Presentation Number: NANO041.07

Topic: G.07. Biological Rhythms and Sleep

Support: NSF BCS1439210

Title: REM alpha bursts refine memory via forgetting

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Abstract: The role of REM sleep in memory remains controversial. REM sleep has been shown to enhance sleep-dependent performance gains in non-hippocampal memory domains, particularly in the rescuing of weaker memories. However, in hippocampal memory domains, distinct REM mechanisms have been implicated in forgetting. For example, REM alpha bursts have recently been associated with overnight forgetting of episodic memories. In the present study, we build on these results and further examine how REM alpha bursts may differentially impact forgetting for weak and strong episodic memories. We hypothesized that increased alpha burst activity would predict greater forgetting of strong memories, thus supporting the rescue of weaker memories. We utilized a validated burst detection algorithm to identify alpha bursts (8-13 Hz) during overnight REM sleep in healthy, young adults (n = 24, 18 - 35 years). Participants completed a Face-Name Association (FNA) task both before and after sleep, with interference introduced via an AB-AC paradigm to a proportion of the face-name pairs prior to sleep. We then examined correlations between REM alpha burst power in four brain regions (frontal, central, parietal, occipital) across the four quartiles of the night and overnight change in task performance for items with and without interference. Participants exhibited forgetting overnight for both interfered and non-interfered memories ($p < .05$), with no difference in the extent of forgetting between the two ($p > .05$). REM alpha burst power predicted forgetting for non-interfered memories in all regions ($p < .05$). In contrast, the relation between REM alpha burst power and interfered memories was not significant for most regions ($p > .05$). A post-hoc non-parametric cluster-based permutation test across all electrodes determined that the alpha-forgetting effect is stronger for non-interfered memories ($p < .01$). These results support the tenets of our recently proposed REM Refining and Rescuing (RnR) Hypothesis. We demonstrate a refining role for REM alpha bursts in episodic memory, specifically via forgetting. We further suggest that differential forgetting between weak and strong episodic memories may facilitate the rescue of weaker memories. This supports the RnR's idea that REM sleep contributes to peak normalization, a process through which strong and weak memories become more equally available at retrieval.

Disclosures: A.E. Shuster: None. **E.A. McDevitt:** None. **S.C. Mednick:** None.

Nanosymposium

NANO041: Sleep Mechanisms Contributing to Cognitive Function

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Presentation Number: NANO041.08

Topic: G.07. Biological Rhythms and Sleep

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Title: Coordinated reactivation across motor cortex, premotor cortex, and the striatum during skill consolidation

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Abstract: Adaptive behavior relies on identifying regularities in the environment to guide movement and maximize reward. Widespread cortical inputs to the striatum allow the brain to identify features that reliably predict reward, and to bind them to the motor routines needed to obtain it. While sleep-dependent processing is known to be associated with this process, it is not fully understood how sleep promotes cross-region communication between disparate cortical regions and the striatum to drive the onset of skilled behavior. Here, we simultaneously record the activity of hundreds of neurons in M1 (primary motor cortex), M2 (premotor cortex analog) and the dorsolateral striatum (DLS) as mice acquire a reaching task along days, as well as during subsequent sleep periods. We find that the crystallization of skill is associated with strong and coherent oscillatory activity between M1, M2 and DLS, during sleep. Synchronized cross-region activity entrained local and cross-region spiking, reactivating task-specific information across a brain-wide network. The degree of cross-region coherence was predictive of next-day performance gains. We conclude that cross-region synchronized activity during sleep may allow the sleep-dependent binding of activity in different nodes of the cortico-striatal system to attain a rich representation of movement context allowing naturalistic skill performance.

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Nanosymposium

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Title: CA2 barrages as a mechanism for network and memory stability

Authors: *L. A. KARABA, H. L. ROBINSON, R. E. HARVEY, A. FERNANDEZ-RUIZ, A. OLIVA;
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Abstract: Memory consolidation requires the reactivation of hippocampal neurons, which were previously active during an experience, in sleep sharp-wave ripples (SWRs). This reactivation coincides with an increase in network firing rate and synchrony which naturally decreases over the course of sleep despite the persistence of memory. The exact mechanism of this reset is not yet understood. To address this, we implanted silicon probes targeting the dorsal hippocampus in mice performing a memory task. We identified a hippocampal network event responsible for regulating network stability in sleep. This event, termed a barrage (BARR), is produced by a subset of CA2 pyramidal cells. This subpopulation of neurons innervates cholecystokinin-expressing (CCK+) basket cells, which, in turn, inhibit CA1 pyramidal cells. We found that CA1 neurons and assemblies that increased their activity during learning were reactivated during SWRs, but were specifically inhibited during BARRs. This trend was abolished by silencing CCK+ basket cells during BARRs, resulting in higher synchrony of CA1 assemblies as well as impaired memory consolidation.

Disclosures: L.A. Karaba: None. **H.L. Robinson:** None. **R.E. Harvey:** None. **A. Fernandez-Ruiz:** None. **A. Oliva:** None.

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Title: Post-conditioning sleep deprivation facilitates fear memory extinction in male and female mice.

Authors: *D. MIYAMOTO[1,2], M. MAHMOUD[1], K. MIYAMOTO[1];
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Abstract: Post-traumatic stress disorder (PTSD), more prevalent in females, is associated with persistent fear memories and sleep disturbances. Sleep plays a crucial role in hippocampus-dependent memory consolidation, but how sleep interacts with sex and memory systems remains unclear. Here, we investigated the three-way interaction among sleep, sex, and hippocampal involvement in fear memory extinction using two auditory cued fear conditioning paradigms: delay conditioning (hippocampus-independent) and trace conditioning (hippocampus-dependent). After fear conditioning, mice underwent 6 h of sleep deprivation (SD), and recent retrieval, extinction, and remote retrieval after extinction were assessed. Female mice showed higher freezing during retrieval in both protocols and exhibited slower extinction in delay conditioning. SD had no effect on recent memory retrieval in either sex or task. However, SD facilitated recent fear memory extinction in trace conditioning and attenuated remote recall after delay fear memory extinction. Our findings highlight distinct temporal roles of sleep in modulating fear memory extinction, depending on hippocampal engagement, and underscore sex-dependent differences in fear memory dynamics. These results may have implications for developing sex-specific, sleep-based interventions for fear-related disorders.

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Nanosymposium

NANO041: Sleep Mechanisms Contributing to Cognitive Function

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO041.11

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R21 MH128740

Title: Statistical learning alters hippocampal responses during awake rest

Authors: *I. ZHOU[1], Y. HUANG[2], Z. BAI[1], E. WIJAYA[1], B. SHERMAN[3], E. V. GOLDFARB[1], N. B. TURK-BROWNE[1];
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Abstract: The hippocampus implements multiple functions along distinct pathways: the trisynaptic pathway supports episodic memory for individual events, whereas the monosynaptic pathway supports statistical learning of regularities across events. Episodic memory is known to be strengthened by replay and consolidation during post-encoding rest and sleep, but what occurs in offline periods after statistical learning is unknown. Based on recent evidence that hippocampal-cortical coupling during rest can facilitate memory integration, we hypothesized that visual statistical learning may alter functional connectivity between the hippocampus and

visual cortex during post-learning rest. We additionally explored how statistical learning affects what information is reinstated in the hippocampus during rest. We collected fMRI data during a task that assesses episodic encoding and statistical learning simultaneously. Participants (N=45) first viewed a series of unique scenes from 12 natural categories in random order, followed by a run of awake rest. They were then exposed to scene sequences containing temporal regularities: scene categories were paired such that certain categories (predictive) were always followed by certain other categories (predictable). Participants were sensitive to these regularities, as shown by enhanced episodic memory for predictive scenes in a follow-up session the next day. These statistical learning runs were followed by another rest run. A final run, in which novel exemplars of the scene categories were again presented in random order, allowed us to take neural snapshots of category representations. We first compared functional connectivity between the hippocampus and visual cortex in the rest runs before and after statistical learning. While both rest runs occurred after scene viewing, only the second occurred after exposure to temporal regularities. We found a significant increase in functional connectivity between hippocampal subfields and lateral occipital and parahippocampal cortices, consistent with consolidation of learned regularities. Next, we used the neural snapshots to measure evidence for scene category reactivation in each timepoint of the rest runs before and after statistical learning. After exposure to temporal regularities, there was increased hippocampal reactivation of predictive categories during rest, suggesting privileged consolidation of information useful for prediction.

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Nanosymposium

NANO041: Sleep Mechanisms Contributing to Cognitive Function

Location: SDCC Rm 33

Time: Tuesday, November 18, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO041.12

Topic: G.07. Biological Rhythms and Sleep

Title: Neurophysiological correlates of sleep and memory in long-term pediatric cancer survivors

Authors: *P. PANDEY[1,2], S. MAHALE[3], C. TORRES ROJAS[2], M. NAVARRETE[2], J. LAI[2], A. SANCHEZ CORZO[2], J. DALBONI DA ROCHA[2], S. GUTHRIE[2], T. BRINKMAN[2], B. MANDRELL[2], K. R. KRULL[2], R. SITARAM[2];
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Abstract: Long-term survivors of childhood cancer experience disproportionate rates of sleep disturbance that correlate with reduced quality of life, emotional distress, neurocognitive deficits, and elevated risk of mortality. Yet the neurophysiology of sleep in this population remains sparsely characterized, and etiology may diverge from that of the general population. We aim to characterize sleep-spindle dynamics during an afternoon nap following a declarative memory

learning task in long-term pediatric cancer survivors. Participants were long-term survivors of childhood acute lymphoblastic leukemia (ALL; n=17, 49.82 ± 8.81 years) or non-central nervous system (non-CNS) solid tumors (n=25, 38.44 ± 10.55 years). They completed a paired-associate card-location task (learning, pre-nap test, post-nap test), making multiple attempts to reach ≥60% accuracy (≥10 of 15 pairs) before a nap (at least 20 min to 2 hours), followed by a single-attempt post-nap test. Subsequently, nap HD-EEG data (128 channels) were down-sampled (1000 to 128 Hz), bandpass filtered (0.3 and 30 Hz), and referenced to averaged mastoids. Sleep stages were scored using the U-Sleep deep-learning network. Spindle amplitude and frequency were extracted (YASA toolbox) from eight channels across frontal (F3, F4), central (C3, C4), parietal (P3, P4), and occipital (O1, O2) regions. ALL survivors made 5.71 ± 4.19 attempts, and non-CNS survivors took 5.04 ± 2.79 attempts to reach the 60% pre-nap criterion. Total nap time averaged 79.47 ± 27.53 minutes for ALL and 92.64 ± 30.66 minutes for non-CNS solid tumor survivors. Memory performance scores changed from pre- to post-nap in both groups: from 10.64 ± 0.70 to 8.47 ± 1.69 (ALL), and from 10.72 ± 0.93 to 9.96 ± 1.79 (non-CNS). In N2, both groups had higher average spindle counts in central (ALL: 186; non-CNS: 279) and parietal (ALL: 206; non-CNS: 317) regions and higher amplitude in the frontal region (ALL: 78.5; non-CNS: 68.1) in the N3 stage. Non-CNS survivors showed ~62% and ~94% more fast (12-16 Hz) than slow (9-12 Hz) spindles in central and parietal regions, respectively. We found no robust correlations between spindle density and post-nap memory performance across the mentioned four cortical regions in either group. However, when stratified by age (20-35 vs. >50 years), only the older group (n = 13) showed a significant positive correlation ($r = 0.70$, $p < 0.05$) between frontal slow-spindle density and memory performance, with an average N2 slow-spindle density of 2.62 ± 1.45 events/min and memory score of 9.23 ± 1.96. Slow-spindle density in older adults may indicate an age-specific mechanism in long-term cancer survivors and warrants further investigation.

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Nanosymposium

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Presentation Number: NANO041.13

Topic: G.07. Biological Rhythms and Sleep

Title: Sodeep: a lightweight causal cnn for real-time slow oscillation detection

Authors: *T. NÄHER[1,2], L. BASTIAN[3], J. BORN[4], P. FRIES[1];
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Abstract: Real-time detection of slow oscillations in sleep EEG remains predominantly reliant on classical algorithmic solutions, which often suffer from limited accuracy and poor temporal resolution. To address these limitations, we introduce a lightweight convolutional deep learning architecture specifically tailored for real-time detection of slow oscillations. Our model employs efficient causal convolutions to accurately capture temporal dependencies while maintaining low inference latency. This architecture demonstrates robust and reliable performance, significantly surpassing traditional filter-only methods. The model's speed and reliability make it highly suitable for real-time interventions, such as slow oscillation-triggered non-invasive brain stimulation or targeted memory reactivation. Moreover, its computational efficiency ensures seamless integration into many systems commonly used in sleep research.

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Nanosymposium

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Presentation Number: NANO042.01

Topic: J.04. Physiological Methods

Support: KAKENHI 23K06344
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Title: Rational engineering of XCaMP-C, a versatile genetically-encoded Ca^{2+} indicator for all-optical interrogation, multiplex imaging, and quantitative Ca^{2+} imaging

Authors: *H. FUJII[1], K. OTA[1], Y. KONDO[1], G. CAI[2,1], R. SONG[3,1], H. SONG[1], M. OKAMURA[1], H. KONDO[1], M. INOUE[4,1], H. BITO[1];
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Abstract: Understanding how neuronal activities and biochemical signaling operate within complex neural circuits remains a fundamental question in neuroscience. The development of G-CaMPs/GCaMPs and further advanced mutants with iterative design improvements have rendered GECI imaging indispensable for quantitative cellular neuroscience.Recently, three new demands have emerged that the state-of-the-art GECIs do not fully address. First, all-optical interrogation, which integrates GECI imaging with optogenetic photomanipulation, requires GECIs that have unambiguously separable two-photon cross-section from light-sensitive channelrhodopsins (ChRs) in order to minimize non-specific stimulation by the imaging laser. Second, advances in single-cell sequencing demand a much higher degree of multiplexity in recording of activities from diverse neuronal types. Third, detailed insights into biochemical

signaling in dendrites and synapses emerging from advanced super-resolution microscopy studies emphasize the need to quantitatively analyze local Ca2+ signaling dynamics in subcellular compartments. To address these challenges, we rationally designed a new cyan variant, XCaMP-C, which offers (1) fast imaging free of crosstalk with optogenetic actuators, (2) enhanced multiplexability that permits co-imaging of more than 4 neuronal types, and (3) precise measurements of Ca^{2+} transient dynamics in sub-neuronal compartments. In vivo two-photon imaging of XCaMP-C at 820 nm, in combination with rsChRmine, a red-shifted ChR variant with a large photocurrent, enabled all-optical single-cell interrogation with minimal crosstalk in the absence of any data averaging. This approach successfully demonstrated horizontal functional connectivities at single-cell resolution in the layer 2/3 of the mouse cortex in vivo. Additionally, XCaMP-C supported 6-plex one-photon Ca^{2+} imaging in cultured neurons in vitro, representing one of the highest multiplexity reports to date. Furthermore, XCaMP-C was compatible with subcellular quantitation of intracellular Ca^{2+} dynamics under fluorescence lifetime imaging mode, resolving a heterogeneity of subthreshold Ca^{2+} levels in dendrites vs soma comparisons. Our progress paves the way towards better understanding at heightened granularity of complex hierarchical neuronal computations in subcellular signaling- and cellular-network levels and offers a unique quantitative framework for future recordings of in vivo Ca^{2+} signaling dynamics in brain health and disease.

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Nanosymposium

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Presentation Number: NANO042.02

Topic: J.08. Methods to Modulate Neural Activity

Support: Academia Sinica AS-CDA-110-L08

Title: Engineering a kalium channelrhodopsin for optogenetic neuropathway inhibition

Authors: S. LOPEZ[1,3], I.-C. LEE[4,5], H.-Y. WANG[1], Y.-J. LIN[1], C.-L. HSU[1,6,2], M.-K. PAN[4,5,1,2], ***W.-C. LIN**[1,2];
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Abstract: Optogenetic inhibition of neuropathways is a powerful approach for functional dissection of neurocircuits. HcKCR1, a light-gated potassium-selective channel from *Hyphochytrium catenoides*, is a promising neuron silencer owing to its large conductance and

high sensitivity to light. However, the gating properties and axonal trafficking of HcKCR1 are unsatisfactory for neuropathway manipulations. To overcome these issues, we first engineered a performance-improved HcKCR1 (piKCR1) that can produce sustained photocurrents for reliable inhibition. piKCR1 was engineered by introducing a mutation in the retinal-binding pocket and the extracellular vestibule, respectively, to reduce current decay under continuous illumination and increase the channel's potassium selectivity. In piKCR1-expressing cultured neurons, action potentials can be robustly suppressed by low-intensity of green (0.0002 mW/mm²) or red (0.16 mW/mm²) light. We next engineered axon-targeted (AT) HcKCR1 and piKCR1 to enable long-range presynaptic control. Using confocal microscopy and electrophysiology, we demonstrated cross-hemispheric trafficking of HcKCR1.AT and piKCR1.AT in hippocampal CA3 neurons. Finally, we validated piKCR.AT-mediated projection inhibition *in vivo*. The opsin was expressed in the cerebellar Purkinje cells (PCs), which innervate the deep cerebellar nuclei (DCN) to control motor function. In a beam walking experiment, illumination in the DCN significantly increased both the number of foot slips and the transversal time of mice, indicating successful suppression of the PC-to-DCN inputs. With its strong inhibitory power and high axonal expression, piKCR.AT may provide new opportunities for probing neuropathway functions in health and diseases.

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Nanosymposium

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Topic: J.08. Methods to Modulate Neural Activity

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Title: Trans-synaptic optical control of user-defined synaptic connections

Authors: P. O'NEILL[1], D. KANKANAMGE[2], S. HEGEL[3], K. BARNES[4], E. M. LEWIS[5], H. WANG[6], C. BOWMAN[7], A. SHIRIAEVA[8], V. KALYANARAMAN[9], H. A. TEJEDA[11], B. A. COPITS[10];
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Abstract: The human brain is estimated to contain over 100 billion neurons that are wired together by more than 100 trillion synapses. These synaptic connections increase the computational capabilities of neural circuits, and are essential for sensation, perception, learning and memory, and the selection and expression of distinct behavioral states. While many tools now exist to activate, inhibit, or modulate specific cell types in the brain, none are currently capable of manipulating activity between user-defined pre- and postsynaptic cell types. As a result, our knowledge of the roles played by specific synaptic connections and how they contribute to information processing and behavior is still quite limited. Here we developed a two-component system to optically control the activity of user-defined synaptic connections between specific cell types. A genetically encoded post-synaptic opto-ligand spans the synaptic cleft and presents a tethered peptide agonist in a photoswitchable manner. The peptide activates an engineered Gi/o coupled receptor in the pre-synaptic neuron for synapse selective inhibition of neurotransmitter release. We developed a platform using the TRUPATH assay to screen for GPCR activation by freely diffusing opto-ligands in crude lysates. Using a lead candidate opto-ligand/GPCR pair from our screen, we further demonstrated trans-cellular control of GPCR signaling in vitro, engineered a Gi/o coupled variant of the receptor, and used it to achieve trans-synaptic inhibition of evoked IPSCs in acute brain slice recordings. Importantly, these genetically-encoded trans-synaptic tools use common AAV gene delivery methods and light sources used for standard optogenetic experiments. Further development to incorporate additional genetic approaches to target different cell types may enable optical control of neurotransmitter release between any user-defined synaptic connections.

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Nanosymposium

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Topic: J.04. Physiological Methods

Support: Weill Neurohub
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Title: Aberration measurement and correction for ultrafast two-photon fluorescence imaging

Authors: *J. ZHU[1], R. NATAN[1], J. ZHONG[2], I. KANG[1], N. JI[2];
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Abstract: Understanding brain function requires the ability to measure neuronal activity *in vivo*. Dynamic processes such as fast neuronal signaling and cerebral blood flow are especially

important for uncovering how the brain works. Imaging these dynamics *in vivo* demands millisecond temporal resolution and subcellular spatial resolution. To meet these demands, using free-space angular-chirp-enhanced delay (FACED), we previously developed an ultrafast two-photon fluorescence microscope (2PFM) capable of megahertz line-scan rates and kilohertz frame rates. This has enabled us to capture mouse cortical activities, such as membrane voltage changes and blood flow, at the millisecond scale *in vivo*. The FACED module achieves rapid scanning by splitting one laser pulse into multiple pulses that are spatially separated and temporally delayed, forming a one-dimensional array of excitation foci at the objective focal plane. Optical aberrations present in the microscope system and/or induced by the biological sample enlarge the volume and reduce the intensity of FACED foci, thereby degrading image signal, contrast, and resolution. To improve spatial resolution *in vivo*, we implemented adaptive optics (AO) in the ultrafast 2PFM. Using AO, we were able to measure and correct these aberrations, recovering diffraction-limited imaging performance by employing a frequency-multiplexed aberration measurement method. We demonstrated benefits of AO in high-resolution studies of neuronal structure, blood flow, and neurotransmitter release in the living mouse brain.

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Nanosymposium

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Title: A versatile platform for two-photon neuronal population voltage imaging

Authors: *J. GUO[1], K. BARBER[1], M. A. FRECHOU[1], S. LU[1], J. DEMAS[1,2], D. CHEN[1], S. YANG[3], A. MCDONALD[4], M. LAND[4], F. ST-PIERRE[4,3], A. VAZIRI[1,2];
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Abstract: Recently genetically encoded voltage indicators have emerged as a powerful tool for resolving neuronal spiking activity with high spatial-temporal resolution within genetically specific populations. However, their fast temporal dynamics, low signal-to-noise ratio (SNR) and fast photobleaching have posed significant demands on imaging methods aimed at large-scale population level recordings in the scattering mammalian brains. Here, we introduce a new

spatiotemporally and energetically optimized, versatile two-photon optical imaging system using Flexible Lateral-Temporal Multiplexing (FlatMux). We demonstrated FlatMux's capability by performing uninterrupted long in vivo GEVI population recordings at 750 Hz with low photobleaching and its flexible reconfigurability for different recording modalities, including for large field-of-views, high speed (2 kHz), deep imaging (≤ 500 μm), dual plane and high SNR recordings in the mouse cortex. Thus, FlatMux meets the challenging demands of multi-photon voltage imaging across the mammalian cortex and can be expected to enable a new range of neurobiological studies of complex brain functions.

Disclosures: J. Guo: None. **K. Barber:** None. **M.A. Frechou:** None. **S. Lu:** None. **J. Demas:** None. **D. Chen:** None. **S. Yang:** None. **A. McDonald:** None. **M. Land:** None. **F. St-Pierre:** None. **A. Vaziri:** None.

Nanosymposium

NANO042: Optical Methodology: Development

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO042.06

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH Grant F32EY035926
NIH Grant RF1NS128772

Title: Balanced two-photon holographic bidirectional optogenetics defines the mechanism for stimulus quenching of neural variability

Authors: *K. SIT[1], C. HUANG[2], J. VEIT[3], H. ADESNIK[4], B. DOIRON[5];
[1]Univ. of California: Berkeley, Berkeley, CA; [2]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [3]Physiol. I, Univ. of Freiburg, Freiburg, Germany; [4]Univ. of California, Berkeley, Berkeley, CA; [5]The Univ. of Chicago, Chicago, IL

Abstract: The onset of a stimulus, whether sensory or non-sensory, quenches trial-to-trial neural vari-ability. The universality of this phenomenon across brain areas and species implies that it is a fundamental strategy employed by the brain to boost the fidelity of stimulus representation. Multiple network models have attempted to explain this phenomenon via circuit interactions, but a recent alternative theory proposes a much simpler cell-intrinsic mechanism: stimulus onset increases membrane conductance which itself is enough to quench output variability. Although this model can explain all of the physiological data, experimentally testing this hypothesis in vivo is currently impossible—requiring a means to artificially increase the membrane conductance of a single neuron while also minimizing any coincident network effects. One way to achieve this would be using bidirectional, single-cell resolution optogenetic manipulation to inject a balanced combination of excitatory and inhibitory conductances; however, no such technology exists. To address this technological gap, we developed a novel multi-color two-photon (2p) holographic microscope with single cell resolution optogenetic manipulation. We

paired this novel optical platform with the ‘somBiPOLES’ construct, which fuses two spectrally separable excitatory and inhibitory opsins: Chrimson and GtACR2. First, we demonstrate that this platform enables flexible bidirectional control of neural activity with single-neuron resolution. Next, we optogenetically inject large balanced excitatory and inhibitory conductances while keeping mean activity constant, demonstrating the fine-scale control of this system. Finally, we show that increasing total conductance to a neuron is entirely sufficient to reduce its trial-by-trail variability in the absence of network effect, strongly supporting a cell-intrinsic model for stimulus-induced quenching of neural variability. These results are the first demonstration of an in vivo bidirectional holographic microscope, and demonstrate its ability to elucidate the mechanism of a fundamental coding principle of neurons.

Disclosures: K. Sit: None. **C. Huang:** None. **J. Veit:** None. **H. Adesnik:** None. **B. Doiron:** None.

Nanosymposium

NANO042: Optical Methodology: Development

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO042.07

Topic: J.03. Anatomical Methods

Support: STI2030-Major Projects 2021ZD0202205

Title: A long-working-distance miniature two-photon microscope enables cross-species imaging from rodents to non-human primates

Authors: *R. WU;
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Abstract: Unrestrained neural imaging across species—from rodents to non-human primates—is essential for understanding the circuit mechanisms underlying complex behaviors and cognition. However, current miniature two-photon microscopes (m2PMs) are limited by short working distances (WDs) and insufficient adaptability to non-human primates, restricting their utility in neuroscience. Here, we developed a long-WD m2PM system featuring three interchangeable objectives (1, 2, and 3 mm WD), enabling high-resolution deep brain imaging in mice and marmosets during free behavior, and in head-unrestrained macaques. In mice, integration with a small cannula enables 700 μm volumetric imaging from CA1 to the dentate gyrus (DG) with axon-level resolution and a 600×500 μm field of view (FOV), while preserving hippocampal integrity. A fast axial-focusing module further allows simultaneous imaging of CA1 and DG neurons in freely moving mice. In marmosets, an optimized cable protection design for the m2PM enables stable calcium imaging of auditory cortical neurons during naturalistic auditory behavior. In macaques, integration of the m2PM with a miniature XYZ motion stage and high-efficiency fluorescence collection enables variable-FOV, high-signal-to-noise, and robust neuronal imaging. These capabilities of long-WD m2PM enable high-resolution, deep-brain, and

flexible in vivo imaging across species, providing a foundation for comparative studies of brain function at neuronal resolution across species.

Disclosures: R. Wu: None.

Nanosymposium

NANO042: Optical Methodology: Development

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Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO042.08

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R00AG056636-04
NIH Grant 1R34NS127103-01
NIH Grant R01NS126076-01

Title: A noninvasive in vivo light source for deep tissue light delivery

Authors: *M. MALINAO[1], S. JIANG[3], G. HONG[2];
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Abstract: Light is a widely used tool for neuroscience research, such as in optogenetics, fluorescence imaging of neural activity, and photoswitchable delivery of neuroactive molecules. However, existing methods for the delivery of light deep into the body are often limited in their penetration depth, require invasive hardware, and/or are confined to a predetermined and fixed location in the body. Here, we present two non-invasive techniques for deep and dynamic visible light delivery in biological systems. First, we introduce a short-wave infrared (SWIR) mediated visible light source based on SWIR-excitable luminescent nanotransducers circulating through the vasculature system for dynamic light delivery in biological systems. We generate consistent and repeatable 490 nm blue light emission under a broadband spectrum of SWIR excitation sources, spanning 785 nm to 1532 nm, which exhibits deep tissue penetration owing to reduced photon scattering in the SWIR spectrum. Moreover, the system presents a high level of spatial and temporal system, as characterized by the small generated spot size, averaging 130 μm, and sub-millisecond emission delay. We measured the emission intensity of our technique, demonstrating a 10-fold improvement in efficiency when compared to SWIR-responsive upconversion nanoparticles, the current standard for non-invasive SWIR-mediated visible light emission. Furthermore, we demonstrate dynamic generation of blue light in multiple locations in the brain and other organs in a single mouse following intravenous delivery. Besides the SWIR-mediated in vivo light delivery, we have also developed an ultrasound-mediated in vivo light source. This light source is enabled by mechanoluminescent nanotransducers that can convert spatiotemporally focused ultrasound into local light emission. We have demonstrated the utility of this ultrasound-mediated in vivo light source with in vivo electrophysiology, immunostaining, and behavioral assays. We envision both in vivo light sources will offer a versatile platform for

non-invasive and precise light delivery for applications in optogenetics and other neuromodulation techniques.

Disclosures: M. Malinao: None. **S. Jiang:** None. **G. Hong:** None.

Nanosymposium

NANO042: Optical Methodology: Development

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO042.09

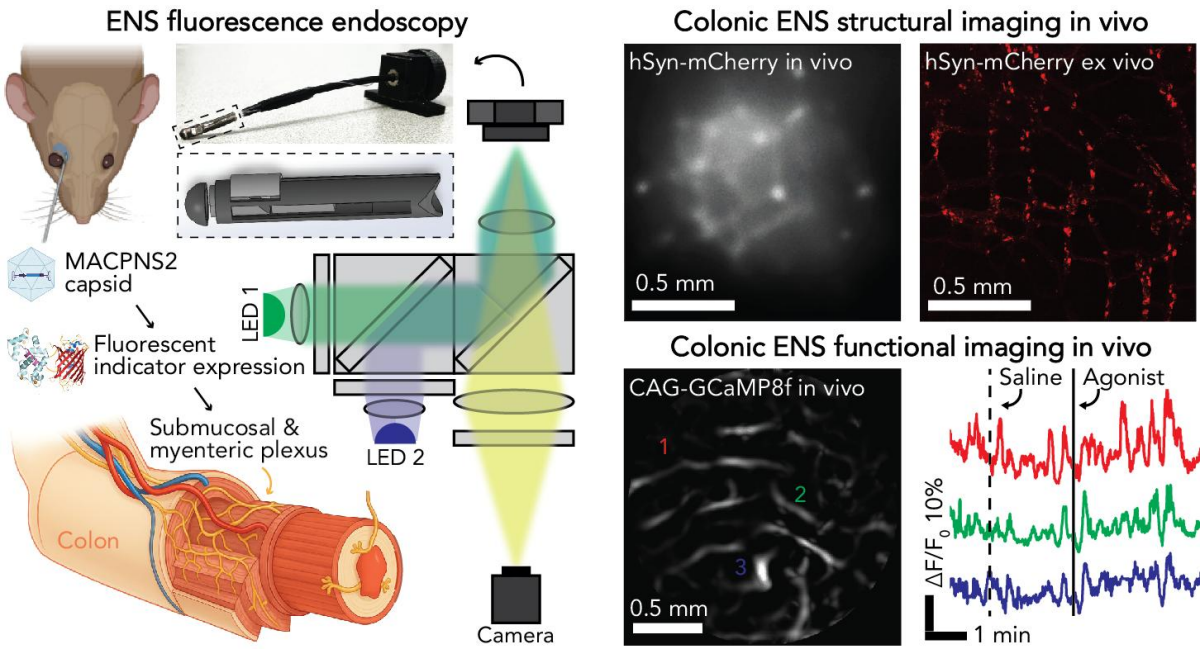
Topic: J.04. Physiological Methods

Support: NIH 1F32MH139162

Title: Optical fiber fluorescence endoscopy for capturing enteric nervous system activity in vivo

Authors: *T. M. CANNON[1], G. L. EBERLY[2], K. NAGAO[3], E. VARGAS PANIAGUA[3], P. ANIKEEVA[4];
[1]Res. Lab. for Electronics, MIT, Boston, MA; [2]MIT, CAMBRIDGE, MA; [4]Brain and Cognitive Sci., [3]MIT, Cambridge, MA

Abstract: Increasing awareness of bidirectional communication between the brain and innervated organs, such as those of the enteric nervous system, has motivated the development of neural monitoring tools to capture and characterize these interactions. Optical imaging of genetically encoded fluorescent indicators reporting the release of calcium or neurotransmitters has transformed our ability to visualize brain function, and stands to similarly empower scalable and specific ENS monitoring. However, barriers to accessing the delicate internal organs of the gastrointestinal (GI) tract have primarily limited previous work to the evaluation of ex vivo tissue, negating opportunities for longitudinal or systemic studies. To address these challenges, we have developed a miniature flexible endoscope using bundled polymer optical fibers to spatially resolve fluorescent indicators expressed by ENS neurons in the distal colon of anesthetized mice. Our minimally invasive approach critically leverages systemic delivery of viral vectors to broadly express a variety of indicators and eliminate the need for localized injections or transgenic animals. By pairing our optical fibers with 3D-printed microlens housing, microfluidics, a multicolor back-end imaging system, and a signal processing pipeline including robust amplification, filtering, and motion correction, we enable the stable recording of multifaceted signaling dynamics in response to delivered stimuli at near-cellular resolution. To date, we have used our endoscopic imaging platform to temporally characterize the colonic expression of genetically encoded indicators and capture calcium and serotonin activity in response to ENS agonists in healthy animals in vivo. We anticipate that our approach will uniquely enable long-term investigations of peripheral nervous system function under normal or pathological conditions and broaden our understanding of the relationship between the ENS, brain, and behavior. Figure 1. Overview of systemic viral delivery, endoscopic imaging system, and preliminary imaging results.



Disclosures: T.M. Cannon: None. **G.L. Eberly:** None. **K. Nagao:** None. **E. Vargas Paniagua:** None. **P. Anikeeva:** None.

Nanosymposium

NANO042: Optical Methodology: Development

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Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO042.10

Topic: J.04. Physiological Methods

Support: PRIN2022_PNRR_PAVONE - SH1 _COoperation and BRAin-Synchrony: a multiscale and translable apporach

Title: Mice-µScope: dual-wavelength cortical imaging of social interaction in freely moving mice

Authors: *J. LUCCHESI[1,2], A. SCAGLIONE[1,2], A. MAZZUCATO[2,1], G. BARBERA[3], D.-T. LIN[3], F. S. PAVONE[1,2];
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Abstract: Deciphering how the cortex orchestrates social behavior demands tools that can capture brain-wide dynamics as animals engage in complex, naturalistic interactions. Despite

growing interest in social neuroscience, how large-scale cortical activity supports and coordinates social behavior remains largely unknown. Genetically encoded calcium indicators such as GCaMP6f, have enabled powerful optical access to neuronal activity; however, their fluorescence signals are inherently vulnerable to hemodynamic artifacts, posing a critical challenge for accurate functional readouts.To overcome this limitation, we developed the MiCe-µScope, a lightweight (<4 g), dual-wavelength miniaturized imaging system capable of stroboscopic acquisition of both calcium-dependent fluorescence and intrinsic optical signals linked to blood volume changes. This design enables stable, bilateral cortical imaging through the intact skull of freely moving mice, with minimal interference to natural behavior.We deployed MiCe-µScope in a social interaction task where mice engaged with either a novel conspecific or an inanimate object. Behavioral dynamics were precisely mapped using SLEAP, a deep learning-based pose estimation framework, enabling high-resolution tracking of interaction-specific dynamics. By concurrently isolating vascular from neural signals, we quantified the influence of hemodynamics on cortical activation patterns and inter-brain synchrony.Our findings reveal large-scale cortical activation during social interactions and demonstrate the MiCe-µScope's ability to correct fluorescence signals for hemodynamic noise in freely behaving subjects. Moreover, neural network-based behavioral analyses uncovered distinct interaction profiles modulated by social context.Together, this work introduces MiCe-µScope as a next-generation platform for brain-wide functional imaging in ethologically relevant conditions, offering a new window into the neural mechanisms of social cognition and inter-brain coordination in health and disease.

Disclosures: J. Lucchesi: None. **A. Scaglione:** None. **A. Mazzucato:** None. **G. Barbera:** None. **D. Lin:** None. **F.S. Pavone:** None.

Nanosymposium

NANO042: Optical Methodology: Development

Location: SDCC Rm 25A

Time: Tuesday, November 18, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO042.11

Topic: J.04. Physiological Methods

Support: NICHD Division of Intramural Research

Title: Calcium imaging of complex behavioral control in free-swimming zebrafish

Authors: *R. H. W. DOCTOR[1], H. A. BURGESS[2];
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Abstract: We are studying the neural dynamics that cause adaptive motor responses to variable sensory cues. While studying command neurons has yielded valuable insights into sensorimotor transforms, more complex circuits are often collaboratively controlled in ways that allow for graded output along several axes. Zebrafish have two startle responses to escape a perceived threat. The short-latency c-start (SLC) is controlled by a command neuron producing stereotyped

behavior, while the long-latency c-start (LLC) is controlled by a cluster of prepontine neurons producing adaptive and energy-efficient behavior at the cost of increased processing time. As the variation in this behavior is graded and multidimensional, yet easy to measure, the LLC circuit is a valuable model to study population encoding at the cellular level in a sensorimotor interface. However, zebrafish rarely produce LLCs when immobilized for calcium imaging using a confocal microscope. To examine this circuit, we have built a 'Swimscope': a low-cost microscope that noninvasively images neuronal activity in free-swimming zebrafish larvae. We first designate a range of starting positions from which a larva is likely to end under the imaging area after performing an escape. Once the larva is correctly positioned, we stimulate an escape using an acoustic stimulus while imaging behavior using a high-speed camera. After completing an escape, larvae pause for several hundred milliseconds. During this period, we use a high-speed z-scan to image GCaMP fluorescence in a whole brain volume in under one second with single-cell resolution using widefield imaging. We image unstimulated brains as references. To compensate for non-signal change in perceived fluorescence between images, we include a pattern of GFP-expressing neurons to normalize to, in addition to deconvolving the images. We combine behavioral and neuronal imaging to determine the schema by which the control cluster codes differing motor output. This system avoids anesthesia, immobilization, and vestibular distortion and minimizes bright light exposure. We have validated this approach by confirming increased activity in the dorsal raphe of larva when aroused. We are now using the swimscope to examine circuits which are intractable to calcium imaging in immobilized samples, notably the LLC circuit.

Disclosures: R.H.W. Doctor: None. **H.A. Burgess:** None.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.01

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

Support: IBB grant VAL120/2023

Title: Investigating the Neuroprotective, Regenerative and Anti-inflammatory Potential of RBM3-inducing Antisense Oligonucleotides

Authors: S.-F. YEN[1,3], E. STAUFFENBERG[1,2,4], A. PAULETTI[1], T. HALTENHOF[2], F. HEYD[2], ***S. C. BROEER**[1,4];
[1]Inst. of Pharmacol. and Toxicology, [2]Inst. of Chem. and Biochem., Freie Univ. Berlin, Berlin, Germany; [3]MedNeuro, [4]Einstein Ctr. for Neurosciences (ECN), Charité – Universitätsmedizin Berlin, Berlin, Germany

Abstract: Neuroinflammation has been identified as a key contributor to neurodegeneration. Upon activation, microglia can exacerbate neuronal damage, and negatively modulate neurogenesis. Studies have suggested that RNA-binding motif 3 (RBM3), a cold-shock protein that is upregulated during hypothermia, can shift microglia toward a neuroprotective phenotype, enhance neuronal survival, and promote healthy neurogenesis. Exploiting the neuroprotective effects of hypothermia is limited by the potentially severe side effects. We have developed an antisense oligonucleotide (ASO) that increases the expression of RBM3 by manipulating alternative splicing, independent of body temperature, and exhibits long-lasting neuroprotective effects. This study aims to investigate whether ASO-mediated RBM3 upregulation can modulate microglia towards a neuroprotective phenotype in a mouse model of kainic acid (KA)-induced hippocampal injury and subsequent neuronal loss. PBS-injected animals served as controls. RBM3-inducing ASO or non-inducing control ASO (ASO-CTR) were injected intracerebroventricularly. Neurodegeneration, -regeneration, and inflammation were assessed histologically. RBM3-ASO treatment prevented KA-induced cell death (Fluorojade C labelling) in the CA1 and CA3 compared to KA only, and compared to ASO-CTR mice at 7 days after KA injection. Reactive microglia were identified by volume fraction analysis. No differences were observed in the non-injected hemispheres, but the volume fraction was significantly higher in the KA-injected hemispheres within all groups, indicating unilateral KA-induced microglial activation. RBM3-ASO treatment reduced microglial activation compared to the KA group. Microglial activation was strongly correlated with neuronal loss. Furthermore, neurogenesis in ASO-treated mice did not differ significantly from that in PBS controls, while neurogenesis was negatively correlated with microglial activation. Further experiments will quantify pro- vs. anti-inflammatory microglial markers to obtain a more comprehensive understanding of the modulatory effect of RBM3 on microglial phenotypes.

Disclosures: S. Yen: None. **E. Stauffenberg:** None. **A. Pauletti:** None. **T. Haltenhof:** None. **F. Heyd:** None. **S.C. Broeer:** None.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.02

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

Support: NS116914

Title: The dose makes the signal: differential effects of interleukin-1 signaling on adult hippocampal neurogenesis and its therapeutic potential in neurodegenerative diseases

Authors: *M. I. SMIRNOVA[1,2], M. C. MONET[1,2], D. P. NEMETH[3,4], H. VAN PRAAG[3,4,1], N. QUAN[3,4,1];
[1]Intl. Max Planck Res. Sch. for Synapses and Circuits, Jupiter, FL; [2]Charles E. Schmidt Col. of

Sci., [3]Charles E. Schmidt Col. of Med., [4]Stiles-Nicholson Brain Inst., Florida Atlantic Univ., Jupiter, FL

Abstract: Adult hippocampal neurogenesis (AHN) is the process by which neural stem cells (NSCs) in the dentate gyrus (DG) proliferate and differentiate into mature granule neurons, integrating into the hippocampal circuitry and contributing to learning and memory. This process is sensitive to inflammatory cues, including signaling by interleukin-1 (IL-1), a proinflammatory cytokine implicated in both physiological brain function and the pathogenesis of neurodegenerative diseases. IL-1 signals through interleukin-1 receptor type 1 (IL-1R1) and plays a vital role in neuroprotection and synaptic pruning; however, its role in AHN remains complex. Using mouse models with cell type-specific IL-1R1 expression, we investigated how IL-1 signaling modulates AHN. Stereotaxic delivery of IL-1 or GFP control via adeno-viral vectors into the DG of ten-week-old mice of both sexes allowed us to control dosage and spatial specificity. A week post-injection, we identified a dose-dependent effect in our global IL-1R1 expression model: low-dose IL-1 enhanced NSC proliferation, while high-dose IL-1 suppressed neurogenesis and triggered neuroinflammation, modeling hallmarks of neurodegenerative pathology. Injecting the DG with adeno-IL-1 receptor antagonist decreased the number of proliferating NSCs, confirming the necessity of physiological IL-1 signaling. In our IL-1R1-knockout, we did not observe differences in NSC proliferation after GFP or IL-1 injection. Strikingly, the selective expression of IL-1R1 in astrocytes was sufficient to recapitulate the pro-neurogenic effects of low-dose IL-1, highlighting the central role of astrocyte-mediated signaling in regulating the neurogenic niche. We are investigating whether IL-1 signaling in astrocytic cultures modulates the secretion of gamma-aminobutyric acid (GABA), given prior evidence that GABA influences the proliferation of NSCs. Our goal is to determine whether this astrocyte-mediated pathway underlies the pro-neurogenic effects of IL-1 on AHN. Using retroviral tagging of proliferating NSCs, we observed increased numbers of newborn neurons one month after the IL-1 injection. We will analyze the morphological complexity of these new neurons. We anticipate that the neurons will have high-order dendritic branching, and that this complex integration into the hippocampal circuitry will have a positive impact on short and long-term memory. Taken together, our findings position IL-1 as a dose- and cell-type-dependent modulator of AHN, underscoring the therapeutic relevance of preserving beneficial IL-1 signaling while mitigating its detrimental effects in neurodegenerative diseases.

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

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.03

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

Support: R01AG071512 BDP
R21AG073684-01 BDP
19PABH134580006 APP

Title: Treatment with neuroprotective aminopropyl carbazole compound mitigates neuroinflammation in a mouse model of Huntington's disease

Authors: *S. J. TRIPATHI[1], S. CHAKRABORTY[1], S. BARKER[2], E. VAZQUEZ-ROSA[3], A. A. PIEPER[4], B. D. PAUL[1];
[1]Dept. of Pharmacol. and Mol. Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Pathology, [3]Psychiatry, Case Western Reserve Univ., Cleveland, OH; [4]Psychiatry, Case Western Reserve Univ., Shaker Hts, OH

Abstract: Huntington's Disease (HD) is a neurodegenerative disorder characterized by a progressive decline in voluntary motor functions, accompanied by psychiatric disturbances and cognitive impairments. HD is caused by CAG repeat expansion in the huntingtin gene (*HTT*), resulting in mutant huntingtin (mHTT) aggregation. These mHTT aggregates elicit widespread neurodegeneration, including general proteinopathy, oxidative stress, neuroinflammation, neuronal cell death, and impaired neurogenesis, which collectively drive the aforementioned symptoms. P7C3-A20 is a neuroprotective aminopropyl carbazole compound that has demonstrated the ability to delay neurodegeneration in a variety of conditions, including Alzheimer's disease and traumatic brain injury. Here, we show that treatment with P7C3-A20 in a cellular and mice model of HD prevents oxidative stress, mHTT aggregation, neuroinflammation, and loss of striatal neurons. Notably, P7C3-A20 mitigates cognitive deficits and improves motor behaviors in HD mice. Our study suggests that P7C3-A20 could be a promising therapeutic approach to prevent neurodegeneration in HD.

Disclosures: S.J. Tripathi: None. **S. Chakraborty:** None. **S. Barker:** None. **E. Vazquez-Rosa:** None. **A.A. Pieper:** None. **B.D. Paul:** None.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.04

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

Support: IBB VAL120/2023

Title: Therapeutic Potential of Cold Shock Proteins: Safety and Efficacy of RBM3-Modulating Antisense Oligonucleotides for Neuroprotection

Authors: *E. SCHENK GRÄFIN VON STAUFFENBERG[1,2,4], T. HALTENHOF[2], A. PAULETTI[3], S.-F. YEN[3,5], F. HEYD[2], S. BRÖER[3,4];

[1]Institue of Pharmacol. and Toxicology, [2]Inst. of Chem. and Biochem., [3]Inst. of Pharmacol. and Toxicology, Freie Univ. Berlin, Berlin, Germany; [4]Einstein Ctr. for Neurosciences (ECN), [5]MedNeuro Berlin, Charité – Universitätsmedizin Berlin, Berlin, Germany

Abstract: Preventing neuronal cell death is a key therapeutic goal in many neurodegenerative diseases. Therapeutic hypothermia (TH) is neuroprotective, but its clinical use is limited by severe cardiovascular side effects. To mimic its positive effects without cooling, we aimed to increase the expression of the cold-shock protein RBM3 (RNA-binding motif 3). The cold induced RNA binding protein RBM3 has been shown to mediate beneficial effects of TH. RBM3, naturally upregulated during mild hypothermia, promotes protein synthesis, exerts anti-apoptotic and anti-inflammatory effects, and protects against synaptic and neuronal loss. In a proof-of-concept experiment, we evaluated potential adverse effects of sustained RBM3 upregulation by generating a mouse line lacking a poison exon in RBM3 leading to its constitutive overexpression. At six months of age male mice showed significantly higher RBM3 levels with up to a 4-fold increase in brain and a 2.5-fold increase in spleen compared to wild-type controls (WT), with no visible organ pathology, or behavioral side effects in a modified Irwin screen. Together, these results support the safety of RBM3 upregulation. In a next step, we developed an antisense oligonucleotide (ASO) that increases RBM3 expression at normothermia by manipulating alternative splicing of the RBM3 poison exon. In a kainic-acid (KA) induced hippocampal injury model in male C57BL/6 mice, ASO-treated mice showed quicker clinical recovery after KA injection. No significant side effects were detected in a modified Irwin screen, indicating a well-tolerated ASO treatment. On day seven a subset of mice was perfused for histological analysis. KA-injected hemispheres displayed a significant reduction in NeuN-positive pyramidal cells compared to PBS-injected controls, which was rescued by ASO treatment, indicating a strong neuroprotective effect of RBM3 upregulation. We conclude that ASO-mediated RBM3 upregulation has a significant neuroprotective potential in hippocampal neurons following acute excitotoxic injury. Importantly, the absence of adverse effects in both genetically and ASO-induced long-term RBM3-overexpression supports the RBM3-inducing ASO as a promising therapeutic strategy for neurodegenerative diseases.

Disclosures: E. Schenk Gräfin von Stauffenberg: None. **T. Haltenhof:** None. **A. Pauletti:** None. **S. Yen:** None. **F. Heyd:** None. **S. Bröer:** None.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.05

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

Title: In vivo preclinical efficacy of NT-0150, a brain penetrant small molecule NLRP3 inflammasome inhibitor

Authors: Z. DIGBY[1], *N. CLARKE[2], P. THORNTON[1], V. READER[1], N. LINDSAY[1], D. HARRISON[1], A. WATT[1];
[1]NodThera, Cambridge, United Kingdom; [2]Nodthera, Cambridge, United Kingdom

Abstract: Low-grade, chronic neuroinflammation is increasingly recognized as a key contributor to the onset and progression of neurodegenerative diseases. Current treatments are largely symptomatic, highlighting the need for novel disease-modifying therapeutics. NOD-like receptor family, pyrin domain containing 3 (NLRP3), a cytosolic pattern recognition receptor, plays a critical role in innate immune responses. Sustained or dysregulated NLRP3 activity is linked to the progression of central inflammatory diseases, including Alzheimer's disease and Parkinson's disease. Therapeutic inhibition of aberrant NLRP3 inflammasome activation in the central nervous system therefore has the potential to treat neurodegenerative diseases.
We investigated the brain penetrance and efficacy of NT-0150, a selective, orally bioavailable small molecule NLRP3 inflammasome inhibitor, in rodent and non-human primate models. CNS exposure was assessed through *in vivo* studies using rodent and non-human primate species. *In vivo* efficacy of NT-0150 was assessed in the experimental autoimmune encephalomyelitis (EAE) chronic neuroinflammation model, and a mechanistic brain LPS-BzATP challenge model. NT-0150 showed high brain and cerebrospinal fluid (CSF) penetration following oral dosing in Sprague-Dawley rats and non-human primates with unbound exposure levels equating to 0.7 CSF-to-plasma partition coefficients ($Kp_{u,u}$) in the rat and 0.94 $Kp_{u,u(AUC)}$ in non-human primates. In EAE mouse model, oral dosing of NT-0150 reduced hind limb paralysis and CSF inflammatory markers, including IL-6 and GFAP. Furthermore, intravenously administered NT-0150 reduced the release of IL-1beta in mouse brain interstitial fluid following brain inflammatory challenge (LPS-BzATP) in a novel cerebral open flow microperfusion model. Collectively, our data demonstrate that NT-0150 is highly brain penetrant following oral dosing and exerts potent anti-inflammatory effects across diverse neuroinflammation models. These findings support the therapeutic potential of NT-0150 for targeting NLRP3-dependent neuroinflammatory mechanisms in chronic neurodegenerative diseases and warrant further clinical investigation.

Disclosures: Z. Digby: A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nodthera. **N. Clarke:** A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. **P. Thornton:** A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nodthera. **V. Reader:** A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nodthera. **N. Lindsay:** A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nodthera. **D. Harrison:** A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nodthera. **A. Watt:** A. Employment/Salary (full or part-time):; NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nodthera. Other; Chair of Babraham Institute Enterprise Board.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.06

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

Support: American Heart Association (957277),
Neuroscience Program
College of Medicine
Department of Chemistry and Biochemistry
, John G. Kulhavi Professorship in Neuroscience, and E. Malcolm Field and Gary Leo Dunbar Endowed Chair in Neuroscience at Central Michigan University and a generous gift from Joan Allinder.

Title: Intraperitoneal Dendrimer-Progesterone Injections alleviate Behavioral Deficits and Inflammation in MCAo Rat Model of Stroke

Authors: *A. POUDEL[1,2,3], S. SCHWIND[1,2,4], A. UPRETY[1,2,4], L. BOLEN[1,2,4], B. SRINAGESHWAR[1,2,3], G. L. DUNBAR[1,2,4], J. ROSSIGNOL[1,2,3];
[1]Program In Neurosci., [2]Field Neurosciences Inst. Lab. for Restorative Neurol., [3]Col. of Med., [4]Dept. of Psychology, Central Michigan Univ., Mount Pleasant, MI

Abstract: Stroke is a major global health burden, ranking as the leading cause of long-term disability and the second leading cause of death worldwide. It occurs when the blood supply to part of the brain is disrupted, depriving brain tissue of essential oxygen and nutrients. This leads to rapid neuronal death and can result in severe neurological impairments, including paralysis, speech difficulties, and cognitive dysfunction. Ischemic stroke, which accounts for approximately 85% of all stroke cases occurs when a blood clot or narrowed artery obstructs cerebral blood flow, leading to neuroinflammation and hypoxic brain injury, ultimately hindering brain function. Progesterone has shown potential as a treatment due to its neuroprotective and anti-inflammatory properties. However, its clinical utility is limited by its inability to cross the blood-brain barrier (BBB). To overcome this, we utilized PAMAM dendrimers, which can cross the BBB and deliver progesterone directly to the brain in a middle cerebral artery occlusion (MCAo) rat model of stroke. Rats underwent stroke or sham surgeries, followed by treatment administration and behavioral testing, including the cylinder test, ladder rung test, and neurological scoring. After euthanasia, brains were collected, frozen, and sectioned into 30 µm slices. We performed hematoxylin and eosin (H&E) staining to assess stroke volume and immunohistochemistry (IHC) to measure expression levels of GFAP (a marker of astrocyte activation) and IBA-1 (a marker of microglial activation). Our results showed 39% reduction in stroke volume in MCAo rats treated with the progesterone-dendrimer complex. Additionally, GFAP and IBA-1 expression were decreased in the progesterone-dendrimer treatment group. Behavioral analysis revealed significant improvement in motor coordination, particularly in the

ladder rung test, among rats treated with the dendrimer-progesterone complex. These findings demonstrate that PAMAM dendrimers can effectively cross the BBB and deliver progesterone to the brain. Furthermore, the dendrimers themselves exhibit anti-inflammatory effects, consistent with our previous findings.

Disclosures: A. Poudel: None. **S. Schwind:** None. **A. Uprety:** None. **L. Bolen:** None. **B. Srinageshwar:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.07

Topic: D.04. Brain Injury and Trauma

Support: NIH grant 1R01NS133233-01A1
NIH grant 1R21AA030625-01

Title: Design and evaluation of PAD4 antagonistic peptides for blocking neutrophil extracellular trap formation after traumatic brain injury.

Authors: *P. ABDUL MUNEER[1], Y. POOVANTHODI[2], S. BHOWMICK[3];
[1]Florida Intl. Univ., Miami, FL; [3]Neurosci., [2]Hackensack Meridian Hlth. JFK Univ. Med. Ctr., Edison, NJ

Abstract: Traumatic brain injury (TBI) disrupts the blood-brain barrier (BBB), allowing inflammatory immune cells to enter the brain. This contributes to neurovascular damage and neuroinflammation, which are the key factors in TBI-related brain injury. Previously, it has been demonstrated that the activation of leukocytes, especially neutrophils causes the release of nuclear and granular contents to form an extensive web-like structure of DNA called neutrophil extracellular traps (NET). Although the mechanism of the formation of NET and its role in exacerbating neurological deficits in stroke is evident, the role of NET in TBI is not yet fully elucidated. Moreover, it is not clear whether blocking of formation of NET provides better outcomes after TBI. Therefore, an approach to suppress the formation of NET would be a valuable therapeutic strategy and to analyze the efficacy of the therapy in the functional recovery level after TBI. We hypothesize that inhibition of peptidyl arginine deiminase type 4 (PAD4), an enzyme required for NET formation, using PAD4 antagonistic peptide (PAP) will attenuate the formation of NET. Using bioinformatics approaches to define the conserved functional elements within the sequence of PAD4, we have designed four small peptides (PAP1-4) to block the critical activity domain of PAD4 and demonstrated that systemic treatment of one of these antagonistic peptides provides better results in attenuating the formation of NET and promoting functional recovery in a mouse model of TBI. All four PAD4 antagonist peptides (PAP) and a random sequence peptide (control peptide, CP) were designed in our lab and synthesized by Life

Technologies Co (Carlsbad, CA). To facilitate their access to cells, we included the transactivator of transcription (TAT) sequence at the C-terminus of all the PAP and CP peptides. The concentration of CP or PAP and cytotoxicity were confirmed by the dose-response study and MTT assay respectively. This study also validates that there are no side effects when blocking the PAD4 gene. We selected one PAP (PAP2) from 4 different PAPs (PAP1-4). We called this selected peptide PAP. We validated the effect of PAP using CRISPR/Cas9 mediated PAD4 gene deletion in human brain microvascular endothelial cells (hBMVEC) and human neutrophil co-culture *in vitro* and PAD4 knockout (KO) mice (*PAD4−/−*) *in vivo*. Therefore, an approach to suppress the formation of NET would be a valuable therapeutic strategy and to analyze the efficacy of the therapy in the functional recovery level after TBI. This work was supported by the NIH grants 1R01NS133233-01A1 and 1R21AA030625-01.

Disclosures: P. Abdul Muneer: None. **Y. Poovanthodi:** None. **S. Bhowmick:** None.

Nanosymposium

NANO043: Targeting Neuroinflammation to Improve Outcomes in Neurodegenerative Disease and Brain Injury

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO043.08

Topic: D.04. Brain Injury and Trauma

Support: National Institutes of Health under Award Nos. 1 R01 NS115994

Title: Inhibition of p38 MAPK after repetitive mild TBI ameliorates immune signaling and behavioral deficits

Authors: *C. LI[1,2], M. GRIFFIN[3], S. TRIPLETT[2], L. WOOD[4,3];
[1]Georgia Inst. of Technology/ Emory Univ., Brookhaven, GA; [2]Emory Univ., Atlanta, GA; [3]George W. Woodruff Sch. of Mechanical Engin., [4]Inst. for Bioengineering and Biosci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Mild traumatic brain injury (mTBI) can cause long-term functional impairments, and repetitive mTBIs within a window of vulnerability can exacerbate these consequences compared to a single mTBI. However, current interventions for mTBI focus on alleviating symptoms, rather than targeting underlying mechanisms. Following the initial mechanical impact, increasing evidence suggests that the brain undergoes an inflammatory cascade consisting of pro-inflammatory intracellular signaling pathways and production of cytokines, ultimately leading to chronic neuroinflammation and persistent neurological deficits. Prior work in severe traumatic brain injury has shown that the p38 MAPK signaling pathway is a key regulator of microglial activation, proinflammatory cytokines, and synaptic dysfunction, but its role in the context of mTBI remains unclear. As such, our current study aimed to determine if acute inhibition of p38 MAPK would attenuate the inflammatory response and longer-term functional deficits following a weight-drop mouse model of repetitive mTBI. Wildtype C57BL/6J male and female mice were

injected with a small molecule p38 MAPK inhibitor (SB239063) after each of 5 once-daily weight-drop closed head injuries (CHIs) or sham injuries. Tail suspension test and rotarod were conducted at 4-weeks post injury to access behavioral changes. Immunoassays were conducted at both 4-hours and 4-weeks post injury to access protein changes associated with the immune response, synaptic function, and microglial phenotype. Bulk RNAseq was conducted to access transcript alterations at 4-weeks post injury. Our data indicates that in females, acute inhibition of p38 MAPK attenuated i) cytokine upregulation and microglial reactivity at 4-hours post injury and ii) antidepressive-like behavior, motor deficit, synaptic loss, and microglial reactivity at 4-weeks post injury. In males, p38 MAPK inhibition also attenuated microglial reactivity and upregulation of specific cytokines, although changes in functional outcomes did not reach significance. Interestingly, bulk RNAseq analysis in both sexes showed that acute p38 MAPK inhibition both normalized the effects of injury and upregulated protective genes and pathways associated with recovery and maintenance of brain homeostasis. Together, these findings suggest a role for p38 MAPK in driving the acute and longer-term consequences post repetitive mTBI in a sex-dependent manner, highlighting the therapeutic potential of p38 MAPK inhibition. To our knowledge, this work is the first to investigate the effects of small molecule inhibitor SB239063 as a potential therapeutic treatment administrated post rmTBI.

Disclosures: C. Li: None. **M. Griffin:** None. **S. Triplett:** None. **L. Wood:** None.

Nanosymposium

NANO044: Peripheral Pain Mechanisms

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.01

Topic: E.02. Somatosensation – Touch

Support: 1R01NS114567-01A1

Title: Genipin for peripheral neuropathy and acceleration of regeneration of severed axons

Authors: *N. ZELTNER;
Univ. of Georgia, Athens, GA

Abstract: The peripheral nervous system (PNS) is essential for proper body function. A high percentage of the world's population suffers nerve degeneration (peripheral neuropathy) or peripheral nerve damage. Yet, there are major gaps in our knowledge of human PNS development and degeneration disorders and therefore, there are no FDA-approved treatments available to patients. Familial Dysautonomia (FD) is a devastating, genetic peripheral neuropathy with many symptomatic similarities to other neuropathies. FD specifically affects the development and causes degeneration of peripheral sensory and sympathetic neurons. We previously employed patient-derived induced pluripotent stem cells (iPSCs) to show that peripheral sensory neurons recapitulate the developmental and neurodegenerative defects observed in FD. Here, we conducted a chemical screen to identify compounds that rescue the

sensory neuron differentiation inefficiency in FD. We identified genipin, a drug that restores neural crest and sensory neuron development in FD, both in the human iPSCs-based model and in two FD mouse models. Genipin further prevented FD degeneration, providing a stepping-stone for the development of genipin as a drug for peripheral neuropathy. We show that genipin's mode af action is via crosslinking of the extracellular matrix (ECM), leading to an increase of the stiffness of the ECM, reorganization of the actin cytoskeleton, and promotion of the transcription of YAP-dependent genes. Finally, genipin is a powerful enhancer of axon regeneration after severing of sensory, sympathetic, and prefrontal cortical neurons in axotomy models. Our results suggest genipin as a promising novel drug candidate to treat neurodevelopmental and neurodegenerative phenotypes, and to enhance neuronal regeneration of severed neurons after injury.

Disclosures: N. Zeltner: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeelaCellTherapeutics.

Nanosymposium

NANO044: Peripheral Pain Mechanisms

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.02

Topic: E.02. Somatosensation – Touch

Support: R01NS043314-19

Title: Methylglyoxal-induced axon degeneration in diabetic peripheral neuropathy requires sarm1

Authors: *G. TOTTA-GRIESE[1], D. E. WRIGHT[2];
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Abstract: Diabetes affects 1 in 10 individuals, with up to 60% of these patients develop diabetic peripheral neuropathy (DPN), a condition characterized by nerve degeneration primarily in the limbs. This degeneration causes symptoms such as pain, numbness, and tingling. Current treatments only alleviate symptoms, highlighting an urgent need for therapies that prevent or stop DPN progression. Methylglyoxal, a metabolite elevated in diabetic patients, causes pain and axon degeneration. Methylglyoxal drives nociception through TRPA1, Nav 1.7, and Nav 1.8. However, the mechanism underlying methylglyoxal induced axon degeneration is not well understood. Characterizing this pathway will lead to better therapeutic targets for DPN treatment. One candidate is SARM1, an enzyme activated in axon degeneration and DPN. We hypothesize that SARM1 mediates methylglyoxal-induced axon degeneration. To test this, SARM1 knockout and wild-type C57BL/6J mice were administered a single intraperitoneal injection of methylglyoxal. Vonfrey behavior was conducted to look for changes in mechanical

hypersensitivity. Intraepidermal nerve fiber (IENF) density in the footpads was assed. We collected spinal cord, dorsal root ganglion (DRG), and sciatic nerve tissues for immunohistochemical analysis of eIF2α (pEIF2α), HIf1a expression, and SARM1 activation. ROS and NMNAT2 levels were assessed *in vitro*. Our results demonstrate methylglyoxal activates SARM1 *in vitro*. We also observed increased ROS production, as well as changes in pEIF2α and HIF1a levels. These findings indicate that SARM1 is essential for methylglyoxal-induced axon degeneration, identifying SARM1 as a promising drug target for DPN. By characterizing the role of SARM1 in methylglyoxal-mediated axon degeneration, this work advances DPN research toward developing effective treatments, potentially benefiting many diabetic patients worldwide.

Disclosures: G. Totta-Griese: None. **D.E. Wright:** None.

Nanosymposium

NANO044: Peripheral Pain Mechanisms

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.03

Topic: E.01. Somatosensation – Pain and Itch

Support: RS-2023-00272846
RS-2021-NR059709
RS-2023-00264409
RS-2024-00441103

Title: Peripheral Nerve Transection Rather Than Crush Injury Determines Sympathetic Nerve Sprouting in Dorsal Root Ganglia

Authors: *S. SHIM[1], H. KIM[2], Y. LEE[3], C. J. WOOLF[4], K. LEE[1], S. OH[5,6];
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Abstract: Sympathetically maintained pain (SMP) is a type of pain that results from increased activity in the sympathetic nervous system. Sympathetic sprouting in dorsal root ganglia (DRG) is a feature of SMP following peripheral nerve injury, yet the factors determining its occurrence remain unclear. Here, we compare transection and crush injury models to determine if injury type or site influence sympathetic remodeling and pain. In mice, L5 spinal nerve transection (SpNT) triggered robust sympathetic fiber sprouting and elevated norepinephrine (NE) levels in the DRG, correlating with a mechanical hypersensitivity reversed by chemical sympathectomy. In contrast, a partial sciatic nerve crush injury (PCI) produced long-lasting mechanical hypersensitivity without sympathetic sprouting or NE elevation and was unaffected by sympathectomy. Further, sympathetic sprouting occurred after both spinal and sciatic nerve

transections, but not after crush injuries at either site, indicating that the injury type and not its location, governs sympathetic remodeling. These findings establish nerve transection, rather than crush, as the driver of sympathetic sprouting and SMP, with implications both for pain subtype identification and treatment strategies.

Disclosures: S. Shim: None. **H. Kim:** None. **Y. Lee:** None. **C.J. Woolf:** None. **K. Lee:** None. **S. Oh:** None.

Nanosymposium

NANO044: Peripheral Pain Mechanisms

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Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.04

Topic: E.01. Somatosensation – Pain and Itch

Support: 5U01EY034693-03

Title: Time-resolved and optogenetically-induced behavioral model of corneal pain in transgenic mice

Authors: *K.-S. JEONG[1], M. T. MCPHEETERS[2], A. CHANDRASEKHARAN[5], I. BEECK[6], A. VEERUBHOTLA[2,3], A. ROY[7], E. Y. LU[2], S. GHOSN[1], M. W. JENKINS[2,4,8], C. Y. SAAB[9]; [1]Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [2]Dept. of Biomed. Engin., [3]Dept. of Comp. and Data Sci., [4]Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH; [5]Wright State Univ., Solon, OH, ; [6]Univ. of Michigan, Ann Arbor, MI; [7]Weldon Sch. of Biomed. Engin., Purdue Univ., West Lafayette, IN; [8]FES Ctr., Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [9]Biomed. Engin., Cleveland Clin. / Case Wester/ Brown, Cleveland, OH

Abstract: Conventional rodent models to study corneal pain are primarily based on eliciting eye blink reflex using pressurized air or “air puff”, which indiscriminately targets both polymodal and mechanoreceptive nociceptors as well as thermoreceptors with sub-second resolution similar to the timescale of underlying neural dynamics. Hence, these models have not enabled the study of specific neural circuits and mechanisms underlying nociceptive encoding of corneal pain. We developed a novel behavioral paradigm in transgenic mice (TRPV1-ChR2-EYFP) that express channelrhodopsin in TRPV1-positive polymodal nociceptors, allowing activation of corneal primary afferents via blue light (490 nm) at unprecedented target specificity and millisecond resolution. Head-restrained mice (N=3) blinked predictably within less than two seconds of corneal stimulation using ultra-short pulse durations (10 ms) of blue light, with low (<20%) at lower light intensity (1.1 mW/mm^2) and maximum response (>90%) at higher intensity (7.9 mW/mm^2), resulting in a typical ‘S’ shape psychometric response curve. In control experiments, mice only blinked in response to red light (638 nm) for any intensity for less than 20% of trials. The dependence of the evoked blink reflex on both the wavelength and intensity of light indicates that brief pulses of optogenetic stimulation of corneal nociceptors reliably evoke nociceptive blink reflexes similar to those evoked by air puff, albeit via more time-controlled and

selective activation of a sub-population of A delta and C fibers primarily composed of polymodal nociceptors. Moreover, we developed a hardware/software platform for the evaluation of blink behavior using high speed video and machine-learning algorithms for automated, high throughput scoring of behavioral data. This model can now be used to study the nociceptive mechanisms of corneal pain in health and disease, potentially incorporating a lick behavior for self-reported pain, which our team previously validated for hindpaw pain in mice.

Disclosures: K. Jeong: None. **M.T. McPheeters:** None. **A. Chandrasekharan:** None. **I. Beeck:** None. **A. Veerubhotla:** None. **A. Roy:** None. **E.Y. Lu:** None. **S. ghosn:** None. **M.W. Jenkins:** None. **C.Y. Saab:** None.

Nanosymposium

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Presentation Number: NANO044.05

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH NCI Grant K00 CA264414

Title: Sphingolipid metabolic flux modulates TRPV1 function in sensory neurons

Authors: *S. A. KERK[1], M. HERNANDEZ[2], J. HERDY[5], Z. WANG[3], R. CHINN[3], A. MCGINNIS[3], F. H. GAGE[4], C. METALLO[3];
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Abstract: Sphingolipids (SLs) are a diverse class of lipids implicated in most facets of cellular biology. Previous work from our laboratory has implicated serine metabolism, and its role in sphingolipid biosynthesis, in peripheral neuropathy, specifically thermal sensing. TRPV1 is a membrane calcium channel expressed in sensory neurons responsive to noxious chemical and thermal stimuli. Given its role in sensation, here we are focus on how altered SL metabolism impacts TRPV1 activity. To do this, we utilize calcium imaging, electrophysiology, and mass spectrometry to examine TRPV1 activity and SL flux in several model systems. As proof of principle, mice fed a serine/glycine-free diet, which impairs sphingolipid production, were less sensitive to hind paw injections of the TRPV1 agonist capsaicin, mimicking the thermal latency observed in other peripheral neuropathy models similarly presenting with low systemic serine. Mechanistically, culturing human sensory neurons induced from pluripotent stem cells in serine/glycine-free media induced an accumulation of non-canonical deoxysphingolipids and perturbed neuronal membrane endocytosis. This suggests that sphingolipids in the neuron are important for proper membrane dynamics which could subsequently impact the activity of membrane ion channels like TRPV1. Future work will measure the impact on TRPV1 calcium transport and electrophysiology during serine/glycine deprivation or with pathologically-relevant

expression of SPTLC1, the rate-limiting enzyme in sphingolipid biosynthesis implicated in hereditary neuropathy.

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Nanosymposium

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Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.06

Topic: E.01. Somatosensation – Pain and Itch

Support: Loyola University Chicago

Title: The neuronal membrane proteasome (NMP) expression is modulated by neuronal activity to regulate pain sensitivity

Authors: *E. KRUEGER[1], E. VILLALÓN LANDEROS[2];
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Abstract: Neuropathic pain affects around 7-10% of the population, significantly impacting patients' quality of life, contributing to disability, psychological distress, and economic burden. Despite extensive research, the molecular mechanisms that regulate pain sensation remain poorly understood. Recently, we identified the neuronal membrane proteasome (NMP), a specialized proteasome localized on the plasma membrane of a subpopulation of somatosensory neurons that sense mechanical and pain sensation. The NMP mediates neuron-to-neuron communication to modulate pain sensitivity; however, the mechanisms regulating its expression remain unknown. Here, we investigated activity-dependent regulation of NMP expression using complementary *in vitro* and *in vivo* approaches. Given that sustained neuronal hyperactivity is a hallmark of neuropathic pain, we hypothesized that hyperactivity increases NMP expression to shape neuronal responsiveness. Using dorsal root ganglion (DRG) neuron cultures, we applied prolonged KCl stimulation to induce hyperactive conditions. We measured NMP expression using membrane fractionation and immunoblotting techniques in tandem with antibody feeding. We found that sustained stimulation increased NMP localization to the plasma membrane, suggesting that neuronal activity influences NMP expression. We then assessed NMP expression changes under neuropathic pain conditions using the chronic constriction injury (CCI) model. Our data show that 20 days post-surgery, NMP levels in the ipsilateral sciatic nerve and L3-L5 DRGs are elevated in the CCI mice compared to the sham mice. To evaluate whether the NMP contributes to pain sensitivity, we administered biotin epoxomicin (BE), an NMP-selective inhibitor, and used a panel of behavioral assays to measure changes in pain sensitivity. We found that acute NMP inhibition attenuated pain hypersensitivity. Taken together, these findings suggest that neuropathic pain drives increased NMP expression, which in turn contributes to pain

development. These results suggest that the NMP is a dynamic modulator of neuropathic pain development and may serve as a promising target for new pain therapies.

Disclosures: E. Krueger: None. **E. Villalón Landeros:** None.

Nanosymposium

NANO044: Peripheral Pain Mechanisms

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.07

Topic: E.02. Somatosensation – Touch

Title: Scn1b is required for mechanosensory signaling and touch behaviors

Authors: *K. NGUYEN[1], Y. BABA[2], J. VALENCIA LESMES[3], D. BAUTISTA[5], E. A. LUMPKIN[4];
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Abstract: Somatosensory neurons transduce sensory inputs into action potentials that represent features of the environment. In dorsal root ganglia (DRG), subpopulations of sensory neurons express distinct pore-forming voltage-gated sodium channels (NaV) and potassium channels that dictate their firing patterns; however, less is known about the role of auxiliary beta subunits in sensory neuron excitability. To address this question, we performed a bioinformatic analysis of NaVβ subunit expression in DRG neurons using published single-cell RNAseq datasets. We found that Scn1b, which encodes the NaV β1 subunit, is highly enriched in myelinated mechanosensory neurons that mediate gentle touch and fast mechanical pain. In situ hybridization of Scn1b transcripts showed that this gene is expressed in 70% of all sensory neurons and 100% of NEFH-positive, myelinated neurons. To determine the role of SCN1B in somatosensory neurons, we generated a somatosensory neuron-specific knockout of Scn1b (PirtCre+;Scn1bfl/fl). These conditional knockout (Scn1bCKO) mice are viable and fertile. In a battery of gentle touch behavior tests (von Frey, dynamic brush and tape test), Scn1bCKO displayed significant reductions in responses to mechanical stimuli across all three tests. To examine behavioral responses to noxious mechanical and thermal stimuli, we performed a randall selitto pressure test followed by hot and cold tail flick. Scn1bCKO mice showed no significant differences in response to these frankly noxious mechanical and thermal stimuli. By contrast, Scn1bCKO mice exhibited attenuated responses to pinprick stimulation delivered via a 27-g hypodermic needle. We next examined the effects of Scn1b deletion on A-fiber excitability using ex vivo skin-nerve preparations. Across all A fibers, the distribution of von Frey mechanical thresholds were significantly elevated in Scn1bCKO mice compared with littermate controls (Control: 1st quartile=0.20, 3rd quartile=1.6; N=13 mice; CKO: 1st quartile=0.25, 3rd quartile=5.9, N=16 mice, P=0.008, Mann-Whitney). Additionally, the conduction velocities of A fibers were 10% lower in Scn1bCKO compared with control mice (Ncont=16 mice, 113 fibers; NCKO=15 mice, 84 fibers; Student's t test, P=0.05). Together, these findings suggest that

SCN1B impacts A fiber-mediated touch and pain behaviors by modulating the initiation and propagation of action potentials in mechanoreceptors.

Disclosures: K. Nguyen: None. **Y. Baba:** None. **J. Valencia Lesmes:** None. **D. Bautista:** None. **E.A. Lumpkin:** None.

Nanosymposium

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Presentation Number: NANO044.08

Topic: E.01. Somatosensation – Pain and Itch

Support: R01NS121533
P30GM145497

Title: Post-transcriptional regulation of sensory neuron excitability and sensitivity by CELF4 RNA-binding protein

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Abstract: Chronic pain is estimated to impact 20-25% of the population worldwide, with many patients experiencing inadequate pain relief from currently available therapeutics. The initiation and maintenance of persistent pain is dependent on *de novo* protein synthesis in sensory neurons and several recent studies have provided evidence that nociceptive sensitivity is regulated by RNA-protein interactions. Therefore, investigating the functions of RNA-binding proteins expressed in sensory neurons may identify new strategies to modulate sensory neuron excitability and to attenuate persistent pain. We previously determined that the RNA-binding protein CELF4 is co-expressed with nociceptive markers in the dorsal root ganglia (DRG) and is highly enriched in peptidergic (TRPV1+) sensory neurons. We generated conditional knockout mice to delete *Celf4* from sensory neuron populations to elucidate the function of CELF4 in sensory neurons in the DRG. We found that loss of CELF4 causes robust mechanical and thermal hypersensitivities accompanied by a dramatic increase in the excitability of capsaicin-sensitive *Celf4* knockout neurons. Additionally, we found *Celf4* KO induces an exaggerated response to low dose intraplantar NGF or capsaicin. Using RNA immunoprecipitation sequencing from bulk DRG homogenates, we found that CELF4 binds to many mRNA transcripts with critical roles in pain signaling. Histology and western blot were used to assess changes in the expression of these nociceptive targets within *Celf4* KO sensory neurons. Together these findings suggest that CELF4 may inhibit the translation of nociceptive mRNAs to regulate sensory neuron excitability and nociceptive sensitivity. We are therefore employing AAV transduction to conditionally overexpress CELF4 in peripheral neurons to determine if

CELF4 overexpression attenuates pain behaviors in inflammatory, post-incisional, and neuropathic pain models.

Disclosures: M. Mueth: None. **P. Neufeld:** None. **T.E. King:** None. **B.J. Harrison:** None.

Nanosymposium

NANO044: Peripheral Pain Mechanisms

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 8:00 AM - 11:00 AM

Presentation Number: NANO044.09

Topic: E.01. Somatosensation – Pain and Itch

Support: MRC DTP
Grünenthal

Title: Diabetic neuropathy in KINGS mice

Authors: *M. KIBRIA MUMU[1], H. SUN[1], A. KING[2], S. BEVAN[1], D. A. ANDERSSON[1]; [1]Wolfson SPaRC, [2]Sch. of Cardiovasc. and Metabolic Med. & Sci., King's Col. London, London, United Kingdom

Abstract: More than 10% of the global population is affected by diabetes. Half of all patients develop diabetic neuropathy (DN), which is characterised by reduced cutaneous innervation and sensory loss, leading to foot amputations. Approximately 60% of DN patients are left with unsatisfactory symptomatic relief, demonstrating a gap in current treatments. The spontaneous Ins2G32S mutation (KINGS) in people causes inherited cases of neonatal diabetes. Heterozygous KINGS mice produce little insulin and become diabetic soon after weaning. Investigations of pathophysiological mechanisms of DN in models like the KINGS mice, which do not rely on the administration of β-cell toxins, may yield conclusions of improved translational validity. Here, we aim to elucidate whether diabetes in KINGS mice leads to sensory abnormalities, indicative of DN. We assessed the behavioural sensitivity of 26-week-old diabetic male KINGS mice (n=10) and wildtype littermates (wt) (n=9) to noxious mechanical and thermal stimuli. We also monitored [Ca2+]i changes in sensory neurons isolated from dorsal root ganglia (DRG) in KINGS (n=2651) and wt (n=3232) mice, upon cold (30-10°C) and heat (30-48°C) stimuli. In addition, we quantified intraepidermal nerve fibre density (IENFD) in glabrous skin sections isolated from hind paws by immunostaining with the pan-neuronal marker protein gene product 9.5 (PGP 9.5). Lastly, we performed bulk RNA-seq of DRG and skin samples, comparing KINGS to wt mice. We showed that KINGS mice displayed reduced sensitivity to mechanical stimuli, both to paw pressure and electronic Von Frey filament (both $p<0.05$, unpaired two-tailed t-test), whereas thermal nociception was preserved. In contrast to in vivo findings, isolated DRG neurons from KINGS mice were hypersensitive to both cold (9% vs 3%) and heat (47% vs 42%) stimuli. Furthermore, IENFD was also reduced in KINGS mice (15.61 ± 0.8 vs 25.02 ± 1.1, $p<0.05$, unpaired two-tailed t-test). Importantly, RNA-seq revealed 913 differentially expressed genes in DRG and 947 in skin. Gene ontology analysis of

upregulated genes in DRG highlighted the enrichment of collagen-containing extracellular matrix (False Discovery Rate (FDR) q value<0.05). In contrast, inflammatory responses and leukocyte migration pathways were enriched in the skin (FDR q value<0.05). In this study, we showed that diabetic male KINGS mice recapitulated the hyposensitivity to mechanical stimuli and reduced IENFD typical of patients with DN. Interestingly, we observed thermal hypersensitivity in DRG neurons from KINGS mice. Ongoing transcriptomic analysis of DRG and skin could elucidate pathways and targets involved in the pathophysiology of DN.

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Nanosymposium

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Support: National Natural Science Foundation of China grants 82101231 (L.S.)
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Title: Dorsal root ganglion neuronal LTBP1 as a novel therapeutic target for the treatment of neuropathic pain

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Abstract: Neuropathic pain (NP) remains a significant global health burden with limited targeted therapies. In this study, we employed the spare nerve injury (SNI) model as the NP mouse model and found that the latent TGF-β binding protein 1 (LTBP1) was significantly upregulated in the L3-5 dorsal root ganglia (DRGs) in NP mice and in the cerebrospinal fluid in NP patients. Immunohistochemistry revealed that LTBP1 was expressed in all-sized DRG neurons as well as satellite glial cells. Specific knockdown of LTBP1 from DRG neurons (*Ltbp1* nKO) but not satellite glial cells (*Ltbp1* gKO) reversed SNI-induced pain. Mechanistically, SNI induced significant increase in the transcription activator octamer 1 (OCT1), which enhanced *Ltbp1* transcription through increased binding with *Ltbp1* promoter region (TSS +908~1270). Elevated LTBP1 expression promoted membrane trafficking of GPR151 in DRG neurons, while *Ltbp1* nKO caused GPR151 retention in the cytoplasm. Our findings reveal a neuron-specific LTBP1-GPR151 signaling axis that drives neuropathic pain, identifying both a novel therapeutic target and potential CSF biomarker for NP.

Disclosures: S. Guo: None. **L. Sun:** None.

Nanosymposium

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Title: Whole body exercise ameliorates sleep induced increases in pain behaviors and inflammation occurring after induction of overuse injuries

Authors: *M. F. BARBE[1], D. M. KLYNE[3], F. L. CHEN[4], M. G. VAN DER BAS[5], A. KEGG[2], B. A. KALICHARAN[6], K. CAESAR[2], P. W. HODGES[7];
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Abstract: We have shown that poor sleep worsened pain behaviors induced by repetitive overuse, in parallel with elevated serum BDNF, and that aerobic exercise ameliorated these changes. We expanded that study to determine if serum biomarkers of inflammation, tissue repair or neuromodulation, correlate with pain-related behaviors. Adult female rats performed an intensive repetitive lever-pulling task for 4 weeks to induce symptoms consistent with acute-onset overuse injury, before being divided into 3 intervention groups (n=8-11/gp) that underwent either voluntary exercise (Ex, running wheel), sleep disturbance (SD), or both (SD+Ex), for 4 weeks after task cessation. Sensorimotor behaviors and 70 serum analytes were assayed longitudinally at pre-injury, post-injury, and post-intervention. Grip strength declined significantly after 4 weeks of repetitive lever pulling and recovered with rest in SD and SD+Ex groups, yet improved significantly in Ex group, compared to baseline. Forepaw mechanical sensitivity to monofilament probing was heightened in each group after 4 weeks of task; Ex with or without SD, ameliorated this sensitivity. Declines in maximum grip strength correlated with higher serum BDNF, CINC-1, Eotaxin, IFNγ, IL-1β, Neuropilin-2, and TNFα. Percent change in grip strength from pre-injury correlated positively with corticosterone, RANTES and TIM-1; and negatively with IFNγ and TCK-1. Enhanced limb withdrawal responses to 1cN and 4cN sized monofilaments correlated moderately with higher BDNF, HGF, and TNFα. Cluster analysis of serum biomarkers identified three clusters with distinct neuroimmune profiles: “low inflammatory” (Cluster 1), “high CINC-1, IFNγ & IL-1β” (Cluster 2), and “high inflammatory” (Cluster 3). Cluster 3 had higher BDNF and neuropilin-2 than other clusters (all $p<0.047$), higher TNF than Cluster 2 ($p=0.057$) and lower TIMP-1 than Cluster 1 ($p=0.02$). Interestingly, Cluster 3 contained 78% SD animals (and 1 SD+Ex, yet 0 Ex animals), the lowest percent change in grip strength, lowest maximum grip strength, and lower limb withdrawals to 1 cN and 4 cN probings.

Thus, chronic sleep disturbance was associated with a high systemic inflammatory profile and significant declines in sensorimotor behaviors; adding exercise reduced these responses.

Disclosures: M.F. Barbe: None. **D.M. Klyne:** None. **M.G. Van Der Bas:** None. **A. Kegg:** None. **B.A. Kalicharan:** None. **K. Caesar:** None. **P.W. Hodges:** None.

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Presentation Number: NANO045.01

Topic: E.03. The Chemical Senses

Title: Activation of Entorhinal Cortex Rescues Olfactory Behavior in Two Mouse Models of Autism Spectrum Disorders

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Abstract: Olfactory deficits in Autism Spectrum Disorders (ASD) limit the food preferences of children, resulting in restricted diets as children reject new food items. Novel odors also cause olfactory deficits in the *Shank3B*$^{-/+}$ and *Cntnap2*$^{-/-}$ mice mouse models of autism. These mouse models of ASD can identify target odors in known background odors but do more error trials when challenged with novel background odors, compared to WT mice (Li et al 2023, Ryndych et al 2023). To explore how novelty impacts olfactory bulb odor representations, we used widefield calcium imaging of glomerular output in *Cntnap2*$^{-/-}$ and *Shank3B*$^{+/-}$ male and female mice while they identified target odors in the presence of novel background odors using a go/no-go head-fixed behavior. Larger neural activity in response to the novel background odors in the olfactory bulb was associated with behavioral errors in five *Cntnap2*$^{-/-}$ and five *Shank3B*$^{+/-}$ mice. The difference of z-score between average error and average correct responses for novel background odors was 0.17±0.07 (mean±s.e.m, n=39 comparisons) and this difference was significantly different from zero (p=0.019, double tailed paired t-test). Electrical activation of the entorhinal cortex can reduce the olfactory bulb effects in their target areas including piriform cortex and basolateral amygdala (Mouly and Scala, 2006). Therefore, we hypothesized that activation of the entorhinal cortex might suppress the excess activity produced by novel background odors in mouse models of ASD, rescuing the olfactory behavior. We have tested this hypothesis by stimulating the entorhinal cortex. The performance of all 7 mice tested (4 *Shank3B*$^{-/+}$ *and 3 Cntnap2*$^{-/-}$) in odor recognition in novel background odors improved with entorhinal cortex stimulation (p=1.7e-5, Fisher exact test). The performance improved from 58.0% (CI: [52.9% 63.0%]) to 74.5% (CI: [68.8% 79.7%]). *Our result demonstrates that activation of the entorhinal cortex can alleviate olfactory symptoms in mouse models of ASD.*

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Nanosymposium

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Presentation Number: NANO045.02

Topic: E.03. The Chemical Senses

Title: Order Code In The Olfactory Bulb

Authors: *K. SAMOILOVA[1], J. S. HARVEY[3], H. NAKAYAMA[4], A. KOULAKOV[2], D. RINBERG[5];
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Abstract: What is the structure of odor representations formed by the olfactory system? In this study, we analyzed glomerular responses in the mouse olfactory bulb to large sets of odorants obtained using calcium imaging. The primacy model suggests that odor identity is encoded by high-affinity sets of olfactory receptors (ORs) called primacy sets. According to this model, ORs that do not belong to any primacy set (null ORs) are eliminated during evolution. The remaining ORs form a low-dimensional structure known as the primacy hull. To test this hypothesis, we embedded recorded OR/glomerular responses into a receptor space using multidimensional scaling (MDS). We found that in the receptor space, ORs/glomeruli form two clusters with distinct odor-tuning properties. The clusters create two independent primacy sets for individual odors, contributing to two distinguishable primacy hulls for odor ensembles. Notably, the OR clusters contain few null ORs (non-primary to any odor), compared to a randomly shuffled dataset, as predicted by the primacy theory. OR activation in response to odors occurs in temporal waves that show orderly patterns in the receptor space. The waves' directions do not align across the two OR clusters. The directions of OR recruitment waves are determined by the odorants and can be used to represent odor identity. Odor representations based on OR recruitment wave directions are consistent across individual animals and allow us to build an accurate cross-animal and cross-concentration odor identity classifier. Together, these findings suggest that ORs form two separate encoding channels, each with its own primacy receptor set (primacy hull). We propose that the order of OR activations generates a robust odor representation that generalizes well across different animals and concentrations.

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Nanosymposium

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Topic: E.03. The Chemical Senses

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Title: A low-dimensional glomerular code for olfactory perception

Authors: *W. G. BAST[1], C. AGHAMOHAMMADI[2], P. GUPTA[1], T. A. ENGEL[2], D. ALBEANU[1];
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Abstract: Despite significant advances in olfaction, the relationship between odorant receptor (OR) activity patterns and odor percepts remains poorly understood. We developed a novel approach to investigate how OR response spectra are related to perceived stimulus similarity. Disentangling this relationship requires controlling olfactory stimuli at the level of OR types; we exploited the anatomical clustering of ORs to individual glomeruli to address this challenge. Using two-photon and widefield imaging in transgenic mice, we identified numerous glomeruli and determined their responses to 121 odorants. We then created synthetic olfactory stimuli by optogenetically activating selected glomerular combinations. To determine perceptual distances between these glomerular sets, we trained mice to report perceived differences between a reference stimulus and other glomerular patterns. We found that individual glomeruli within the reference set differ in perceptual relevance, as some glomeruli contribute more to the perception of the reference stimulus than others. This distinct relevance emerged as a property of the entire glomerular activity pattern rather than being solely defined by odorant receptor (OR) identity. The same glomeruli (ORs) embedded in different glomerular patterns could have widely different perceptual weights, revealing a pattern-dependent glomerular hierarchy that depended on the response spectra of the entire glomerular reference set. To investigate how odorant responses determine the perceptual similarity of sets of photo-activated glomeruli, we developed an unsupervised, autoencoder-based method to extract latent factors from glomerular response profiles. This approach captured a ~12-dimensional manifold that represents the response spectra of about 40 glomeruli used as inputs in each animal with 90% accuracy. We further trained a model of behavioral performance on these latent factors, which successfully predicted individual animals' responses to novel glomerular sets. Together, these findings provide new insights into how OR activation patterns are mapped to olfactory percepts. Our results suggest that olfactory perception is low-dimensional and inherently structured for efficient odor representation.

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Nanosymposium

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Topic: E.03. The Chemical Senses

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OIST Graduate University

Title: Linking stimulus encoding with behaviour using synthetic olfactory stimuli

Authors: *I. FUKUNAGA;
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Abstract: The olfactory system is a well-established model for studying the temporal encoding of sensory stimuli, owing to its rhythmic stimulus delivery through respiration. Sniff-locked activity is pervasive in the primary olfactory area, the olfactory bulb, and is considered critical to structuring the output of its computation.
We tested the behavioural importance of these temporal features using simple, temporally precise, closed-loop optogenetics embedded in custom behavioural paradigms. Male and female mice that express ChR2 in the output neurons of the olfactory bulb (Tbx21-Cre::Ai32 mice) were used, and 450 nm light from a laser diode coupled into a fibre optic cannula targeted to the anterior portion of the lateral olfactory tract.
We found that the mice perceive differences in evoked spike counts and discriminate between synchronous vs. asynchronous activations of the output neurons. Surprisingly, they failed to distinguish the timing of evoked activity relative to the sniff cycle. The mice were also unable to perceive the latency of stimulation relative to the sniff cycle when optogenetic stimulation occurred in the olfactory bulb.
These results suggest that, beyond the initial steps of olfactory processing, sniff rhythms play a more nuanced role, with a greater reliance on spike rate and synchrony for the neural encoding of the environment, consistent with a gradual transformation of encoding format at successive stages of sensory processing.

Disclosures: I. Fukunaga: None.

Nanosymposium

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Topic: E.03. The Chemical Senses

Title: Causal manipulations of odor perception

Authors: *S. CEBALLO, M. KARADAS, D. RINBERG;
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Abstract: Understanding how the brain transforms sensory inputs into behavioral outputs is a central question in sensory neuroscience. Biasing sensory-guided decisions is a powerful approach to causally link specific neuronal activity features to behavior. In this context, the mammalian olfactory system transforms inputs from olfactory sensory neurons (OSNs) into glomerular activity patterns in the olfactory bulb (OB), relaying odor-specific signals to cortical and limbic areas. Yet, how these signals drive distinct perceptual decisions remains unclear. We developed a two-odor categorization (2OC) task, training mice to lick left or right spouts in response to odorants A or B. In probe trials, we presented binary odor mixtures with varying ratios and measured behavioral choices. Using fast and high-resolution optical imaging, we recorded glomerular responses to odor stimuli and identified spatiotemporal patterns correlated with categorization boundaries. We observed that behavioral decisions are correlated with identity of a small number of the early activated glomeruli. To establish causal links between neural features and behavior, we used optogenetics to perturb these glomeruli during the 2OC task, focusing on trials with stimuli near the decision boundary. Perturbations of a small glomerular set during the first inhalation were sufficient to bias perceptual decisions, proving their causal role in behavior.

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Title: Locomotion modulation of neural representations of odors in the rodent main olfactory bulb

Authors: *K. P. SZYMULA[1,2], A. C. KOLSTAD[1,2], K. PADMANABHAN[3];
[1]Biomed. Engin., [2]Med. Scientist Training Program, [3]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Olfactory behaviors like navigating towards an odor source or tracking an odor trail (Khan et al 2012) require locomotion; this movement both assists in the tracking of chemosensory cues (Findley et al, 2021) but also alters those cues due to self-generated motion. While odors are encoded through spatiotemporal patterns of activity across populations of mitral and tufted (M/T) cells in the main olfactory bulb (MOB), recent work has demonstrated that M/T

cells are also capable of encoding locomotor information, independent of odor encoding (Chockanathan et al, 2021). This adds to an existing body of literature demonstrating that M/T cell activity and responses to odors can be modulated by behavior (Kay and Laurent, 1999). An open question is thus how M/T cell encoding of behavior shapes the stability and variability of the neural responses to odors. To address this question, we performed high-density recordings of M/T cell responses to an array of monomolecular odors (N=22 odors) in the MOB of awake head fixed mice (N=6) on a running wheel. In tandem, we monitored other aspects of behavior such as the animal's sniffing, which is affected by running, and has a strong modulatory effect on the precise timing of neural activity in response to odor presentation. We found that both single neuron and population responses to odors were variable across different time scales due to behavior. While some neurons encoded odors independently of the locomotor behavior, other cells showed significant modulation of trial-to-trial responses to the same odor in neural activity due to locomotion. Thus, odor encoding by M/T cells in the bulb requires consideration of not only the identity or concentration of those odors, but also the locomotor behavioral state of the animal.

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Title: Olfactory coding in ventral CA1 region of the hippocampus

Authors: *A. C. KOLSTAD[1,2], K. P. SZYMULA[1,2], K. PADMANABHAN[3];
[1]Med. Scientist Training Program, [2]Dept. of Biomed. Engin., [3]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: While the ventral CA1 region of the hippocampus (vCA1) has historically been associated with memory, navigation, and anxiety, increasing evidence suggests that chemosensory stimuli may influence vCA1 neural responses. The olfactory cortex sends direct projections to vCA1 and vCA1 sends monosynaptic feedback projections to multiple olfactory areas including the main olfactory bulb. Despite this reciprocal connectivity and the increasing

evidence for use of olfaction in hippocampal-related behaviors, vCA1's role in encoding and processing olfactory stimuli remains poorly understood. A necessary first step toward resolving this question is to identify whether features of olfactory stimuli such as odorant identity are encoded for in the firing of neurons in vCA1. To address this, we performed high-density extracellular electrophysiology recordings in vCA1 in awake head-fixed 2-4-month-old C57BL/6J mice as they ran on a wheel while passively exposed to volatile monomolecular odorants. When we examined neural activity in vCA1 in four female mice (n = 33 single units), we found 55% of units exhibited odorant tuning. Odorant tuned units were responsive to anywhere between one and three odorants out of a ten-odorant panel, and both excitatory and inhibitory responses were observed. Furthermore, responses were observed across the population to several classes of odorants as defined by chemical functional groups. Understanding olfactory representations in healthy ventral hippocampal circuits will lay the groundwork for studying what role changes in hippocampal circuitry may play in mediating the co-occurring changes in cognition, emotional regulation, and olfactory perception seen in neurodegenerative diseases.

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Nanosymposium

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Kavli fellowship

Title: State-dependent olfactory navigation and information processing in C.elegans

Authors: *K. S. CHEN[1], J. W. PILLOW[2], A. M. LEIFER[3];
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Abstract: Olfactory-guided navigation is essential across many species to find food and resources. To successfully navigate chemical environments, animals must flexibly interpret odor signals in light of their learned experiences and internal states. However, how these factors shape sensory processing and navigation strategies remains less understood. Here, we focus on the nematode worm *C.elegans* to investigate state-dependent navigation behavior and olfactory processing. We developed a novel apparatus to precisely measure both the olfactory landscape and the worm's behavioral kinematics during navigation. We measured the worm's odor navigation in this controlled environment. We addressed two main questions: (1) How are

navigation strategies altered by prior associative learning experiences? and (2) How do time-varying behavioral states support navigation?
To quantify learning-dependent effects, we developed a statistical model that describes sensorimotor transformation along the navigation path. The model revealed that worms up- or down-regulate weights on the odor signal depending on its past association with food or starvation, respectively. By delivering optogenetic stimulation to the sensory neuron and conducting genetic ablation of interneurons, we concluded that the learning-dependent changes are distributed across the neural circuit, rather than localized in specific neurons.
To characterize how worms dynamically employ behavioral strategies along the navigation path, we further developed a latent variable model that accounts for discrete state-switching. This model uncovered two dominant states—turn-enriched and steer-enriched states—that persist over time and whose transitions are driven by sensory input. This sensory-driven state-switching provides a mechanism for previously unexplained directed turning behavior observed during worm navigation. Together, our work demonstrates that navigation behavior in *C.elegans* is experience-dependent and state-modulated. These findings provide new insight into the flexible behavioral algorithms and distributed neural computations that underlie olfactory navigation in compact nervous systems.

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Title: The construction of odor representations via intrinsic network dynamics

Authors: *T. A. CLELAND;
Cornell Univ., Ithaca, NY

Abstract: Spike timing matters. This is clear simply based on the integration time constants of synapses, as well as on more sophisticated synaptic mechanisms such as spike timing-based plasticity. Given the enormous effects that fine-timescale spike timing exerts on neural computation, do brain systems regulate spike timing accordingly, or leave it to chance? And, assuming the former, by what dynamical mechanisms can the necessary regulation of spike timing be maintained? And how can information be represented in patterns of neural activity while respecting the constraints imposed by these dynamical systems? Using optogenetically programmed fictive odorant stimuli applied to olfactory bulb explants, contextualized in the broader literature, I discuss how the early olfactory system constructs its representations within a dynamical systems framework rendered robust by both glomerular-layer preprocessing

mechanisms and PRING dynamics. Briefly, we find that a small proportion of olfactory bulb principal neurons phase-locks strongly to the fast oscillations evoked by fictive odorants, and exhibits tightly coupled spike-spike synchrony on the gamma timescale during this stimulation. Moreover, the specific population of synchronized neurons differed based on the “quality”, but not the “concentration”, of the fictive odorant presented, and was conserved across multiple presentations of the same fictive odorant. This synchronization-based metric discarded nearly all concentration-based variance, whereas mean spike rate metrics still were contaminated by concentration. Finally, given that neural activity and fine-timescale spike timing profiles even in the olfactory bulb are influenced by learning, neuromodulation, and other behavioral state variables, I further argue that a framework of “odor coding” is limiting; instead favoring “representational cascades” as a more robust and generative framework with which to understand activity in brain networks.

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Title: Encoding of position and odor concentration by neuronal activity in dorsal CA1 in mice engaged in odor plume navigation.

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Abstract: In nature, an animal's survival hinges on its ability to process and integrate sensory information, which is a critical skill for finding sustenance, avoiding threats, and selecting mates. An ethologically relevant behavior of particular interest is an animal’s ability to detect, pursue, and ultimately reach target scents, termed “odor plume navigation”, as this behavior relies on complex integration of present and past information of spatial and contextual experiences with intermittent olfactory information. Previous research suggests that the hippocampus, particularly the dorsal CA1 (dCA1) region, would be potentially a key player in odor plume navigation, because it is involved in spatial navigation and processes olfactory input from the lateral entorhinal cortex, a key location for odorant processing. In this work, we implanted GRIN lenses

to image dCA1 pyramidal cell activity in Thy1-GCaMP6f mice using a miniscope as outlined in our previous publication (Simoes de Souza et al, JoVE doi:10.3791/67039, 2024), while recording mouse position in the arena. We found that following odor release, a mouse would consistently navigate towards one side wall, turn towards the spout, and increase velocity when it had chosen the side where the odorant was being released. We performed decoding of position and the logarithm of average odorant concentration using a binary decision tree machine learning algorithm. The algorithm was trained with z-normalized ΔF/F from all ROI activity in all but one trial and position and odorant concentration were predicted in the remaining trial (leave one out approach). For position decoding there were no differences in the goodness of fit between hit and miss trials (goodness of fit was quantified as the correlation, R1, between predicted and actual values). In contrast, for prediction of odorant concentration R1 was higher for hits compared to misses. Interestingly, when ROIs were ranked in prediction importance the top 5% ROIs were largely non-overlapping between prediction of position vs. odorant concentration. The data indicates that dorsal CA1 participates in combined computation of position and odorant concentration in the odor plume navigation task.

Disclosures: K. Steinke: None. **F.M. Simoes de Souza:** None. **D. Restrepo:** None. **E. Gibson:** None.

Nanosymposium

NANO045: Olfactory Behavior and Perception

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Presentation Number: NANO045.11

Topic: E.03. The Chemical Senses

Support: National Institute of Health grant U19NS112953
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Title: Respiration coordinates the olfactory cortical code

Authors: *R. M. BLAZING[1], E. CHONG[2], D. RINBERG[3], K. M. FRANKS[4];
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Abstract: A central goal of systems neuroscience is to understand how the brain decodes temporally structured sensory signals. In the olfactory system, odor identity is represented by the response timing of olfactory bulb (OB) glomeruli relative to the respiration cycle. However, it remains unclear whether and how downstream brain regions read out this temporal code. To address this, we optogenetically stimulated glomerulus-sized spots on the OB at defined respiration phases while recording from the piriform cortex (PCx), the primary OB projection

target, in awake, head-fixed mice. PCx neurons exhibited robust phase tuning, responding preferentially to glomerular stimulation at specific sniff phases. Notably, different cells responded to stimulation of the same spot at distinct preferred phases, while individual cells retained consistent phase preferences across spots, suggesting that phase tuning in PCx is independent of glomerular identity. Across the population, preferred phases tiled the respiration cycle, indicating that PCx transforms the OB's temporal code into a distributed, rate-based spatial code. We next investigated the mechanisms underlying this phase tuning. PCx neurons showed spontaneous, respiration-locked fluctuations in firing rate, with each neuron exhibiting a preferred phase of spiking. Evoked responses to OB stimulation were aligned to each neuron's spontaneous preferred phase, suggesting that respiration-driven oscillations gate PCx responses to phase-coded input. Consistent with this, ipsilateral naris occlusion strongly attenuated both spontaneous phase locking and phase tuning in PCx. Interestingly, OB mitral and tufted cells showed only weak, inhalation-biased phase preferences, implying that PCx phase tuning emerges within the cortex. Supporting this, silencing recurrent PCx circuits with tetanus toxin caused phase preferences to shift toward those in the OB, while preserving overall selectivity. These results suggest that feedforward computations enforce phase selectivity, while recurrent circuitry refines phase tuning and redistributes phase preferences across the respiration cycle. Together, these findings demonstrate that PCx circuitry transforms a bulb-derived temporal code into a sparse, distributed ensemble code, enabling the storage and recognition of phase-encoded odor information.

Disclosures: R.M. Blazing: None. **E. Chong:** None. **D. Rinberg:** None. **K.M. Franks:** None.

Nanosymposium

NANO045: Olfactory Behavior and Perception

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Topic: E.03. The Chemical Senses

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R01DC022320

Title: Multiscale interrogation of neural connectivity in vivo using precision optogenetics

Authors: *J. V. GILL[1,2], M. KARADAS[2], S. SHOHAM[2,4,3], D. RINBERG[2,5,6];
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Abstract: Neural circuits achieve their computational power through their connectivity. Behaviors linking sensation to action are governed by the precise interplay of neurons

communicating over multiple spatial scales, such as between brain areas and layers of processing, as well as within local populations of neurons. Yet, circuit computations are usually only inferred by observing the activity of neurons, not the connections between them. Here, we describe a system based on a custom 2-photon microscope that permits multiscale optogenetic interrogation of the effective connectivity between neurons. This system combines a pathway for large scale patterned illumination using a digital micromirror device (DMD), along with a pathway for precise holographic 2-photon stimulation at a fine spatial scale using a spatial light modulator (SLM). Using this combined approach, we demonstrate our ability to identify neurons by both their tuning to sensory stimuli, as well as the effective input they receive from other circuits. We demonstrate this using two model systems, the mouse olfactory bulb and the somatosensory cortex. In the olfactory bulb, we used DMD pattern stimulation to activate individual glomerular channels expressing ChR2 and demonstrate that we can identify groups of mitral and tufted cells receiving direct input from individual glomeruli. We then interrogated the effective connections between individual mitral and tufted cells using holographic stimulation. Surprisingly, we observed a mixture of excitatory and inhibitory influence, even though only inhibitory interneurons are thought to mediate the anatomical connections between mitral cells. In ongoing experiments we attempt to relate the sign and strength of coupling between mitral cells to distances in odor tuning and differences in glomerular input to determine how local connectivity reshapes odor representations, as well as extend this technique to study the connections between and within whisker barrels in the somatosensory cortex. Together, these advances provide a versatile platform for uncovering how multiscale connectivity shapes neural computation, offering new opportunities to link circuit architecture to behavior and perception.

Disclosures: J.V. Gill: None. **M. Karadas:** None. **S. Shoham:** None. **D. Rinberg:** None.

Nanosymposium

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Presentation Number: NANO045.13

Topic: E.03. The Chemical Senses

Support: R01DC018075

Title: Spatiotemporal dynamics of odor feature processing in the human brain

Authors: *S. CORMIEA[1], G. N. DIKECLIGIL[2], J. M. STEIN[1], H.-C. I. CHEN[3], K. DAVIS[1], J. A. GOTTFRIED[2];
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Abstract: We live immersed in a sea of smells; innumerable volatile molecules eddying around us as we breathe, move, and talk. Each odor carries with it a wealth of information on the nature of its source as well as its relevance to the smeller. And with every sniff the human olfactory system works to answer myriad questions pertaining to the odor: “what is this smell?”, “do I like

it?", "can I eat it?". It remains unknown whether the olfactory system resolves all possible dimensions of odor information to address these varied questions and whether it does so in parallel or in a hierarchical manner. To investigate the spatiotemporal dynamics of distinct odor features in the human brain, we paired an odor feature rating task with high temporal resolution intracranial EEG recordings in patients undergoing invasive monitoring for treatment of intractable epilepsy. Local field potentials were recorded via surgically implanted depth electrodes throughout the behavioral paradigm. On each trial, participants evaluated a real-world odor (e.g., cheese, dirt, lemon, shampoo) on one of three dimensions: (i) pleasantness, (ii) edibility, or (iii) identity. Participants' ratings reliably differentiated pleasant and unpleasant odors as well as edible and inedible odors. Participants also overwhelmingly endorsed the true label of an odor versus an incorrect foil label. Consistent with prior work, we observed robust changes in the oscillatory activity of piriform cortex in response to odors. Odor-evoked oscillations were not observed in control regions. Piriform cortex electrodes were selected for further analysis. A set of support vector machine classifiers were trained to decode neural responses on the basis of odor identity, valence, or edibility. Our preliminary results show above-chance level decoding on all three dimensions. Furthermore, time-resolved decoding analyses reveal that the three odor features emerge and evolve along distinct timelines. These results suggest that oscillations in primary olfactory cortex maintain overlapping yet separable codes for odor identity, pleasantness, and edibility. Ultimately, this work seeks to uncover the contents, sequence, and dimensionality of odor stimulus information embedded in olfactory neural signals as they unfold over time.

Disclosures: S. Cormiea: None. **G.N. Dikecligil:** None. **J.M. Stein:** None. **H.I. Chen:** None. **K. Davis:** None. **J.A. Gottfried:** None.

Nanosymposium

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Title: Tuning consistency across luminance contrasts: Evidence for object-centered coding in primate V4

Authors: *T. NAMIMA[1], A. K. PASUPATHY[2];
[1]The Univ. of Osaka, Suita, Japan; [2]Neurobio. & Biophysics, Univ. Washington, Seattle, WA

Abstract: There has been a longstanding debate about what V4 neurons encode. Prior studies have demonstrated selectivity for boundary curvature. But because in these studies stimuli were presented in one contrast, it has not been incontrovertibly demonstrated to be an object-centered code. Specifically, is the preference for convex versus concave contours with the same boundary shape just a preference for a specific contrast polarity? We hypothesized that consistent tuning across contrast reversals for boundary shape would provide evidence supporting an object-centered code of V4 neurons. To test this hypothesis, we used a high-density Neuropixels probe in two awake macaque monkeys (one male and one female) to target area V4 and characterized contrast-invariance in the responses to shapes from dozens of neurons recorded simultaneously across layers. A set of 120 2D shape silhouettes was presented at two contrast levels: either darker or brighter (4 cd/m^2 or 12 cd/m^2) than the gray background (8 cd/m^2), centered on the aggregate receptive field of the recorded neurons. We assessed the response invariance across contrast reversals for each neuron by computing the correlation coefficient between the responses of a neuron to dark and bright shapes. More than half of our neurons exhibited highly contrast-invariant shape responses (median of correlation coefficient was 0.6228, n = 1728). This contrast-invariance was not fully dependent on tuning similarity across nearby neurons, indicating a salt-and-pepper structure of shape tuning in V4. When the response invariance was related to the tuning for boundary curvature as quantified by the angular position and curvature model, response invariance and the tuning for boundary curvature were mildly but statistically correlated (Spearman's $r = 0.300$, $p < 0.0001$). Moreover, neurons well-fit by a Gaussian function in angular position x curvature space (n = 656, GOF > 0.5) showed statistically greater contrast-invariance than neurons poorly-fit (n = 1072, GOF ≦ 0.5, two-sample Welch's t-test $p < 0.0001$). Our results demonstrate that tuning of V4 neurons to boundary curvature reflects object-centered code, rather than preference for a specific contrast polarity. High-yield sampling using high-density electrodes allowed us to evaluate response trends across large populations of neurons.

Disclosures: T. Namima: None. **A.K. Pasupathy:** None.

Nanosymposium

NANO046: Insights From Large Scale Recording of Primate Visual Cortex

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Presentation Number: NANO046.02

Topic: E.06. Vision

Support: RO1 EY018839
RO1 EY029601
NS131810

Title: Integration of prospective signals with visual inputs in V4 neurons

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Abstract: In natural vision, the retinal image is dramatically shifted during eye movements, altering the visual input within each neuron's receptive field (RF). Despite this, our perception of the visual world remains stable. Prospective remapping, which is a shift of a neuron's RF toward its future post-saccadic location (postRF) just before a saccade, is thought to support perceptual stability by bridging the visual input across eye movements. Although prospective encoding in V4 has been previously observed, it is not known whether these anticipatory signals are stimulus selective, nor how they might interact with visual input after saccade completion. Using high-density Neuropixels probes, we measured the responses of V4 neurons as a macaque made saccades to a target dot, while stimuli were presented in the postRF. For all experiments, RF position was determined using an automated RF mapping procedure, and stimulus position was determined by the aggregate RF of all neurons recorded simultaneously. The experimental paradigm manipulated stimulus identity at the time of saccade onset, allowing us to examine how prospective signals in the V4 population influence post-saccadic visual processing and how these signals evolve differently depending on stimulus identity. We calculated population metrics and used dimensionality reduction techniques to study how the V4 population represents a visual stimulus across saccades and how prospective signals evolve over time. Across 15 recording sessions, we found that when a stimulus was extinguished at saccade onset, V4 responses still emerged after saccade completion, suggesting the presence of anticipatory input in the postRF. Additionally, when a stimulus unpredictably appeared or changed identity, the V4 population exhibited different response dynamics compared to conditions where the stimulus predictably remained the same. We also developed a simple biologically plausible model of a V4 neuron that can explain these findings, with an assumption that anticipatory signals can interact with stimulus adaptation to influence the generation of an excitatory postsynaptic potential by the sensory input. Our experimental results suggest that prospective signals in V4 are stimulus selective, and adapt responses to incoming visual information. These signals may be important for the smooth representation of objects across saccades, and can emerge from mechanisms already present in the V4 population.

Disclosures: R. Kamath: None. **S. Beaufrand:** None. **T. Kim:** None. **A.K. Pasupathy:** None.

Nanosymposium

NANO046: Insights From Large Scale Recording of Primate Visual Cortex

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Presentation Number: NANO046.03

Topic: E.06. Vision

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Title: Microstimulation of V4 domains modulates visual perception in non-human primates

Authors: *Y. VALIBEIGI[1], C. C. PACK[2];
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Abstract: In primates, cortical area V4 plays a key role in visual object recognition. It is organized into millimeter-scale domains that represent specific stimulus features such as shape, texture, and color. These domains are thought to serve as a critical intermediate stage between early visual areas that process simple features and higher-order regions involved in complex object representation. However, the causal influence of these domains on shape perception remains unknown. To investigate this, we trained a non-human primate to perform a delayed match-to-sample task involving the discrimination of circular and radial gratings—stimuli known to evoke distinct patterns of neural activity in V4—and applied electrical microstimulation to V4 domains. We used bipolar stimulation to confine current spread to domains with similar stimulus preferences. We found that microstimulation of V4 domains significantly biased perceptual decisions toward the stimulus that evoked the strongest neural response in the targeted region. Importantly, this bias was stronger when we stimulated larger domains, even though the total amount of current was identical across all experiments. Specifically, behavioral effects were weak for stimulation of domains smaller than 0.5 mm, while strong effects were found for stimulation of domains greater than 1.5 mm in diameter. These findings suggest that the topographical organization of V4 has functional significance for perceptual decision-making that extends beyond the selectivity of individual neurons. More generally, these results show that applications aimed at artificially inducing specific percepts may benefit from distributing electrical stimulation across multiple sites with similar feature coding.

Disclosures: Y. Valibeigi: None. **C.C. Pack:** None.

Nanosymposium

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JSPS KAKENHI for Grant-in-Aid for Early-Career Scientists 24K20883

Title: Comparing Neural and Perceptual Representations of Translucency in Macaques and Humans via Large-Scale Electrode Recording in the Inferior Temporal Cortex

Authors: H. NAKADA[1], S. FUJIMOTO[2], T. MATSUO[3], K. KAWASAKI[4], S. NISHIDA[5], ***R. HAYASHI**[1];
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Abstract: Introduction The appearance of translucency —how much light passes through a material—depends on multiple visual features. Therefore, its neural representation is likely better understood at the population level rather than single-cell level activity. In this study, we recorded neural activity from the macaque inferior temporal (IT) cortex using large-scale electrode arrays while presenting object images that varied in shapes and degrees of translucency. We then compared the resulting neural population activity with translucency ratings obtained from human behavioral experiments using the same stimuli. **Visual Stimuli** The first image set was generated using an unsupervised model (Liao et al., 2023) that encodes object shape and translucent appearance in latent variables, consisting of object images that varied across 27 shapes and 7 translucency levels. Pixel-scrambled versions of these images served as a control condition. The second image set consisted of CG-rendered images varying across 4 shapes and 13 material appearances, created by manipulating multiple optical parameters based on prior translucency research. **Human behavioral experiments** Twenty participants per experiment completed an online task. After providing informed consent, they viewed each image for 500 ms and rated its appearance on a scale from 0 (transparent) to 100 (opaque) scale using a scroll bar. **Macaque electrophysiological experiments** Neural responses were recorded from the IT cortex of two male macaques using four Utah arrays (512 channels in total). In both experiments, each trial began with a 500 ms fixation period, followed by a 500ms image presentation in random order, repeated 10 times per image. **Results** Human ratings showed that the translucency range of model-generated images spanned that of CG-rendered images used in prior studies. From these ratings, we constructed representational similarity matrices (RSMs) reflecting the perceptual representation of translucency. We found a significant correlation between the perceptual RSM and the neural RSM computed from macaque IT responses to the model-generated images (No such correlation was observed for the pixel-scrambled images). Additionally, an SVM trained on neural data from the model-generated images generalized well to the CG-rendered images, with classification outcomes aligning with human translucency ratings. Neural responses also partially depended on object shape. **Discussion and Conclusion** Our results demonstrate that neural population activity in the macaque IT cortex encodes the translucent appearance of objects in a partially shape-dependent manner that is consistent with human perception.

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Nanosymposium

NANO046: Insights From Large Scale Recording of Primate Visual Cortex

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Topic: E.06. Vision

Support: NWO VENI VI.Veni.222.217
NWO OCENW.XS22.2.097

Title: Deep generative networks uncover the tuning of neurons in the primate inferotemporal cortex and predict their influence on visual perception

Authors: *P. PAPALE[1], P. R. ROELFSEMA[2];
[1]Netherlands Inst. of Neurosci., Amsterdam, Netherlands; [2]Vision & Cognition, Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Despite appearing effortless, making sense of a daily visual scene is a complex and computationally intensive process for our brain. Thus, finding the tuning of visual neurons has kept neuroscientists busy for decades. The traditional approach to this problem has been to spend a lot of time on the blackboard, thinking of a possible property of interest, then build a set of "artificial" stimuli that vary along that property (e.g. orientation or color) and then record neural responses to those stimuli. Here, we introduce a data-driven approach that leverages deep generative networks to uncover the tuning properties of visual neurons in the inferotemporal cortex (IT) of macaque monkeys.
Utilizing a Generative Adversarial Network (GAN), we established a linear mapping between the latent space of the GAN and the recorded neuronal activity from chronically implanted electrodes in IT. This mapping enabled the reconstruction of visual stimuli from neural responses, providing insights into the features that drive neuronal activation. By perturbing the latent vectors along specific directions corresponding to individual cortical sites, we generated (offline) sequences of naturalistic images that systematically modulated the activity of targeted neurons.
These generated image sequences revealed interpretable tuning dimensions of IT neurons. Inspired by these sequences we produced fully controlled stimuli varying along previously unknown tuning properties in IT, and then recorded their evoked responses in the same IT neurons. We observed that the generated image sequences predicted the response to the fully controlled stimuli.
To further validate the functional relevance of these findings, we conducted electrical microstimulation experiments. Stimulating neurons with known preferred images induced predictable warps in the monkeys' perceptual judgments, confirming that the identified tuning properties have a causal influence on visual perception.
In sum, our approach enables the discovery of previously unknown tuning properties of high-level visual neurons that are easily interpretable and can be used to predict the effects of microstimulation on perception. By allowing the brain to tell us what it cares about, we are no longer limited by our experimental imagination.

Disclosures: P. Papale: None. **P.R. Roelfsema:** None.

Nanosymposium

NANO046: Insights From Large Scale Recording of Primate Visual Cortex

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UC San Diego Social Sciences Research Grant

Title: Interpreting Visual Percepts from Macaque Multi-unit Spike Data

Authors: *T. FEI[1], S. RAVISHANKAR[1], V. R. DE SA[2];
[2]Cognitive Sci., [1]UCSD, La Jolla, CA

Abstract: Visual neural decoding from macaque visual iBCIs has seen substantial progress in recent studies. While prior work has primarily emphasized optimizing decoding architectures for high-fidelity image reconstruction, comparatively little attention has been given to understanding the source and structure of the decoded information.We introduce a unified framework that enables image reconstruction from spike data using a simple linear decoder, while also isolating interpretable spike feature maps relevant to visual perception. In contrast to previous methods that depend on deep, opaque neural networks, our approach maintains linearity by leveraging the inherent structure of CLIP embeddings.Specifically, we train a linear decoder to project spiking activity into CLIP's latent space, followed by image synthesis using a fixed, pretrained diffusion model. Beyond reconstruction, we apply an existing analysis framework to extract latent-filtered spatiotemporal patterns, revealing feature maps aligned with distinct visual attributes—such as semantic category. This allows us to systematically probe spiking signals across different levels of the visual information hierarchy.

Disclosures: T. Fei: None. **S. Ravishankar:** None. **V.R. de Sa:** None.

Nanosymposium

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Presentation Number: NANO046.07

Topic: E.06. Vision

Support: EY014924
NS116623

Title: Optimal processing of high-density electrophysiological data and deployment of real-time spike sorting

Authors: *S. MURALIDHARAN[1], C. LENG[1], T. MOORE[2];
[1]Stanford Univ., Stanford, CA; [2]Neurobio., Stanford University/HHMI, Stanford, CA

Abstract: The advent of high density electrophysiological tools such as the Neuropixels 1 probe has enabled improved spike sorting and monitoring of large populations of neurons. This improved spike sorting enhances the ability to increase the efficacy of brain machine interfaces and experimental neuroscience. We compare the decoding ability of stimulus and task information from recordings in primary visual cortex (V1), prefrontal cortex (PFC), and primary motor cortex (M1), for different types of visual, motor and memory signals. We find substantial benefits for decoding with populations of spike sorted neurons when compared to current threshold crossing based spike extraction methods used in brain machine interface applications. We also find that many fewer spike-sorted features are required to exceed the maximum performance achieved with threshold crossing. To realize the benefits of sorting in a real-time scenario, we present a real-time spike sorting implementation based on Kilosort 4 2 . We first estimate template waveforms and locations using a training recording period. We perform the optimal matching pursuit portion of the spike retrieval algorithm on batches of data. We propose a modification to Kilosort's clustering algorithm that utilizes the cluster positions found in our initial training step. Our algorithm is able to sort spikes over the entire Neuropixels probe with an average latency of 15 milliseconds. Our implementation produces normalized spiking activity with a high level of similarity to Kilosort's results. We characterize the performance of the real-time spike sorter under various drift conditions, assessing key metrics such as ISI violations and stability of cluster position over the duration of the recording. Decoding from real-time spike sorting output achieves virtually all of the benefits of offline spike sorting when compared to threshold crossing, suggesting benefits for closed loop brain machine interface applications. 1. Steinmetz NA, et al. Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings. Science. 2021 Apr 16;372(6539):eabf4588. doi: 10.1126/science.abf4588. 2. Pachitariu, M. et al. Spike sorting with Kilosort4. Nat Methods 21, 914-921 (2024). https://doi.org/10.1038/s41592-024-02232-7

Disclosures: S. Muralidharan: None. **C. Leng:** None. **T. Moore:** None.

Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO047.01

Topic: H.08. Drugs of Abuse and Addiction

Support: 1UM1DA052244-01

Title: A comparative multi-omic and spatial atlas for the effects of opioids and stimulant drugs in the ventral striatum of humans, macaques, rats, and mice

Authors: *B. HERB[1], L. M. WILLIS[2], J. RECEVEUR[4], O. WHITE[4], P. J. KENNY[3], S. A. AMENT[5];
[1]Pharmacol. and Physiol., Univ. of Maryland Baltimore, Baltimore, MD; [3]Neurosci., [2]Icahn Sch. of Med. At Mount Sinai, Tarrytown, NY; [5]Dept. of Psychiatry and Inst. for Genome Sci., [4]Univ. of Maryland Baltimore, Baltimore, MD, United States, Baltimore, MD

Abstract: The nucleus accumbens (NAc) mediates rewarding properties of addictive substances and undergoes lasting changes in substance use disorders (SUDs). The NAc is structured into discrete functional domains with medium spiny neurons (MSNs) projecting topographically to downstream brain regions. However, the molecular diversity, spatial organization, and functional adaptations of MSNs in SUDs remain elusive. To address this, we constructed a comparative multi-omic and spatial atlas of the NAc in humans, macaques, rats, and mice, integrating single-cell multi-omic and spatial transcriptomic data from over one million NAc cells. Our analyses revealed deep evolutionary conservation of many MSN subtypes, as well as human- and primate-specific specializations. Projection mapping revealed previously unrecognized, topographically arranged MSN projections to brain regions regulating reward and aversion. In humans, the dynamics of MSN subtype-specific gene networks reflected complex patterns of polysubstance use that are inherent to SUDs. Comparisons to animal models enabled us to link SUD-associated gene networks to specific substances and stages of addiction. Selective manipulation of these gene networks in mice altered opioid-related behaviors, offering potential therapeutic targets. This work provides a blueprint for identifying key molecular and circuit mechanisms underlying addiction and developing targeted interventions.

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Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO047.02

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA

Title: H2a.z dysregulation during cocaine withdrawal modulates drug relapse behavior.

Authors: *E. P. CHEN[1], V. KONDEV[1], Y. YIM[2], B. T. KIPP[1], E. KAHN[3], E. J. NESTLER[4];
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Abstract: Human and animal studies on drug addiction have demonstrated distinct behavioral and neuronal gene expression changes that occur during periods of prolonged abstinence, suggesting a role for stable epigenetic mechanisms that mediate long-lasting gene priming and

desensitization that contribute to drug relapse. Recently, we demonstrated that H2A histone family member Z (H2A.Z), a variant of histone 2A, is dysregulated after prolonged cocaine withdrawal in the mouse nucleus accumbens (NAc). However, H2A.Z's mechanism and role in addiction and relapse behavior has yet to be elucidated. Here, we aim to characterize H2A.Z dynamics in the NAc over the course of chronic drug exposure and withdrawal and assess how direct manipulation of H2A.Z in the NAc affects behavioral paradigms. We performed qPCR and Western blotting to determine relative H2A.Z mRNA and protein abundance 24 hours and 4 weeks after chronic cocaine exposure. To directly assess the causal role of H2A.Z in cocaine conditioning behavior, we performed viral knockdown (KD) of H2A.Z in the mouse NAc and analyzed the animals in a cocaine conditioned place preference (CPP) paradigm. We demonstrate that there is downregulation of H2A.Z mRNA after both 24 hours and 4 weeks of withdrawal. Interestingly, there is a significant increase in H2A.Z protein abundance at 24 hours withdrawal but not at 4 weeks withdrawal, suggesting the presence of posttranslational mechanisms dysregulating H2A.Z protein abundance. Direct cell-type-specific viral manipulation of H2A.Z in the NAc bidirectionally influences cocaine conditioning behavior in D1 and D2 medium spiny neurons, demonstrating H2A.Z's potential to modulate the rewarding effects of cocaine. Taken together, we demonstrate that dysregulation of H2A.Z during prolonged cocaine withdrawal influences relapse behavior. Future studies focus on investigating the underlying molecular mechanism of drug regulation of H2A.Z and its downstream effects on behavior.
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Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

Location: SDCC Rm 33

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Presentation Number: NANO047.03

Topic: H.08. Drugs of Abuse and Addiction

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NIH Grant F32DA055438
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Title: The mechanistic role of NR4A2 function in nucleus accumbens cholinergic interneuron regulation of synaptic plasticity and cocaine-associated behaviors

Authors: *T. L. FETTERLY[1,2,3], J. E. CHILDS[1,2,3], G. SANDOVAL[4,3], E. A. KRAMAR[1,2,3], J. ROUNDS[1,2,3], D. P. MATHEOS[1,2,3], J. DÍAZ-ALONSO[4,3], M. A. WOOD[1,2,3,5];
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Learning and Memory, [4]Anat. & Neurobio., [5]Inst. for Memory Impairments and Neurolog. Disorders (MIND), Univ. of California, Irvine, Irvine, CA

Abstract: Many difficulties in treating substance use disorder (SUD) arise from the chronic nature of the condition, which is characterized by a lifelong risk of relapse. This persistent vulnerability to relapse is in part driven by the formation of abnormally strong drug/reward-associated memories. Epigenetic regulation plays a major role in supporting persistent changes in cell function, that give rise to altered circuit plasticity, and ultimately lead to long-lasting changes in behavior. HDAC3 has been identified as a negative regulator of gene expression that is important for long-term memory formation. Downstream, we have identified *Nr4a2*, a transcription factor, as a key target gene of HDAC3. The Wood Lab has demonstrated an important role of NR4A2 in cholinergic neurons within the medial habenula in regulating relapse to cocaine-seeking. However, cocaine exposure has also been shown to alter *Nr4a2* expression within the nucleus accumbens (NAc), a key reward-related brain region. Cholinergic interneurons (ChIns) within the NAc express *Nr4a2* and have been shown to regulate medium spiny neuron (MSN) activity and thus overall NAc output. We hypothesized that altering NR4A2 function specifically in NAc ChIns would alter NAc synaptic plasticity and ultimately cocaine-related behaviors. To test this, we expressed a transcriptionally inactive dominant negative form of NR4A2 (NURR2C) exclusively in NAc ChIns using ChAT-Cre mice. We observe that reducing NR4A2 function with NAc ChINs alters synaptic plasticity. Using local field potential recordings, there is an increase in NAc long-term potentiation in NURR2C expressing mice. Additionally, we observe altered glutamate plasticity in NAc MSNs using whole-cell patch-clamp electrophysiology to measure spontaneous activity, paired-pulse ratios, and AMPA/NMDA ratios. At the behavioral level, mice expressing NURR2C within NAc ChIns have enhanced acquisition of both conditioned place preference and operant cocaine self-administration. Overall, this suggests NR4A2 within NAc ChIns plays an important role in regulating NAc activity, plasticity, and cocaine-associated behaviors. Future work will investigate the mechanisms linking cholinergic interneuron NR4A2 function with changes in MSN plasticity and how these circuits may be altered following exposure to cocaine.

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Topic: H.08. Drugs of Abuse and Addiction

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Title: Estradiol Depletion Modulates Social Motivation During Cocaine Abstinence in Female Rats

Authors: *V. M. KIMBLE[1,2], E. J. NUNES[1], N. A. ADDY[1,2,3,4];
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Abstract: Cocaine abstinence is associated with social withdrawal and reduced social motivation in both clinical and preclinical models. In rodents, these effects are particularly pronounced in females, where estradiol plays a critical role in regulating social behavior. In this study, we investigated how estradiol depletion via ovariectomy (OVX) influences social motivation during cocaine withdrawal in female rats. Adult female Sprague Dawley rats underwent OVX or sham surgery alongside i.v. catheterization surgery. Following a 5-7 day surgical recovery period, female rats underwent 10-day cocaine self-administration training where they learned to self-administer intravenous cocaine (~ 0.7 mg/kg/infusion) or saline. Social preference was assessed after 17 days of abstinence using a three-chamber social interaction test, measuring time spent investigating a novel rat versus a novel object. As hypothesized, Sham + cocaine rats showed blunted social preference relative to saline controls, consistent with a cocaine-induced social deficit. Surprisingly, OVX + cocaine rats exhibited significantly greater preference for the novel rat compared to sham + cocaine controls, suggesting a potential protective effect of estradiol depletion on social behavior during withdrawal. Uterine weights confirmed successful OVX and estradiol depletion. This work highlights the complex and context-dependent role of estradiol in modulating social behavior during abstinence and the findings highlight the importance of understanding female-specific mechanisms contributing to relapse vulnerability.

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Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

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Presentation Number: NANO047.05

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant R00DA039991
Osteopathic Heritage Foundation Grant OHFE-E-2023-31

Title: Psychosocial stress-induced cocaine seeking in rats is inversely correlated with activation of medial prefrontal cortical neurons projecting to the rostral periaqueductal gray

Authors: N. M. HINDS, I. WOJTAS, C. A. GALLAGHER, M. E. CONCANNON, ***D. F. MANVICH**;
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Abstract: Cocaine Use Disorder is characterized by a high risk of relapse even after prolonged abstinence. Although psychosocial stress is a well-established trigger for craving and relapse in humans, it remains understudied in preclinical relapse models. Consequently, the neurobiological mechanisms and neural circuits by which psychosocial stressors promote cocaine-seeking behavior are not well understood. Previous work from our lab identified the periaqueductal gray (PAG) as a brain region that may contribute to social stress-induced reinstatement of cocaine seeking. To further investigate the PAG's role, adult male Long-Evans rats received injections of a retrograde viral tracer into the lateral and ventrolateral aspects of the rostral PAG (rPAGl/vl) and were trained to self-administer cocaine (0.5 mg/kg/infusion, IV) over 20 daily 2-hour sessions. On sessions 11, 14, 17, and 20, a discrete tactile cue was introduced in the operant chamber and was conditioned to signal either impending social defeat stress or footshock stress in separate groups. Following extinction training, rats were re-exposed to their respective stress-predictive cue and tested for cocaine-seeking behavior under extinction conditions. Re-exposure to either a social stress- or footshock-predictive cue elicited reinstatement of cocaine-seeking behavior. Social stress-induced cocaine-seeking behavior was selectively associated with activation of the rPAGl/vl as assessed by Fos expression analysis. Furthermore, we found significant negative correlations between psychosocial stress-induced cocaine seeking magnitude and the number of Fos-positive rPAGl/vl-projecting neurons within the prelimbic and anterior cingulate cortices. These findings suggest that the contributions of the rPAGl/vl to psychosocial stress-induced cocaine seeking may be modulated by top-down frontal cortical input.

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Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO047.06

Topic: H.08. Drugs of Abuse and Addiction

Title: Evidence for oxytocin control of social buffering of cocaine aversion in rats

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Abstract: A growing body of research shows that social interactions influence motivation for drugs of abuse. Despite this, investigations into how social interactions shape neurobiological

responses to drugs that may drive motivation are few. Our laboratory uses a cocaine place conditioning model to assess cocaine induced reward and aversion. Human and animal studies demonstrate that cocaine (COC) administration produces initial reward which then gives way to dysphoria. Indeed, rats develop a conditioned place preference (CPP) to the immediate effects of COC (0-5 min after administration) and a conditioned place aversion (CPA) to the delayed effects of COC (15 min after). It is likely that the rewarding and aversive effects of COC contribute to drug taking via positive and negative reinforcement mechanisms, respectively. To assess how social interaction affects COC conditioned reward and aversion we used a procedure in which rats learn to associate a unique environment with either the immediate or delayed effects of one of three doses of COC (0.1mg/kg, 0.25mg/kg, and 1.0mg/kg, IV). Further, during each conditioning session, rats were either alone in the environment (as is typical in these procedures) or they were paired with a same-sex cage mate that never received drug. All animals underwent a post-conditioning test. We replicated previous studies showing that 1.0mg/kg COC produces robust CPP and CPA in male rats, and extended current knowledge to show that while female rats developed COC CPP similar to male rats, there was greater heterogeneity in CPA expression in female rats. Further, while 1.0mg/kg COC produced a CPA in males conditioned alone, rats conditioned with a conspecific failed to develop a CPA, evidencing social buffering of cocaine aversion. Interestingly, conspecifics did not develop a CPP or CPA, suggesting that cocaine reward and aversion are not socially transferred. Because the neurochemical oxytocin is shown to be involved in social behavior, we sought to determine if oxytocin was required for social buffering of cocaine averion. Activation of an inhibitory DREADD targeted at oxytocin receptor-possessing neurons of the paraventricular nucleus of the thalamus (PVN) abolished the ability of a conspecific to attenuate CPA for the delayed effects of COC. These data show that oxytocin signaling is required for the development of social buffering of COC-induced aversion.

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Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

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Title: Sex differences in cocaine-induced plasticity of D1R- and D2R-MSNs in the mouse nucleus accumbens core

Authors: *H. M. MCMULLAN[1,2,3], A. D. CHAPP[4,3], C.-M. PHAN[4], P. JAGTAP[4], P. G. MERMELSTEIN[4,3,5];
[2]Clin. and Translational Sci. Inst., [3]Med. Discovery Team on Addiction, [4]Neurosci., [5]Ctr. for Neural Circuits in Addiction, [1]Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Previous work has shown that cocaine-induced changes in nucleus accumbens shell medium spiny neurons (MSNs) differ between D1- and D2-expressing MSNs, as well as the sex of the animal, and for females, the phase of the estrous cycle. Whether MSNs of the nucleus accumbens core (NAcC), which regulate different aspects of addiction, will exhibit similar sex and estrous cycle effects following cocaine administration was investigated. Mice underwent a 5-day locomotor sensitization paradigm via daily cocaine administration (15 mg/kg, s.c.) followed by a 1- to 4-day drug-free abstinence period. We examined NAcC MSN excitability by obtaining *ex vivo* whole-cell recordings from a transgenic mouse line with differentially labeled D1-receptor (Drd1a-tdTomato) and D2-receptor (Drd2-eGFP) expressing MSNs. In this genetic background of mice, both male and female mice sensitized to cocaine in a similar manner. In males, there were no cocaine-induced changes in D1R- or D2R-MSN excitability. In saline-treated females, D1R-MSN excitability fluctuated across the estrous cycle, with increased excitability during estrus. Following cocaine, estrous cycle-dependent D1R-MSN excitability was locked into an intermediate excitable state between estrus and diestrus when compared to saline controls. D2R-MSNs did not change either across the estrous cycle or following cocaine. When comparing across MSN subtypes, in diestrus, D2R-MSNs were more excitable under saline conditions, but indistinguishable from D1R-MSNs following cocaine. In contrast, during estrus, D1R- and D2R-MSN excitability ran similar in saline treated animals, but with cocaine, D2R-MSNs displayed heightened excitability. These relative changes in D1R- versus D2R-MSN excitability following cocaine are opposite when compared to what occurs in the nucleus accumbens shell. These data indicate fundamental sex differences in the neuropharmacological effect of cocaine in males versus females that are shell and core specific.

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Nanosymposium

NANO047: Cocaine Addiction: From Cells to Experiences That Shape Drug-Seeking Behavior

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Topic: H.08. Drugs of Abuse and Addiction

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F31DA055445

Title: Region-specific regulation of cocaine seeking by astrocyte calcium

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Abstract: Accumulating evidence indicates that astrocytes actively modulate cocaine-seeking behavior, yet the specific roles of astrocyte Ca^{2+} signaling within dorsal striatum subregions remain unclear. To address this, we suppressed astrocytic Ca^{2+} signaling via targeted overexpression of the human plasma membrane Ca^{2+} ATPase pump (hPMCA) in discrete dorsal striatum subregions in rats that were trained to self-administer cocaine or saline. Suppression of astrocyte Ca^{2+} in the central striatum significantly increased cocaine self-administration and enhanced cue-induced reinstatement of cocaine seeking when compared to control animals. Afterwards, brain slices were collected from each animal and e*x vivo* neuronal Ca^{2+} imaging using GCaMP6f demonstrated increased amplitude and reduced duration of neuronal Ca^{2+} transients in rats trained to self-administer cocaine. Subsequently, we further determined subregion-specific effects on cocaine-related behaviors by either suppression of astrocyte Ca^{2+} via hPMCA2 or stimulation of astrocyte Ca^{2+} via GqDREADDs. Using fiber photometry, we monitored neuronal Ca^{2+} dynamics *in vivo* during active lever presses. Preliminary results indicate that increased reinstatement of cocaine-seeking can be specifically attributed to targeted suppression of astrocytic Ca^{2+} signaling within the dorsolateral striatum. Interestingly, suppression of Ca^{2+} in dorsomedial striatum (DMS) astrocytes significantly reduced cocaine self-administration, while during extinction sessions, lever pressing was paradoxically increased. DMS astrocyte Ca^{2+} suppression had no effect on cue-induced reinstatement behavior although neuronal Ca^{2+} signaling during active lever presses continued to be elevated relative to GqDREADD- animals and animals expressing the control, tdTomato, virus. These data collectively highlight distinct and regionally specific roles for astrocyte Ca^{2+} within the dorsal striatum, demonstrating critical astrocytic influence over neuronal circuits that shape cocaine-seeking behavior.

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NANO048: Human Intracranial Recordings: Cognitive and Clinical Science

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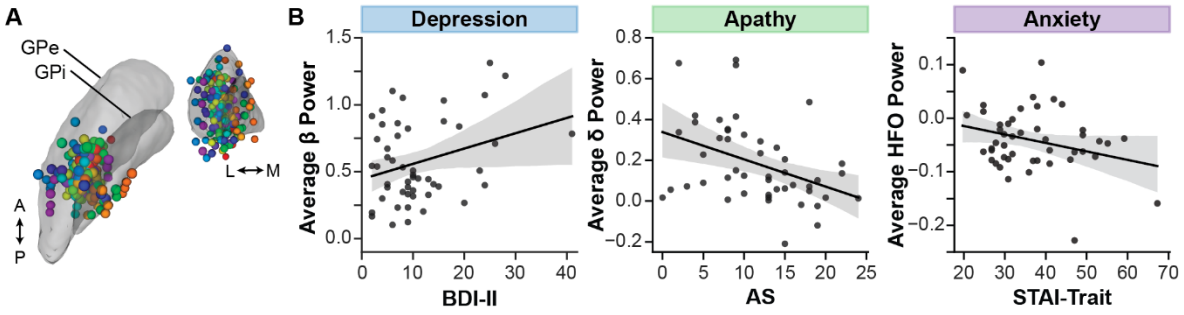
Topic: H.05. Mood Disorders

Support: NIH K99NS137249

Title: Basal ganglia neurophysiological activity differentiates depression, apathy, and anxiety in Parkinson's disease

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Abstract: Depression, apathy, and anxiety are common debilitating nonmotor symptoms of Parkinson's disease (PD), but the underlying pathophysiology is unclear. Deep brain stimulation (DBS) has been leveraged to record neural activity from the basal ganglia associated with depression in PD. However, the results have been inconsistent and focused exclusively on the subthalamic nucleus (STN), leaving a gap in knowledge about the role of the pallidum. The objective of this study was to investigate neurophysiological markers of depression, apathy, and anxiety in PD in a large retrospective cohort undergoing pallidal DBS for PD.
In N=50 patients with PD (N=33 male), we acquired 30 seconds of intraoperative local field potential recordings at 22 kHz sampling from all monopolar contacts on the DBS lead while the patient was off-medication and at rest. The power spectral density was computed and normalized across patients. Preoperative off-medication Beck Depression Inventory (BDI-II), Apathy Scale (AS), and State-Trait Anxiety Scale (STAI-Trait) scores were acquired. Generalized linear models were used to identify spectral frequency bands associated with each symptom while controlling for potential confounds.
A substantial proportion of patients exhibited clinically elevated preoperative depression (26.0%), apathy (37.5%), and anxiety (34.0%). Depression was associated with elevated beta (13-30 Hz) power (p=0.038), apathy with reduced delta (1-4 Hz) power (p=0.027), and anxiety with reduced high-frequency oscillations (HFO) (200-400 Hz) (p=0.034), even when controlling for demographics, disease duration, motor symptoms, levodopa dosage, other psychiatric symptoms, psychiatric medications, and neurophysiological variables.
Oscillatory neural activity in the pallidum in distinct frequency bands may contribute to the pathophysiology of depression, apathy, and anxiety in PD. These signals could serve as objective biomarkers to guide DBS therapy, including adaptive algorithms. Future work will compare these results with the STN and determine the anatomical localization.



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Topic: H.05. Mood Disorders

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Title: Neurocomputational basis of goal-directed decision making: Dopamine, deep brain stimulation, and intracranial recordings from human fronto-basal ganglia circuits

Authors: *C. W. HOY[1], P. A. STARR[2], W. KOOL[3], S. LITTLE[4];
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Abstract: Adaptive behavior requires overriding impulses and habits with goal-directed decision making, and deficits in this process predict the severity of neuropsychiatric symptoms, including apathy and impulsivity issues common in Parkinson's disease (PD). Goal-directed control relies on top-down frontal cortex signals, and the basal ganglia (BG) integrates goal-directed and habitual systems under dopaminergic influence. Theta and beta oscillations help coordinate these functions, and dopaminergic medications and BG deep brain stimulation (DBS) treatments for PD motor symptoms are known to modulate theta, beta, and goal-directed decision making. Therefore, identifying fronto-basal ganglia signals underlying these decision-making strategies could yield biomarkers to tailor precision therapies for neuropsychiatric symptoms. Here, we use novel sensing-enabled DBS devices and chronic electrocorticography to record intracranially from human premotor cortex (PMC), frontopolar cortex (FPC), and BG while 14 people with PD perform a modified two-step reward learning task repeatedly at home (3-18 runs each), both ON/OFF dopamine and BG DBS. Behaviorally, reinforcement learning models showed that dopamine and DBS worsened learning (DA: $p=0.001$; DBS: $p=0.001$), revealing cognitive side effects of motor therapies. Dopamine also increased habitual decision making ($p=0.022$), which was mediated by impaired cognitive flexibility (higher state switching costs, $p=0.042$). Neural recordings showed that single-trial low-frequency power in PMC, FPC, and BG tracked key learning and decision-making variables, including choice values and reward prediction errors (RPEs). PMC beta power was reduced after difficult choices between similarly valued options, when the habitual system was surprised at the second task stage, and after surprising outcomes at feedback (all $p<0.05$, cluster-corrected), collectively supporting a role for beta in internal model

updating. Theta power increased for negative RPEs in PMC and BG but for unsigned RPEs (i.e., non-valence surprise) in FPC, potentially reflecting cognitive control adaptations. In contrast, BG delta power increased for positive RPEs (all $p<0.05$, cluster-corrected), much like the EEG reward positivity linked to mood disorders. These findings demonstrate how dopaminergic and DBS treatments for motor symptoms can cause learning and decision-making deficits linked to apathy and impulsivity and reveal the neurocomputational basis of these processes in human reward circuits, thereby informing future therapies for neuropsychiatric conditions.

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Title: Multi-temporal intracranial biomarkers tracking symptom domains in depression

Authors: *A. ALLAWALA[1], A. G. TREMBLAY-MCGAW[4], N. STAPPER[2], L. P. SUGRUE[4], K. K. SELLERS[5], A. KHAMBHATI[3], K. SCANGOS[6], A. KRYSTAL[3], E. F. CHANG[7]; [1]Univ. of California San Francisco, San Mateo, CA; [2]Univ. of California San Francisco, San Diego, CA; [3]Univ. of California San Francisco, San Francisco, CA; [5]Neurolog. Surgery, [4]UCSF, San Francisco, CA; [6]Univ. of Pennsylvania, Philadelphia, PA; [7]Univ. of California, San Francisco, Piedmont, CA

Abstract: Personalized deep brain stimulation (DBS) has gained traction to address heterogeneity in symptoms and circuit dysfunction in treatment-resistant depression (TRD). Our study aims to identify neurophysiological biomarkers of symptom state across temporal scales, and evaluate biomarker stability across time during closed-loop DBS. We analyzed data from five subjects with TRD as part of a clinical trial (NCT04004169). Subjects were implanted bilaterally with stereo-EEG electrodes across limbic regions for 10 days, during which time we collected intracranial recordings and symptom ratings. Using L1 LASSO regression and permutation testing (N=1000), we identified biomarkers (power spectra) of symptom state in the ambulatory state. The ambulatory state was subsequently followed by implantation of a chronic DBS system for therapy and we obtained daily at-home intracranial recordings from the DBS leads alongside symptom ratings. We then implemented a Hidden Markov Model and permutation testing (N=1000), to identify neural state transitions representative of symptom states across time. In the ambulatory state, neurophysiological circuits of symptom severity across regions were identified in three of five individuals. Subject A, amygdala-hippocampus

(RMSE=0.5); subject B, hippocampus (RMSE=0.1, non-predictive); subject C, amygdala-subcallosal cingulate (RMSE=0.75); subject D, hippcampus-bed nucleus strias terminalis (RMSE=0.6); subject E, hippocampus-subcallosal cingulate (RMSE=0.1, non-predictive).Following the chronic implant, neural state transitions representative of symptom severity were found in 3/5 patients (Subject A, C, D), in at least one recording site. Across subjects, these transitions were representative of changes in depression severity. Permutation testing revealed significant alignment between neural states identified via HMM and independently assessed behavioral symptom clusters ($p < 0.01$). State-specific spectral features were identified in each participant, indicating biomarkers of specific symptom types and severity. Ongoing work will investigate whether specific neural trajectories, or changes in brain state may serve as predictive biomarkers of symptom change, enabling early detection of clinical relapse, or identifying a stable therapeutic response state. Our insights on multi-scale biomarkers across limbic circuits underscore the importance of personalized biomarker assessment strategies to predict symptom state, and potentially informs future algorithmic strategies for closed-loop or adaptive neuromodulation.

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Topic: H.05. Mood Disorders

Support: Foundation for OCD Research
AE Foundation
NIMH R25MH119043

Title: Acute stimulation of the nucleus accumbens and ventral pallidum in obsessive-compulsive disorder produces quicker approach-avoidance decisions

Authors: *P. M. LAURO[1], N. VARDALAKIS[2], R. L. SEILHEIMER[1], L. QIU[2], Y. NHO[2], A. CHANG[2], K. SCANGOS[1], C. H. HALPERN[2];
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Abstract: Background Obsessive-compulsive disorder (OCD) is characterized by distressing obsessive thoughts and compulsive behaviors, in addition to heightened sensitivity to the risk of aversive outcomes (harm avoidance) and deficits in goal-directed action control (incompleteness). While deep brain stimulation (DBS) of the nucleus accumbens and ventral pallidum (NAc-VeP) can improve OCD symptoms, its effects on approach-avoidance decision making underlying these improvements are unknown.
Methods Three patients (2 female, 1 male) with OCD undergoing stereo-EEG as part of a DBS for OCD trial (NCT05623306) performed a probabilistic approach-avoidance task with and without acute NAc-VeP stimulation. In each trial, patients chose between two distributions containing both rewarding and aversive outcome probabilities. To better characterize their decisions, each patient's behavior was fit with a drift-diffusion model, producing distributions of behavioral parameters such as drift rate (*d*), boundary separation (*a*), and starting point (*z*). Resulting behavioral model parameters and reaction times (times to decision) were compared across stimulation conditions using linear mixed models.
Results With acute NAc-VeP stimulation, all patients demonstrated decreased reaction times (OFF v. stim reaction times, fixed effects of stimulation condition, random effects of subject, beta=0.474, Z=7.639, p=10^{-14}). When comparing behavioral parameters, all patients demonstrated smaller boundary separation with NAc-VeP stimulation (OFF v. stim decision boundaries - *a*, fixed effects of stimulation condition, random effects of subject, beta=0.235, Z=2.135, p=0.033). Two patients demonstrated YBOCS-II improvement with sustained NAc-VeP DBS (Baseline v. DBS, P1: 40 v. 22, P2: 44 v. 21), with the third still undergoing DBS titration.
Conclusions Acute NAc-VeP stimulation produces quicker approach-avoidance decisions with smaller decision boundaries, suggesting that clinically effective DBS may result from changes in risk sensitivity and speed/accuracy decision tradeoffs (i.e. mitigating harm avoidance and incompleteness that can prolong real-life decisions in patients).

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Nanosymposium

NANO048: Human Intracranial Recordings: Cognitive and Clinical Science

Location: SDCC Rm 23A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO048.05

Topic: H.05. Mood Disorders

Support: NIMH R01MH124761

Title: Intracranial Neural Dynamics of Contextual Fear Learning During Ambulatory Virtual Reality in Humans

Authors: *A. I. JANG[1], J. GILL[8], M. VALLEJO[9], R. MUSTAPHA[2], J. BAHAM[1], H. N. ZUBAIR[3], S. HILLER[4], J. A. SCHNEIDERS[2], D. BATISTA[2], M. JENKENS-DRAKE[9], C.

ORAGWAM[9], B. BARTHOLOMEW[11], U. TOPALOVIC[9], M. SEEBER[5], M. STANGL[12], C. S. INMAN[13], M. S. FANSELOW[6], M. CRASKE[2], A. ADHIKARI[7], R. KOEK[2], J.-P. LANGEVIN[14], N. A. SUTHANA[10];
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Abstract: Exaggerated fear responses, as seen in anxiety disorders and PTSD, often occur in contexts where threat is no longer present. Rodent studies suggest that contextual fear learning depends on interactions between the amygdala and hippocampus, but it remains unclear how these mechanisms operate in humans in naturalistic settings. To address this, we designed immersive ambulatory virtual reality (VR) environments including a grocery store, library, and museum to test fear conditioning in settings that closely mimic real-world contexts, marking a naturalistic approach unprecedented in human intracranial EEG (iEEG) studies. Fourteen participants with treatment-resistant epilepsy completed a two-day ambulatory VR fear conditioning task while undergoing iEEG recording in the hippocampal-amygdala circuit via the Responsive Neurostimulation (RNS) device. The task consisted of three phases across distinct virtual environments: (1) fear acquisition, where a conditioned stimulus (CS+) such as a green light predicted an aversive unconditioned stimulus (US) such as the appearance of a spider, while another stimulus (CS-) such as a blue light did not; (2) fear extinction, where both conditioned stimuli were presented without the US; and (3) fear renewal, an additional extinction session conducted the following day in a different context. These phases model the development, recovery, and relapse of fear responses seen in anxiety disorders. We observed increased iEEG theta power in the amygdala ($p < 0.05$) and elevated skin conductance responses ($p < 0.001$) to the CS+ compared to CS-, consistent with successful fear conditioning. Notably, we observed a decrease in amygdala theta power after the US ($p < 0.001$), suggesting a role in fear anticipation. Neural and physiological responses diminished following extinction, but skin conductance re-emerged during fear renewal ($p < 0.05$). To assess whether fear altered environmental context encoding, we analyzed iEEG power by spatial location and time, comparing pre- and post-fear conditioning activity using representational similarity analysis (RSA). The amygdala and hippocampus encoded representations of spatiotemporal context, with this encoding selectively disrupted in the fear acquisition environment (e.g., library) relative to the neutral environments (e.g., museum, store), as measured by a decrease in RSA. These findings reveal how the human amygdala-hippocampal circuit supports fear learning and dynamic context representation during naturalistic ambulatory virtual reality experiences, providing insights into maladaptive fear relevant to anxiety disorders and PTSD.

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Nanosymposium

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Location: SDCC Rm 23A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO048.06

Topic: H.05. Mood Disorders

Title: Computational and behavioral predictors of transcranial magnetic stimulation treatment response in individuals with major depressive disorder

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Abstract: Major depressive disorder (MDD) is a pernicious disorder that has been notoriously difficult to effectively treat. Over one-third of MDD patients fail to improve after multiple antidepressant treatments and are classified as having treatment-resistant depression (TRD). Transcranial magnetic stimulation (TMS) of the dorsolateral prefrontal cortex has emerged as a viable solution for TRD though such protocols are effective in one out of two patients. Several TMS protocols already exist (deep, high-frequency, low frequency) and while it is understood that such protocols therapeutically modulate relevant brain networks, it is unclear what the optimal protocol is for individual patients. In this observational clinical study, we leverage existing behavioral paradigms to track patients undergoing a 7 week TMS treatment plan. By deploying an array of computational models to previously validated behavioral tasks, we quantify relevant and distinct decision making constructs (effortful discounting, delay discounting, reward learning) that are putative representations of psychological function. Here, we highlight which computational parameters 1) best serve as a proxy for patients' clinical status 2) are associated at baseline with a successful TMS treatment outcome. As such, this work presents a roadmap for clinicians to prescribe the most effective TMS treatment protocol and expounds the neural infrastructure that is therapeutically modulated by current TMS treatments.

Disclosures: G.J. Pagnier: None. **M. Razafsha:** None. **S. Petelis:** None. **J. Brown:** None. **P. Kumar:** None.

Nanosymposium

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Topic: H.05. Mood Disorders

Support: NIH Grant 1R21MH127009-01A
DARPA Cooperate Agreement Number W911NF-14-2-0045

Title: Causal evidence for distributed fronto-temporo-limbic oscillations in cognitive conflict encoding using intracranial electrical stimulation

Authors: *A. SHEKARA[1], A. ROSS[1], A. C. PAULK[3], A. S. WIDGE[4], S. S. CASH[5], P. K. SHEAR[6], I. BASU[2];
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Abstract: Cognitive control, the ability to flexibly adapt behavior to thoughts and behavior, is crucial for achieving longitudinal goals and frequently compromised in neuropsychiatric disorders. Tasks measuring the response inhibition aspect of cognitive control elicit theta (4-8Hz) and high-gamma (70-200Hz) oscillations in prefrontal cortex (PFC) in response to cognitive conflict. Stereotactic EEG (sEEG) studies of cognitive control show conflict-related neural responses are widely distributed beyond the PFC in the lateral temporal lobe (LTL), amygdala, and hippocampus. However, a role of these temporo-limbic structures in conflict encoding has not been established. Here, we used a previously recorded sEEG dataset during intracranial electrical stimulation to determine a potential role for fronto-temporo-limbic oscillations in encoding cognitive conflict.
We analyzed sEEGs from fronto-temporo-limbic regions of patients with intractable epilepsy (n=6) performing a Multi-Source Interference Task (MSIT) with and without 130Hz electrical stimulation of the ventral capsule/ventral striatum (VC/VS). Stimulation was delivered to either left or right VC/VS on 50% of trials in stimulated trial blocks. Spectral power was estimated in theta, alpha (8-15Hz), beta (15-30Hz), gamma (30-50Hz) and high-gamma bands during non-stimulated trial blocks, and unstimulated trials within stimulation blocks. Generalized linear mixed-effects models (GLME) were used to determine effects of conflict (low, high) and stimulation (off, on) on response times and spectral power during task performance.
Left VC/VS, but not right VC/VS stimulation reduced conflict-related response slowing compared to non-stimulated trial blocks. Stimulation of left VC/VS increased conflict-related theta power in left dorsal anterior cingulate cortex (dACC) and right ventrolateral PFC (vlPFC), high-gamma (70-110Hz) power in the left LTL, and theta-gamma (4-50 Hz) in left hippocampus. Single-trial response times were predicted by theta power in the right dorsolateral PFC, right vlPFC, left dACC, and left hippocampus, and theta-beta (4-30Hz) power in the left LTL.
Our findings provide initial evidence for a causal role of distributed fronto-temporo-limbic oscillations encoding conflict encoding during cognitive control. Further investigation and biophysical modeling of large-scale network dynamics of conflict encoding electrical stimulation can inform neuromodulation approaches to restore impaired cognitive function in neuropsychiatric patient populations.

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Nanosymposium

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Location: SDCC Rm 23A

Time: Wednesday, November 19, 2025, 8:00 AM - 10:00 AM

Presentation Number: NANO048.08

Topic: H.05. Mood Disorders

Title: Close-loop neuromodulation for decision-making under risk and uncertainty

Authors: *L. WANG;
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Abstract: Decision-making under risk and uncertainty is integral to daily life and closely tied to impulsivity. While subthalamic nucleus deep brain stimulation (STN-DBS) effectively treats Parkinson's disease and obsessive-compulsive disorder, it can also induce impulsivity and hypomania. Previous work shows that cognitive state-dependent, intermittent STN stimulation can reduce risk-taking and enhance evidence accumulation, but it remains unclear whether this modulation is inherently state-dependent and whether intracranial decoding can effectively guide closed-loop control. In the first experiment, we tested the effects of continuous STN stimulation (OFF, 5 Hz, 130 Hz) on risky decision-making in 20 Parkinson's patients performing a card gambling task. Despite a double-blind, counterbalanced design and sufficient washout periods, repeated-measures ANOVA revealed no significant effects of stimulation frequency on reaction time or betting behavior, suggesting the influence of stimulation may be state-dependent.
In a second experiment, we implemented closed-loop STN stimulation in 8 patients, triggered by elevated theta power. Stimulation thresholds were either fixed (2 SD above baseline) or adaptively adjusted. Compared to sham, theta-triggered stimulation significantly reduced betting in high-uncertainty trials ($p = 0.031$), especially when delivered 2-3 s after cue onset ($p = 0.043$). Stimulation enhanced theta activity in high-uncertainty trials and suppressed beta in low-uncertainty trials.
In summary, continuous theta-frequency STN stimulation had no behavioral impact, whereas closed-loop theta-contingent stimulation effectively modulated risky decision-making. These findings support personalized, physiology-guided DBS approaches for treating impulsivity-related disorders.

Disclosures: L. Wang: None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.01

Topic: A.03. Axon and Dendrite Development

Support: Stanford University

Title: In vivo cell-surface tethering of Netrin1 protein implicates diffusible Netrin1 in long-range axon guidance in developing spinal cord

Authors: *C. PAN[1], R. CAI[1], M. ONESTO[2], M. TESSIER-LAVIGNE[1];
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Abstract: The canonical axonal attractant Netrin1 steers many classes of axons to their targets during nervous system development. Two sources of Netrin1 have been proposed to guide commissural axons from dorsal spinal cord to ventral midline floor plate cells, with floor plate-derived Netrin1 acting at a distance of a few hundred micrometers (chemotaxis) and ventricular zone-derived Netrin1 acting locally (haptotaxis). The relative contributions of the two mechanisms have been a subject of ongoing debate. To illuminate this, we created a mouse model in which Netrin1 is cell-surface tethered, which blocks long-range action while preserving local guidance. In this model, we found that optic disc entry of retinal ganglion cell axons, known to be guided by local Netrin1, is largely normal. Projection of spinal commissural axons to the floor plate, by contrast, is severely disrupted. These results support long-range chemotaxis as a significant contributor to guidance of commissural axons in the spinal cord.

Disclosures: C. Pan: None. **R. Cai:** None. **M. Onesto:** None. **M. Tessier-Lavigne:** A. Employment/Salary (full or part-time):; Stanford University (on leave) and Xaira Therapeutics. Other; MTL is a director and shareholder of Denali Therapeutics, and a director, shareholder and employee of Xaira Therapeutics.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.02

Topic: A.03. Axon and Dendrite Development

Support: NSTC Grant 113-2628-B-001-009-MY3

Title: Compartment-specific axonal innervation of dopaminergic neurons in drosophila mushroom body

Authors: *S. MAGESH[1,2], M.-T. TSAI[3], S. LIN[1];
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Abstract: The precise subcellular organization of neural circuits in large brain regions is poorly understood. The *Drosophila* mushroom body (MB), crucial for learning and memory, serves as an excellent model. It comprises ~2,000 Kenyon cells (KCs) of different subtypes (γ, α/β, α′/β′) that form five axonal lobes (γ, β, β′ medially; α, α′ vertically). These lobes are precisely wired into 15 distinct compartments by extrinsic neurons, including MB output neurons (MBONs) and dopaminergic neurons (DANs). We are investigating the PPL1-α′2α2 DANs, which specifically innervate the α′2 and α2 compartments, to uncover the molecular basis of this compartmentalized innervation. Loss of the guidance receptor *frazzled* in these neurons disrupted their axonal targeting (Adult n=12, 100%). Furthermore, overexpression of *frazzled* in MBONs (MBON-α3 and β2β′2a) but not in DANs redirected dendrites toward the lobe base, suggesting a guidance cue may be present in this region. Supporting this, flies lacking *Netrin-A* (NetA) and *Netrin-B* (NetB) displayed similar targeting defects, indicating that Netrins guide PPL1-α′2α2 axons (Adult n=8,90%). NetB, but not NetA, is expressed in the MB during pupal stages, time-locked expression in newly born KCs shifting from γ to α′/β′ to α/β, and forming a stronger gradient from the base to the tip of the vertical lobes. This gradient likely creates an attractive zone for Frazzled-positive neurites in the lower and middle regions. Consistent with this idea, loss of *frazzled* also impaired targeting by PPL1-γ2α′1 DANs, while PPL1-α3 and PPL1-α′3 DANs targeting the tip remained unaffected. Interestingly, *NetA* was upregulated in *NetB* mutants, suggesting functional compensation. Despite its different expression pattern, innervation appeared largely normal, indicating an alternative innervation process. Overexpression of *NetB* in KCs led to neurite retention at the tip, likely due to a disrupted gradient. A membrane-tethered *NetB* failed to support proper targeting, confirming the need for diffusible Netrin. Overexpression of the repulsive receptor *unc-5* in PPL1-α′2α2 DANs misdirected axons to the tip, further supporting Netrin's attractive role. Knockdown of *NetB* in KCs impaired PPL1-α′2α2 targeting to the MB, confirming that KCs provide Netrin essential for compartment-specific innervation (Adult n=15, 100%). Our findings suggest that a stronger Netrin gradient originating at the base of the lobe attracts incoming Frazzled-positive PPL1-α′2α2 axons downward, guiding them to their appropriate compartments. Our findings advance the understanding of mechanisms underlying subcellular innervation and MB wiring during neural development.

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Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

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Presentation Number: NANO049.03

Topic: A.03. Axon and Dendrite Development

Support: NICHD R01 HD105946
NINDS R35 NS097340
NSF IOS-1853719
NIGMS K12 GM081259

Title: Dissecting the transcriptional networks regulated by the Fra/DCC intracellular domain.

Authors: *C. BARRIOS CAMACHO, E. YU, D. JIMENEZ, G. J. BASHAW;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Midline circuit assembly relies on the precise spatiotemporal regulation of axon guidance receptors. In *Drosophila*, the Frazzled (Fra) receptor regulates midline crossing through transcription-independent and -dependent mechanisms. In the former, commissural axons receive cues from Fra's chemoattractive ligand Netrin to project towards the midline. In the latter, γ-secretase cleaves the Fra intracellular domain (ICD), allowing it to translocate to the nucleus and induce the expression of *commisureless* (*comm*), supporting midline exit. It is unknown if this *in vivo* transcriptional output of Fra is conserved in the human homolog, DCC, despite evidence that the DCC ICD also undergo γ-secretase-mediated cleavage and enter the nucleus to regulate transcription *in vitro.*The P3 domain of the ICD functions as a transcriptional activation domain; in *Drosophila*, specific point mutations within this region render the receptor transcriptionally inactive, unable to induce *comm* expression. However, it is not known whether the role of these residues is conserved in the DCC ICD. To test this, we generated HA-tagged constructs overexpressing1) wild-type ICD 2) a point mutant L1433A 3) a point mutant Q1426A 4) a dual point mutant L1433A/Q1426A, 5) a ΔP3 domain mutant and 6) an HA-tag negative control. We generated and bulk RNA-sequenced stably transfected HEK293 cell lines (n=4/condition). Bioinformatic analyses show that transcriptomes of the ICD mutants are equivalent to that of the HA-tag negative control; however, the WT ICD induces a distinct transcriptomic profile defined by 609 differentially expressed genes (adj. p-value < 0.01, Benjamini-Hochberg false discovery rate correction; $\log_2 FC \neq 0$).We next subjected this DCC ICD signature to bottleneck gene analysis using cytoHubba, which identified the transcription factors *Tshz1* and *Tshz2* as bottleneck genes maintaining the downregulated components of the DCC ICD signature. To test their functional roles in the nervous system, we tested the fly orthologue *teashirt (tsh*) in a Fra-sensitized background in which 50% of Eagle-positive commissural neurons fail to cross the midline. Reducing *tsh* function dominantly enhances midline crossing defects, and this effect is specific to a loss-of-function *tsh* allele (tsh^{8}), but not tsh^{3}, an allele retaining TSH expression (n=87 embryos, $p > 0.01$, one-way ANOVA with Tukey post-hoc correction). Together, these data strongly imply that the ability for these residues to confer the transcriptional activity of the ICD is conserved between flies and mammals and identifies *tsh* as a novel regulator of axon guidance.

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Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

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Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.04

Topic: A.03. Axon and Dendrite Development

Support: R01NS116992

Title: A stem cell-derived neuronal culture model for mouse species variation in intrinsic neurite growth

Authors: *J. VALENCIA LESMES[1], E. A. LUMPKIN[2], D. BAUTISTA[3];
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Abstract: Axon regeneration and repair is driven by both extrinsic and intrinsic factors. However, the regenerating adult mammalian central nervous system (CNS) displays limited ability to regenerate and restore function after injury. Recent studies have identified both intrinsic and extrinsic regulators of regeneration. However, a better understanding of the intrinsic mechanisms that promote and suppress axonal regeneration are needed to develop effective therapeutics that stimulate repair in the adult CNS. Here we examined the exceptional regenerative capacity of the Southeast Asian mouse Mus musculus castaneus (CAST/EiJ) CNS. Previous work has shown that CAST/EiJ sensory neurons isolated from CNS-injured animals display more robust axon growth in vitro than neurons isolated from commonly used inbred mouse strains. Here we asked whether the enhanced ability of CAST/EiJ CNS neurons to extend axons is a cell-intrinsic property. To address this, we compared neurite outgrowth and branching in motor neurons generated from stem cells derived from C57BL/6, 129S6/SvEv, and CAST/EiJ mice. Stem cell-derived motor neurons were differentiated, stained with beta-III Tubulin Antibodies, and neurite outgrowth and branching was quantified on culture days 3, 5, and 7. Overall, CAST/EiJ neurons showed significantly greater neurite growth and branching than C57BL/6 and 129S6/SvEv. Two-way ANOVA showed significant effects of day ($p=0.0039$) and strain ($p=0.0432$) on neurite outgrowth with no significant day-by-strain interaction ($p=0.60$). Branching exhibited significant effects of day, strain, and their interaction ($p=0.0010$, 0.0049, 0.0263). To assess heritability of the CAST/EiJ neuronal phenotype, we examined stem cell-derived motor neurons from F1 CAST/EiJ×129 hybrids, and found that they exhibited neurite growth comparable to CAST/EiJ-derived neurons, consistent with dominant inheritance. Together, these findings suggest CAST/EiJ harbors heritable, cell-intrinsic mechanisms promoting neurite outgrowth in multiple neuronal types. We are now applying integrative genetic and multiomic approaches to identify candidate molecules that promote the remarkable regenerative capacity of the Mus musculus castaneus CNS.

Disclosures: J. Valencia Lesmes: None. **E.A. Lumpkin:** None. **D. Bautista:** None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

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Title: Exploring cytoskeletal architecture and function in Drosophila class IV sensory neurons

Authors: *J. LIAO[1], M. JALAL[2], J. HOWARD[2,3];
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Abstract: Neuronal morphology is regulated by the continuous remodeling of the cytoskeleton, which provides structural support and facilitates intracellular transport. Recent work from our lab on *Drosophila* larval class IV nociceptive sensory neurons has demonstrated that the tips of growing dendrites undergo stochastic transitions between growing, shrinking, and paused states, resulting in the highly branched dendritic arbors characteristic of these cells. To elucidate the roles of the cytoskeleton in generating dendrite morphology, we individually knocked down *α-tubulin84B* and *actin5C* using RNAi in class IV cells. Downregulation of α-tubulin and actin progressively attenuated dendritic branching, leading to reductions in branch number, arbor size, and total dendritic length as the larvae developed. Furthermore, we found that tubulin knockdown results in reduced dendritic diameter. To test whether cytoskeletal dynamics drive tip dynamics, we expressed the fluorescently tagged microtubule-binding proteins jupiter and tau. We found that they labeled both punctate and filamentous structures throughout the dendritic arbor. The puncta migrated along dendrites in both distal and proximal directions at rates of up to 5 μm/min. The filamentous structures stochastically converted between growing and shrinking phases, the hallmark of microtubule dynamic instability, strongly indicating that the fluorescent proteins indeed label microtubules. In addition, we visualized the distribution and dynamics of actin filaments using fluorescent actin reporters and found that the growth of new tips (monitored by CD4 membrane marker) strongly correlates with actin polymerization. Together, our findings suggest that the cytoskeleton drives the dynamics of dendritic tips, which in turn lead to the highly branched dendritic arbors.

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Nanosymposium

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Topic: A.03. Axon and Dendrite Development

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Title: Neurons exploit stochastic elongation, retraction, and branching of dendrite tips to rapidly and economically build dense neuronal arbors

Authors: *X. OUYANG[1], S. SUTRADHAR[1], O. TROTTIER[2], S. SHREE[1], Q. YU[3], Y. TU[4], J. HOWARD[1];
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Abstract: During larval development in Drosophila melanogaster, class IV sensory neurons form a dense, planar meshwork of dendrites that tile the body surface beneath the cuticle. This structure enables detection of localized noxious stimuli, such as penetration by parasitoid wasps. The dendrite tips exhibit highly dynamic behaviors—undergoing transitions between growth, shrinkage, and pause states, branching laterally, and retracting upon contact with other dendrites. A key question is whether such stochastic tip dynamics is sufficient to form highly branched dendritic morphologies. Alternatively, does dendrite geometry depend on signals from other cells or from the topological hierarchy of the growing network? To answer these questions, we developed an isotropic and homogenous mean-field model in which branch dynamics depends only on average lengths and densities so that no external cue or tree topology was present. Despite its simplicity, the model predicted several key features of class IV dendritic arbors, including the exponential distributions of branch lengths, the parabolic scaling between dendrite number and length densities, the tight spacing of dendritic meshwork, and the radial orientation of branches. Stochastic growth also accelerated the overall expansion rate of the arbor. These results show that complex dendritic structures can emerge from simple, local stochastic rules without the need for global guidance cues or hierarchical control. Our work therefore provides a general theoretical framework for understanding how macroscopic branching patterns emerge from microscopic dynamics.

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Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

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Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

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Topic: A.03. Axon and Dendrite Development

Support: DeNardo Education and Research Foundation

Title: Ctbp1 mutation dysregulates early neurite formation

Authors: *S. LEE[1], U. EZEKIEL[2];
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Abstract: C-terminal binding protein 1 (CtBP1), a transcriptional corepressor with a heterozygous missense mutation (c.991C > T, p.R342W), has been linked to a rare neurodevelopmental disease called Hypotonia, Ataxia, Developmental Delay, and Tooth Enamel Defects (HADDTS). The HADDTS patients also exhibit congenital hypotonia, delayed psychomotor development, and intellectual disability and is often associated with cerebellar atrophy. The mutation is located within the major protein-interaction cleft, the PXDLS-binding cleft, which is involved in binding to various transcriptional repression molecules. To eliminate genetic variability associated with patient-derived induced pluripotent stem cells (iPSCs), we developed isogenic iPSC cell lines harboring heterozygous and homozygous mutations for the pathogenic *CTBP1* p.R342W allele. In our earlier work, we found that at day seven, both mutant isogenic neurons exhibited significantly longer neurites than wild-type neurons. In this study, we conducted a time course experiment to evaluate how early neurites are formed in the mutants compared to the wild type. We differentiated the neural stem cells (NSCs) into neurons and measured average neurite length from day two to seven. We found that on day two, most of the mutant neurons formed neurites compared to the wild type and that on day 7, the average neurite lengths were significantly longer than the wild-type counterparts. Likewise, our transcriptome analysis of isogenic neurons revealed downregulation of several genes associated with neurite dysregulation, including GATA3 and ISL1. Genes that regulate the Sonic Hedgehog Pathway, known to be involved in neurite dysregulation, were also found to be downregulated. These in-vitro results indicate that CTBP1 mutation dysregulates neurite formation.

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

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.08

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant 1R21NS133564

Title: Let-7 sustains cortical projection neuron migration by targeting RBX2

Authors: *S. DECKER[1], K. HINO[2], A. C. LA TORRE[1], S. SIMO[2];
[1]Cell Biol. and Human Anat., Univ. of California-Davis, Davis, CA; [2]Cell Biol. and Human Anat., Univ. of California Davis, Davis, CA

Abstract: In the central nervous system, there is a tightly coordinated relationship between the fate and migration of projection neurons, ensuring that specific neuronal fates settle in precise spatial locations. This is particularly evident in the mammalian neocortex, where early-born projection neurons predominantly settle in the deeper layers of the cortical plate, whereas later-born neurons localize more superficially. However, it remains unclear whether neuronal fate acquisition directly determines projection neuron positioning, or whether fate and positioning are regulated independently. MicroRNAs have emerged as key regulators of cell fate determination in the neocortex. Among them, let-7 is known to influence neural progenitor competence and promote the neurogenesis of late-born projection neurons. Here, we show that let-7 also regulates projection neuron migration by targeting RBX2, a core component of the E3 ubiquitin ligase CRL5, which has been previously shown to inhibit neuron migration by terminating the Reelin/DAB1 signaling pathway. Let-7 directly binds a conserved motif in the 3′ UTR of RBX2, reducing its translation and thereby diminishing CRL5 activity. Importantly, restoring RBX2 levels in the context of let-7 expression rescues the positioning of pyramidal neurons without altering let-7-induced effects on neuronal fate. Furthermore, we demonstrate that let-7 enhances pyramidal neuron migration by increasing locomotion speed and prolonging migratory activity. Together, these findings reveal that let-7 coordinates neuronal fate specification and migration via distinct molecular pathways, thereby ensuring the proper laminar positioning of late-born pyramidal neurons in the neocortex.

Disclosures: S. Decker: None. **K. Hino:** None. **A.C. La Torre:** None. **S. Simo:** None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.09

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH MIRA grant R35GM118183652
Flora Lamson Hewlett Chair in Biochemistry

Title: Ploidy and neuron size impact nervous system development and function in Xenopus

Authors: *X. LIU;
Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: Neuron size varies significantly over evolution, contributing to diverse nervous systems of variable complexity, while aberrant neuron size is associated with neurodevelopmental and degenerative diseases. How do neuron soma and neurite size and organization impact nervous system development and function? To systematically study effects of neuron size on the vertebrate nervous system, we characterized triploid *Xenopus* tadpoles that possess a 1.5-fold increase in genome size compared to diploids. Triploid neurons displayed a

scaling increase in total volume and a superscaling increase in membrane surface area. Imaging, flow cytometry, and RNA-seq analyses revealed that triploid brains were morphologically and transcriptionally similar to diploid brains, but less proliferative, containing fewer neurons and displaying increased global activity. Interestingly, physiological differences at the neuron and nervous system levels affected swimming behavior in tadpoles. Our findings thus establish a framework to link genome size, neuron size, and nervous system development and function in vertebrates.

Disclosures: X. Liu: None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.10

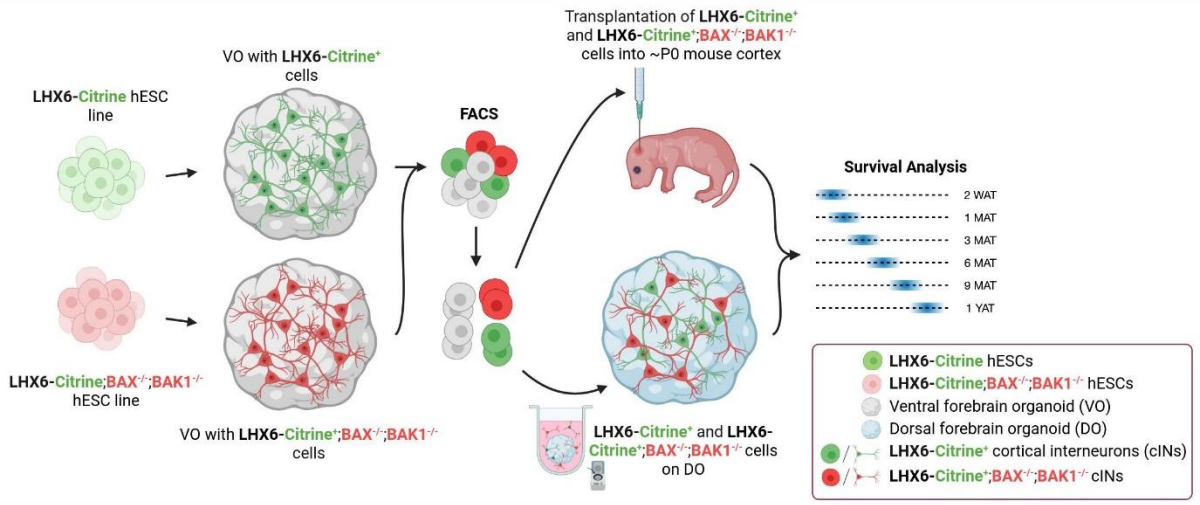
Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome Trust Grant #218461/Z/19/Z

Title: Investigating human cortical interneuron cell death using neural organoids and a xenotransplantation model

Authors: *G. HERRERA-OROPEZA[1], O. MARIN[2];
[1]King's Col. London, London, United Kingdom; [2]Ctr. for Developmental Neurobio., Kings Col. London, London, United Kingdom

Abstract: Correct functioning of the cerebral cortex requires the coordinated activity of excitatory neurons and inhibitory interneurons. Dysfunction or changes in the number of cortical interneurons is a hallmark of several neuropsychiatric disorders. During mouse embryonic development, excess numbers of cortical interneurons are produced in the ventral forebrain. These immature interneurons migrate dorsally into the cortex and form connections with locally produced excitatory neurons. About 30% of these cells are eliminated through programmed cell death during the first two weeks of postnatal development. The surviving population of interneurons comprise 1/6 of the total number of neurons in the adult mouse cortex. Interestingly, in the adult human cortex, the excitation/inhibition balance is achieved by a different ratio of interneurons to excitatory neurons, 1/2.5, respectively. This suggests that the regulation of human cortical interneuron numbers might differ from that in mice. To understand how this process occurs in a human context, we are employing two different approaches to model human cortical interneuron development: a neural organoid system and a xenotransplantation model. Human cortical interneurons are generated in ventral forebrain organoids, isolated and co-cultured with human dorsal forebrain organoids or transplanted into the mouse cortex, and their survival is subsequently analysed at different time-points.



Disclosures: G. Herrera-Oropeza: None. **O. Marin:** None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.11

Topic: A.01. Neurogenesis and Gliogenesis

Title: The role of REEP1 and ER-mitochondrial membrane contacts in brain development

Authors: *Y. LIM;
Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: REEP1 (Receptor expression enhancing protein1) is involved in shaping the endoplasmic reticulum (ER) membrane and also in modulating ER-mitochondria contact sites (ERMCs). Mutations in *REEP1* are linked to hereditary spastic paraplegia (HSP), a rare group of genetically heterogeneous neurodegenerative diseases affecting lower limbs. Recent studies suggest that HSPs are not just affected by neuronal degeneration but also brain development. However, the role of REEP1 in brain development remains poorly understood. To explore the function of ERMCs in neurogenesis and cellular processes, we introduced *Reep1* into the developing mouse neocortex and HeLa cells. Remarkably, *Reep1* expression promoted the formation of sheet-like ER over tubular ER. This effect requires the C-terminal region of Reep1, as its deletion results in a shift toward tubular ER morphology. Moreover, REEP1 enhances ER-mitochondria contacts, leading to increased mitochondrial calcium influx and potentially elevated mitochondrial enzyme activity. In the developing mouse brain, *Reep1* is expressed not in the proliferating cells in the ventricular zone, but in differentiating/differentiated neurons. Overexpression of *Reep1* disrupts radial migration and alters the balance between cell cycle

progression and differentiation. Taken together, these findings suggest that ER-mitochondria contacts may regulate neurogenesis through mitochondrial function.

Disclosures: Y. Lim: None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.12

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01NS131151
NIH R01NS126680

Title: Recombinant Adeno-Associated Virus (AAV) Depletes PARP1 and Other DNA Damage Response Proteins Required forNeural ProgenitorCell Division

Authors: *M. SHTRAHMAN;
Univ. of California San Diego (UCSD), La Jolla, CA

Abstract: Recombinant adeno-associated virus (rAAV) is a replication-defective viral vector used in hundreds of human gene therapy trials, resulting in five FDA-approved therapies. Despite this success, rAAV-base gene therapies suffer from dose-limiting toxicities, resulting in a number of severe adverse reactions including death. Previously, we discovered that rAAV rapidly kills mouse NPCs *in vitro* and *in vivo*. This vector contains a minimal genome comprised of 145-base pair inverted terminal repeats (ITRs) with T-shaped hairpin structure that appear to be necessary and sufficient for this toxicity. However, the mechanism for AAV ITR toxicity is not known and there have been few attempts to engineer ITRs to attenuate rAAV toxicity. In the current study, we explore the molecular mechanisms that drive dose-dependent rAAV toxicity in dividing human NPCs (hNPCs) and test whether disrupting these mechanisms mitigates this toxicity. Recombinant AAV infection induces aberrant cell cycle progression with activation of the ATM /CHK1/CHK2 pathway and expression of the DNA damage markers γH2AX and 53BP1. Affinity-based proteomics indicate that AAV ITRs bind to Poly-(ADP-Ribose)polymerase 1 (PARP1) and other DNA damage response (DDR) proteins involved in single strand break repair (SSBR). Recombinant AAV infection attenuates poly-(ADP-ribose) (PAR) formation and mimics the antiproliferative effects of pharmacological PARP inhibitors used in cancer therapy. Moreover, treatment of hNPCs with PARP inhibitors is sufficient to reproduce many features of rAAV-induced toxicity. Finally, we demonstrate that eliminating the T-shaped hairpin within the AAV ITR reduces binding to SSBR proteins and resulting rAAV toxicity. These findings suggest that rAAV infection induces replication stress and cell death in dividing hNPCs by functionally depleting PARP1 and other proteins DDR proteins that are essential for DNA replication. This work fills substantial gaps in the understanding of the

mechanisms of rAAV toxicity and has important implications for the development of safer rAAV-based human gene therapies.

Disclosures: M. Shtrahman: None.

Nanosymposium

NANO049: Mechanisms of Axon Guidance and Neurite Development Across Species and Models

Location: SDCC Rm 11

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO049.13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS/NIH R01 RNS107428

Title: Llgl2 protein participate in the brain development and cognitive function, likely through regulation of glutamate neurotransmission

Authors: *A. A. ZAIB[1], Z. M. AHMED[1], Y. JI[2], M. C. KRUER[3], J. MAYER[4], S. ZEIDLER[5], S. RIAZUDDIN[6], S. RIAZUDDIN[2];
[1]Otorhinolayrngology, [2]Otorhinolaryngology Dept., Univ. of Maryland Baltimore, Baltimore, MD; [3]Phoenix Children's Hosp., Phoenix, AZ; [4]Univ. Children's Hosp., Salzburg, Austria; [5]Erasmus MC, Rotterdam, Netherlands; [6]Jinnah Burn and Reconstructive Surgery Centre, Allama Iqbal Med. Res. Univ. of Hlth. Sci., Lahore, Pakistan

Abstract: LLGL2 (MIM: 618483) encodes Lethal giant larvae 2 protein that establishes cell polarity, asymmetric division, and migration. In this study we identified 8 individuals from 4 unrelated families harboring a splicing and 3 missense variants in the LLGL2 segregating with neurodevelopmental disorder (NDD), epilepsy, and microcephaly. Over-expression of constructs harboring NDD variants impacted the subcellular localization of encoded LLGL2 as well as cytoskeleton of cells. Using HDR-mediated CRISPR-Cas9 genome editing technique, we introduced a c.1456G>A LLGL2 variant in the HEK293T cells. Intriguingly, during cell division, deficits in centrosome assembly, multipolar mitotic spindles, increased frequency of multinucleated cells and abnormal cytokinesis were noted in the knockin cells along with altered numbers and diameters of centrosomes. Knockin cells also showed a significantly increased number of gamma-H2AX foci, indicating DNA damage, compared to control cells. Finally, we also noted reduced proliferation potential in the knockin cells. Flow cytometric analysis further validated significant alterations in all the cell cycle phases in the knockin cells. Furthermore we observed significant dysregulation of the glutaminergic pathway and ERK1/2 regulator effect networks, which are known to control excitatory neurotransmission and several physiological functions of the brain, including behavior, and cognitive abilities in the bulk mRNA sequencing of knockin cells. Deficits in glutamatergic pathways are involved in many brain disorders, including depression, epilepsy, schizophrenia, and ischemic brain damage. To decipher role of LLGL2 in brain development and function, we generated *llgl2* knockdown in zebrafish larvae.

Comparable to human patients, we observed significant developmental deficits in the *llgl2* morphants. Upon behavioral assessment, morphants showed significantly reduced locomotion patterns. These deficits were significantly rescued with the co-injection of human wildtype *LLGL2* mRNA but not the NDD variants harboring mRNAs, further validating their pathogenic nature. In parallel, we generated the c.1456G>A knockin human induced pluripotent stem cells (iPSCs) lines and differentiated into neural progenitor cells (NPCs) followed by cortical neuronal differentiation. In our ongoing experiments we are using these NPCs to understand the effect of mutation in cortical glutamatergic neurons morphology, and functions. In conclusion, our study demonstrates critical role of LLGL2 in neurodevelopment and cognitive function

Disclosures: A.A. Zaib: None. **Z.M. Ahmed:** None. **Y. Ji:** None. **M.C. Kruer:** None. **J. Mayer:** None. **S. Zeidler:** None. **S. Riazuddin:** None. **S. Riazuddin:** None.

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.01

Topic: B.09. Glial Mechanisms

Support: AMED ADI06343
Kao Foundation for health science
The Sumitomo Foundation
Ono Pharmaceutical Foundation for Oncology, Immunology, and Neurology

Title: Exploring the physiological role of micronuclei propagation to microglia

Authors: *F. TSURUTA;
Univ. of Tsukuba, Tsukuba, Japan

Abstract: Microglia, the resident immune cells of the central nervous system (CNS), orchestrate a wide array of biological processes, including neurogenesis, synaptogenesis, efferocytosis, and the maintenance of CNS interfaces. Fate mapping analyses have revealed that microglia originate from erythro-myeloid precursors and infiltrate the brain primordium during early embryonic development. Subsequently, microglia colonize specific regions and differentiate into homeostatic microglia. Recent single-cell analyses have shown that microglia exhibit a wide variety of subcellular populations, particularly during postnatal and aging stages. Accordingly, microglia are thought to acquire diverse cellular characteristics in response not only to genetic factors but also to stimuli from the surrounding environment. However, the mechanisms underlying microglial characteristic alterations remain largely unknown.Previously, we found that the propagation of micronuclei (MN) regulates microglial characteristics. Neuronal MN are generated by mechanical stress as neurons pass through narrow spaces. These MN are then secreted into the extracellular space via the autophagy secretion machinery and subsequently

taken up by microglia. Using two-photon excitation microscopy, we also observed that microglia incorporating MN underwent morphological changes. MN incorporation into microglia induced alterations in gene expression patterns. Notably, several MN were found not only in the parenchyma but also at CNS interfaces, such as the meninges and blood vessels. These findings suggest that MN serve as a novel mediator regulating microglial characteristics.Recently, we have been investigating the phenomena and significance of microglial transformation via micronuclei propagation in both the brain parenchyma and CNS interfaces. We also observed that micronucleus propagation occurs not only during development but also in aging. Based on our recent finding, we would like to discuss comprehensively the biological significance of why MN propagation occurs and the impact of MN propagation on the brain homeostasis.

Disclosures: F. Tsuruta: None.

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.02

Topic: B.09. Glial Mechanisms

Support: DoD Grant W81XWH1910353
TSC Alliance Crossref DOI: 1340014

Title: mTOR-LPL-driven dysregulation of lipid metabolism in human microglia of tuberous sclerosis complex leads to aberrant neuronal development and hyperexcitability

Authors: *W. NIU[1], S. YU[2], M. CHU[3], J. GUO[2], C. MICHALSKI[2], X. LI[2], Z. WANG[3], M. GAMBELLO[2], J. PENG[3], Z. WEN[2];
[1]Psychiatry and Behavioral Sci., Emory Univ., Decatur, GA; [2]Emory Univ., Atlanta, GA; [3]St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by heterozygous pathogenic variants in either *TSC1* or *TSC2*. Emerging evidence suggests a connection between microglia activation and epilepsy as well as cognitive impairment in TSC patients. However, the impact of the causal variants of *TSC1*/*2* genes on human microglia and their contribution to TSC's neurological symptoms remain largely unexplored. In this study, we generated human microglia from induced pluripotent stem cells (iPSCs) from a TSC patient cohort. Through extensive multi-omic and cellular analysis of TSC microglia, including transcriptomics, proteomics/phosphoproteomics, and lipidomics, we found that heterozygous *TSC2* pathogenic variants were sufficient to cause aberrant lipid metabolism marked by increased glycerophosphocholines and fatty acyls. These metabolic changes resulted in enhanced phagocytosis and inflammation. Strikingly, the dysregulated lipid metabolism in TSC microglia is driven by a hyper-activated mTOR-lipoprotein lipase (LPL) pathway. Further, cellular and electrophysiological assessments of neuron/microglia co-cultures revealed that TSC microglia

directly affect neuronal development, excitability, and neuronal network activity, which could be largely ameliorated by mTOR/LPL inhibition. Collectively, our research unveiled the molecular and cellular abnormalities in TSC microglia affecting neuronal development and function, and highlighted the mTOR-LPL pathway as a novel potential therapeutic target for the neuropathology of TSC.

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

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.03

Topic: B.09. Glial Mechanisms

Support: Utensu Grant

Title: The 22q.11.2 Deletion Mediates Dysfunction of iPSC derived Microglial Models

Authors: *K. L. COOK;
Psychiatry, Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: 22q.11.2 Deletion Syndrome (22qDS) is a prevalent chromosomal abnormalities and occurs in approximately 1 in 2,000 live births. The approximately three kilobase deletion encompasses 45 genes on the long arm of chromosome 22. Clinical manifestations include craniofacial abnormalities, cardiac malformations, immune dysfunction, and neuropsychiatric sequalae including cognitive impairment and a 25-fold increased risk in developing psychosis. Nine of the canonically deleted genes in 22qDS are associated with mitochondrial or metabolic function. Microglia, the resident macrophage of the brain, contribute to the establishment, upkeep, and refinement of neurocircuitry in both direct and indirect capacities. They achieve their dynamic functions largely through signaling and phenotypic variation dictated by their ability to reprogram their metabolism. Given the enrichment of metabolic effector genes in the deletion region, our study seeks to investigate how 22qDS mediates microglial functions which impact CNS function. Our work utilizes a CRISPR-CAS9 edited isogenic pair of hESC derived microglial models (22q iMG) to investigate relevant microglial functions *in-vitro* and *in-vivo*. Transcriptomic and phenotypic data point to aberrant activation and altered metabolic function in both naïve and stimulated 22q iMG. *In-vitro,* naive 22q iMG upregulate genes in GO pathways such as “Inflammatory Response”, “Microglial Cell Activation”, “Respiratory Burst” and “Icosanoid Metabolic Process”. Stimulation with 2ng/ml IFNg induces increased expression of transcripts in GO pathways indicative of ‘hyper-activation’ and metabolic dysfunction such as “Cell Activation Involved in Immune Response”, “Phagocytosis”, “Positive Regulation of Nitrogen Compound Metabolic Process”, “Lipid biosynthetic Process”. Protein level studies corroborate naïve 22q iMG inflammatory activation as CD68 and TREM2 are significantly

increased (P=0.023, P=0.033). Fluorometric assays further reveal increased production of reactive oxygen species, and dysregulation of phagocytic activity in 22q iMG. Xenotransplanted 22q iMg transcriptomes reflected increased metabolic pressure and immune activation with upregulated transcripts enriched in GO pathways such as “Signaling by Interluekins”, “Primary Metabolic Processes” and “Cellular Response to Stress”. *Ex-vivo* iMG also uptake host synaptic material, further validating their microglial functionality. Ongoing studies aim to quantify *ex-vivo* synaptophagy and 22q iMG’s impacts on synaptic function. Our study implicates fundamental differences in microglial state and function mediated by the 22q deletion.

Disclosures: K.L. Cook: None.

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.04

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01NS110564

Title: The effect of microglia depletion and repopulation on neural activity and connectivity across visual cortex, white matter, and hippocampus.

Authors: A. GORMALEY[1], N. P. WILLIAMS[1], X. HU[2], A. VAZQUEZ[3], T. CUI[4]; [1]Bioengineering, [2]Neurol., [3]Radiology, [4]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The objective of this study is to examine the role of microglia in neuronal network organization using awake 2-Photon (2P) and wide-field imaging. Microglia depletion using a CSFR-1 inhibitor followed by repopulation has been found to reverse cognitive and synaptic deficits in aged Alzheimer’s Disease (AD) model mice [Wanbing Wang et al 2023]. This implies that microglia may be involved in a contributing mechanism of cognitive deficits in AD by affecting neuronal function. Previous studies have examined microglia and neuronal calcium activity separately and within the superficial layers (Layer 1-Layer 2/3) of the cortex due to limited imaging depth of traditional cranial windows [Yong-Jun Liu et al 2021, Linh Le et al 2024]. In this study we utilize healthy, transgenic mice expressing CX3CR1-GFP (microglia) and Thy1-jRGECO1a (neuronal calcium) to examine how microglia-neuronal interactions impact the network behavior. We utilize a microprism implant to reflect light horizontally which allows for imaging of all cortical layers, white matter, and portions of the hippocampus within a single plane. We capitalize on this view to examine network connectivity and depth-dependent effects. After the implant has healed (>5 weeks) the mice were given PLX5622 chow for two weeks. The microglia and neuronal calcium activity were imaged prior to treatment and weekly during depletion and repopulation. Overall, 2P imaging shows that spontaneous neuronal activity decreases after depletion and repopulation. However, the max firing rate increases suggesting

some neurons had increased excitability. Superficial and deep neurons decreased activity compared at 7d of depletion and after repopulation, but not at 14d of depletion. This suggests there is a difference between the initial surviving microglia activity and the remaining microglia population after 14d of depletion. Wide-field imaging shows that depletion and repopulation induced positive and negative correlation changes between local and distant regions of the cortex, white matter, and hippocampus. In conclusion, neuronal activity is impacted by length of PLX5622 treatment and repopulation promotes changes in connectivity between brain regions. Future work will compare these results with aged AD mice.

Disclosures: A. Gormaley: None. **N.P. Williams:** None. **X. Hu:** None. **A. Vazquez:** None. **T. Cui:** None.

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.05

Topic: B.09. Glial Mechanisms

Title: Thik-1 channel mediates microglial glucose sensing and modulates agrp neurons

Authors: *Q. LIU, H. LIU, Y. XU;
Baylor Col. of Med., Houston, TX

Abstract: Microglia play essential roles in maintaining energy homeostasis, and their dysfunction contributes to metabolic disorders. High-fat diet (HFD) exposure results in microglial activation and hypothalamic inflammation; however, the underlying mechanisms remain poorly defined. Here, we identify a novel role for THIK-1 potassium channels in mediating microglial responses to dietary cues. Using ex vivo brain slice electrophysiology, we show that HFD exposure leads to hyperpolarization of microglia in the arcuate nucleus of the hypothalamus (ARH), accompanied by enhanced THIK-1 mediated outward potassium currents. Meanwhile, elevation of extracellular glucose levels similarly increases THIK-1 current in ARH microglia, leading to microglia hyperpolarization. Tetrapentylammonium (TPA), a selective THIK-1 channel blocker, blunts the microglia hyperpolarization induced by high glucose level, indicating that THIK-1 channel mediates glucose-sensing and activation in microglia. Interestingly, perfusion of TPA to the brain slice suppresses the activity of neighboring AgRP neurons, while TPA puffing directly onto AgRP neurons has no effect. Since there is no THIK-1 channel expression in AgRP neurons, these findings suggest a microglia-to-neuron communication pathway. We found that TPA treatment enhances microglia-mediated phagocytosis, leading to reduced perineuronal nets (PNNs) in the ARH. Digestion of PNN suppresses AgRP neuron activity, indicating that microglia inhibit AgRP neurons at least partly through engulfing PNNs. In addition, intraperitoneal TPA administration reduces food intake and attenuates body weight gain in both chow- and HFD-fed mice. Together, our results identify THIK-1 channel as a critical glucose sensor in hypothalamic microglia and uncover a novel

mechanism by which microglia regulate energy balance through modulation of AgRP neuron activity. Therefore, targeting microglial THIK-1 may offer a therapeutic strategy for diet-induced obesity.

Disclosures: Q. Liu: None. **H. Liu:** None. **Y. Xu:** None.

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.06

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

Title: Dissecting Microglia-Neural Circuit Interactions Under Spaceflight Conditions Using Human Brain Assembloids and Spatial Proteomics

Authors: L. COELHO[1], *T. CONKLIN[2], R. MIHANI[3];
[1]UCSD, SD, CA; [2]Akoya Biosci., Exeter, NH; [3]Akoya Biosci., Marlborough, MA

Abstract: Spaceflight-associated stressors, including microgravity and cosmic radiation, pose significant risks to human health, particularly to the central nervous system (CNS), which is highly susceptible due to its limited regenerative capacity and vulnerability to accelerated inflammation. As the brain's resident immune cells, microglia play a critical role in maintaining neural homeostasis, responding to injury, and modulating inflammatory processes. Understanding microglial responses under spaceflight conditions, and their potential to mitigate CNS damage, is therefore essential. To evaluate the neuroprotective potential of microglia during spaceflight, we generated human cortical assembloids, three-dimensional brain organoid models that incorporate microglia, and exposed them to a 30-day long mission to the International Space Station (ISS). Spaceflown microglia-containing and microglia-depleted organoids were compared to matched Earth-based controls through bulk proteomics analysis using Astral-Orbitrap mass spectrometry and high-dimensional spatial proteomics using Akoya Biosciences' PhenoCycler®-Fusion platform in combination with the PhenoCode™ Human Neurobiology Panel. This multimodal approach enables spatially resolved mapping of neuroinflammatory signaling, synaptic remodeling, and developmental trajectories influenced by microglia in the space environment. Early proteomics data suggest differential pathway engagement in assembloids with microglia, particularly in oxidative stress, cytokine signaling, NFkB signaling and synaptic pruning mechanisms. This work establishes a novel platform for studying brain-immune interactions under Low Earth Orbit stress, revealing how microglia may shape vulnerability or resilience in developing neural circuits exposed to spaceflight. The resulting molecular atlas will inform strategies to mitigate CNS risks during long-duration missions and offer disease modelling insights into accelerated aging mechanisms in the human brain.

Disclosures: **L. Coelho:** A. Employment/Salary (full or part-time):; UCSD. **T. Conklin:** A. Employment/Salary (full or part-time):; Akoya Biosciences, UCSD. **R. Mihani:** A. Employment/Salary (full or part-time):; Akoya Biosciences.

Nanosymposium

NANO050: Microglial Function Within Neural Circuits

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Presentation Number: NANO050.07

Topic: B.09. Glial Mechanisms

Support: NIH Grant P50AA024333
Bodossaki Foundation Postdoctoral Fellowship
NIH Grant R01NS112526

Title: Microglia-mediated extracellular matrix remodeling promotes neuronal dysfunction, motor impairments and sedation following alcohol abuse

Authors: ***E. PAOURI**[1], S. STANKO[1], N. GASMI[1], G. GLUSAUSKAS[1], V. MAO[1], A. KWAK[1], S. SARMADI[1], E. HUANG[1], C. HERSHBERGER[1], Q. WATERCUTTER[1], H. BURTON[1], A. CROCKETT[1], A. GJOJDESHI[1], M. MCMULLEN[1], R. HANAMSAGAR[2], D. ROTROFF[1], R. PRAYSON[3], S. BILBO[4], S. VALADKHAN[5], H. DANA[1], L. NAGY[1], D. DAVALOS[1];
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Abstract: Alcohol abuse is the primary risk factor for alcohol use disorder (AUD), a leading cause of preventable morbidity and mortality, characterized by systemic inflammation, multi-organ damage, and neurological impairments. While direct effects of alcohol on brain function are well-established, the role of microglia in acute and chronic neurological dysfunction in AUD remains unclear. We investigated how alcohol abuse affects microglial responses using mouse models of human binge and high-intensity drinking patterns. Through longitudinal *in vivo* imaging of individual microglia during a repeated course of ethanol abuse in adult mice, we found that microglia underwent gradual morphological changes that preceded the onset of, yet paralleled the recovery from, ethanol-induced sedation. Repeated sedative ethanol doses caused progressively worsened morphological microglial reactivity, accompanied by prominent matrisome/phagocytosis transcriptional signatures. These microglial responses were also associated with structural network changes including microglia-mediated engulfment of peri-synaptic extracellular matrix (ECM), elimination of synaptic structures, and dose-dependent neuronal loss. Remarkably, genetically disrupting key components of microglial Toll-like receptor (TLR) signaling completely prevented both acute and chronic alcohol effects, blocking ECM disruption, aberrant synapse loss, and neuronal death throughout the entire alcohol abuse regimen. Critically, preventing microglial responses also protected against acute decreases in

neuronal activity *in vivo* and significantly reduced ethanol-induced sedation and motor impairments. These findings identify microglia as cellular drivers of both acute and chronic brain dysfunction following alcohol abuse, highlighting novel therapeutic targets for AUD's detrimental neurological consequences.

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Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO050.08

Topic: B.09. Glial Mechanisms

Support: NIH R01 DA053070
T32 DA035200

Title: Evaluating the role of microglia in the PFC on fentanyl escalation using PLX 3397 in Sprague-Dawley rats

Authors: *J. SHAYKIN[1], M. WARDELL[1], J. MARTIN[2], D. LUO[2], R. TRIVEDI[2], T. E. PRISINZANO[2], P. I. ORTINSKI[3], J. R. TURNER[2], M. BARDO[1];
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Abstract: Purpose: In people with opioid use disorder (OUD), chronic drug use causes microglial reactivity and subsequent release of proinflammatory cytokines leading to inflammation, potentially weakening one's executive control over drug taking and ultimately leading to greater use. Previous research in our laboratory pointed to a positive correlation between *Treml1* expression in the PFC and mean infusions on the last 3 days of fentanyl self-administration ($p < 0.05$). Thus, it is our hypothesis that depletion of microglia using PLX 3397 will result in a reduction in escalation of fentanyl intake, and a reduction of inflammatory signaling, compared to controls. **Methods**: Male and female Sprague-Dawley rats underwent

daily 1h acquisition sessions for i.v. fentanyl self-administration (2.5 µg/kg; FR1) for 7 days. Starting experimental day 8, rats received once daily injections of PLX 3397 (25 mg/kg, s.c.) until the end of the study. On experimental days 15-35, rats underwent daily 6h escalation sessions. Approximately 20h after the last self-administration session, acute symptoms of fentanyl-withdrawal were assessed, blood plasma was collected to assess peripheral cytokine and chemokines, and tissue from prefrontal cortex was collected for immunohistochemistry (IHC) and qPCR. **Results:** Using IHC, IBA1 was evaluated to verify knockdown of microglia. Rats that received PLX 3397 and vehicle showed similar rates of fentanyl SA during acquisition and escalation sessions, and mRNA transcripts of inflammatory targets within the PFC were assessed. **Conclusion**: Ongoing investigations including greater statistical power will continue to assess the inflammatory markers that may drive fentanyl escalation. Supported by: NIH R01 DA053070; T32 DA035200

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Nanosymposium

NANO050: Microglial Function Within Neural Circuits

Location: SDCC Rm 33

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Presentation Number: NANO050.09

Topic: B.09. Glial Mechanisms

Support: National Natural Science Foundation of China (No. 82271477 to X.X.)
Shanghai Science and Technology Rising Star Program (No. 23QA1408400 to Y.W.)
Tongji University Original Basic Research Program (No. 22120220596 to X.X.)

Title: Neural stem cell-derived extracellular vesicles inhibit microglia dysfunction in depression through targeting miR-16-5p/Cxcr4 axis

Authors: *X. LI[1], M. SHI[1], X. XIA[1], J. ZHENG[2];
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Abstract: Neural stem cell-derived extracellular vesicles inhibit microglial dysfunction in depression through targeting miR-16-5p/Cxcr4 axis Xiangyu Li[1‡], Meng Shi[1‡], Xiaohuan Xia[1*], Jialin C. Zheng[1*]Translational Research Center, Shanghai Yangzhi Rehabilitation Hospital affiliated to Tongji University School of Medicine, Shanghai 201613, China.Purpose:Major depressive disorder (MDD) is a widespread and disabling condition, with limited treatment efficacy in many patients. Neuroinflammation and microglial dysfunction are increasingly recognized as key pathological factors. Neural stem cell-derived extracellular vesicles (NSC-EVs) possess immunomodulatory properties and can cross the blood-brain barrier (BBB),

offering potential for therapeutic delivery. This study aimed to evaluate the antidepressant effects of NSC-EVs in a chronic restraint stress (CRS) mouse model and explore their underlying mechanisms.Methods:NSCs were isolated from E13.5 mouse embryos and cultured to produce EVs, which were characterized and labeled for intravenous administration. CRS mice received NSC-EVs, and behavioral assays assessed depression-like phenotypes. Brain tissues were analyzed via immunostaining, molecular assays, and electrophysiology. EV miRNA content was profiled by sequencing, and downstream targets were identified using RNA-seq, luciferase assays, and in vivo genetic/pharmacological manipulation.Results:NSC-EVs crossed the BBB and were predominantly taken up by microglia in the prefrontal cortex and hippocampus. Treatment significantly improved depressive behaviors and surpassed fluoxetine in reducing immobility. NSC-EVs suppressed microglial activation, reduced pro-inflammatory cytokines, and preserved synaptic structure. Mechanistically, EV-delivered miR-16-5p targeted Cxcr4, a receptor elevated in activated microglia. Loss of miR-16-5p abrogated therapeutic effects, while Cxcr4 knockout or inhibition (AMD3100/Motixafortide) restored behavioral and neurobiological improvements. Cxcr4-mediated phagocytic activation was further linked to its interaction with Spp1.Conclusion:NSC-EVs alleviate depression-like behaviors by modulating microglial activity through the miR-16-5p/Cxcr4 axis. These findings highlight NSC-EVs as a promising cell-free therapeutic and identify Cxcr4 as a novel target for regulating microglia-driven neuroinflammation in MDD.

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Nanosymposium

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R00 HL165026

Title: Pulmonary Hypertension Disrupts Inhibitory Synapse Integrity and Engages Synapse-Specific Microglial Remodeling in the Paraventricular Nucleus of the Hypothalamus

Authors: *S. KHANFAR, R. ABDELNOR, F. DA SILVA, A. OLIVEIRA;
Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Pulmonary hypertension (PH) is a progressive cardiopulmonary disorder characterized by elevated pulmonary arterial pressure, right heart dysfunction, and high mortality. In addition to vascular remodeling, PH is marked by sustained sympathetic overactivation, which exacerbates disease progression and worsens clinical outcomes. While this autonomic imbalance is well recognized, its underlying basis remains unclear. The paraventricular nucleus (PVN) of the hypothalamus plays a critical role in regulating sympathetic tone, and disruptions in the balance of excitatory and inhibitory inputs within this region may contribute to exaggerated

output. To investigate this, we used a chronic hypoxia model (10% O_2 for 4 weeks) and performed immunohistochemistry on 30 μm-thick brain sections. High-resolution confocal microscopy and 3D reconstruction in Imaris were then used to quantify discrete pre- and post-synaptic puncta representing excitatory and inhibitory synaptic terminals on PVN neurons. When comparing mice with PH to normoxic controls, inhibitory inputs showed a compartment-specific dissociation: presynaptic terminals expressing glutamic acid decarboxylase (GAD) were preserved ($p = 0.5031$), while post-synaptic terminals—marked by Neuroligin-2 (NLGN2)—were significantly reduced ($p = 0.0181$), indicating selective disruption at the post-synaptic component of inhibitory synapses in PH mice. Conversely, excitatory presynaptic input was elevated, as reflected by a significant increase in vesicular glutamate transporter 2 (vGLUT2)-labeled terminals ($p = 0.0046$). Building on our observation of altered excitatory-inhibitory balance in the PVN, we next asked whether microglia contribute to or respond to this remodeling. Prior work from our lab has shown that chronic microglial activation alone induces PH, suggesting that microglia may contribute to circuit-level dysfunction through targeted engagement with synaptic elements. Thus, we quantified microglia-synapse interactions in the PVN by assessing puncta colocalization with reconstructed microglial surfaces. Microglial engagement with inhibitory synapses was significantly reduced at both presynaptic ($p = 0.0100$) and post-synaptic ($p = 0.0271$) terminals, while engagement with excitatory presynaptic terminals was significantly elevated in PH mice ($p = 0.0278$). Altogether, these findings indicate that PH is associated with distinct patterns of synaptic remodeling and differential microglial engagement in the PVN, offering new insight into the structural and neuroimmune mechanisms underlying central autonomic dysfunction in cardiopulmonary disease.

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Nanosymposium

NANO051: Aging: Models to Mechanisms

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Presentation Number: NANO051.01

Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA grant 5R01AG066018-05

Title: Cell-type-specific transposable element demethylation and TAD remodeling in the aging mouse brain

Authors: *R. ZENG[1], W. TIAN[2], H. LIU[3], B. REN[5], M. BEHRENS[6], J. R. ECKER[4];
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Abstract: Aging is a major risk factor for neurodegenerative diseases, yet underlying epigenetic mechanisms remain unclear. Here, we generated a comprehensive single-nucleus cell atlas of

brain aging across multiple brain regions, comprising 132,551 single-cell methylomes and 72,666 joint chromatin conformation-methylome nuclei. Integration with companion transcriptomic and chromatin accessibility data yielded a cross-modality taxonomy of 36 major cell types. We observed that age-related methylation changes were more pronounced in non-neuronal cells. Transposable element methylation alone distinguished age groups, showing cell-type-specific genome-wide demethylation. Chromatin conformation analysis demonstrated age-related increases in TAD boundary strength with enhanced accessibility at CTCF binding sites. Spatial transcriptomics across 895,296 cells revealed regional heterogeneity during aging within identical cell types. Finally, we developed novel deep-learning models that accurately predict age-related gene expression changes using multi-modal epigenetic features, providing mechanistic insights into gene regulation. This dataset advances our understanding of brain aging and offers potential translational applications.

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Nanosymposium

NANO051: Aging: Models to Mechanisms

Location: SDCC Rm 23A

Time: Wednesday, November 19, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO051.02

Topic: C.01. Brain Wellness and Aging

Title: Decoding Regional Drivers of Microglia Aging Via Heterochronic Myeloid Cell Replacement

Authors: C. GIZOWSKI, G. POPOVA, ***O. HAHN**;
Hahn Lab., Calico Life Sci. LLC, South San Francisco, CA

Abstract: Aging, the key risk factor for cognitive decline, impacts the brain in a region- and cell type-specific manner, with microglia in white matter and cerebellum considered among the fastest aging cells. Yet so far, it remains untested if microglia aging is driven through intrinsic mechanisms or is instead primarily influenced by aging in neighboring cells. Here, we describe a scalable, genetically modifiable system for heterochronic myeloid cell replacement, enabling the in vivo reconstitution of young and aged mouse brains with intrinsically young immune cells. We find that reconstituted myeloid cells in the brain adopt region-specific microglia expression, morphology and tiling profiles including a low-density, immune-vigilant state specialized to the cerebellum. Strikingly, in aged brains, these reconstituted cells rapidly acquire aging phenotypes, particularly in the cerebellum—implicating the local environment, rather than cell-autonomous programming, as the principal driver of microglia aging. To this end, we identify STAT1-mediated interferon signaling as one axis controlling microglia aging in the cerebellum, and loss of STAT1 prevents formation of cerebellar aging trajectories in reconstituted cells. Spatial transcriptomics analysis combined with genetic cell ablation models identify rare, regionally restricted Natural Killer cells that serve as the source of interferon signaling critical for triggering

microglia aging signatures in the cerebellum.
Together, our data identify that shifts in microglia with age can be largely attributed to changes in the local brain environment, providing a platform to systematically identify the signaling pathways driving their functional impairment in aged and diseased brains.

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Nanosymposium

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Presentation Number: NANO051.03

Topic: C.01. Brain Wellness and Aging

Support: NSF Grant 2233539
CNU's Office of Undergraduate Research and Creative Activity

Title: The Impact of Intestinal Barrier Integrity on Aging, Muscles, and the Brain

Authors: C. AUBY, H. MOORE, S. LE, D. FOMBY, ***A. SALAZAR**;
Christopher Newport Univ., Newport News, VA

Abstract: Recent experiments have revealed that altered expression of intestinal epithelial junction proteins in *Drosophila melanogaster* can lead to various hallmarks of aging, including modulation of intestinal homeostasis, variations in microbial dynamics, changes in immune activity, stem cell perturbation, and alterations in lifespan. Loss of a specific occluding junction protein, Snakeskin (Ssk), leads to rapid and reversible intestinal barrier dysfunction, altered gut morphology, dysbiosis, and a dramatically reduced lifespan. Remarkably, restoration of Ssk expression in flies showing intestinal barrier dysfunction rescues each of these phenotypes previously linked to aging. Intestinal up-regulation of Ssk protects against microbial translocation, improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. These findings indicate that intestinal occluding junctions may represent prolongevity targets in mammals, in addition to their possible roles in intestinal dysfunction, aging, and disease. This project is investigating the impact of intestinal barrier modification on tissue outside of the gut and address communication between the gut and the brain and muscles in disease models. Results show that perturbing the gut barrier leads to an increase in age-related changes in the muscles and the brain, with accompanying changes in behavior. Current work utilizes cellular and molecular biological methodologies to build upon current knowledge to address crucial questions at the intersection between microbial dysbiosis, epithelial integrity, inflammation, protein aggregation, neurodegeneration, and disease, with the ultimate goal of discovering novel therapies that may enhance barrier function, healthspan, and lifespan.

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Presentation Number: NANO051.04

Topic: C.01. Brain Wellness and Aging

Support: Blavatanik Sensory Disorders
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Title: A genetic dissection of neuronal vulnerability in the cochlea

Authors: *J. A. FRANCO[1,2], T. G. COPELAND[1,3], R. MERROW[1], L. V. GOODRICH[1]; [1]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [2]Dept. of Cancer Biol. and Pediatrics Oncology, Dana-Farber Cancer Inst., Boston, MA; [3]Speech, Hearing, and Biotech. PhD Program, Harvard Univ., Cambridge, MA

Abstract: Neurodegenerative disorders often target specific types of neurons, suggesting that molecular differences determine selective vulnerability. Decoding this relationship typically requires comparing functionally distinct neurons—presenting a serious technical limitation. However, the type I spiral ganglion neurons (SGNs, the primary sensory neurons for hearing) provide a unique system for evaluating neuronal vulnerability among functionally similar subtypes. SGNs undergo age-related synaptopathy that appears to target an anatomically defined cohort preferentially. This synaptopathy mirrors what occurs after excessive noise exposure, where high frequency regions of the cochlea lose synapses, accompanied by diminished auditory nerve activity. With the identification of molecularly distinct SGN subtypes, a clear mapping between this purported anatomical subgroup and the IC subtype emerged, allowing confirmation that there is a disproportionate loss of IC SGNs in aged mice. Here, we tested whether IC SGNs are also more vulnerable to age-related and noise-induced synaptopathy.
To do this, we leveraged a *Ntng1*^{Cre}*;Ai14* mouse line to label IB and IC SGNs. Quantification of synapses revealed a significant reduction of tdTomato associated synapses in old mice relative to young controls, suggesting greater vulnerability of IB/C SGNs over 1As. Since age-related and noise-induced synaptopathy present in a similar fashion, we then asked if SGN subtypes show differential susceptibility to synapse loss following traumatic noise exposure. To better distinguish effects among the three subtypes, we introduced two additional reporter lines (*Calb2*^{CreERT2} *and Lypd1*^{CreERT2}*)* to label IA/B and IC SGNs, respectively. In all cases, noise exposure led to a significant decrease in the total number of high frequency synapses. When assayed for subtype identity, only IC synapse counts were significantly reduced whereas IA/Bs remained unchanged. These findings establish the presence of genetically defined selective vulnerability in SGNs. This leads to the hypothesis that shifting the genetic makeup of SGNs towards that of a more IA like population will expand resilience and reduce synaptopathy. To test this hypothesis, we tested the effects of noise exposure in *Runx1* conditional knock-out (CKO) mice, in which the IA population is significantly expanded at the expense of IB and IC SGNs. We found that *Runx1* CKOs have more synapses following acoustic trauma relative to

controls. This work shows that controlling intrinsic gene expression can shift selective vulnerability and provides an ideal entry point for identifying cellular mechanisms of neurodegeneration.

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Title: The extracellular matrix as a dynamic mediator of synaptic remodeling in aging and brain pathologies

Authors: *C. CODAZZI[1], M. MOROTTI[2], F. D'ALELIO[2], C. CALIGIURI[1], C. FEROLETO[2], C. D'AMELIO[1], I. PAOLETTI[2], G. DE CHIARA[3], C. GRASSI[1,2], L. LEONE[1,2], M. V. PODDA[1,2];
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Abstract: The extracellular matrix (ECM) of the brain, once regarded as a passive structural element, is increasingly recognized as a dynamic regulator of neurobiological processes. However, its role in neuronal vulnerability and synaptic dysfunction during aging and in brain disorders, remains inadequately understood. To investigate this, we used mouse models including: i) aging animals of both sexes at three distinct ages (4, 14, and 24 months); ii) focal ischemic injury induced by photothrombosis; and iii) Alzheimer's disease (AD)-like phenotype induced by Herpes Simplex Virus type 1 infection with recurrent reactivation via thermal stress. To characterize ECM alterations across these models, we employed multidisciplinary methodologies, including immunohistochemistry, Western blotting, and ELISA. Physiological aging prompted complex, sex-dependent ECM remodeling across brain regions. Notably, the expression of metalloproteases (MMPs) 2 and 9 was preserved in aged males but significantly reduced in females, indicating sex-specific regulation of ECM proteolysis. Inhibitory circuits exhibited region- and age-dependent dynamics. VGAT expression demonstrated biphasic modulation, increasing in adult mice and decreasing in older ones. In the hippocampus, parvalbumin (PV) interneurons were significantly reduced with aging, although their association with perineuronal nets (PNNs) was maintained. Conversely, in the prefrontal and motor cortices, aging was associated with increased PNN recruitment around PV cells, suggesting region-

specific stabilization of inhibitory circuits. In the ischemic stroke model, multiple post-ischemic time points were analyzed. During the acute phase, there was intense MMP-2 and MMP-9 activity, leading to ECM degradation and increased blood-brain barrier (BBB) permeability. In the chronic phases, ECM remodeling was characterized by an upregulation of components that inhibit neuronal plasticity. Additionally, PNNs initially showed a reduction followed by a disorganized structure at later stages, similar to observations in aging. In the mouse model of sporadic AD, we observed early ECM remodeling. Initially, dysregulation of ECM-remodeling proteases became evident, contributing to PNN degradation and synaptic instability. In later stages, a compensatory or dysfunctional activation of structural ECM proteins, emerged, notably in association with BBB disruption. Collectively, these findings underscore the ECM as a critical and dynamic interface between aging, injury, and neurodegeneration, revealing both shared and distinct mechanisms that impact synaptic remodeling across different conditions.

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Neuroscience Innovation Foundation
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Title: Meningeal lymphatics-microglia axis regulates synaptic physiology

Authors: *K. KIM[1], J. KIPNIS[2];
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Abstract: Meningeal lymphatics serve as an outlet for cerebrospinal fluid, and their dysfunction is associated with various neurodegenerative conditions. Previous studies have demonstrated that dysfunctional meningeal lymphatics evoke behavioral changes, but the neural mechanisms underlying these changes have remained elusive. Here, we show that prolonged impairment of meningeal lymphatics alters the balance of cortical excitatory and inhibitory synaptic inputs, accompanied by deficits in memory tasks. These synaptic and behavioral alterations induced by lymphatic dysfunction are mediated by microglia, leading to increased expression of the interleukin 6 gene (Il6). IL-6 drives inhibitory synapse phenotypes via a combination of trans-

and classical IL-6 signaling. Restoring meningeal lymphatic function in aged mice reverses age-associated synaptic and behavioral alterations. Our findings suggest that dysfunctional meningeal lymphatics adversely impact cortical circuitry through an IL-6-dependent mechanism and identify a potential targetfor treating aging-associated cognitive decline.

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Nanosymposium

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Presentation Number: NANO051.07

Topic: C.01. Brain Wellness and Aging

Support: UCSF-NORC 2P30DK098722-06

Title: Metabolic Syndrome-Induced Neuroinflammation Drives Lipid Droplet-Accumulating Microglia and Cerebral Small Vessel Disease Pathology

Authors: *M. ZHANG[1], A. LETIAN[2], E. L. GOLDBERG[3], K. ARKELIUS[1], N. SINGHAL[4]; [1]UCSF, San Francisco, CA; [2]Physiol., Univ. of California San Francisco, San Francisco, CA; [3]Univ. of California, San Francisco, San Francisco, CA; [4]Neurol., Univ. of California San Francisco Neurosci. Grad. Program, San Francsico, CA

Abstract: Objective: Metabolic syndrome significantly increases risk for cerebral small vessel disease (CSVD) and vascular cognitive impairment, yet mechanisms linking systemic metabolic dysfunction to cerebrovascular pathology remain unclear. We investigated whether PCSK9 overexpression combined with high-fat diet (HFD) induces CSVD-like pathology and characterized microglial lipid metabolism in disease progression. Methods: C57BL/6J mice were randomized to four groups: control, PCSK9 overexpression, HFD, and PCSK9+HFD. After 4 months, neurovascular coupling was assessed using laser speckle contrast imaging during whisker stimulation. Immunofluorescence evaluated microvascular density (lectin), microglial activation (Iba1), phagocytic activity (CD68), lipid droplet accumulation (BODIPY, Perilipin), astrocyte reactivity (GFAP), blood-brain barrier integrity (ZO-1), perivascular amyloid-β deposition, and white matter damage (Luxol Fast Blue). Three-dimensional cerebrovascular architecture and glial cells were analyzed using light sheet microscopy. Cognitive function was assessed via novel object recognition and open field tests. Results: PCSK9+HFD mice developed severe metabolic dysfunction with elevated serum cholesterol and weight gain compared to controls. Neurovascular coupling was significantly impaired with markedly attenuated cerebral blood flow responses during whisker stimulation.3D light sheet microscopy demonstrated reduced capillary density and diminished vascular complexity. Concurrent 3D imaging revealed increased ramified Iba1+ microglia in close proximity to the altered vasculature. We identified

lipid droplet-accumulating microglia (LDAM) abundant in PCSK9+HFD mice, a novel microglial phenotype. LDAM exhibited amoeboid morphology with Perilipin+ lipid droplets. Additional CSVD pathology included perivascular amyloid-β accumulation, microglial activation, enhanced phagocytic activity, severe astrogliosis, ZO-1 decrease indicating blood-brain barrier disruption, and white matter injury. PCSK9+HFD mice demonstrated impaired recognition memory and decreased exploratory behavior. Conclusions: This study demonstrates that metabolic syndrome converges on neuroinflammatory pathways to induce comprehensive CSVD pathology. The novel LDAM phenotype represents a unique microglial response to metabolic stress, potentially linking systemic lipid dysregulation to cerebrovascular dysfunction. This model provides a robust platform for investigating metabolic syndrome-CSVD mechanisms and testing therapeutic interventions targeting microglial lipid metabolism.

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Topic: C.01. Brain Wellness and Aging

Support: NIA Grant R01AG075000

Title: Attentional plasticity and associated neurophysiological changes in locus coeruleus-salience network connectivity between domain-general and unimodal training paradigms

Authors: *Y.-Y. CHEN[1], E. SEAGO[2], B. KATZ[2], T.-H. LEE[1,3];
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Abstract: Emerging evidence suggests that the locus coeruleus (LC) is intrinsically connected to the salience network (SN), which includes the anterior insula (aINS) and dorsal anterior cingulate cortex (dACC), and that this circuit plays a critical role in processing selectivity. Our recent task-based study further demonstrates that heightened LC-SN connectivity supports the maintenance of attentional performance in the face of distraction, suggesting a compensatory mechanism. However, no studies to date have experimentally modulated LC-SN connectivity through behavioral intervention to test its causal role in selective attention, a critical step toward identifying this pathway as a potential therapeutic target in reducing age-related distractibility. To address this gap, we recruited 45 older adults (M_{age} = 65.29 years, range = 55-75) to complete two attentionally demanding tasks, the Attention Network Test (ANT) and the Place Discrimination Task (PDT), across three fMRI sessions. Between Visit 2 and Visit 3, participants were randomly assigned to one of two 10-day-at-home attention training conditions. One group (domain-specific, n = 23) completed a unimodal training program consisting of tasks closely resembling the PDT. The other group (domain-general, n = 22) completed a multimodal training

program that included adaptive versions of visual search tasks, go/no-go tasks, and processing speed tasks. Following training, the task-dependent LC-dACC and LC-aINS connectivity were examined.
A 3 (visit) × 2 (group) repeated-measures ANOVA revealed a significant interaction between visit time and training group for LC-aINS connectivity during the ANT ($F = 3.87$, $p = .025$), with the domain-general group showing a marked increase only from Visit 2 ($M = -0.014$) to Visit 3 ($M = 0.039$), while the domain-specific group showed no such increase (Visit 2: -0.020; Visit 3: -0.027). Behaviorally, a main effect of visit was found ($F = 11.99$, $p < .001$), but no significant interaction between visit time and training group ($F = 1.10$, $p = .339$), suggesting that the observed connectivity differences were driven by training type rather than changes in task performance. A similar pattern of LC-aINS connectivity was observed for the PDT, although it did not reach statistical significance due to greater variability ($F = 0.84$, $p = .436$).
Consistent with our previous work, this cognitive training study demonstrates that multimodal, domain-general training may enhance LC-SN connectivity, providing preliminary evidence that targeted cognitive interventions may promote neuroplasticity in the LC-SN circuit, potentially leading to improved attentional behavior.

Disclosures: Y. Chen: None. **E. Seago:** None. **B. Katz:** None. **T. Lee:** None.

Nanosymposium

NANO051: Aging: Models to Mechanisms

Location: SDCC Rm 23A

Time: Wednesday, November 19, 2025, 1:00 PM - 3:45 PM

Presentation Number: NANO051.09

Topic: C.01. Brain Wellness and Aging

Support: CIHR MOP180400
CIHR MOP179758

Title: Changes in resting-state functional connectivity of the hippocampus along the longitudinal axis in healthy cognitive aging

Authors: *M. CAHILL[1], J. SERRANO[2], S. HRYBOUSKI[3], P. SERES[2], N. MALYKHIN[2]; [1]Neurosci. and Mental Hlth. Inst., [2]Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. of Pennsylvania, Wallingford, PA

Abstract: Background: The hippocampus (HC) is a vital structure for episodic memory and has been shown to be affected by healthy aging. Hippocampal functional connectivity (FC) to other cortical areas has been shown to differ along its longitudinal axis. Previous structural Magnetic Resonance Imaging (MRI) studies demonstrated that healthy aging differentially affects anterior (aHC) and posterior (pHC) HC. Therefore, the goal of the present study was to examine if healthy aging differentially affects resting-state FC of both the aHC and pHC with cortical regions in a large cohort of cognitively healthy adults.
Methods: 117 healthy participants (20-93 years old, 52 male, 65 female) were recruited. MRI

data acquisition was performed at 3T on a Siemens Prisma scanner. 320 functional volumes were acquired using a T2*-sensitive Gradient Echo Planar Imaging pulse sequence [voxel size: 1.75 x 1.75 x 1.74 mm^3], registered to an anatomical T1-weighted Magnetization-Prepared Rapid Gradient Echo image [voxel size: 0.75 mm^3 isotropic]. These datasets were co-registered to MNI standard space using the Advanced Normalization Tools (ANTs) library. The HC was manually segmented into its aHC (head) and pHC (body+tail) divisions using reliable volumetric methods. FC of the aHC and pHC regions of interest (ROI) was assessed using the CONN functional connectivity toolbox with a voxel threshold of $p < 0.001$ and a cluster threshold of $p < 0.05$ for cluster mass p-FDR-corrected with a minimum cluster size of 10 voxels. The effect of age was also assessed with nonparametric correlations using this toolbox.
Results: Several brain areas were functionally connected to both the aHC and pHC, including the parahippocampal gyrus, amygdala, anterior and posterior cingulate cortex, precuneous, lingual gyrus, middle and superior temporal gyrus, fusiform cortex, temporal pole and the medial and orbital frontal cortex. The aHC was uniquely connected with the inferior, middle and superior frontal gyri, central opercular cortex, left supramarginal gyrus and the left supplementary motor cortex. In contrast, the pHC was uniquely connected with the frontal operculum cortex and the right pallidium. There were no significant effects of age on the aHC's FC. However, increased age was associated with reduced FC of the pHC s with the right middle frontal gyrus ($p<0.047$) and increased FC of the pHC with the left occipital pole ($p<0.047$) and left lingual gyrus ($p<0.047$).
Conclusion: Our results demonstrated that healthy cognitive aging differentially affects FC of the HC along its longitudinal axis and that the significant effects of age on HC FC were found in pHC.

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Nanosymposium

NANO051: Aging: Models to Mechanisms

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Presentation Number: NANO051.10

Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA Grant AG067781
NIH/NIA Grant AG07315
NIA Grant AG072977

Title: Cortical White Matter Integrity in Cognitive SuperAgers

Authors: *A. ZOURIDAKIS[1], I. A. AYALA[1], R. J. CASTELLANI[2], P. JAMSHIDI[2], M.-M. MESULAM[1], E. J. ROGALSKI[3], T. GEFEN[1], C. GEULA[1];
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Abstract: Introduction: SuperAgers are individuals over the age of 80 who demonstrate superior episodic memory equal to that of individuals 20-30 years younger. Previously we observed significantly lower density of activated microglia in the white matter of SuperAgers compared to their cognitively normal peers. Preliminary observations also showed increased immunohistochemically visualized myelin in SuperAger cortical white matter. In this study we utilized western blot analysis to further investigate the integrity of white matter, measured through markers of myelin and axonal integrity. We hypothesized that SuperAgers would display enhanced white matter integrity compared to age-matched cognitively average peers.
Methods: Western blot analysis was performed to detect levels of myelin basic protein (MBP, Abcam EPR21188, rabbit monoclonal, 1:1,000 concentration) and phosphorylated neurofilament H (SMI-31, Millipore Sigma NE1022, mouse monoclonal, 1:1,000 concentration), a marker of axons, in 10 Caucasian participants; 5 SuperAgers and 5 controls. The SuperAging group consisted of 2 females and 3 males, and the control group consisted of 4 females and 1 male. Frozen postmortem human white matter harvested from matching frontal cortex centrum semiovale in coronal slabs was homogenized for western blot, and protein concentrations were measured through BCA protein assay. Optical density of the protein bands was analyzed in ImageJ and expressed as a percentage of the housekeeping protein GAPDH. Students' t-tests were used to compare protein levels across groups.
Results: The SuperAging and control groups did not differ significantly in postmortem interval (PMI) or mean age at death (SuperAgers: M = 90.6 years; controls: M = 88.8 years). Quantitative analysis of the MBP western blots revealed an expected band at 18 kDa, and an additional prominent band at 45 kDa. Both bands appeared darker in the SuperAgers than controls. Levels of the 18 kDa MBP band were significantly higher in the frontal cortex white matter of SuperAgers compared to controls ($p<0.05$). Analysis of the axonal marker SMI-31 revealed a prominent band at the expected molecular weight of ~180 kDa, which was significantly higher in SuperAgers compared to controls ($p<0.05$).
Conclusion: These preliminary results suggest enhanced myelin and axon integrity in the white matter of SuperAgers compared to controls in the frontal cortex, which plays a crucial role in working memory. Further quantitative analysis will include additional specimens and white matter regions, including white matter tracts such as the fornix and corpus callosum.

Disclosures: A. Zouridakis: None. **I.A. Ayala:** None. **R.J. Castellani:** None. **P. Jamshidi:** None. **M. Mesulam:** None. **E.J. Rogalski:** None. **T. Gefen:** None. **C. Geula:** None.

Nanosymposium

NANO051: Aging: Models to Mechanisms

Location: SDCC Rm 23A

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Presentation Number: NANO051.11

Topic: C.01. Brain Wellness and Aging

Support: AG073153
AG067781
AG045571
AG072977

Title: Stable Levels of MAP2K3 in Frontal Cortex of Cognitive SuperAgers and Normal Controls

Authors: T. HILL[1], R. TAEFI[3], E. TAEFI[4], I. A. AYALA[1], A. BAHRAMI[5], E. J. ROGALSKI[6], T. GEFEN[7], M.-M. MESULAM[2], ***C. GEULA**[8];
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Abstract: Mitogen Activated Protein Kinase Kinase 3 (MAP2K3), a member of the MAP kinase signaling family, is a dual specificity serine/threonine cell-signaling kinase activated by stress. The MAP2K3 pathway has been implicated in processes such as embryonic development, cell proliferation, inflammation, β-Amyloid (Aβ) deposition associated with Alzheimer's Disease (AD), and learning and memory. Evidence from the mouse brain indicates high MAP2K3 expression in microglia, which is associated with production of inflammatory proteins. Consistent with the role of MAP2K3 in learning and memory, in a small cohort, we observed lower frequency of two variants of the MAP2K3 gene in cognitive SuperAgers, individuals 80 years or older with memory performance equal to or better than individuals 20-30 years their junior, when compared with age-matched cognitively normal peers with similar demographics. Using two commercially available antibodies to human MAP2K3 in western blots, we were able to detect the protein in different cells. One antibody (Abcam, rabbit monoclonal, 1:2000) preferentially detected MAP2K3 protein in primary human microglia, while the other (ProteinTech, rabbit polyclonal, 1:500) detected the protein preferentially in iPSC derived cortical neurons. The purpose of the present study was to investigate whether levels of the MAP2K3 protein in the frontal cortex distinguish SuperAgers from their cognitively average peers. The two antibodies were employed in western blot analysis of frozen tissue from the middle frontal gyrus of SuperAgers (n=12) and normal controls (n=9). Optical density of bands at the correct molecular weight were quantified using the Image J software. Levels of MAP2K3 were expressed as percentage of the housekeeping protein GAPDH. Both antibodies visualized MAP2K3 in all participants. Overall, levels of MAP2K3 detected with the antibody that recognizes the protein in microglia were significantly higher (M=105.8, SD=30.2) when compared with the levels detected with the antibody that recognizes the MAP2K3 in neurons (M=81.1, SD=32.6) ($p<0.02$). There were no significant differences between the levels of MAP2K3 detected in SuperAgers when compared with normal controls. A comprehensive survey of various cortical areas is required to determine whether levels in the two groups differ preferentially in specific cortical regions. Levels of MAP2K3 demonstrated a great deal of variability in both groups. It remains to be determined if levels of the protein in SuperAgers or controls are linked to inheritance of specific variants of the MAP2K3 gene.

Disclosures: T. Hill: None. **R. Taefi:** None. **E. Taefi:** None. **I.A. Ayala:** None. **A. Bahrami:** None. **E.J. Rogalski:** None. **T. Gefen:** None. **M. Mesulam:** None. **C. Geula:** None.

Nanosymposium

NANO052: Auditory Processing at Multiple Scales

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.01

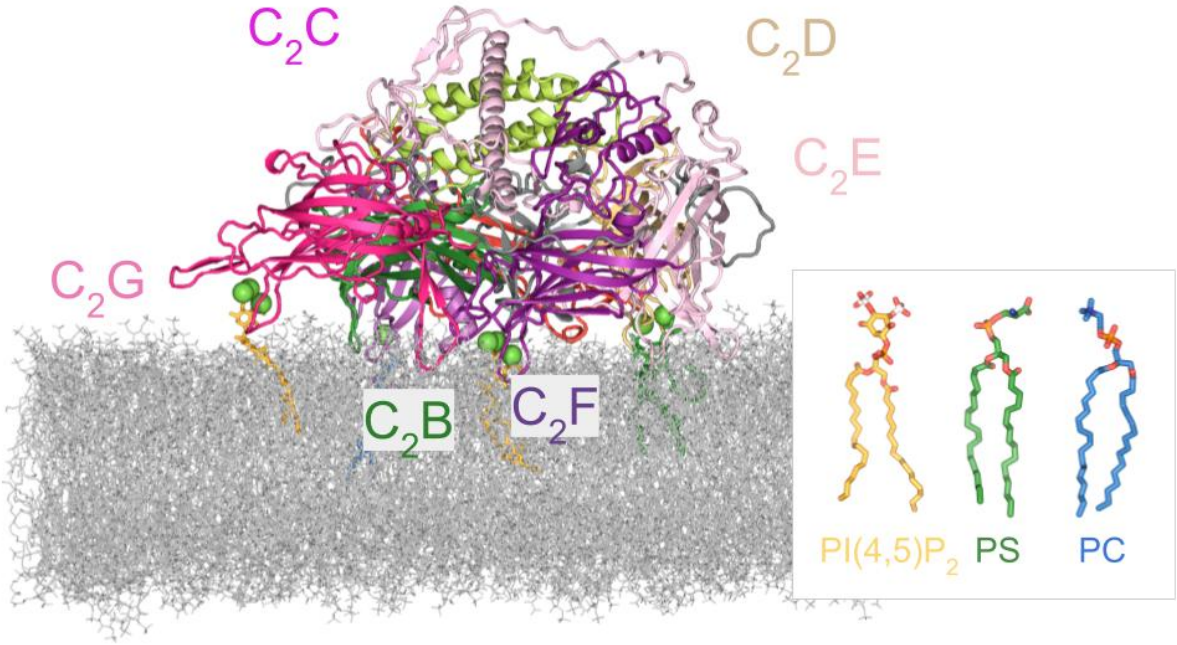
Topic: E.05. Auditory and Vestibular Systems

Support: DFG Grant EXC 2067/1-390729940
DFG Grant CRC 889
DFG Grant CRC 1690
Boehringer Ingelheim Fonds fellowship

Title: Mechanisms of lipid binding by otoferlin studied by molecular dynamics simulations

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Abstract: Hearing is essential for human social integration and quality of life. Up to 7% of hereditary nonsyndromic hearing loss cases are caused by mutations in the *OTOF* gene. Otoferlin, the product of *OTOF*, is expressed in inner hair cells and is implicated in synaptic vesicle fusion. It consists of at least seven C_2 domains arranged in a ring-like shape. Because the C_2 domains were shown to bind Ca^{2+} and lipids in biochemical assays, otoferlin is hypothesized to be the Ca^{2+} sensor for synaptic vesicle release and to serve vesicle replenishment in inner hair cells. However, mechanistic details of otoferlin function remain unknown. In this work, we aim to bridge the gap between otoferlin structure and function by investigating how otoferlin interacts with a model membrane. To this end we set up atomistic molecular dynamics simulations starting from the Ca^{2+}-bound otoferlin cryo-EM structure (domains C_2B-C_2G). At the start of simulations, the structure was positioned above the membrane which consisted of 60% phosphatidylcholine (PC), 30% phosphatidylserine (PS), and 10% phosphatidylinositol 4,5-biphosphate ($PI(4,5)P_2$). Otoferlin reproducibly bound the membrane with all modelled C_2 domains at microsecond timescales. Surprisingly, the number of lipid contacts established by a domain did not correlate with the number of Ca^{2+} ions bound to it. Instead, many contacts were established via direct polar and hydrophobic interactions between the protein and the membrane. We then started an additional set of simulations to test the importance of Ca^{2+} and $PI(4,5)P_2$ for lipid binding. While removal of Ca^{2+} decreased the number of contacts between otoferlin and membrane, masking of $PI(4,5)P_2$ headgroup charges in the parallel simulation did not have an effect. Our biochemical data also suggested that Ca^{2+} plays a more important role than anionic lipids in liposome binding by otoferlin. Overall, our study provides atomistic information on nature and mechanisms of lipid binding by otoferlin. In the future, we plan to investigate the role of Ca^{2+} in otoferlin-membrane interaction in a larger system.



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Nanosymposium

NANO052: Auditory Processing at Multiple Scales

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.02

Topic: E.05. Auditory and Vestibular Systems

Support: Deutsche Forschungsgemeinschaft, CRC 1286, Project A04

Title: Activity-dependent structural changes of murine endbulbs of Held

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Abstract: The auditory pathway involves different kinds of synapses, which are highly specialized in terms of both structure and function. From the peripheral inner hair cells, spiral ganglion neurons form the calyx-shaped endbulbs of Held that project onto spherical bushy cells in the cochlear nucleus (CN). Endbulbs of Held comprise hundreds of individual active zones (AZs) that enable firing with high fidelity. Loss of the hair cell protein otoferlin abolishes exocytosis at the cochlea and results in profound deafness (Roux et al., 2006). Downstream in the brainstem, the input-deprived endbulbs of Held are reduced in size and branching (Wright et

al., 2014) and at individual endbulbs AZs of otoferlin mutant (*Otof*$^{e/-}$) mice, synaptic vesicles (SVs) decline in numbers at 6 months of age (Hintze et al., 2021). Despite showing some degree of degeneration the question remains, if such activity-deprived AZs are still functional throughout life span. Therefore, we investigated structural correlates of endo- and exocytosis upon acute activation comparing wild-type and deaf animals.
Freshly prepared vibratome sections of the anteroventral CN were subjected to chemical stimulation using a high-K^+ solution. We analyzed synapses of wild-type and *Otof*$^{e/-}$ mice at different ages (P40-60, 6 months and ~1.5 years) and at different activity states (stimulated or resting). For a near-to-native structural preservation, we employed high-pressure freezing followed by freeze-substitution. After embedding, endbulbs were imaged using electron tomography. Finally, morphometric parameters such as SV pools were quantified in 3-4 mice per condition.
First results showed a decline of SVs per AZ in *Otof*$^{e/-}$ endbulbs starting at 6 months of age, in line with previous findings (Hintze et al., 2021). Upon chemical stimulation, we observed an elevated count of omega-shapes in the periphery of AZs, especially in young and 6 months old *Otof*$^{e/-}$ endbulbs, which could indicate some degree of functionality. However, docked SVs were already reduced in young *Otof*$^{e/-}$ endbulb AZs, generally implicating a smaller readily releasable SV pool in the mutant. Overall, these results suggest that activity-deprived synapses remain functional over months but might deteriorate at older ages.

Disclosures: J.N. Bahr: None. **C. Wichmann:** None.

Nanosymposium

NANO052: Auditory Processing at Multiple Scales

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.03

Topic: E.05. Auditory and Vestibular Systems

Support: DynaHear (ERC AdG 101054467)
Else Kröner Fresenius Center for Optogenetic Therapies
Volkswagen Foundation and State of Lower Saxony (ZN 3872)

Title: Multiscale analysis of mouse cochlear morphology with high resolution isotropic light sheet fluorescence microscopy and deep-learning segmentation

Authors: *A. DINIZ[1,4,5], L. ROOS[1,5,2], E. KOERT[1,5], M. SCHILLING[6], M. AAKHTE[7], J. HUISKEN[7,8], J. NEEF[1,5], C. PAPE[6], T. MOSER[1,5,9,3];
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Res., Madison, WI; [9]Auditory Neurosci. and Optogenetics Group, German Primate Ctr., Göttingen, Germany

Abstract: The cochlea's complex three-dimensional (3D) architecture poses significant challenges for conventional histological techniques. These techniques require physical sectioning and flattening, which can obscure spatial relationships essential to understanding sensorineural hearing loss. Recent improvements in light sheet fluorescence microscopy (LSFM) allow examination of the intact immunolabeled cochlea with sub-micrometer resolution.
Here, we used a custom-built LSFM with an isotropic resolution of 850nm to perform a multiscale analysis of cochlear morphology from afferent synapses to inner hair cells and spiral ganglion neurons across the organ. We immunolabelled and cleared the intact mouse cochlea with a customized iDISCO$^+$ protocol. We then developed a deep learning-based segmentation model for spiral ganglion neurons (SGNs), inner hair cells (IHCs), and synapses (CochleaNet). The high isotropic resolution of the CTLSM and accurate analysis methods allow for robust quantification of the number and distribution of synapses, IHCs, and SGNs, as well as their molecular subtypes throughout the cochlea. Our preliminary results show 9877+/-2634 SGNs, 782+/-47.34 IHC, and 14+/-3.73 ribbons per IHC in the native mouse cochlea. These results align well with the previous publications.
The combination of robust tissue processing, innovative LSFM, and deep-learning-based image segmentation enables multiscale analysis of cochlear morphology. In addition to deciphering the afferent and efferent connectome of the cochlea, this analysis will facilitate studies of cochlear disease mechanisms and the preclinical development of innovative hearing restoration approaches such as gene therapy and optogenetic cochlear implants.

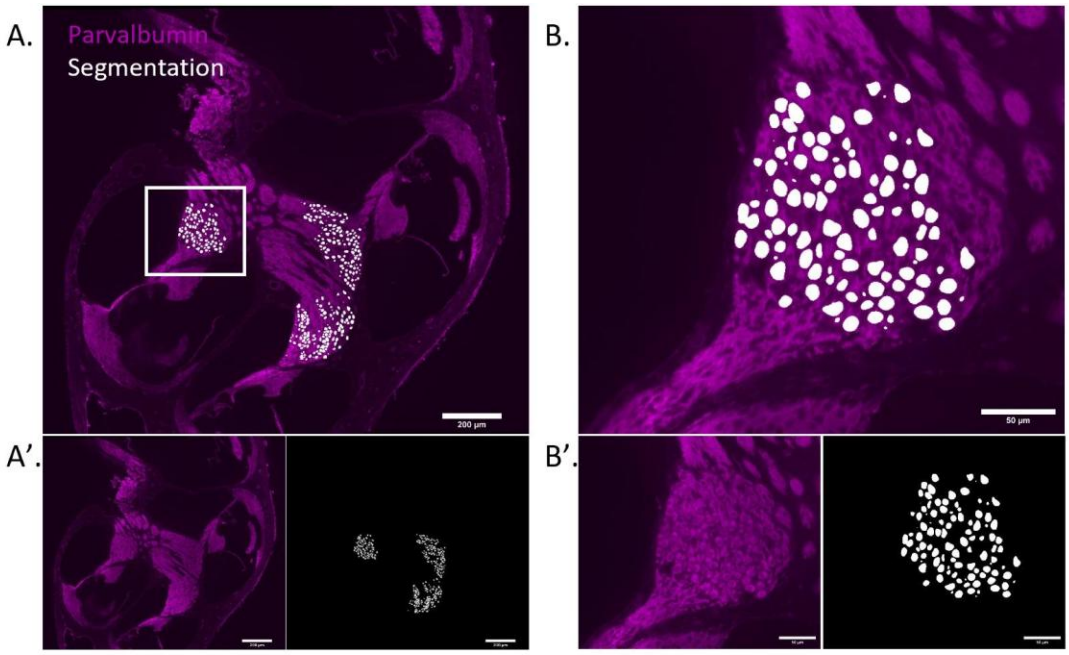


Figure1. A. Optical slice of the cleared tissue light sheet microscope (CTLSM) imaged mouse cochlea labelled with context marker parvalbumin (PV, magenta) labelling all spiral ganglion neurons (SGNs) with the deep-learning-based segmentation mask (white). A'. On the left: PV channel and on the right: segmentation mask. B. 5-times zoomed in region of interest in A. at the basal turn of the mouse cochlea with SGNs. B'. On the left: PV channel and on the right: segmentation mask on the 5-times zoomed in region of interest in A.

Disclosures: A. Diniz: None. **L. Roos:** None. **E. Koert:** None. **M. Schilling:** None. **M. Aakhte:** None. **J. Huisken:** None. **J. Neef:** None. **C. Pape:** None. **T. Moser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OptoGenTech GmbH.

Nanosymposium

NANO052: Auditory Processing at Multiple Scales

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Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.04

Topic: E.05. Auditory and Vestibular Systems

Support: ERC Advanced Grant 101054467

Title: Perturbing Runx1-dependent SGN identity alters presynaptic Ca^{2+}channel gradient via transsynaptic signaling

Authors: *L. BÖSCHE[1,3,4], L. ŠOŠE[1,3,4], N. STRENZKE[1,3,2], B. R. SHRESTHA[6], T. MOSER[1,3,4,5];
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Abstract: The auditory system responds to an astonishing range of sound pressures covering over six orders of magnitude. However, our understanding of the underlying mechanism remains incomplete. While the receptor potential of inner hair cells (IHCs) covers the full range of audible sound pressures, their postsynaptic partners - spiral ganglion neurons (SGNs) - alter their firing only within limited subranges. However, physiological SGN subtypes collectively cover the entire dynamic range. Functional SGN diversity is believed to arise by a combination of heterogeneous IHC active zones and molecularly distinct profiles. Single-cell transcriptomics has revealed three SGN subtypes - I_a, I_b, and I_c - believed to correspond to high, medium, and low spontaneous rate (SR) fibers, respectively. Importantly SGN subtype composition is believed to shape presynaptic IHC active zone properties via transsynaptic signaling, thereby contributing to presynaptic heterogeneity along the modiolar-pillar axis of individual IHCs - essential for sound encoding. To test how altering SGN molecular subtype influences presynaptic heterogeneity, we investigated a Runx1 conditional knockout ($Runx1^{cKO}$) mouse model in which I_b and I_c subtype identities are substantially shifted towards I_a. We hypothesized that this shift would result in an altered transsynaptic signaling at IHC-SGN synapses, thus affecting presynaptic active zone properties along the modiolar-pillar axis. Using spinning disk confocal Ca^{2+} imaging and whole-cell patch-clamp in IHCs, we found that overall amplitude and voltage-dependence of whole-cell Ca^{2+} influx remained unchanged. However, the previously shown spatial gradient of voltage of

half-maximal activation across active zones - typically pillar synapses activating at lower voltages - was abolished in Runx1^{cKO}. This is, to our knowledge, the first evidence that transsynaptic signaling modulates the spatial gradient of voltage dependence of presynaptic Ca^{2+} influx. Unexpectedly, preliminary results from *in vivo* juxtacellular recordings of single SGNs indicated reduced spontaneous and evoked firing rates in Runx1^{cKO} mice, while further work is warranted. Together, our findings indicate that Runx1 not only governs SGN subtype specification but also likely influences presynaptic heterogeneity through transsynaptic signaling. This supports a model in which neuronal identities contribute to shaping synaptic properties crucial for sensory encoding.

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Nanosymposium

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Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.05

Topic: E.05. Auditory and Vestibular Systems

Support: NIH R01DC013826-07
NIH F32 DC018721-01A1

Title: Brain-wide survey of neuroanatomical connections to the auditory cortex of hearing and deaf mice

Authors: *T. HARMON[1], R. D. MOONEY[2];
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Abstract: Advances in technologies for hearing restoration have made it possible to establish hearing to deaf patients with no previous auditory experience. However, fully restoring auditory function requires that neuroanatomical connections to the auditory system in deaf patients are preserved. In addition, expansion of some connections may help compensate for the loss of auditory drive while also endowing the auditory system with new functions. For instance, the auditory cortical neurons in deaf animals can show strong responses to non-auditory sensory stimuli, a phenomenon called cross-modal plasticity. Injection of retrograde neuroanatomical tracers into the auditory cortex of deaf animals reveals expanded connections from adjacent sensory cortical regions, though the same techniques show only small effects on long-distance connections. We took advantage of genetic strategies available in mice to measure neuroanatomical connections to the primary auditory cortex throughout the brains of hearing and deaf mice. Specifically, we injected retrograde viral vectors into the primary auditory cortex of reporter mice that also featured a nullifying mutation to the TMC1 locus, rendering them deaf from birth. We used generalized multivariate linear regression to estimate the influence of auditory experience on presynaptic neuron counts gathered from brain regions in hearing or deaf

mice. This analysis revealed that the size of most regional auditory afferent neuron populations does not change in congenital deafness. However, deafness was predictive of expanded neuroanatomical connections from subregions of the orbitofrontal, ectorhinal, perirhinal, insular, somatosensory, and visual cortex and reduction of neuroanatomical connections from the basomedial amygdala and most thalamic nuclei. In summary, we have found previously unknown neuroanatomical changes to auditory cortical presynaptic neurons in animals with no auditory experience, raising new considerations for efforts to restore full auditory function to congenitally deaf patients.

Disclosures: T. Harmon: None. **R.D. Mooney:** None.

Nanosymposium

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Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.06

Topic: E.05. Auditory and Vestibular Systems

Support: Z01 DC000091

Title: Biocomputational Modelling of MOC Neurons Based on Pharmacologically Informed Ex-Vivo Voltage-Clamp Recordings.

Authors: *S. AMER;
NIH, Bethesda, MD

Abstract: The medial olivocochlear (MOC) neurons are the final efferent neuron in the mammalian auditory system. They are the brain cells that directly innervate the outer hair cells (OHC) of the cochlea, which are implicated in enhancing cochlear vibrations. By inhibiting OHCs, MOC neurons have roles in auditory gain control. In vitro recordings of MOC neurons in mice have shown that these neurons have a capacity for extremely rapid and high amplitude spiking, making them an interesting target to study intrinsic cellular electrical properties. Here we discuss the work that has been done to characterize the behavior of the medial olivocochlear neurons based on selective pharmacological blocking of voltage-gated potassium channels and recreating those behaviors in silico.MOC neurons were identified for patch-clamp electrophysiology experiments in ChAT-IRES-Cre;tdTomato transgenic mice. Cells were recorded in voltage clamp to extract current responses in control conditions as well as with pharmacological blockading of specific potassium channels. Key metrics such as the activation threshold for the channel and peak conductance were described across a population of MOC neurons. Preliminary work is being done extracting channel activity, comparing them to existing models of these channels and examining how they replicate the behaviors seen in MOC neurons. The NEURON software (Carnevale & Hines 1997) was used as the simulation environment to test the extracted features and total cellular response; the morphology of in silico MOCs was defined using information from the literature (Brown and Levine 2008). Current endeavors focus

on modelling the responses of voltage-gated potassium channels including Kv7, Kv4, and Kv2 to better capture the action potential waveform of MOC neurons and recreate the high spiking rate that these cells can produce in vitro and in vivo. Further questions on how to better leverage the population level data that has been collected on these cells, the technical limits of the modelling accuracy we can reasonably extract from whole cell recordings, as well as what kinds of statistical methods we can leverage to better quantify the validity of our outcomes are topics of current investigation. We believe that this technique will help to inform the patch-clamp experimentation in the lab and further our understanding of the role of these cells in the broader efferent auditory system.

Disclosures: S. Amer: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NIDCD.

Nanosymposium

NANO052: Auditory Processing at Multiple Scales

Location: SDCC Rm 24A

Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.07

Topic: E.05. Auditory and Vestibular Systems

Support: NIH Grant R15DC017616

Title: Contributions of sound localization cues to azimuth tuning in the auditory midbrain

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Abstract: Neurons in the central nucleus of the inferior colliculus (ICC), which is a major brain area along the central auditory pathway, exhibit broad, often complex, spatial receptive fields, meaning their firing rates are sensitive to sound source location. Their firing rates are also sensitive to individual cues that vary with source location, including interaural time difference (ITD), interaural level difference (ILD), and spectral shape and acoustic gain in the left and right ears. What remains unknown is how individual cues contribute to a neuron's spatial receptive field when all cues covary together with spatial location. In the present study, the contributions of individual cues to a neuron's azimuth tuning curve (i.e., the receptive field within the front horizontal plane) were determined and analyzed with respect to the neuron's characteristic frequency (CF). Broadband noise stimuli were presented to awake rabbits of both sexes over earphones in virtual acoustic space using the rabbit's own head-related transfer functions. Transfer functions were digitally manipulated in several conditions to fix some cues while allowing others to vary naturally with azimuth in order to assess the contributions of each cue to azimuth tuning. Sound localization cues contributed to a neuron's azimuth tuning curve in a complex manner that depended on both the CF of the neuron and the azimuth of the source. In general, rate coding of azimuth in most low-CF neurons (< 2.8 kHz) was determined by the joint

combination of ITD and one or more of ILD and contralateral-ear gain. Rate coding of azimuth in most high-CF neurons (> 2.8 kHz) was determined by either ILD alone; the joint combination of ILD and contralateral-ear spectrum; or the joint combination of ITD, ILD and contralateral-ear spectrum depending on whether the source was ipsilateral to the recording site, contralateral, or straight-ahead, respectively. The CF transition boundary (~2.8 kHz) between neurons strongly or weakly influenced by ITD corresponded to the acoustic frequency above which ILD range becomes large. Binaural interaction tended to increase the maximum firing rates of low-CF neurons and decrease the minimum firing rates of low-CF neurons, both of which expanded the functional range of rates over which neurons code azimuth. Despite CF-dependent differences in the contributions of localization cues and their effects on maximum or minimum firing rates, rate sensitivity to azimuth was approximately the same, on average, across the tonotopic axis.

Disclosures: E. Garretson: None. **M.L. Day:** None.

Nanosymposium

NANO052: Auditory Processing at Multiple Scales

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Time: Wednesday, November 19, 2025, 1:00 PM - 4:15 PM

Presentation Number: NANO052.08

Topic: E.05. Auditory and Vestibular Systems

Support: MRC MR-Y014693-1

Title: Distinct auditory cortical temporal processing abnormalities contributed by hearing loss and genetic risk for schizophrenia in the Df1/+ mouse

Authors: *C. LU[1], J. F. LINDEN[1,2];
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Abstract: Central auditory processing deficits, such as abnormally elevated duration thresholds for detection of a brief gap in noise, are common in schizophrenia patients (Moschopoulos et al., 2020). However, schizophrenia patients tend to have hearing impairment (Saperstein et al., 2023), and hearing impairment is itself associated with elevated gap-detection thresholds (Fitzgibbons and Wightman, 1982). Do temporal processing deficits associated with hearing impairment and genetic risk for schizophrenia arise from the same mechanisms?
Here, we used the *Df1*/+ mouse model of the 22q11.2 chromosomal microdeletion to address this question. In humans, the 22q11.2 deletion confers ~30% risk of developing schizophrenia and ~60% risk of hearing impairment (Bassett and Chow, 2008; Verheij et al., 2018). The *Df1*/+ mouse has a homologous chromosomal microdeletion and recapitulates many features of the human deletion syndrome, including susceptibility to developmental middle ear problems (Fuchs et al., 2013; Lu & Linden, 2025).
We sought to disentangle the effects of hearing impairment and 22q11.2 deletion on auditory temporal processing by comparing cortical responses to gap-in-noise stimuli between *Df1*/+ mice

with or without naturally occurring hearing impairment and WT mice with or without induced hearing impairment (by removing the malleus bone on P11). We recorded spiking activity of auditory cortical neurons using Neuropixels in awake, head-fixed adult mice passively listening to gap-in-noise stimuli with variable gap durations.
We found distinct effects of hearing impairment and genetic risk for schizophrenia on different measures of temporal processing in the auditory cortex. In mice of either genotype with hearing impairment, both single-unit and population-level measures of neural sensitivity to brief gaps in noise revealed poorer temporal acuity, confirming results from human studies. Meanwhile, in *Df1*/+ mice with or without hearing impairment, fast-spiking units (putative parvalbumin-positive interneurons) exhibited increased sensitivity to noise onsets and offsets.
Our findings demonstrate that hearing impairment and the 22q11.2 deletion generate distinct effects on auditory cortical processing: hearing impairment broadly disrupts temporal acuity, while the *Df1*/+ deletion specifically alters fast-spiking interneuron dynamics. These results highlight separable mechanisms by which hearing impairment and genetic risk for schizophrenia may alter auditory temporal processing.

Disclosures: C. Lu: None. **J.F. Linden:** None.

Nanosymposium

NANO052: Auditory Processing at Multiple Scales

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Topic: E.05. Auditory and Vestibular Systems

Support: MRC Grant MR-Y014693-1

Title: Contributions of sound offsets and onsets to auditory cortical population dynamics evoked by brief gaps in noise

Authors: *Z. LI[1,2], C. LU[3], M. SAHANI[1], J. F. LINDEN[2,3];
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Abstract: Humans and mice can detect brief silent intervals ("gaps") lasting only a few milliseconds within continuous sound. Minimum duration thresholds for gap detection are commonly used as an index of auditory temporal processing ability, but the underlying neural mechanisms are not understood. Previous studies have argued that onset responses to the post-gap sound are most critical for gap detection, while offset responses to the pre-gap sound might amplify cortical sensitivity to the shortest gaps [1,2]. However, in short-gap conditions, neural responses to sound offsets and onsets overlap in time, making it difficult to distinguish their contributions to the brain's representation of the interruption of a continuous sound. Here, we sought to isolate and track onset-specific and offset-specific population dynamics in auditory cortex of awake mice listening passively to gap-in-noise stimuli with varying gap durations, in

order to delineate the relative contributions of sound onsets and offsets to auditory cortical representations of brief gaps in noise. We recorded auditory cortical population activity with single-unit resolution and high temporal precision using Neuropixels probes. Examining well-separated onsets and offsets, we found that population responses evoked by the different events explored different, although non-orthogonal, activity subspaces. We then used normalized-covariance-based techniques to ask how population activity evoked by brief gaps in noise aligned with these subspaces. These methods revealed strong contributions of both onset-specific and offset-specific neural population dynamics to cortical representations of gaps in noise, even when the gap duration was so short (e.g., 2-8ms) that offset and onset responses would have been indistinguishable at a single-neuron level. Further application of the method to data from mouse groups varying in genotype (wild-type or 22q11.2 deletion model) and hearing condition (with or without conductive hearing loss) revealed that peripheral hearing loss impaired both offset-specific and onset-specific neural population dynamics evoked by gaps in noise. These results show that covariance-based population analysis can be used to resolve population coding of closely timed events that evoke overlapping patterns of neural activity. We conclude that both offset and onset responses contribute to auditory cortical population dynamics evoked by very brief silent gaps in noise.[1] Weible, Moore, Liu, DeBlander, Wu, Kentros & Wehr M (2014). *Curr Biol* 24:1447-1455. [2] Weibel, Yavorska and Wehr (2020). *Cereb Cortex* 30:3590-3607.

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Nanosymposium

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Presentation Number: NANO052.10

Topic: E.05. Auditory and Vestibular Systems

Support: NIH R01 DC004290

Title: The human insula reimagined: Single neurons respond to simple sounds during passive listening

Authors: *J. I. BERGER[1], H. KAWASAKI[1], M. I. BANKS[2], S. KUMAR[1], M. A. HOWARD, III[1], K. V. NOURSKI[1];
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Abstract: There are a limited number of previous studies suggesting that posterior insula is sensitive to sounds. However, these studies almost always involved behaviorally-relevant stimuli. Further, auditory response properties of the insula have not previously been studied at the level of single neurons, while reports of single neurons in human auditory cortex are extremely rare, with only a few published studies to date. Here, we provide the first report of human single neuron data recorded from the insula and provide comparative data from the adjacent primary auditory cortex, recorded intracranially in human participants during passive

listening. Participants were neurosurgical patients undergoing intracranial-EEG monitoring for medically-intractable epilepsy. Stimuli were click trains and pure tones (0.25-8 kHz). Over 370 single neurons were recorded in 12 participants to each stimulus type from auditory cortex and insula. In auditory cortex, 84% of all recorded neurons were click-responsive and 96% were responsive to tones. Remarkably, over a quarter of neurons in posterior insula and a smaller subset in anterior insula also responded to clicks and pure tones. Responsive neurons were distributed throughout posterior and anterior insula and showed preferred frequency tuning, as did those in auditory cortex. Onset latencies in the insula were similar to those in the primary auditory cortex but response durations were significantly shorter. The data add to the highly limited literature of human single neuron auditory cortex recordings and show that insula neurons respond to auditory stimuli even in non-behaviorally relevant contexts. The results change our understanding of the insula cortex in the context of audition, suggesting that processing basic auditory stimuli is an important integrative function of insular cortex, which may result from direct connections from auditory thalamus.

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Nanosymposium

NANO052: Auditory Processing at Multiple Scales

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Presentation Number: NANO052.11

Topic: E.05. Auditory and Vestibular Systems

Support: NSF Grant 2319491

Title: Pre-stimulus network dynamics track trial-by-trial behavioral uncertainty during auditory learning

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Abstract: Understanding the neural underpinnings of individual variability in learning requires examining brain states beyond simple task-evoked responses. The pre-stimulus period, rather than being a neutral baseline, may harbor critical neural dynamics influencing subsequent cognition. We hypothesized that network-level spatiotemporal patterns like cortical traveling waves (Muller et al. 2018; Zhang et al. 2018) may reflect dynamic brain states linked to evolving internal belief states that predict upcoming perceptual encoding and learning outcomes.Here, we analyzed high-density electrocorticography (ECoG) data from English-speaking human participants performing a challenging perceptual category learning task where they learn to identify Mandarin tone categories (Yi et al. 2021). Behaviorally, participants exhibited highly variable, non-monotonic learning of the tone categories. To understand the factors that underlie

these learning dynamics, we developed a belief updating model and quantified trial-by-trial behavioral uncertainty as Shannon entropy. We hypothesized that behavioral uncertainty on each trial would be related to pre-stimulus (500ms before sound onset) brain states, which we characterized as theta band (4-7Hz) traveling waves.We found distinct patterns of theta traveling waves propagating across widespread cortical regions in the pre-stimulus period. Importantly, these wave patterns changed dynamically on a trial-by-trial basis. Critically, specific pre-stimulus traveling wave patterns significantly correlated with, and predicted, concurrently modeled trial-specific behavioral uncertainty (entropy). Specifically, one wave pattern across the lateral sensorimotor and auditory cortex consistently preceded high-entropy (low certainty) trials, while a distinct pattern preceded low-entropy (high certainty) trials. Furthermore, modeled belief states evolved dynamically as learning progressed.Our findings suggest the pre-stimulus period contains rich, dynamic network-level neural activity, specifically theta traveling waves, reflecting trial-by-trial fluctuations in an individual's internal belief state during learning. This framework moves beyond traditional accuracy metrics, enabling tracking of more nuanced cognitive dynamics. Understanding these pre-stimulus ECoG patterns and their link to subjective uncertainty provides a novel avenue for investigating neural mechanisms of individual learning variability and cognitive performance.

Disclosures: D. Lee: None. **A. Qin:** None. **M.K. Leonard:** None.

Nanosymposium

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Presentation Number: NANO052.12

Topic: E.05. Auditory and Vestibular Systems

Support: NIH Grant R01-DC04290

Title: Differential processing of clear and spectrally degraded speech in the human cortex revealed by intracranial electrophysiology

Authors: *K. V. NOURSKI[1], M. STEINSCHNEIDER[2,1], A. E. RHONE[1], A. J. BILLIG[3], E. R. DAPPEN[1], I. S. JOHNSRUDE[4], M. A. HOWARD, III[1];
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Abstract: Cochlear implants (CIs) are the treatment of choice for severe to profound hearing loss. Despite progress in CI technology, there is considerable variability in outcomes. The function and plasticity of central auditory pathways contribute to this variability. While assessing cortical processing in CI users is methodologically difficult, spectrally degraded sounds presented to normal-hearing listeners can approximate the CI’s input to the central auditory system. A previous intracranial electroencephalography (iEEG) study used spectrally degraded (noise-vocoded) speech tokens in a phonetic behavioral task (Nourski et al., 2024, Front Hum

Neurosci 17:1334742). Differential responses to clear and vocoded stimuli could present as a "clear-preferred" or a less common "vocoded-preferred" pattern. This study sought to further characterize the vocoded-preferred pattern using clear and vocoded sentences. Participants were adult neurosurgical epilepsy patients undergoing chronic iEEG monitoring. Speech sentences (1.3-3.7 s duration) were degraded using a 3-band noise vocoder. Cortical activity was recorded using depth and subdural electrodes. Electrode coverage included superior temporal plane, superior temporal gyrus, dorsal and ventral auditory-related areas, prefrontal, and sensorimotor cortex. Analysis of iEEG data focused on event-related band power (ERBP) in canonical bands (theta through high gamma). Differences between responses to clear and vocoded sentences were established by cluster-based permutation tests. Differential responses to clear and vocoded sentences typically manifested as ERBP increases in high and low gamma bands and decreases in beta, alpha and theta bands. These responses could persist throughout the sentence duration. The vocoded-preferred pattern was less common than clear-preferred. Vocoded-preferred responses occurred bilaterally in the superior temporal plane, to a lesser extent in posterior regions of the superior and middle temporal gyri and inferior parietal cortex and were virtually absent in prefrontal cortex. The results emphasize the role of auditory cortex and the dorsal stream in processing degraded speech. Clear-preferred responses are likely driven by spectral complexity and intelligibility. Vocoded-preferred responses may reflect processing demands associated with challenging listening conditions. Cortical regions that are differentially activated by clear and vocoded speech may have diagnostic and prognostic utility and present potential targets for neuromodulation-based CI rehabilitation strategies.

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Nanosymposium

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Topic: E.05. Auditory and Vestibular Systems

Support: NIH Grant R01-GM109086
NIH Grant R01-DC04290

Title: Intracranial electrophysiology of cortical responses to speech in delirium

Authors: *E. R. DAPPEN[1], A. J. BILLIG[2], A. E. RHONE[1], M. STEINSCHNEIDER[3], M. I. BANKS[4], K. V. NOURSKI[1];
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Abstract: Speech processing involves multiple hierarchically organized regions. Higher-order cortical areas involved in speech comprehension are sensitive to changes in level of consciousness. Delirium, an acute disorder of consciousness, is characterized by fluctuating symptoms that include inattention, disorganized thinking, confusion, emotional changes, hallucinations, hypo- or hyper-active behaviors, and impaired speech communication. This study examined cortical responses to conversational speech during delirium. Participants were adult neurosurgical patients who were diagnosed with delirium while undergoing intracranial electroencephalography (iEEG) monitoring for medically refractory epilepsy. Delirium assessments were verbally administered twice daily and following seizures. Audio and iEEG data were collected simultaneously during assessments. Suppression indices (SI) were calculated as the difference between the average broadband gamma (30-150 Hz) neural activity during the interviewer's speech and participant's own speech, divided by their sum. SI at each recording site were compared between delirium-negative and -positive conditions. Cortical processing was examined using a linear modeling approach (temporal response function, TRF). TRF output (model prediction accuracy) was compared between delirium-positive and -negative conditions for acoustic, sub-lexical, lexical, and semantic features of speech. Sites with a SI near zero in the delirium-negative condition often exhibited significantly higher or lower SI during delirium, reflecting a global imbalance in processing of self-generated speech. Auditory areas within superior temporal cortex, as well as middle temporal and inferior frontal cortices showed greater SI during delirium. Auditory core cortex, middle frontal gyrus, and sensorimotor cortex showed similar SI in delirium-positive and -negative conditions. TRF analysis revealed lower prediction accuracy in delirium for lexical and semantic speech features in frontal and parietal regions. Changes in SI and TRF model prediction accuracy may reflect delirium-related impairments impacting speech comprehension. SI changes during delirium may reflect impaired executive control and contribute to broader deficits in processing of self-related stimuli. Reduced TRF prediction accuracy suggests disruptions in speech processing beyond acoustic attributes. This study expands our knowledge of the impact of delirium on cortical auditory processing.

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Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

Location: SDCC Rm 30

Time: Wednesday, November 19, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO053.01

Topic: E.06. Vision

Title: Macaque gaze behavior reflects sensitivity to local statistical structure

Authors: *M. TIULENEVA[1], C. R. PONCE[2];
[1]Harvard, Boston, MA; [2]Neurobio., Harvard Univ., Boston, MA

Abstract: Visual exploration in primates is well-adapted to natural scenes, yet it is unclear whether gaze behavior is primarily guided by local, low-level features or by higher-order semantic representations. Here, we used a preferential-viewing paradigm in macaque monkeys to disentangle these influences. Monkeys freely viewed pairs of images while eye movements and neuronal activity in V1, V4, and PIT were recorded. Stimuli comprised (1) real-world photographs of conspecifics, other mammals, non-primate animals (e.g., mammals, amphibians), and inanimate objects; (2) generative network-based images containing natural textures and photorealistic object-like features; (3) and unstructured images (white noise). We found that monkeys showed a graded preference for photorealistic images based on the visual similarity to images of conspecifics. When presented with generative textures versus white noise, monkeys strongly preferred the generative stimuli, indicating sensitivity to visual regularities. However, monkeys did not consistently prefer randomly sampled photographs over generated images, suggesting that local cues alone could attract gaze independently of semantic content. To probe the role of global configuration, we disrupted the overall shape of the photorealistic images using CNNs, preserving local patches. Monkeys still favored images containing visual patches related to conspecifics, demonstrating that local features drive viewing bias even when global form is disrupted. We also targeted local features while keeping global structure intact. This changed gaze patterns, suggesting these features directly modulated saliency. Collectively, our findings indicate that macaque gaze preferences are primarily influenced by local image statistics rather than by semantic object identity. Although preferences for conspecifics remain robust, they appear to rely on mid-level feature patches rather than holistic configurations. These results suggest that the primate visual system's emphasis on local features for guiding spontaneous gaze. Next, we ask to what extent visual behavior can be predicted by neuronal representations in the ventral stream, and whether neural saliency maps based on the firing rates of local feature detectors can account for this behavior.

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Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

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Kenzi Valentyn Vision Research Award, McPherson Eye Research Institute

Title: The primary visual cortex plays a crucial role in motion transparency

Authors: B. GHIMIRE[1], S. WIESNER[1], *X. HUANG[2];
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Abstract: Motion transparency refers to the perception of overlapping stimuli moving transparently against each other as segregated surfaces. Understanding the mechanism underlying motion transparency can provide insight into the fundamental visual processes of motion segmentation and integration. In the hierarchical motion processing pathway, area MT is thought to be important for motion transparency. However, the role of V1 has been controversial. Previous psychophysics studies have shown that when random dots moving in two directions are locally paired, motion transparency is abolished. When the direction separation (DS) between two component directions is less than 120º, the paired-dot stimuli elicit the perception of an integrated, vector-averaged (VA) direction. In comparison, unpaired-dot stimuli elicit the perception of the component directions. In a psychophysics study, we discovered that the critical spatial separation between a paired dots moving in different directions that changes the perception from VA direction to motion transparency closely matches the receptive field (RF) sizes of V1 neurons at corresponding eccentricities. These perceptual findings raised the possibility that V1 may be essential for motion transparency. To test this hypothesis, we recorded from direction-selective neurons in V1 of awake macaque monkeys performing a fixation task. First, we varied the VA direction of paired- and unpaired-dot stimuli with a DS of 90º to characterize the tuning to the bi-directional stimuli. The random dots had a pathlength of 0.4º and a lifetime of 80 ms. We found V1 neurons' responses to paired- and unpaired-dot stimuli changed from better representing the VA direction to component directions, consistent with perception. Second, we used unpaired-dot stimuli that had a lifetime as long as the stimulus duration and with DSs of 60º and 90º. We found that V1 response to the bi-directional stimuli followed a soft-max operation, roughly corresponding to the stronger response to the individual component direction when presented alone. This allows V1 neurons to represent the component directions that match their preferred directions (PDs), thereby facilitating motion segmentation. Third, we recorded neuronal responses across many trials to the unpaired-dot stimuli, consisting of two components moving in the PD and 90° away from the PD. We found that the spike-count distribution to the bi-directional stimuli across trials closely followed the PD component and was only slightly influenced by the non-PD component. Taken together, these results suggest that V1 plays a crucial role in motion transparency, which was previously underappreciated.

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Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

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Topic: E.06. Vision

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Title: Functional and causal roles of macaque areas MT and FST in 3D motion perception

Authors: *Z. ZHU[1], L. W. THOMPSON[3], N. SRIRAM[4], M. ABDALAZIZ[5], R. DOUDLAH[2], B. KIM[6], A. ROSENBERG[7];
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Abstract: Visual perception of three-dimensional (3D) object motion begins with the processing of a pair of eye-specific patterns of two-dimensional (2D) retinal motion. In the macaque middle temporal (MT) area, most neurons are selective for 2D retinal motion. Downstream of MT, in the fundus of the superior temporal sulcus (FST), 2D and 3D motion are represented by similar proportions of neurons. We previously introduced a hierarchical model for computing 3D motion in which ocular dominance (OD) serves to label the output of 2D retinal-motion selective neurons, providing downstream neurons with the eye-specific 2D retinal velocities necessary to compute 3D motion.
Here we applied functional and causal techniques to test this labeled-line model while two monkeys performed a 3D motion (toward/away) discrimination task. A key prediction of the model is that OD is essential for 2D retinal-motion selective neurons to contribute to 3D motion computations. First, we trained a linear decoder to classify toward/away motion based on the responses of 2D or 3D selective MT and FST neurons. Second, we calculated choice probabilities (CPs) for the same neurons using the monkeys' behavioral ('toward'/'away') reports. Consistent with the model, neurons with both stronger 2D direction selectivity and stronger OD had larger decoder weights and CPs, implicating them in the downstream computation of 3D motion. For 3D motion-selective neurons, stronger direction selectivity predicted larger decoder weights, independent of OD, and the average CP was significantly greater than chance. We then causally tested the model by applying electrical microstimulation (EM) to 2D or 3D selective neurons in MT or FST during the toward/away discrimination task. Supporting the model, behavioral biases elicited by the EM of 2D selective neurons were determined by the combination of direction preference and OD. Importantly, the biases were inconsistent with an alternative model in which utrocular (eye-of-origin) signals could allow 3D motion to be directly inferred from the responses of 2D selective neurons. These results imply that neurons which carry eye-specific 2D retinal velocity signals subserve the downstream computation of 3D motion. Finally, behavioral biases elicited by the EM of 3D selective neurons were predicted by stronger direction selectivity, unmoderated by OD. These results collectively support a hierarchical, labeled-line model for the computation of ecologically relevant 3D object motion across macaque areas MT and FST.

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Nanosymposium

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Title: Integration of Motion and Form Cues in the Human Brain

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Abstract: Although brain areas responsible for processing motion and form cues have been well identified, the neural mechanism serving the integration of these two cues remains largely unknown. To address this question, we examined the influence of perspective cues on perceived speed during object motion and the brain areas involved in this process. We conducted an fMRI experiment to identify cortical areas that respond to the perceived speed influenced by perspective cues. We tested two display conditions: in the moving condition, a sphere object moved on a textured ground plane that provided perspective information for depth; in the static condition, the sphere object remained static throughout the trial. The sphere object was placed at either a near (9.1° below the horizon) or a far (0.6° below the horizon) distance from the observer but moved at the same speed. We adopted a block design, and each block corresponded to one of four experimental conditions (2 display conditions × 2 object distances). A task-irrelevant fixation color discrimination task was used during scanning. The stimulus was validated in a psychophysical experiment before scanning, confirming that the perceived speed was effectively modulated by perspective cues. For each participant, typical visual ROIs and the ROIs previously reported to respond to motion and form processing were identified using standard localizers (i.e. V1, V2, V3d, V3v, V3a, KO/V3b, V7, LOC, V4, MT, and MST). We first performed the general linear model (GLM) analysis for all ROIs. Then, we performed ROI-based multivoxel pattern analysis (MVPA) to identify brain areas involved in integrating motion and form cues. MVPA results found that only the ventral visual area LOC showed significantly higher decoding accuracy of near versus far backgrounds for the moving-object than the static-object condition, indicating that LOC is specifically sensitive to perceived speed modulated by perspective cues rather than to background differences alone. No such difference was found in the GLM analysis. Our results provide direct evidence showing that area LOC does not simply respond to motion and form cues but integrates these two cues for the perception of object motion.

Disclosures: X. Shen: None. **H. Liang:** None. **Y. Lian:** None. **L. Li:** None.

Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

Location: SDCC Rm 30

Time: Wednesday, November 19, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO053.05

Topic: E.06. Vision

Support: STI 2030 Major Projects 2021ZD0200401
The National Natural Science Foundation of China (U20A20221 to A.W.R)
The National Natural Science Foundation of China (819611280292 to A.W.R)

Title: From sensation to action: distributed mesoscale dynamics in the primate brain

Authors: ***S. SHI**[1], M. CHEN[1], Z. QUAN[1], M. QIAN[2], H. TANIGAWA[1], K. JIA[1], X. ZHANG[1], G. PAN[1], A. W. ROE[2,3];
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Abstract: Understanding how neural activity supports perceptual decisions is a fundamental question in systems neuroscience. While single-neuron studies in primates have uncovered choice-related signals, how such information integrates across mesoscale networks remains poorly understood. To address this, we used high-resolution 7T fMRI in macaques performing a contrast discrimination task, providing a framework to quantify mesoscale dynamics that link sensation to action. Each trial began with 2 s of fixation, followed by 6 s of bilateral gratings: one fixed at 50% contrast, the other varied (10-90%). After fixation offset, subjects made a saccade to the higher-contrast side. Imaging was performed at 0.8-1 mm isotropic resolution with custom coils. Trials (n = 1,761 across 18 sessions) were modeled using GLM with FDR correction. Inter-trial intervals of 10 s minimized overlap between hemodynamic responses. To characterize task-phase dynamics, we modeled voxelwise timecourses using GLM regressors and fixation-period slopes, which revealed three distinct functional response groups. Group A encompassed early visual areas (V1, V2 and V4), showing rapid stimulus-locked responses that scaled with test contrast. Even for identical stimuli, contralateral areas responded more when that side was chosen, suggesting that behavioral choice may shape early sensory representations. Group B included associative regions such as the intraparietal sulcus (e.g., LIP) and lateral prefrontal cortex (45a/b), where activity ramped throughout the fixation period and encoded the subject's eventual choice. These signals were strongly lateralized and extended into subcortical structures like the superior colliculus and caudate nucleus, forming a distributed network that may encode decision variables prior to action. Following saccade onset, Group C regions became engaged, including posterior IPS, M1, and anterior PFC (e.g., FEF, 46v). Spatial gradients across IPS, premotor cortex, and PFC reflected a transition from fixation- to action-related signals, with overlapping zones in mid-IPS and F4 suggesting dynamic reconfiguration. Action-related areas, including M1, also exhibited choice-specific encoding, thereby closing the loop from sensory

evidence to motor output. Our results reveal spatiotemporally organized mesoscale dynamics that link sensation to action in the primate brain. By identifying potential decision-relevant signals prior to action, this noninvasive approach offers a functional map for targeting distributed circuits in brain-machine interface applications.

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Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

Location: SDCC Rm 30

Time: Wednesday, November 19, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO053.06

Topic: E.06. Vision

Support: STI2023-Major Projects(2022ZD0208200)

Title: Learning-induced uniform background facilitation in primate primary visual cortex

Authors: *R. GUO[1], W. LI[2], Y. YAN[3];
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Abstract: It is widely accepted that perceptual learning enhances neuronal responses to task-relevant target figure while suppressing responses to task-irrelevant background noise. Training can induce a push-pull response pattern in V1 neurons. However, this pattern is typically defined relative to neuronal responses to background stimuli presented without a target. It remains unclear whether and how, learning alters this baseline response to target-absent background stimuli.Monkeys were trained to perform saccadic responses to orientation singletons embedded in backgrounds of uniformly oriented bars. V1 neuronal activities were recorded using implanted microelectrode arrays throughout the training period.Building on our finding that V1 neurons' initial burst response remains stable during learning, we normalized each neuron's daily responses using the peak response amplitude during this early epoch. We found that the normalized V1 responses to the background pattern increased significantly during the delayed response epoch as training progressed. Notably, this learning-related enhancement was orientation-specific. After the training phase, we rotated the orientation of the bar elements by 90°. The V1 response amplitude of the delayed epoch dropped significantly, indicating that the learning effect did not generalize to untrained orientations. This specificity helps to rule out the possibility that this learning effect is caused by non-specific factors.Together, learning effects in the orientation singleton detection task comprised two components: (1) changes in the contrast between push-pull modulation. (2) changes in the neural response to the background pattern that serves as the baseline reference. To isolate the influence of the latter, we analyzed changes in neuronal encoding under behaviorally saturated conditions—where the contrast between push-pull modulation remained constant throughout learning. As neuronal responses to the

background pattern increased, the encoding performance of push-group neurons declined, while that of pull-group neurons improved. This shift may reflect that elevated background responses provide a greater dynamic range for suppression-based encoding. Furthermore, model simulations revealed that an elevated baseline reference improves target location encoding accuracy. Consistent with this, eye movement data showed that the deviation in saccade landing positions toward the target decreased after training.These findings reveal an important and previously overlooked component of visual perceptual learning and shed new light on the neural mechanisms underlying its orientation specificity.

Disclosures: R. guo: None. **W. Li:** None. **Y. Yan:** None.

Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

Location: SDCC Rm 30

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Presentation Number: NANO053.07

Topic: E.06. Vision

Support: National Science and Technology Innovation 2030 Major Program 2022ZD0204802

Title: From decision to perception: generalized visual learning through sensory reactivation

Authors: *Y. SONG, Q. WANG, F. FANG;
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Abstract: Despite requiring thousands of training trials, traditional perceptual learning yields limited transfer effects, constraining both practical applications and ecological validity. This limitation may originate from the learning paradigm's reliance on early-stage cortical plasticity. Challenging this paradigm, we demonstrate that just 5 trials/day of visual motion direction discrimination training over 8 days elicits significant behavioral improvements (>40%)—achieving comparable effects to extensive protocols while exhibiting superior generalization. This striking efficiency advantage raises a central question: How does such brief reactivation (5 trials/day) support robust, generalized learning?
To address this, functional MRI reveals that learning is initially driven solely by plasticity in the intraparietal sulcus (IPS), a region associated with perceptual decision-making. Surprisingly, at two weeks after training—without any further practice—learning effects emerge in early visual cortex (EVC), suggesting delayed functional reorganization in a top-down hierarchy.
Extending these observations, MEG temporally resolves the plasticity with millisecond precision: learning initially engages only late IPS processing (250-300 ms) but spontaneously shifts to early EVC processing (50-110 ms) two weeks post-training, accompanied by accelerated encoding. The data further reveal the transfer mechanism: decorrelation of motion-direction representations expands neural distances across all tested directions without altering mean activity and noise level.

These findings converge on a reverse hierarchical two-stage plasticity model: immediate higher-order circuit optimization precedes delayed sensory refinement, which may emerge implicitly through natural visual experience during post-training daily life. This framework redefines minimal requirements for visual learning, proposing a dynamic model where offline sensory reactivation enables the transfer of visual learning.

Disclosures: Y. Song: None. **Q. Wang:** None. **F. Fang:** None.

Nanosymposium

NANO053: Visual Cognition in Higher Areas: Motion Perception to Decision-Making

Location: SDCC Rm 30

Time: Wednesday, November 19, 2025, 1:00 PM - 3:00 PM

Presentation Number: NANO053.08

Topic: E.06. Vision

Support: BioTechMed-Graz Young Researcher Group Grant

Title: Early visual cortex represents illusory content in the composite face illusion

Authors: L. GÖNITZER[1], *N. ZARETSKAYA[1,2];
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Abstract: Holistic face perception is the tendency of the brain to process faces as unified wholes rather than isolated features. It serves as a well-established hallmark of human social cognition. One of the demonstrations of this holistic face perception is the composite face effect, where aligning the top half of one face with the bottom half of a different face makes it difficult to perceive each half separately, leading us to perceiving a completely new, distinct face. While higher order visual brain regions like the fusiform face area are well known to be involved in holistic face perception, predictive coding models suggest that early visual regions may also contribute to holistic face perception by processing the feedback signals from higher areas. The present functional Magnetic Resonance Imaging (fMRI) study tested this using multivoxel pattern analysis. 28 human participants viewed upright and inverted composite faces during scanning, with stimuli precisely controlled for low-level visual features. A functional localizer was used to define regions of interest (ROIs) representing the top and bottom face halves in areas V1-V3. Support vector machine classification was used to decode information about face identity from early visual cortex activation patterns in these ROIs. Classification accuracies were significantly above chance in the top visual field representation for two identical face parts when their bottom parts were different. Presenting an upside-down analogue of this configuration (i.e., identical mouths and different eyes of an upside-down face) significantly reduced classification accuracy in accord with the disruption of holistic face processing for inverted faces. No corresponding effects were found in the ROI representing the bottom half of the face. An additional behavioral task outside of the scanner confirmed that participants indeed experienced the composite face effect. An additional control analysis ruled out that the effect stems from a generally better decodability of eyes compared to mouths in the top ROI. Together, the findings

demonstrate that the early visual cortex carries decodable signals related to holistic face perception, supporting interactive models of vision in the context of face processing.

Disclosures: L. Gönitzer: None. **N. Zaretskaya:** None.

Nanosymposium

NANO054: Novel Biochemical and Molecular Techniques

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO054.01

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: Elsa U. Pardee Foundation
Central Michigan University Office of Research and Graduate Studies

Title: Developing and optimizing bioluminescent neurotransmitter sensors and neurotransmitter dependent neuromodulators

Authors: *K. TAYLOR[1], H. GALVIN[1], E. D. PETERSEN[2];
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Abstract: Many neurological diseases such as Alzheimer's Disease, Parkinson's Disease, and autism spectrum disorder have been shown to be associated with neurotransmitter disfunction or imbalance. Expanding on the types of neurotransmitters and methods that can be used to study them is important for revealing disease mechanisms and creating new treatments. In this study, we focus on gamma-aminobutyric acid (GABA) and acetylcholine (ACh), which are neurotransmitters found throughout the brain and are involved in many neurological disorders. We developed a variety of genetically encoded bioluminescent GABA and ACh sensors that are an attractive alternative to using fluorescent sensors because they do not require an excitation light source, allowing deeper areas of the brain to be recorded without damaging tissue and improving signal-to-noise ratio due to the lack of autofluorescence. We created a library of bioluminescent GABA and ACh sensor variants based on our prior glutamate sensors and tested them for responses to GABA and ACh respectively. Taking bioluminescence readings on a plate reader, we found that the sensors with a mutated GABA or ACh binding domain as well as optimized linkers and transmembrane domains have higher responses to saturating amounts of neurotransmitter than our initial prototypes. To further improve the response of the sensors to neurotransmitters with the goal of using them to image brain activity in rodents, we are using rational design and further linker optimization to improve response amplitude and signal-to-noise ratio. We also paired these light emitting sensors with light sensitive optogenetic channels to excite or inhibit neurons based on the presence of a specific neurotransmitter. For example, the ACh sensor was paired with an excitatory channel to depolarize cells in the presence of ACh and luciferin. This showed a 500 pA response to ACh and luciferin compared to a 180 pA response

to luciferin alone, giving a SNR of 2.8. Our sensors will allow scientists to modulate and correct over- or underactive neural circuits to treat neurodegenerative disease.

Disclosures: K. Taylor: None. **H. Galvin:** None. **E.D. Petersen:** None.

Nanosymposium

NANO054: Novel Biochemical and Molecular Techniques

Location: SDCC Rm 25A

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Presentation Number: NANO054.02

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: Brain Canada Platform Support Grant
France Canada Research Fund

Title: Engineering a green fluorescent protein-based sensor for D-serine

Authors: *R. DALANGIN[1], R. MORIN-PELCHAT[2], L. PAQUET[5], A. G. GODIN[3], E. S. RUTHAZER[6], M.-E. PAQUET[4];
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Abstract: The last two decades have seen a growing interest in the role of D-amino acids within the nervous system. In particular, D-serine is now recognized as a key neuromodulator in its role as a more potent co-agonist than glycine for N-methyl-D-aspartate receptors (NMDARs), which are widely recognized as the key receptor responsible for synaptic plasticity. It has also been historically considered as a key molecule released by astrocytes in the tripartite model of the synapse. Accordingly, aberrations in D-serine signalling have been consistently associated with several pathological conditions, including schizophrenia, Alzheimer's disease, and epilepsy. However, despite our understanding of D-serine's role in the nervous system, the molecular mechanisms that govern its dynamics remain unclear, with recent works challenging its role as a gliotransmitter. Thus, a more thorough understanding of D-serine dynamics is necessary to properly understand its role in both healthy and disease states. To address these gaps in knowledge, tools with the requisite spatiotemporal resolution, such as genetically encoded fluorescent protein-based indicators, are necessary to monitor D-serine dynamics. To date, the only genetically encoded indicator for D-serine is a FRET-based indicator, called DserFS, based on a bacterial periplasmic binding protein (PBP) that was computationally redesigned to bind D-serine. PBPs are ideal scaffolds for sensor engineering because they are orthogonal to neurons, offer large changes in fluorescence in response to ligand binding, and can be targeted to arbitrary cellular compartments. However, DserFS shows a limited dynamic range relative to single fluorescent protein-based indicators and requires exogenous addition of purified protein for imaging in brain slices, significantly limiting its potential use in vivo. Here, we present our work

on engineering a genetically encoded single fluorescent protein-based indicator for D-serine using the redesigned PBP from DserFS. Indeed, our results indicate that our D-serine sensor is bright, shows large fluorescence changes in the presence of D-serine, and has good membrane localization. We anticipate that our new D-serine indicator will open new avenues for investigating D-serine dynamics within the nervous system.

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Nanosymposium

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Presentation Number: NANO054.03

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH New Innovator Award DP2MH129956
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Chan Zuckerberg Initiative

Title: Red-shifted chemigenetic voltage indicators for deep-tissue imaging

Authors: *E. W. SALTER[1], J. CHEN[2], P. PARK[3], J. XU[3], M. SEYEDOLMOHADESIN[4], F. GALEAZZI[1], D. SHEINBERG[2], B. PARADISO[2], K. AMIN[2], M. TEST[2], K. PODGORSKI[4], A. E. COHEN[3], A. S. ABDELFATTAH[2];
[2]Neurosci., [1]Brown Univ., Providence, RI; [3]Chem. and Chem. Biol., Harvard Univ., Cambridge, MA; [4]Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: Transmembrane voltage is a hallmark of all cells. In the nervous system, membrane voltage fluctuations are the primary mode of communication. Traditional measurements of transmembrane voltage using electrodes are invasive, low throughput and technically demanding. Conversely, optical readout of transmembrane voltage using genetically-encoded voltage indicators (GEVIs) has the potential to permit non-invasive voltage measurement from many cells and/or subcellular compartments simultaneously. Current GEVIs perform optimally with green/yellow fluorophores, which imposes severe constraints on the depth, duration, and sensitivity of *in vivo* recordings. This is because the green/yellow spectral window is not well suited for imaging biological tissue due to high scattering, autofluorescence and absorption-induced tissue heating. Also, red-shifted GEVIs enable paired use with blue-shifted reporters and optogenetic actuators. We developed GEVIs that perform optimally in the red and far-red spectral window to realize the promise of robust *in vivo* voltage imaging. We rationally engineered Voltron[1,2] to enhance its performance with red and far-red fluorophores. Voltron is a chemigenetic voltage indicator that couples an organic fluorescent dye with a sensor domain

protein. Dye fluorescence is modulated by voltage-dependent changes in the sensor domain's absorption. While Voltron is extremely bright and has fast kinetics, it has poor voltage sensitivity with red and far-red dyes[1,2]. We developed an assay to evolve red-shifted Voltron variants by co-staining primary neuron cultures with both a yellow dye, Janelia Fluor $(JF)_{525,}$ and a red dye, JF_{608}. Through measuring action potential-induced fluorescence changes in both yellow and red channels, we assayed the relative spectral sensitivity of each variant on a cell-by-cell basis. We evolved *re*d-shifted *Volt*ron (ReVolt) variants that exhibit maximal voltage sensitivity with red/far-red dyes. ReVolt enables reliable voltage imaging in both mouse acute brain slices and *in vivo*. Additionally, we use ReVolt for simultaneous one-photon voltage imaging and two-photon glutamate imaging of iGluSnFR4[3] to map the synaptic input patterns that dictate membrane voltage dynamics. Efforts are underway to further improve voltage sensitivity of ReVolt, and to enhance trafficking to neuronal processes for subcellular voltage imaging. 1. Abdelfattah, A. S., ... & Schreiter, E. R. (2019). *Science*, *365*(6454), 699-704. 2. Abdelfattah, A. S, ... & Kolb, I. (2023). *Neuron*, *111*(10), 1547-1563.3 3. Aggarwal, A., ... & Podgorski, K. (2025). *bioRxiv*, 2025-03.

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Nanosymposium

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Presentation Number: NANO054.04

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R21EB034494
NIH 1R35GM156968

Title: Engineering a bioluminescent sensor for neuroinflammation detection and control

Authors: *O. DUBEY[1], M. CHATTERTON[1], K. TAYLOR[2], J. ROSSIGNOL[3], J. BAKKE[1], E. D. PETERSEN[4];
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Abstract: Chronic inflammation is the cause of numerous diseases, including autoimmune and neurodegenerative disorders. In the central nervous system, it exacerbates conditions such as Alzheimer's and Parkinson's disease. New therapies, including JAK inhibitors, lack cell specificity and lead to severe side effects. To circumvent these limitations, we are developing a Bioluminescent Kinase Sensor (BlinKS) coupled with a synthetic gene circuit for real-time

inflammation monitoring and control. BlinKS is designed to detect activation of the JAK/STAT and NF-κB pathways and convert it into a bioluminescent signal that regulates gene expression via the light-sensitive transcription factor EL222. The system uses a split luciferase that is reconstituted upon activation, a phospho-amino acid binding domain (PAABD) to detect phosphorylation events, and kinase substrates phosphorylated by activated JAK1, JAK2, STAT1, STAT3, and candidate kinases within the NF-κB pathway. To further increase the sensitivity and specificity of BlinKS, we are optimizing luciferase variants, tuning sensor architecture (including linkers and PAABD-substrate pairs), and expanding the repertoire of kinase targets. We will validate the system in vitro using cell-based assays to assess whether BlinKS can detect inflammatory signaling and test the ability of the light emitting sensors to induce optogenetic gene circuit activation through EL222. This strategy offers a novel tool for investigating the dynamics of inflammation and enabling precise, self-regulating therapeutic interventions with potential applications extending beyond neuroinflammation.

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Nanosymposium

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Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO054.05

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: F31 DA056121

Title: Multi-color fluorescent sensor suite for contextual monitoring Hydrogen Peroxide

Authors: *J. D. LEE[1], A. MOGHADASI[1], Y. WANG[1], W. WON[3], C. NEISWANGER[2], C. LEE[3], C. I. CHAVKIN[4], A. BERNDT[1];
[1]Bioengineering, [2]Univ. of Washington, Seattle, WA; [3]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of; [4]Pharmacol., Univ. of Washington Sch. of Med., Seattle, WA

Abstract: Hydrogen peroxide is a key endogenous reactive oxygen species (ROS) in mammalian systems. Recent studies have highlighted its roles in neurobiology, including synaptic plasticity, neuronal excitability, and receptor signaling. Traditionally, hydrogen peroxide has also been considered a hallmark of oxidative stress, as its accumulation is often associated with brain aging and neurodegenerative disorders. Monitoring hydrogen peroxide dynamics alongside key biological interactants is essential for understanding the physiological outcomes of cellular redox regulation. Here, we present a multicolor sensor suite, oROS, a set of highly sensitive and fast genetically encoded fluorescent indicators for hydrogen peroxide. The oROS suite enables simultaneous, compartment-specific measurements of hydrogen peroxide at multiple subcellular sites, providing spatially resolved insights into its roles in biology both *in vivo* and *in vitro*. For

example, multiplexed peroxide imaging with oROS allows differential visualization of hydrogen peroxide pools at the inner or outer surfaces of the plasma membrane, in the cytoplasm, mitochondria, and mitochondrial intermembrane space, thereby helping to dissect the causal roles of oxidative stressors at subcellular resolution. In addition, the multicolor design of the oROS suite facilitates simultaneous monitoring of hydrogen peroxide alongside critical cellular interactants such as pH, redox potential, and calcium levels. Using this platform, we captured acute, real-time changes in hydrogen peroxide levels in parallel with intracellular redox potential and Ca^{2+} dynamics in response to auranofin, an inhibitor of antioxidant enzymes. We showcased use of the sensor in oxidative stress models, highlighting the sensor's relevance in validating neurodegenerative disease models. Lastly, we explored the use of the oROS sensor for fiber photometry to directly monitor acute peroxide response to Nalfurafine in vivo, confirming activation of JNK-PRDX6-PLA2-NOX in KOR-positive neurons in the Ventral Tegmental Area. Altogether, the multicolor oROS sensor suite provides a powerful tool for investigating intricate intracellular and intercellular hydrogen peroxide dynamics and their interactions, through real-time, spatially resolved imaging. We envision that the oROS sensors and the use cases discussed here will stimulate new interest and discovery in the redox biology of neurological systems.

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Nanosymposium

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Presentation Number: NANO054.06

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant R01 NS121073

Title: Next-generation NOSTIC probes enable high-contrast brain-wide functional imaging of cells and circuits

Authors: *Y. KE[1], S. MODIANO[1], C. LIN[2], B. STALLINGS[1], T. KAISE[1], V. GRADINARU[2], A. JASANOFF[1];
[1]MIT, Cambridge, MA; [2]Caltech, Pasadena, CA

Abstract: Advancing our understanding of brain-wide neuronal dynamics requires noninvasive tools capable of precise cellular and circuit-level imaging in intact organisms. Previously, we introduced hemogenetic imaging using NOSTIC probes, genetically engineered enzymes that convert neuronal calcium signaling into localized functional magnetic resonance imaging (fMRI)-detectable hemodynamic responses. This approach enables whole-brain imaging of genetically targeted cells and circuits. First-generation NOSTICs relied on large viral vectors and offered limited contrast over background, however, highlighting an urgent need for smaller, higher-sensitivity probes. Here we report on second-generation NOSTIC probes with enhanced

activity, specificity, and versatility. Using high-throughput mutagenesis and computational structural modeling, we identified multiple mutants designed to function effectively during suppression of endogenous fMRI signals. We also engineered compact NOSTIC variants by introducing targeted truncation and deletion mutants that reduce probe size while preserving function. Shortened mutant constructs could be packaged into adeno-associated viral vectors that produce high expression efficiency and low cytotoxicity. We apply these vectors to examine circuit- and cell-type-specific contributions to integrated brain activity dynamics in rodents. We also describe a related effort to create drug-inducible NOSTICs that can be switched on and potentially multiplexed using small molecule activators. Together, these new NOSTIC probes will enable noninvasive investigations of a wide range of neural phenomena at brain-wide scale in health and disease.

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Nanosymposium

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Topic: J.01. Molecular, Biochemical, and Genetic Techniques

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BJC Investigators Program
HHMI Investigator Program

Title: Tagless MitoIP for the immunocapture of neuronal mitochondria from peripheral tissues

Authors: *C. FECHER, D. J. PAGLIARINI;
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Abstract: Mitochondria are essential organelles in virtually all eukaryotes, involved in apoptosis, Ca^{2+} buffering, and metabolism. Their functions and proteome differ across tissues and cell types due to transcriptional and translational differences in ~1,100 nuclear-encoded mitochondrial proteins (Pagliarini DJ*, Calvo SE* *et al.* 2008). Additionally, mitophagy and active transport shape mitochondria, particularly in dendrites, axons, and synapses of neurons. Mitochondrial dysfunction is frequently observed in neurodegeneration and aging, highlighting the importance of this organelle for neuronal fidelity. Strikingly, our current understanding of mitochondrial heterogeneity across neuronal CNS and PNS populations and their mitochondrial adaptation/dysfunction during neurodegeneration is limited due to the lack of appropriate *in-vivo* tools.
Recent work has filled this gap by enabling cell-type-specific mitochondrial profiling from genetically defined cells *in vivo* (Fecher C*, Trovo L* *et al.* 2019; Bayraktar EC *et al.* 2019). This MitoTag approach combines the expression of an outer mitochondrial membrane (OMM)

epitope with the immunocapture of tagged mitochondria (MitoIP) from mouse tissues. Nonetheless, the method faces limitations: (1) the need for complex genetic breeding schemes to faithfully express the tag, sometimes in combination with genetic disease models; and (2) its inadequacy for human patient materials (de Mello NP*, Fecher C* *et al.* 2023).
Here, we advance this technique by systematically evaluating the OMM proteome to identify endogenous proteins that facilitate the MitoIP. We trypsinized intact mitochondria from multiple rodent tissues to enrich accessible OMM proteins and identified them by mass spectrometry. This analysis yielded >30 candidates with varying degrees of tissue and cell type specificity. Among them, we identified an uncharacterized OMM protein specific to the brain and enriched in neurons. Further validation experiments with this candidate confirmed a good mitochondrial isolation yield from brain tissue. Additionally, preliminary data indicated the successful isolation of mitochondria from PNS neurons and their axons, which are particularly difficult to study due to their sparsity within peripheral tissues and traditionally require cumbersome genetic tools. Using lipidomics and proteomics, we are currently profiling PNS mitochondria from several peripheral organs under metabolic challenges and in the context of neurodegeneration.
We anticipate that this tool will accelerate future studies of neuronal mitochondria in both health and disease.

Disclosures: C. Fecher: None. **D.J. Pagliarini:** None.

Nanosymposium

NANO054: Novel Biochemical and Molecular Techniques

Location: SDCC Rm 25A

Time: Wednesday, November 19, 2025, 1:00 PM - 3:30 PM

Presentation Number: NANO054.08

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

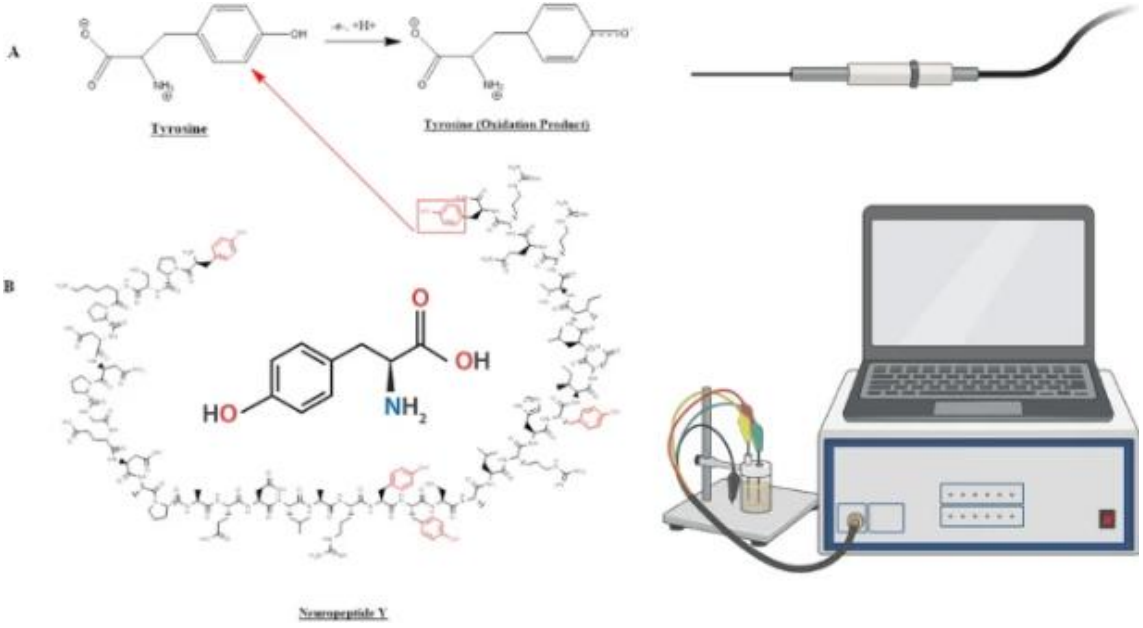
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Title: Multiplexed neuropeptide detection with carbon electrodes and fast-scan cyclic voltammetry

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Abstract: Carbon fiber microelectrodes (CFMEs) have been used to detect neurotransmitters and other biomolecules using fast-scan cyclic voltammetry (FSCV) for the past few decades. These assays typically measure small molecule neurotransmitters such as dopamine and serotonin. The carbon fiber is relatively small, biocompatible, and makes minimally invasive measurements at high spatial and temporal resolution. Carbon Fiber Multielectrode arrays have been utilized to measure multiple neurotransmitters in several brain regions simultaneously with

multi-waveform application on each electrode. We have extended this work to measure larger molecule neuropeptides such as endorphins, galanin, Neuropeptide Y and Oxytocin, a pleiotropic peptide hormone, is physiologically important for adaptation, development, reproduction, and social behavior. This neuropeptide functions as a stress-coping molecule, an anti-inflammatory agent, and serves as an antioxidant with protective effects especially during adversity or trauma. Here, we measure tyrosine using the Modified Sawhorse Waveform (MSW), enabling enhanced electrode sensitivity for the amino acid and peptide, decreased surface fouling, and codetection with other catecholamines. As both oxytocin and Neuropeptide Y contain tyrosine, the MSW was also used to detect these neuropeptides. Additionally, we demonstrate that applying the MSW on CFMEs allows for real time measurements of exogenously applied neuropeptides on rat brain slices. These results may serve as novel assays for neuropeptide detection in a fast, sub-second timescale with possible implications for *in vivo* measurements and further understanding of the physiological role of neuropeptides such as beta endorphins, Neuropeptide Y, galanin, oxytocin. Further work will be discussed on extending these assays to develop novel enzyme-modified glutamate biosensors for the rapid measurement of glutamate in the brain.



Disclosures: A.G. Zestos: None.

Nanosymposium

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Presentation Number: NANO054.09

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R01NS136181

Title: Expression of Small Peptide (SP7737) Stops mTORopathies-related Seizures

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Abstract: Rationale Somatic mutations in the mechanistic target of rapamycin (mTOR) pathway genes have been frequently identified in patients with focal cortical dysplasia type II (FCDII), the most common underlying pathologies in children with drug-resistant epilepsies (DRE). Although often curative, destructive lesionectomy could only be offered to less than one-fourth of patients. New therapeutic development to treat FCDII has significantly lagged behind our staggering advances in understanding of mTORopathies at genetic and cellular levels. For example, oral Rapalogs have been proposed as the prevision medicine but only showed equivocal benefits due to its systemic side effects, low blood brain barrier (BBB) penetration, and of feed-back loop activation. **Methods** We first used *in-utero* electroporation (IUE) of the human pathogenic RHEB mutant (P37L) or mTOR mutant (S2215Y) to establish mouse models of mTORopathies. We then applied a conditional co-expression IUE system to gate the expression of a small peptide (SP7737), a micro-peptide that negatively modulates MTORC1, at postnatal day (PND), and 60 to test its therapeutic efficacy at different disease stages. Finally, we screened a AAV capsid that was rationally designed with direct evolution for cortical excitatory neurons and stereotactically injected the SP7737-expressing virus in the dysplastic cortex to test its clinical translatability. **Results** Mice with RHEBp.P37L or MTORp.S2215Y IUE displayed the pathological signatures in the human dysplastic cortex and developed frequent clinical seizures. SP7737effectively inhibited mTOR hyperactivation in neurons, rescued cytomegaly, re-engaged neuronal migration and restored cortical lamination, and stopped seizures. In addition, the stereotactic injection of novel AAV expressing SP7737 significantly improved the clinical seizure outcome in FCDII mice. **Conclusions** Our findings suggest that the SP7737 is a promising universal therapeutic target for mTORopathies.

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Nanosymposium

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Presentation Number: NANO054.10

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: A Novel Optogenetic Inhibitor of Protein Kinase C Reveals the Role of PKC alpha in Prefrontal Cortex-Mediated Cue Association Learning

Authors: *G. OZ, L. A. COLGAN, S. MAJUMDER, Y. HAYANO, I. SUPONITSKY-KROYTER, H. INAGAKI, R. YASUDA;
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Abstract: Protein kinase C alpha (PKCa) plays a critical role in learning, memory, and associative learning, yet the lack of isozyme-specific tools has hindered precise investigation of individual PKC isozymes in these processes. The medial prefrontal cortex (mPFC), a brain region implicated in reward-based cue association, provides a key context for studying PKCa's functional specificity. To address this limitation, we developed a novel photoactivatable inhibitory system that selectively targets PKC isozymes. Using this optogenetic tool, we demonstrate that transient inhibition of PKCa in the PFC disrupts auditory cue-reward learning *in vivo*. These results were confirmed using full knockout PKCa mice, and local re-expression of PKCa in the PFC of these knockout mice restored cue-reward association, demonstrating sufficiency. This effect was not observed in another PKC isozyme, PKCd—known to be important for synaptic plasticity —underscoring PKCa's unique necessity for this form of associative learning. Our findings resolve longstanding ambiguities caused by non-selective pharmacological agents and establish PKCa as indispensable for reward-related plasticity in the mPFC. This approach enables spatiotemporally precise interrogation of PKC isozyme contributions to neural circuits and cognitive processes, offering a framework to dissect their divergent roles in health and disease.

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