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**Nanosymposium**

**NANO01: Neurodevelopmental Disorders: Genetic and Molecular Mechanisms**

**Location:** WCC 143

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO01.01

**Topic:** A.07. Developmental Disorders

**Support:** Taconic Biosciences, Academic Grant

**Title:** Examining behavioral and molecular effects after sub-chronic administration of an NMDA receptor partial agonist in a 22q11.2 deletion syndrome mouse model

**Authors: S. POTTERS**[1], B. GOODMAN[1], C. NEFF[2], L. KOWALSKI[2], A. BUNTING[2], S. BURDEN[2], N. TRUVER[2], A. THOMPSON[1], S. SMAW[2], D. CALVANO[2], J. SANCHEZ[2], D. MITRANO[1], J. BOGENPOHL[1], *J. BURKET[1];
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**Abstract:** Deletions and duplications of the chromosome 22q11 region are associated with high rates of psychiatric disorders. The 22q11.2 microdeletion (Df(h22q11)/+) mouse model displays both genetic and behavioral phenotypes present in the 22q11 deletion syndrome (DS) clinical population. Initial characterization studies of this model show deficits related stress and prefrontal cortex connectivity including alterations in behavioral tasks and immunostaining of parvalbumin (PV) in 10-13 week old male mice. Df(h22q11)/+ mice display increased sensitivity to NMDA receptor (NMDAR) antagonism and age-dependent increases in prepulse inhibition (PPI) after puberty. The NMDAR regulates social and cognitive processes and represents a novel therapeutic target in neurodevelopmental disorders, relevant in the 22q11.2 deletion syndrome. This study characterized the 22q11DS mouse model within the 4-6 week age range and compared gender differences . Additionally, we tested the ability of a NMDAR agonist intervention to promote beneficial effects in adolescent male and female Df(h22q11)/+ mice. Specifically, 4-6 week old C57BL/6-Del(16Dgcr2-Hira01Tac mice (n=60) were administered a 1-week sub-chronic dose of the NMDAR partial agonist, D-cycloserine (DCS, 30 mg/kg, i.p.), or saline. A battery of behavioral tests to assess impairments on anxiety, sociability, cognition, stereotypic behaviors were employed, including light/dark box, elevated plus maze (EPM), three-chamber sociability, y-maze, and marble burying task. Following behavioral testing, a subset of brains were perfused for immunostaining of PV and perineuronal nets (PNN). Using PV antibody (1:1000-10,000) and WFA lectin (1:1000), which selectively labels residues of glycoproteins within neuronal extracellular matrix, cerebellar tissue was immunostained to identify PV+ neurons and visualize PNN expression within the dentate nucleus (DN) of the deep cerebellar nuclei (DCN). The DN projects to regions that regulate social behaviors. Preliminary analysis on EPM measures in male mice showed differences in genotype on total arm entries ($F(1, 46) = 30.5$, $p < 0.0001$). Male Df(h22q11)/+ mice showed a higher percentage of marble burying behavior compared to WT mice, suggesting increased stereotypic behavior in this model. DCS treatment had no effect on weight over the 7-day injection paradigm and current analyses are being conducted to determine treatment effects within this model. Overall, these data

encourage exploration of a selective therapeutic targeting of receptors and pathways involved in social-cognitive domains, such as NMDAR activation in 22q11.2 deletion syndrome.

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**Presentation Number:** NANO01.02

**Topic:** A.07. Developmental Disorders

**Support:** NRF-2017M3C7A1026959
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Cooperative Research Program of Basic Medical Science and Clinical Science from Seoul National University College of Medicine

**Title:** Association of altered αCaMKII $Thr^{286}$ autophosphorylation with sex-specific auditory sensory processing impairments in a mouse model of Noonan syndrome

**Authors: S. KIM**[1], S. PARK[2], H. VU[3], Y. KIM[4], G. PARK[1], M. KANG[1], J. AN[4], M.-S. KIM[3], S. KIM[1], M. PARK[5], *Y.-S. LEE[1];
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**Abstract:** Rasopathy is a group of disorders caused by germline mutations in genes related to the RAS signaling pathway. Noonan syndrome (NS) is the most common among Rasopathies and exhibits a high prevalence of ASD. However, the neural mechanisms underlying ASD-like behavioral phenotypes in NS are not well understood. *Ptpn11*$^{D61G/+}$ mice, which display NS-like symptoms such as short stature, heart deficits, and learning and memory impairments, have been used as a mouse model of NS. In this study, we report that *Ptpn11*$^{D61G/+}$ mice show abnormalities in sensory processing, which are one of the key features of ASD. While *Ptpn11*$^{D61G/+}$ mice exhibited lowered sensitivity in auditory brainstem responses, they showed increased startle responses to acoustic stimuli, reduced habituation of startle response, and impaired sensorimotor gating in the pre-pulse inhibition test. Notably, only male, not female mutant mice, showed significantly disrupted acoustic startle habituation and sensorimotor gating. As a potential mechanism underlying sex-specific impairment of auditory processing in NS mice, we found that the phosphorylation of αCaMKII at Thr286 is significantly decreased in male mutant mice compared to wild-type littermates but not in female mutants. Our results suggest that sex-specific alterations in αCaMKII Thr286 autophosphorylation may lead to sensory processing impairments in male *Ptpn11*$^{D61G/+}$ mice. Our study provides new insights into the neural mechanisms underlying ASD-like behaviors in NS mice.

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**Presentation Number:** NANO01.03

**Topic:** A.07. Developmental Disorders

**Support:** The Peter O'Donnell Jr. Brain Institute Sprouts Program grant
University of Texas Southwestern Medical Center
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**Title:** The chromatin remodeler KDM5A specifies hippocampal cell identity

**Authors: *L. EL HAYEK**, D. DEVRIES, A. GOGATE, A. AIKEN, K. KAUR, M. CHAHROUR;
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**Abstract:** Epigenetic chromatin regulation is essential in establishing and maintaining cellular identity and differentiation. It is required for normal brain development and proper gene expression as well as wiring of neuronal circuits. Disruptions in chromatin remodelers lead to several diseases, notably neurodevelopmental disorders. For example, many of the top causative genes in autism spectrum disorder (ASD) are chromatin remodelers (e.g., *ARID1B*, *CHD8*, *KMT5B*). By unraveling the role of chromatin remodelers in neurodevelopmental disorders like ASD, we gain insight into their function in early brain development, including their cell-type specific roles. Patients with ASD often present with learning and memory deficits, two cognitive tasks mediated primarily by the hippocampus. The hippocampus is composed of a variety of different cell types, each unique in its functions and transcriptome. However, the specific cell types in the hippocampus that are affected in ASD as well as the cell-type specific transcriptional programs that are disrupted in this disease are unknown. We recently identified a novel ASD gene, *KDM5A*, which encodes a chromatin remodeler and histone H3 methyl 4 demethylase. Patients with pathogenic variants in *KDM5A* present with severe ASD, cognitive impairments, and absence of speech. We showed that *Kdm5a* knockout (*Kdm5a*$^{-/-}$) mice have severe neurobehavioral phenotypes including deficits in hippocampal-dependent learning and memory and dysregulation of the hippocampal transcriptome. We therefore hypothesized that KDM5A mediates hippocampal development. To investigate this, we performed single-nuclei RNA sequencing from wildtype (WT) and *Kdm5a*$^{-/-}$ hippocampal tissue. We found that loss of KDM5A leads to a more mature cellular identity and that specific subtypes of hippocampal cells are particularly sensitive to the loss of KDM5A. We also found that KDM5A is essential in establishing hippocampal cell identity by controlling a differentiation switch early in development. Our findings define a role for the chromatin remodeler KDM5A in establishing hippocampal cell identity and, more broadly, advance our knowledge of the role of epigenetic chromatin regulation in dictating cellular identities in the brain.

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**Presentation Number:** NANO01.04

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant HD042182

**Title:** Cortical Neural Precursors as Potential Targets in a Mouse model of Polygenic Neurodevelopmental Disorder

**Authors: *S. RUKH**[1,2], D. W. MEECHAN[1], T. M. MAYNARD[1], Z. ERWIN[1], C. SIGGINS[1], A. S. LAMANTIA[1,3];
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**Abstract:** Layer 2/3 projection neurons (PNs) make cortico-cortical connections, especially those between association cortices, and are key pathogenic targets in multiple neurodevelopmental disorders (NDDs) including Intellectual disability (ID), autistic spectrum disorder (ASD), and Schizophrenia (Scz). Aberrant regulation of cortical precursor proliferation, specifically basal progenitors (bPs) that are primary precursors of layer 2/3 PN, has been proposed as a potential pathogenic mechanism underlying NDDs, leading to altered layer 2/3 PN frequency as well as quantitative or qualitative changes in connectivity. Nevertheless, there is limited evidence for altered cortical progenitor proliferation in most NDDs. Previously, we have shown that bP proliferative activity is diminished in the genomically valid *LgDel* mouse model of 22q11.2 Deletion Syndrome (22q11DS), a polygenic NDD associated with behavioral changes that parallel those in ASD and Scz. We have now found that this diminished proliferation reflects premature neurogenesis that depletes the bP pool in mid-gestation *LgDel* embryos. Premature neurogenesis among *LgDel* bPs *in vivo* is paralleled by increased *LgDel* bP neurogenic divisions (Neuron-Neuron) and reduced proliferative divisions (Precursor-Precursor or Precursor-Neuron divisions) *in vitro*, suggesting a potential shift in mode of cell division from self-renewing towards terminal neurogenic divisions. Changes in neurogenesis and mode of division are matched by divergent *LgDel* vs. wild type (WT) bP transcriptomes. Both *LgDel* and WT bPs express substantial numbers of 22q11 deleted genes; however, in *LgDel* bPs these genes are uniformly down-regulated, most by 50%. An additional 74 genes are up-regulated in *LgDel* bPs vs. WT, and most are associated with neuronal differentiation consistent with premature neurogenesis and increased terminal neurogenic divisions due to 22q11 deletion. These expression differences were validated in vivo using RNAscope. Mean expression differences in *LgDel* bPs in vivo, however, reflect selective modification of the range of expression of candidate transcripts per cell in *LgDel* bPs rather than shifts in a single expression mean between the two genotypes. Thus, premature neurogenesis, divergent modes of bP division, and dynamic regulation of gene expression levels in *LgDel* diminish L2/3 PN frequency, likely contributing to association cortico-cortical under-connectivity. Apparently, 22q11 genes modulate timing and mode of bP division, and their deletion targets bPs neurogenic capacity and transcriptional state resulting in suboptimal cortical development associated with NDD pathology.

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**Title:** Upregulation of SYNGAP1 in neurons by redirecting alternative splicing

**Authors: *R. YANG**[1], X. FENG[1], A. ARIAS-CAVIERES[2,3,4], R. M. MITCHELL[5], A. POLO[2,3,4], K. HU[1], R. ZHONG[1], C. QI[1], R. S. ZHANG[1], N. WESTNEAT[1], C. A. PORTILLO[6,5], M. A. NOBREGA[1], C. HANSEL[5,4], A. J. GARCIA III[2,3,4], X. ZHANG[1,4];
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**Abstract:** The synaptic Ras GTPase-activating protein SYNGAP1 is essential for synaptic plasticity. Hundreds of *de novo SYNGAP1* mutations are reported to be the causes of multiple neurological conditions, such as epileptic encephalopathy, autism, and intellectual disability. However, the regulation of *SYNGAP1* during development remains unclear, and there is no treatment for *SYNGAP1*-associated haploinsufficiency. Here, we characterize an alternative 3' splice site (A3SS) of *SYNGAP1* intron 10 that induces nonsense-mediated mRNA decay (A3SS-NMD) in mouse and human neural development. We demonstrate that PTBP1 directly binds to and promotes *SYNGAP1* A3SS inclusion. Overexpression of *PTBP1* in mouse primary neurons decreases Syngap1 protein. Genetic deletion of the Syngap1 A3SS in mice upregulates Syngap1 protein in cortices and hippocampi, alleviating the long-term potentiation and membrane excitability deficits caused by a *Syngap1* knockout allele. We further develop a splice-switching oligonucleotide (SSO) that converts *SYNGAP1* unproductive isoform to the functional form in human induced pluripotent stem cell (iPSC)-derived neurons and improves SYNGAP1 protein expression in iPSC-derived cerebral organoids. This study describes the regulation and function of *SYNGAP1* A3SS-NMD, the genetic rescue of heterozygous *Syngap1* knockout mice, and the development of an SSO to potentially alleviate *SYNGAP1*-associated haploinsufficiency.

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**Topic:** A.07. Developmental Disorders

**Support:** NICHD grant # R37 HD29957
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**Title:** 7q11.23 gene dosage effects on measures of cerebellar volume

**Authors: *D. A. ADAMS**[1], T. A. NASH[1], J. S. KIPPENHAN[1], O. R. KLINE[1], A. K. ILSLEY[1], C. J. PFISTER[1], M. R. HAMBORG[1], M. D. GREGORY[1], P. D. KOHN[1], D. P. EISENBERG[1], C. B. MERVIS[2], K. F. BERMAN[1];
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**Abstract: INTRODUCTION:** While the cerebellum is classically associated with motor coordination, there is recent appreciation that the "little brain" may also be involved in emotion, cognition, and sociability. Williams syndrome (WS) and the 7q11.23 duplication syndrome (Dup7) are rare neurodevelopmental disorders resulting from a hemi-deletion and duplication, respectively, of ~25 genes on chromosomal locus 7q11.23. These copy number variations (CNV) result in alterations in fine motor skills, cognition, and socioemotional processing, which may be due, in part, to alterations in cerebellar structure. In order to test for structural differences in the cerebellum and their relationship with 7q11.23 gene dosage, we measured cerebellar volume in people with WS and Dup7. **METHODS:** T1-weighted MRI images were longitudinally collected on a 3T scanner from 68 participants aged 5-26 at 203 visits, including 24 individuals with WS (80 timepoints: mean age=14.1±4; 17 females), 12 Dup7 (37 timepoints: mean age=14.7±3; 5 females), and 32 typically developing (TD) participants (86 timepoints: mean age=13.0±4; 18 females). Volumetric segmentations of the whole brain and cerebellum, encompassing gray and white matter volume, were generated using Freesurfer7 software. We used R's lme4 to test for differences between each CNV group and TDs in total cerebellar volume, relative cerebellar volume (normalized by total brain volume), relative cerebellar gray matter volume (GMV), and relative cerebellar white matter volume (WMV). All models included fixed effects for age and sex, and reported findings are significant at Bonferroni-corrected $p<0.006$. **RESULTS:** While both CNV groups exhibited reduced absolute cerebellar volume compared to TDs (WS, $p=0.0055$; Dup7, $p=0.0018$), a gene dosage effect emerged when considering cerebellar volume relative to brain size: individuals with WS had larger cerebellar volumes relative to brain size ($p=0.0007$), whereas those with Dup7 had smaller relative volumes ($p=0.000002$). Similar patterns were observed when cerebellar GMV was examined separately, with WS>TD ($p=0.0002$) and TD>Dup7 ($p=0.000002$), but no significant group effects were found for cerebellar white matter volumes ($p>0.05$). **CONCLUSIONS:** Our study revealed an effect of 7q11.23 gene dosage on the relative total volume and relative gray matter volume of the cerebellum in individuals with WS and Dup7. Future investigations focusing on specific regions of the cerebellum may help elucidate these structural differences as they relate to the neurobehavioral phenotypes of the CNVs.

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**Presentation Number:** NANO01.07

**Topic:** A.07. Developmental Disorders

**Support:** SFARI Sex Differences in Autism
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**Title:** Mapping neuropsychiatric disorders through spatial transcriptomics of the mouse brain reveals convergent gene enrichment

**Authors: *D. M. YOUNG**[1,2], R. SHARMA[1], L. LIANG[1], B. DEVLIN[3], D. MANOLI[1], S. J. SANDERS[1,4];
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**Abstract:** The past decade has seen tremendous progress in the identification of genes associated with complex neuropsychiatric disorders, including autism spectrum disorder (ASD) and schizophrenia. Expression patterns of these genes in single cell data strongly implicate excitatory and inhibitory neurons; however, there are limited data on the brain regions involved - a critical question for experimental design. Spatial transcriptomics provide an opportunity to perform systematic multi-regional analyses to provide insights into this question. Here, we have generated a spatial transcriptomics dataset encompassing the diverse anatomical territories of the adult mouse brain sagittal midsection. We then applied a gene enrichment metric. Critically, we controlled for neuronal proportion, given the strong enrichment observed in these cells and the widely differing proportions across regions. ASD genes, identified from exonic rare variants, were most enriched in the thalamus, followed closely by the cortex and hippocampus. Similar regions were enriched for genes identified by GWAS for schizophrenia, although the enrichment was greatest for cortex. GWAS-identified genes for Alzheimer's disease also yielded a similar pattern, with the greatest enrichment in the hippocampus. As a positive control, we assessed genes identified by GWAS for height, which yielded highly specific enrichment in the hypothalamus, where growth hormone-releasing hormone is produced. Together, these findings represent the first systematic multiregional, cross-disorder analysis of convergent gene expression in the brain. The results highlight shared and distinct patterns for pleiotropic brain disorders that could elucidate common underlying mechanisms and circuitry.

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**Presentation Number:** NANO01.08

**Topic:** H.07. Long-Term Memory

**Support:** NIH Grant HD103360
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**Title:** Autism- and epilepsy-associated EEF1A2 mutations lead to translational dysfunction and altered actin bundling

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**Abstract:** Protein synthesis is a fundamental cellular process in neurons that is essential for synaptic plasticity and memory consolidation. Here, we describe a neuron- and muscle-specific translation factor, Eukaryotic Elongation Factor 1a2 (EEF1A2), that is mutated in patients with autism, epilepsy, and intellectual disability. We characterize three most common patient mutations, G70S, E122K, and D252H, and demonstrate that all three mutations decrease *de novo* protein synthesis and elongation rates in HEK293 cells. In mouse cortical neurons, the *EEF1A2* mutations not only decrease *de novo* protein synthesis, but also alter neuronal morphology, regardless of endoges levels of eEF1A2, indicating that the mutations act via a toxic gain of function. We also show that eEF1A2 mutant proteins display increased tRNA binding and decreased actin bundling activity, suggesting that these mutations disrupt neuronal function by decreasing tRNA availability and altering the actin cytoskeleton. In human stem cell-derived excitatory neurons, polysome profiling shows altered initiation that may compensate for decrease elongation rates. Whole-cell electrophysiological recordings show increased resting membrane potential and firing rates in eEF1A2 mutant neurons. More broadly, our findings are consistent with the idea that eEF1A2 acts as a bridge between translation and the actin skeleton, which is essential for proper neuron development and function

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**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant R25MH060482
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**Title:** Slow progress of learning in rats haploinsufficient for a high-risk ASD gene

**Authors: *D. KASTNER**[1], C. HOLOBETZ[1], N. YOKOTOA[1], G. WILLIAMS[1], J. P. ROMANO[2], P. DAYAN[3];
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**Abstract:** Behavior forms the foundation upon which we interpret neurophysiology and model neuropsychiatric disease in animals. However, we have a limited understanding of the causes underlying the complex and dynamic decision-making processes used by animals. Although it is common to use hypothesis driven or model-based approaches to identify differences in behavior, those approaches are often based on simple and unverifiable assumptions. Furthermore, standard methods for analyzing behavior can often be too coarse to provide information about statistically subtle differences that occur during learning. Therefore, we present the choice-wide behavioral association study (CBAS) as a new approach for analyzing group differences in learning. This is motivated by data-driven approaches prevalent in genomics, such as the genome-wide association study (GWAS), which solved related problems in genetics. CBAS breaks down behavior into shorter sequences of choices, and then uses powerful, resampling-based, multiple comparison corrections to identify choices that differ in prevalence between groups of animals. First, we apply CBAS to a set of structurally different Reinforcement Learning (RL) agents;

CBAS discovers many, interpretable, sequences of choices that distinguish the agents. Then, we apply CBAS to a cohort of 240 rats to discern the effect of *Scn2a* haploinsufficiency (*Scn2a*$^{+/-}$) on the learning of a succession of spatial alternation contingencies. *Scn2a* expresses a neuronal sodium channel, and its disruption has been strongly associated with autism spectrum disorder (ASD). CBAS finds many choice sequences that differ between *Scn2a*$^{+/-}$ rats and their wild-type littermates, revealing a persistence of choices related to the prior contingency. This indicates that *Scn2a*$^{+/-}$ rats are slower to transition from a prior state of knowledge to current goals. Through developing a data-driven behavioral analysis, CBAS, we can richly phenotype the effect of a high-risk ASD gene.

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**Nanosymposium**

**NANO02: Effects of COVID-19: Physiology and Cognition**

**Location:** WCC 150

**Time:** Saturday, November 11, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO02.01

**Topic:** H.05. Working Memory

**Support:** NIAID Grant 3U19AI159822-02S1

**Title:** Sex differences in SARS-CoV-2 during acute and post-acute infection in a mouse model of COVID-19

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**Abstract:** Males are at increased risk for severe COVID-19 and experience greater rates of mortality during acute SARS-CoV-2 infection, whereas females are more likely than males to experience long-term symptoms (i.e., post-acute sequelae (PASC) or long COVID) despite similar rates of infection. The mechanisms underlying differential disease severity between sexes remain poorly understood. The premise of this study was to interrogate molecular mechanisms that contribute to differential outcomes during acute and post-acute SARS-CoV-2 infection. A mouse model of SARS-CoV-2 infection was developed by intranasally inoculating 30 week old male and female C57BL/6J wildtype mice with $10^6$ $TCID_{50}$ of SARS-CoV-2 (mu variant B.1.621 with N501Y mutation) or DMEM (mock infection) and were monitored acutely at 2, 4, or 7 days post infection (dpi). Male mice exhibited greater morbidity (i.e., body mass loss and clinical disease scoring) compared to females following infection, despite having comparable viral titers across respiratory tissues between sexes. To uncover the sex-specific mechanism of SARS-CoV-2 disease outcomes, we used the transgenic XY* mouse model to determine if sex chromone

dosage contributed to protection among female mice, with XY* breeding resulting in XY and XXY gonadal males, and XX and X0 gonadal females. Following infection, XX females and XXY males were protected against severe morbidity, with XY males displaying the greatest morbidity following acute infection, X0 females display an intermediate phenotype. These data illustrate that a double dosage of X chromosome regardless of gonadal sex is protective, whereas a single dosage of X in the presence of testes but not ovaries is detrimental to acute SARS-CoV-2 outcome. Next, we developed a mouse model to explore whether persistent viral RNA was associated with long-term symptoms following recovery from acute infection. Wildtype mice were inoculated at 30 weeks and monitored for viral titers and viral RNA persistence as well as behavioral phenotypes at 1, 6, or 12 weeks post infection. Male and female SARS-CoV-2 infected mice displayed anosmia (i.e., olfactory impairments) by 6 weeks after infection compared to mock infected mice. SARS-CoV-2 infected female mice displayed greater hippocampal-dependent spatial working memory deficits than either infected males or mock-inoculated mice, suggesting that females have greater post-infection cognitive impairment, reflected in PASC patients. Collectively, these findings illustrate that using the mu-variant of SARS-CoV-2 we developed a mouse model that replicates male-biased acute disease severity and female-biased long-term cognitive impairment.

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**Presentation Number:** NANO02.02

**Topic:** H.05. Working Memory

**Support:** Margaret Milam McDermott Foundation

**Title:** Optimization of paradigms for assessing cognitive performance within a hypoxic environment

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**Abstract:** Chronic Obstructive Pulmonary Disease, anemia, and obstructive sleep apnea are examples of chronic hypoxic conditions that often adversely affect aging populations and are associated with cognitive impairment. The gold-standard for assessing cognitive outcomes has been behavioral testing paradigms in rodent models. However, their usage in understanding chronic hypoxic conditions has been hindered by the inability to perform these tests under stable hypoxic conditions. Changes to behavior tests impact the validity of the paradigm. We present validated testing paradigms that can be performed in hypoxic chambers to model hypoxia-inducing diseases and allow for better understanding of chronic conditions. 3 month old (3 mo) wild-type male and female C57Bl6/J mice (n=6 each sex) were housed, trained and tested within enclosed hypoxia chambers from Biospherix, Ltd. Spontaneous Alternation (SA) and Spatial Object Recognition (SOR) paradigms were selected as tests of working memory that could fit within the chambers. Mice were housed and acclimated to specific oxygen (O2) conditions in one chamber and transferred to a separate chamber for testing under the same O2 level.

Following one day of acclimation to either 21% or 15% oxygen, protocols were conducted as outlined in D'Isa, et al., 2021 (SA) and Leger, et at., 2013 (SOR). Animal movements and interactions were tracked using an overhead camera and EthoVision tracking software. To determine validity, thresholds for chance were set at greater than 50% alternation over 7 trials for SA, and greater than 60% novel location and 0.25 D.I. for SOR. The total number of object contacts in a familiar and a novel location were quantified and used to calculate the percentage of contacts in the novel location and a discrimination index (D.I.) using the following formulas:
% novel = (# novel contacts / total number of contacts) * 100
D.I. = (# novel contacts - # familiar contacts) / total # contacts
In SA, 3 mo mice exhibited a percentage of alternation 61.11%, which is above the expected threshold for chance. As expected, testing under 15% O2 reduced alternation to 33.33%. In the SOR, both the % novel (66.57%) and D.I. (0.33) were also above chance for 3 mo normoxic control mice. The results indicate both the SA and SOR paradigms can be adapted to assess behavior under mild-moderate hypoxic conditions. In conclusion, these paradigms more accurately mimic cognitive performance and functional conditions for people who suffer from chronic hypoxic conditions.

**Disclosures: S. Wazir:** None. **B. Snyder:** None. **T.F. Floyd:** None.

**Presentation Number:** NANO02.03

**Topic:** C.01. Brain Wellness and Aging

**Support:** Grant from MeitY (Government of India) under grant 4(16)/2019-ITEA
Cadence Chair Professor fund awarded to Prof. Tapan Kumar Gandhi
Prime Minister Research Fellowship Grant awarded to Sapna S Mishra

**Title:** Neural Correlates of Post-COVID Symptoms: Insights from Analysis of Subcortical Structures using T1-weighted MRI

**Authors:** P. YADAV[1], S. S. MISHRA[1], ***T. K. GANDHI**[1], B. B. BISWAL[2];
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**Abstract:** Patients who have recovered from COVID-19 are more frequently experiencing post-COVID symptoms such as brain fog, memory loss, headache, anxiety, altered smell or taste, and other issues. In order to evaluate the neurological correlates of these post-COVID symptoms, we conducted a cross-sectional study where 48 Healthy Controls (10F, 31.82+/-9.098 years) and 74 COVID-19 recovered patients (CRPs) (17F, 32.43+/-11.78 years) were scanned for T1-weighted (T1-w) MRI volumes. These CRPs were imaged within 6 months of their recovery.
T1-w images were collected utilizing the BRAVO sequence of a 3T GE scanner equipped with 32 channel head coil. To remove non-brain tissues and isolate the brain region, we utilized the "*mri_watershed*" algorithm available in FreeSurfer software. Subsequently, the volumes of subcortical regions were extracted using the Integrated Registration and Segmentation Tool (FIRST) Algorithm. It segments and generates features of 15 subcortical structures, including the thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens-area, and brain stem. Each structure is individually identified and delineated within the T1-w image space, and volumes of subcortical structures are provided as independent image-derived phenotypes (IDPs) for further analysis. Each of these IDPs was then subjected to quantile normalization prior to

testing. Finally, we performed an ANCOVA test over these normalized IDPs where the group of subjects was the variable of interest, whereas age and sex were considered covariates of no interest. We performed multiple comparison error correction by controlling the false discovery rate (FDR) rate for $p_{FDR}<0.05$.
The analysis revealed statistically significant differences in the volumes of several subcortical brain regions of the right hemisphere. Components of the basal ganglia: the caudate, putamen, and pallidum showed reduced volume in CRPs, suggesting possible degeneration, which could affect learning, emotion, and reward processing in patients. The thalamus, amygdala, and hippocampus also showed reduced volumes in CRPs, indicating an impact on smell/taste processing, emotion regulation, and memory, respectively. These inferences are consistent with the post-COVID symptoms being experienced by the CRPs. Overall, our findings suggest that the basal ganglia and limbic system show abnormalities in CRPs and provide a foundation for further studies investigating the long-term post-COVID symptoms.

**Disclosures: P. Yadav:** None. **S.S. Mishra:** None. **T.K. Gandhi:** None. **B.B. Biswal:** None.

**Presentation Number:** NANO02.04

**Topic:** C.01. Brain Wellness and Aging

**Support:** Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) - Grant E-26/110.305/2014
R.P. is an Atlantic Fellow of the Global Brain Health Institute
A.L.G is fellow from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

**Title:** Impact of the covid-19 pandemic on older adults and its association with cognition, mood, and quality of life

**Authors: *A. DO VALE GUIMARÃES**[1], K. REUWSAAT[1], N. EMELE[1], B. COSTA POLTRONIERI[1], Y. OLIVEIRA[1], C. CARVALHO[1], A. PELEGRINO[2], F. LIN[3], R. PANIZZUTTI[1];
[1]Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; [2]McGill Univ., Montreal, QC, Canada; [3]Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

**Abstract:** The COVID-19 pandemic has had a profound and enduring impact on various aspects of people's lives, including social interactions, physical and mental health, and technology usage. This impact is particularly significant among vulnerable populations, such as older adults. In this study, we measured the global impact of the COVID-19 pandemic on older adults, both with and without mild cognitive impairment (MCI), and investigate its association with cognition, depression/anxiety, and quality of life. The pandemic impact was measured using a comprehensive questionnaire that assessed living arrangements, physical health, pandemic knowledge, behaviors adopted during restrictions, financial situation, mental health, and changes in daily activities. Additionally, we explored whether the impact differed between participants assessed before and after vaccination. Results showed that among cognitively healthy older adults, a higher pandemic impact was associated with poorer cognitive performance (r= -.31, p= .02), reduced quality of life (r= -.67, p< .00), and increased symptoms of anxiety (ρ= 0.50, p<

0.00) and depression (ρ= 0.37, p< 0.00). In older adults with MCI, a higher pandemic impact was linked to elevated levels of depressive symptoms only (ρ= 0.35, p= 0.05). Notably, only cognitively healthy older adults demonstrated a significant reduction in pandemic impact scores after vaccination, suggesting that more vulnerable populations, such as those with MCI, may continue to experience the consequences of lifestyle changes, even after vaccination. Currently, participants are engaged in a remote cognitive training protocol, and our future research aims to investigate the relationship between learning, cognition, engagement, social isolation, mood, and quality of life in older adults with and without cognitive impairment. By understanding the factors that mediate the pandemic's impact on older adults, clinicians can better protect this population during future periods of social isolation.

**Figure 1**.Correlations between total pandemic impact score and (a) cognition, (b) anxiety, (c) depression, (d) quality of life in HOA, and between pandemic impact and depression in MCI (e).

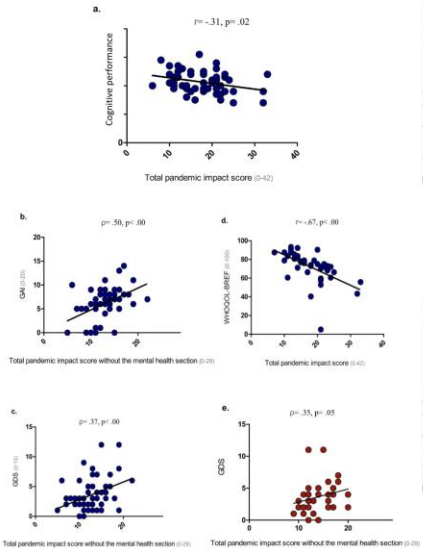
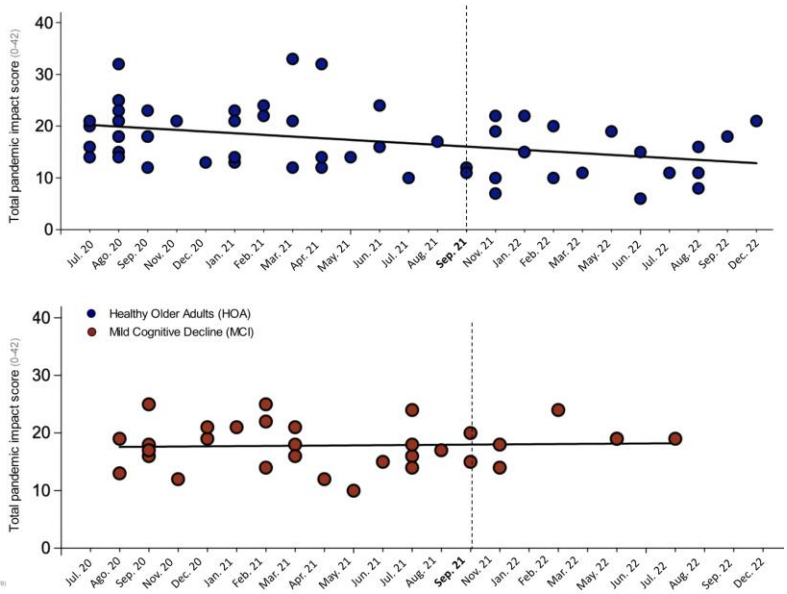



**Figure 2**. Individual Covid-19 pandemic scores of healthy older adults (HOA) and individuals with mild cognitive impairment (MCI).

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**Disclosures: A. do Vale Guimarães:** None. **K. Reuwsaat:** None. **N. Emele:** None. **B. Costa Poltronieri:** None. **Y. Oliveira:** None. **C. Carvalho:** None. **A. Pelegrino:** None. **F. Lin:** None. **R. Panizzutti:** None.

**Presentation Number:** NANO02.05

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIEHS
LSUHS COVID award

**Title:** Neural Exposome Converges on Brain Aging Pathways to Drive Post-Acute Neurologic and Cognitive Sequelae of COVID-19

**Authors:** J. LI[1], X. TIAN[2], H. ZHANG[2], L. JIN[2], Y. WANG[2], A. YUROCHKO[3], ***X.-H. LU**[2]; [1]Louisiana State Univ. Hlth. Sci. Pharmacoloy, Toxicology & Neurosci., Louisiana State Univ. Hlth. Scienc Pharmacoloy, Toxicology & Neurosci., Shreveport, LA; [3]Micobiology, [2]LSU Hlth. Shreveport, Shreveport, LA

**Abstract:** The dramatic heterogeneity of the clinical outcomes of COVID-19, including highly prevalent long-lasting neurological and neurocognitive deficits in post-acute sequelae of COVID-19 (PASC or long-COVID), highlights the dire need to understand the pathogenic role of noninheritable environmental factors or the neural exposome in mediating virus and host interaction to initiate and propagate chronic neuronal dysfunction with the possibility of progressing to cognitive decline and neurodegenerative conditions. Our work and others have provided mechanistic insights to link environmental toxicant exposure to long-lasting neurologic sequelae of virus infection. We have recently identified a strong association of the accumulative COVID-19 death per capita by the parish with agriculture herbicide use in Louisiana. Our autopsy study in human COVID-19 patients identified a significant increase in genotoxic stress (telomere attrition and increased DNA damage response (DDR) pathway). These findings are consistent with our previous findings that persistent genotoxic stress is a prominent feature in aged human brains, Parkinson's disease (PD), and Huntington's disease (HD). Persistent genotoxic stress can trigger an overzealous DDR signaling cascade orchestrated by Ataxia-telangiectasia mutated (ATM), dictating the fate of cells into cell cycle arrest or senescence. To examine the hypothesis in vivo, we have successfully developed a human ACE2 Bacterial artificial Chromosome (BAC) transgenic mouse model with full-length human ACE2 regulatory regions that faithfully recapitulated the structure, tissue distribution, and gene regulation of the human gene. Furthermore, to facilitate the analysis of PASC in an ABSL-2 facility, we developed a strategy to model the acute or senescence induction phase of SARS-CoV-2 infection that recapitulates the signature cytokine storm and acute respiratory distress syndrome (ARDS). Known cell senescence-inducers: Doxorubicin or Bleomycin, and environmental genotoxic agents (e.g., Parkinson's disease (PD)-associated herbicide, Paraquat) exerted strong genotoxic stress to facilitate the virus infection, which can be blocked by an ATM inhibitor. Exploiting knowledge on neural exposome in disease susceptibility, severity, and the manifestation of neurological and neurocognitive PASC will enhance our ability to address the long-term clinical outcome of viral infection, as it will shed light on the underlying mechanisms of pathobiology at molecular virus-host interfaces.

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**Presentation Number:** NANO02.06

**Topic:** C.01. Brain Wellness and Aging

**Support:** Spanish Instituto de Salud Carlos III Ref. PI21/00010.
EU REACTUE funds for regional development, regional government (Madrid, Spain)
Wellcome Trust, Advanced Investigator Grant to RGMM

**Title:** Post-covid brain changes associated with selective decline in everyday memory, executive function and age: the albacovid registry

**Authors:** V. SERRANO-DEL-PUEBLO[1], G. NAVARRO[2], C. M. ROMERO-SÁNCHEZ[3], P. PIQUERAS[3], L. ROJAS[3], I. FERIA[3], B. STRANGE[5], F. MANSILLA[4], B. CASTRO-ROBLES[2], L. ARIAS-SALAZAR[2,6], R. MORRIS[6,5], R. INSAUSTI[1,2], T. SEGURA MARTÍN[3], ***M. MUNOZ**[1];

[1]Lab. of Human Neuroanatomy, Univ. of Castilla-La Mancha, Albacete, Spain; [2]Res. Unit, [3]Neurol. Service, [4]Radiology Service, Albacete Univ. Hosp. Complex, Albacete, Spain; [5]The Lab. for Clin. Neurosci., Madrid Polytechnic Univ., Madrid, Spain; [6]Edinburgh Neurosci., Univ. of Edinburgh, Edinburgh, United Kingdom

**Abstract:** Between 2.5 and 27% of SARS-CoV-2 survivors suffer persistence of symptoms for months after infection (post-COVID syndrome, PCS) and among these symptoms cognitive complaints are particularly frequent. However, the brain changes underlying the cognitive symptoms are still unclear and key to guide rehabilitation. This study aims to determine potential brain changes in PCS and their possible relationship with neuropsychological decline. Participants were scanned with MRI to determine integrity of the grey/ white matter (Freesurfer/ FSL-TBSS) and assessed with a clinical interview and a standardized neuropsychological protocol to determine cognitive status. Patients with PCS (N=83, mean± SEM, 50.68± 1.03 years old; 59, 71% female) had mean overall cognitive function significantly below standard norms and matched controls (N=22, ACE III, $p< 0.01$; 50% patients $z\leq 0$). Executive function and memory were most affected. Attention was below 8$^{th}$ pc in 28% of patients; 21% WAISIII-digit span; 17% Verbal Fluency and 10% TMT-A attentional switch. Memory and episodic memory were below 8$^{th}$ pc in 24%. This was consistent with the families' report on everyday and spatial memory ($z= -1.04\pm 0.23$, $t_{72}= 2.61$, $p<0.01$, 31%). Patients had decreased cortical thickness in polymodal association areas and decreased white matter integrity in frontal, temporal and deep white matter regions, and this was associated with cognitive decline ($p<0.05$). Especially, age was negatively correlated ($p<0.01$) with cortical thickness in widespread cortical areas, and with decreased white matter integrity in frontal, temporal and deep white matter regions. Presence of ageusia during the acute infection was associated with worst episodic memory in patients. Post COVID insomnia, but not depression/anxiety, was associated with episodic memory loss. In sum, PCS is characterised by significant brain changes associated with a specific decline in episodic memory and executive function and age. Funded by the Spanish Instituto de Salud Carlos III Ref. PI21/00010.

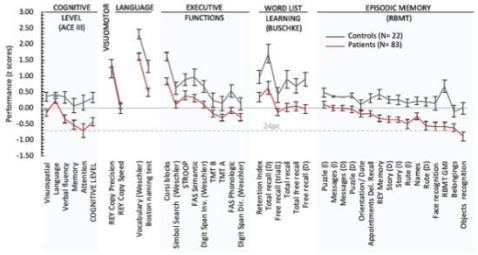



**FIGURE 1 |** Neuropsychological profile is characterized by specific executive function and memory deficits in post COVID. Differences in *neuropsychological scores between patients (red) and controls (grey)* **B.** Differences *neuropsychological scores between patients with overall. Abbreviations: D: delayed; Dir.: direct; GMI: general memory index; I: immediate; TMT A, B: trail making test versions A and B.*

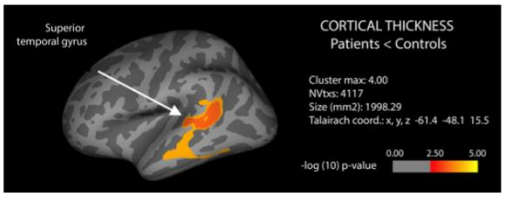



**FIGURE 2 |** **A.** *Differences in cortical thickness between patients and controls, light orange cluster p< 0.05 and dark orange cluster p< 0.01 in the caudal two thirds of the left superior temporal gyrus region. Corrected for intracranial volume, age, and gender.*

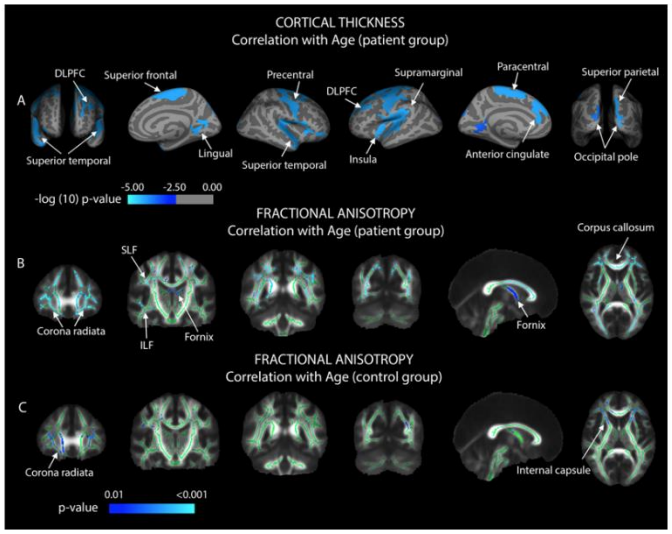



**FIGURE 3 |** **A.** *Spatial negative correlation of age with cortical thickness and age in the patient group (blue). No correlation was found in control group* **B.** Negative correlation of FA values and age in patient group (blue) **C.** Negative correlation of FA values and age in control group (blue). *Corrected for intracranial volume and gender ($p \leq 0.01$).*

**Disclosures: V. Serrano-del-Pueblo:** None. **G. Navarro:** None. **C.M. Romero-Sánchez:** None. **P. Piqueras:** None. **L. Rojas:** None. **I. Feria:** None. **B. Strange:** None. **F. Mansilla:** None. **B. Castro-Robles:** None. **L. Arias-Salazar:** None. **R. Morris:** None. **R. Insausti:** None. **T. Segura Martín:** None. **M. Munoz:** None.

**Presentation Number:** NANO02.07

**Topic:** F.04. Neuroimmunology

**Title:** Post-covid-19 brain-immune-genome clinical profile in Taiwan

**Authors: *P.-Y. CHUANG**;
Univ. of Maryland, Bethesda, MD

**Abstract:** Society of Neuroscience Abstract (< 2300 characters)**Post-COVID-19 Brain-Immune-Genome Clinical Profile in Taiwan.**Authors**P.Y. CHUANG**, EMBA, Ph.D., MSN, University of Maryland, Baltimore, MDCOVID-19 Statistic System, Taiwan Centers for Disease Control, Taiwan, ROC**Disclosures**:**P.Y. CHUANG**: None.**AbstractMOTIVATION/PROBLEM STATEMENT**: The COVID-19 operational structure in Taiwan was establishedon January 20, 2020, and coordinated personalized mobilization resources from a cross-ministry perspective asprivate stakeholders on January 23, 2020. The main neurological sign/symptom seeking emergency medicalattention was new confusion based on hospital admissions. This study **AIMED** to detect the Brain-Immune-COVID-19 Genome (BIG) clinical profile insights of the personalized predisposition.**METHODS/APPROACH:** 22,260,456 individuals (children and adults) with 9,970,937 post-COVID-19positive confirmed cases and 17,672 deaths from January 2020-March 2023. Inclusion Criteria: 1) diagnosiswith severe acute respiratory syndrome (SARS) following fever (> 38°C [100.4F]), respiratory signs/symptoms(>3), and chest radiology results with positive SARS-CoV pathology; 2) two or more positive SARS-CoV virustests (molecular bio-nucleotide and serological antibodies [ELISA or IFA]) from the specimen (saliva, sputum,blood, and stool) at two different timelines was to aim for genomic surveillance and types of variant proportion;and 3) demographics (age, gender, race, vaccination, hospitalizations, death, emergency visits, andtraveling). **RESULTS:** Older age (+65 years), male with chronic diseases, had a positive impact with severepositive COVID-19 comorbidity. 91.84% (n=21,872,466) of the population received at least one dose of theCOVID-19 vaccine. 2115 post-COVID-19 positive cases had fever (including headaches, muscle aches,dizziness, tastes/smell)**.** New Taipei City (n=270,163) and Taichung City (n=166,274) remain higher spreaderCOVID-19 novel pathogens in 2023. D614G strain and Alpha variant of SARS_CoV2 have been identified.The mechanisms of BIG had been linked with systematic inflammatory and blood-brain-barrier biomarkers.**CONCLUSION/IMPLICATIONS**: Immediate prevention strategies (surveillance and laboratory diagnosis,border, and community transmission control, medical response and preparedness of PPE, and health educationand disinformation management via daily media) had shown a significantly lower risk of communitytransmission via transportation, foreign affairs, economics, labor, and education in Taiwan. We continue torespond to international collaboration on the epidemic situation.

**Disclosures: P. Chuang:** None.

**Nanosymposium**

**NANO03: Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease**

**Location:** WCC 146C

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO03.01

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

**Support:** Alzheimer's Association Grant AARF-21-48972
NIH Grant P30-AG072979
NIH Grant RF1-AG069474
NIH Grant R01-AG056014
NIH Grant R01-AG055005
NIH Grant R01-AG072796
NIH Grant R01-AG070592

**Title:** Aging and Alzheimer's Disease Have Dissociable Effects on Medial Temporal Lobe Connectivity

**Authors: *S. HRYBOUSKI**[1], S. DAS[2], L. XIE[1], L. WISSE[5], M. KELLEY[3], J. LANE[3], M. SHERIN[3], M. DICALOGERO[3], I. M. NASRALLAH[1], J. A. DETRE[2], P. A. YUSHKEVICH[4], D. A. WOLK[2];
[1]Radiology, [2]Neurol., [3]Penn Memory Ctr., [4]Univ. of Pennsylvania, Philadelphia, PA; [5]Diagnos. Radiology, Lund Univ., Lund, Sweden

**Abstract: Introduction:** The extent to which pathological processes in aging and Alzheimer's disease (AD) relate to functional disruption of the medial temporal lobe (MTL)-dependent networks is poorly understood. Here, we examined functional connectivity (FC) alterations between the anterior and posterior regions of the MTL and in MTL-associated functional communities - the Anterior-Temporal (AT) and Posterior- Medial (PM) networks - in normal agers ($N$ = 116), individuals with preclinical AD ($N$ = 23), and patients with Mild Cognitive Impairment (MCI) or mild dementia due to AD ($N$ = 40). **Methods:** In this cross-sectional study, we analyzed data from 179 individuals from the Aging Brain Cohort study of the Penn ADRC. For intra-MTL FC comparisons, the MTL subregions were segmented using the ASHS-T1 pipeline. When modeling the MTL's interactions with the rest of the cortex, we employed anterior/posterior MTL ROIs derived from an *ex vivo* atlas of tau accumulation in the MTL. Functional datasets were preprocessed using a customized fMRIprep pipeline. Sparse network estimation and modularity-based clustering were used to reconstruct the AT and PM networks. Age effect analyses and AD group comparisons were performed using the General Linear Model. **Results:** The preclinical stage of AD was characterized by excessive FC between the perirhinal cortex and other regions of the MTL (Fig. 1), as well as between the anterior MTL and its direct neighbors in the AT network. This effect was not present in symptomatic AD. Instead, symptomatic patients displayed reduced hippocampal and intra-PM connectivity. For normal aging, our results led to three main conclusions. First, intra-network connectivity of both the AT and PM network systems is negatively correlated with age. Second, FC between the anterior and

posterior segments of the MTL declines with age. Finally, within the MTL, we observed greater vulnerability of the posterior MTL subregions, particularly the parahippocampal cortex, to age-associated FC decline. **Conclusion:** Together, the current results highlight evolving MTL dysfunction in aging and AD.

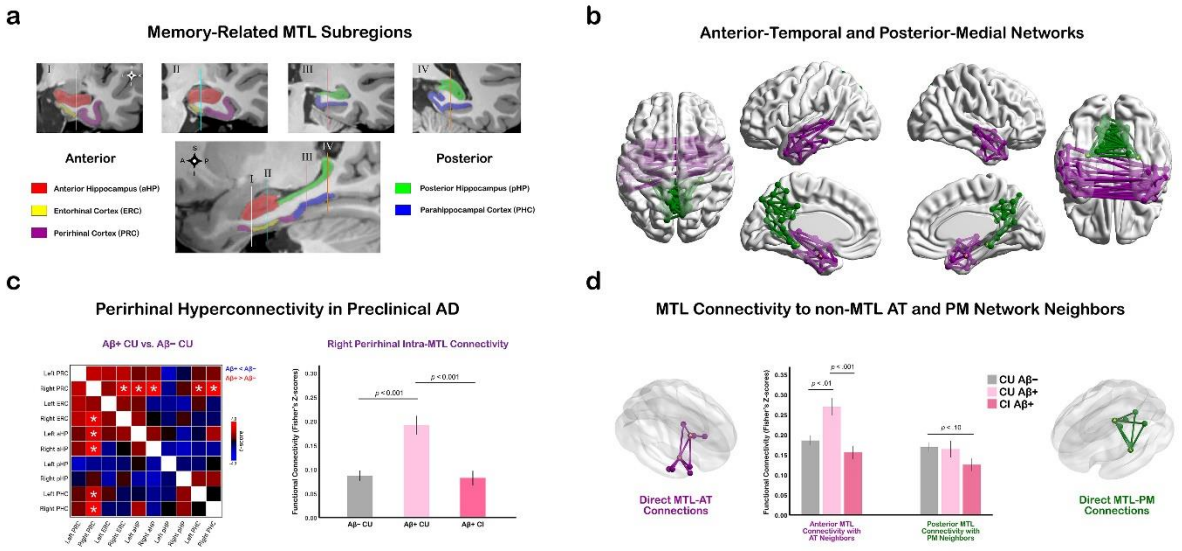



**Figure 1. (a)** Example ASHS-T1 segmentation of memory-related MTL subregions. **(b)** MTL-affiliated AT and PM brain networks. AT nodes and edges are in purple; those of the PM network are in green. **(c-d)** Functional connectivity differences between age- and sex-matched amyloid-negative normal agers (Aβ− CU group), amyloid-positve cognitively normal individuals with preclinical AD (Aβ+ CU group), and amyloid-positive patients with symptomatic AD (Aβ+ CI group). Purple/green nodes with a yellow asterisk represent anterior/posterior MTL, respectively. Abbreviations: AD = Alzheimer's disease; AT = anterior-temporal; PM = posterior-medial; CU = cognitively unimpaired; CI = cognitively impaired; MTL = medial temporal lobe.

**Disclosures: S. Hrybouski:** None. **S. Das:** None. **L. Xie:** None. **L. Wisse:** None. **M. Kelley:** None. **J. Lane:** None. **M. Sherin:** None. **M. DiCalogero:** None. **I.M. Nasrallah:** Other; I.N. serves on the Scientific Advisory Board for Eisai. **J.A. Detre:** None. **P.A. Yushkevich:** None. **D.A. Wolk:** Other; D.A.W. has served as a paid consultant to Eli Lilly, GE Healthcare, and Synapse. He serves on a DSMB for Functional Neuromodulation. He receives research support paid to his institution from Biogen.

**Presentation Number:** NANO03.02

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

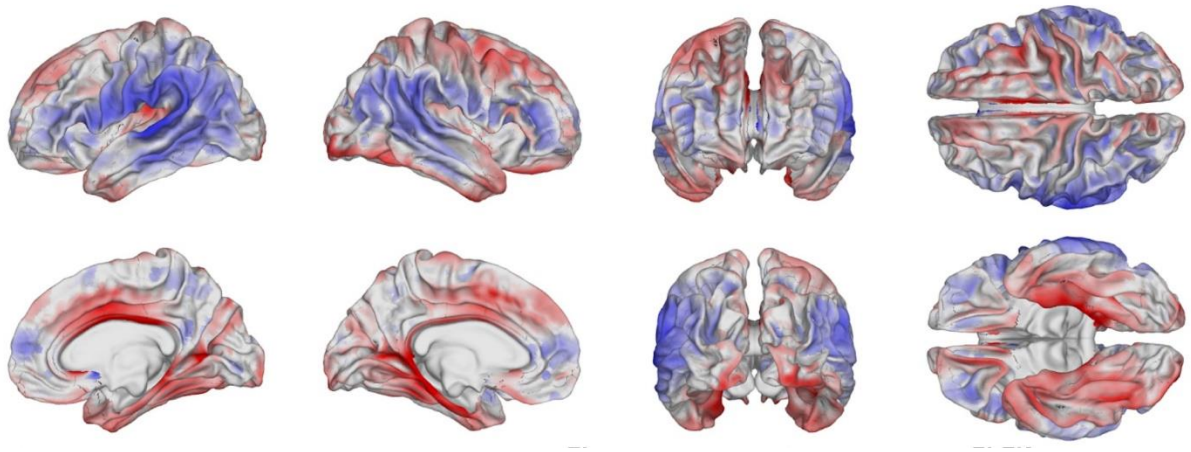
**Support:** NIH Grant R01 AG 079957

**Title:** Brain age estimation using anatomically interpretable deep neural networks to reveal domain-specific cognitive impairment

**Authors: *A. IRIMIA**[1], C. YIN[2], P. E. IMMS[3], A. AMGALAN[3], N. CHOWDHURY[3], N. CHAUDHARI[3], P. BOGDAN[3];
[1]Gerontology, [2]Electrical & Computer Engin., [3]USC, Los Angeles, CA

**Abstract:** The gap between chronological age (CA) and biological brain age, as estimated from magnetic resonance images (MRIs), reflects how individual patterns of neuroanatomic aging deviate from their typical trajectories. MRI-derived BA estimates are often obtained using deep learning models that may perform relatively poorly on new data or that lack neuroanatomic

interpretability. This study introduces a novel convolutional neural network (CNN) to estimate BA after training on the MRIs of 4,681 cognitively normal (CN) participants and testing on 1,170 CN participants from an independent sample. BA estimation errors are notably lower than those of previous studies. At both individual and cohort levels, the CNN provides detailed anatomic maps of brain aging patterns that reveal sex dimorphisms and neurocognitive trajectories in adults with mild cognitive impairment (MCI, N = 351) and Alzheimer's disease (AD, N = 359). In individuals with MCI (54% of whom were diagnosed with dementia within 10.9 years from MRI acquisition), BA is significantly better than CA in capturing dementia symptom severity, functional disability, and executive function. Profiles of sex dimorphism and lateralization in brain aging also map onto patterns of neuroanatomic change that reflect cognitive decline. Significant associations between BA and neurocognitive measures suggest that the proposed framework can map, systematically, the relationship between aging-related neuroanatomy changes in CN individuals and in participants with MCI or AD. Early identification of such neuroanatomy changes can help to screen individuals according to their AD risk.



**Disclosures: A. Irimia:** None. **C. Yin:** None. **P.E. Imms:** None. **A. Amgalan:** None. **N. Chowdhury:** None. **N. Chaudhari:** None. **P. Bogdan:** None.

**Presentation Number:** NANO03.03

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIH Grant RF1-AG059869

**Title:** Alzheimer's disease genetic risk and long-term trajectories of neurodegeneration and white matter hyperintensities among cognitively normal individuals

**Authors: *A. SOLDAN**[1], C. PETTIGREW[2], J. WANG[2], M. BILGEL[3], C. DAVATZIKOS[4], G. ERUS[4], S. C. JOHNSON[5], C. MASTERS[6], J. C. MORRIS[7], S. M. RESNICK[3], M. ALBERT[2]; [1]Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]NIA, NIH, Baltimore, MD; [4]Univ. of Pennsylvania, Philadelphia, PA; [5]Med., Univ. of Wisconsin, Madison, WI; [6]The Florey Institute, Univ. of Melbourne, Melbourne, Australia; [7]Washington Univ., St. Louis, MO

**Abstract: Background:** Neurodegeneration due to Alzheimer's disease (AD) and cerebrovascular disease, measured by white matter hyperintensities (WMH) on MRI, are is present many years prior to the onset of cognitive impairment. It remains unclear whether rates of atrophy and WMH accumulation among cognitvely normal adults differ as a function of genetic risk for late-onset AD, as most prior studies have been cross-sectional. To address this gap, we examined the long-term trajectories of brain atrophy and WMH change in relationship to APOE genotypes (N=1,541) and AD-polygenic scores (AD-PRS, N=1,093) among indivdiuals with normal cognition at baseline. **Method:**Data were derived from the Preclinical AD Consortium (PAC), which includes harmonized data from five longitudinal cohorts: ACS, AIBL, BIOCARD, BLSA, and WRAP. Longitudinal MRI atrophy patterns associated with standardized measures of brain aging (SPARE-BA), Alzheimer's disease (SPARE-AD), hippocampal volume, and WMH volumes were derived by machine learning and harmonized across scanners and data sets using the previously validated MUSE platform. Mean MRI followup=5.3 years (max=24 years); mean baseline age=66.2 years. **Results:** In linear mixed models, atrophy in the SPARE-AD pattern and the hippocampus increased more among APOE ε4/ε4 carriers (N=63) relative to ε3/ε4 participants (p=0.028), who had greater increases than ε3/ε3 carriers (*p*=0.008). Relationships between APOE-ε4 status and atrophy did not differ by sex, but males had greater age-related and AD-related atrophy than females (sex x time interactions, p<=0.009). Higher education was associated with a reduced impact of APOE-ε4 on rate of change in SPARE-AD and hippocampal volume (education x ε4 x time interactions, both p<0.039). APOE-ε2 carrier status was not associated with levels or change in atrophy. However, both APOE-ε2 and ε4/ε4 carriers had greater increases in WMH volumes (p=0.013) relative to the other groups. Higher AD-PRS scores were associated with greater AD-related and hippocampal atrophy (both p<=0.035), as well as with greater increases in WMH volumes (p=0.009), indepenent of APOE. **Conclusion:** Among cognitively normal individuals, APOE-ε4 status is mostly associated with greater atrophy in AD-vulnerable regions, and this association may be lower among those with more education. APOE-ε2 status does not appear to protect against brain atrophy, but increases WMH accumulation. APOE-ε4/ε4 carriers also also had higher increases in WMH volumes, as did those with higher AD-PRS scores, supporting the view that AD pathology may contribute to WMH formation. Higher AD-PRS scores contributed to atrophy in AD-vulnerable regions.

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**Title:** Neurophysiological trajectories in Alzheimer's disease progression

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**Abstract:** Alzheimer's disease (AD) is characterized by early-stage accumulation of amyloid-β and misfolded tau proteins causing synaptic dysfunction and progressive neurodegeneration, resulting in cognitive decline. Altered neural oscillations associated with abnormal accumulation of proteins have been consistently demonstrated in electrophysiological imaging AD studies. However, the specific trajectories of abnormal neuronal oscillations in AD progression with respect to neurodegeneration and cognitive decline is unknown. Here we deployed robust event-based sequencing models (EBMs) investigate the trajectories of long-range and local neural synchrony across AD stages, estimated from resting-state magnetoencephalography (MEG) imaging. The long-range and local synchrony were quantified by amplitude-envelope correlation (AEC) and spectral power, respectively. Stages of AD progression were first estimated from an EBM that included neurodegeneration and cognitive decline, quantified respectively by parahippocampal gyrus (PHG) volume loss and mini mental state examination (MMSE) score decline. Both long-range and local neural synchrony increases in delta-theta (2-7 Hz) and decreases in alpha (8-12 Hz) and beta (15-29 Hz) bands showed progressive changes along the EBM stages of AD. Especially, the alpha- and beta-band synchrony were found to be abnormal during preclinical stages of AD, preceding both neurodegeneration and cognitive decline [Fig.1: a profile of the alpha-band long-range synchrony (AEC) along the EBM stages]. Furthermore, long-range synchrony effects were larger when compared to local synchrony, indicating that connectivity metrics involving multiple brain regions are more sensitive to disease progression. These findings demonstrate that frequency-specific abnormalities in neural synchrony are early manifestations of AD pathophysiology.

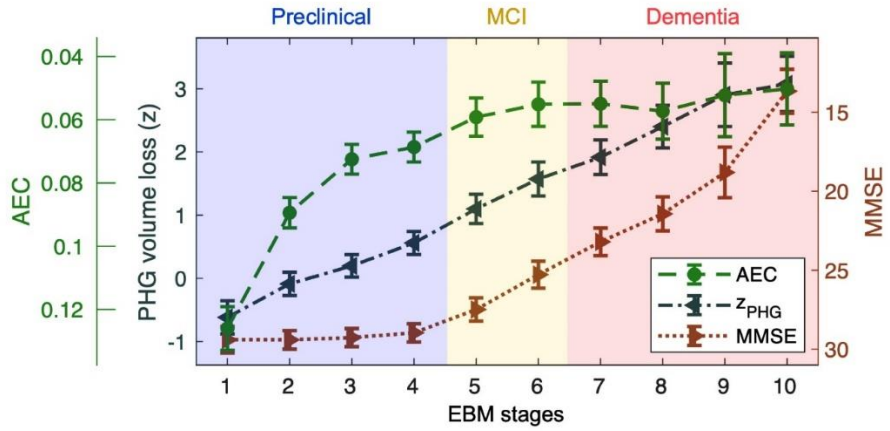


Fig. 1 Trajectories of long-range neural synchrony in alpha, hippocampal volume loss and MMSE.

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**Title:** Links between cognition and multivariate brain white matter differences in individuals with family history of Alzheimer's disease and APOE4 genetic risk

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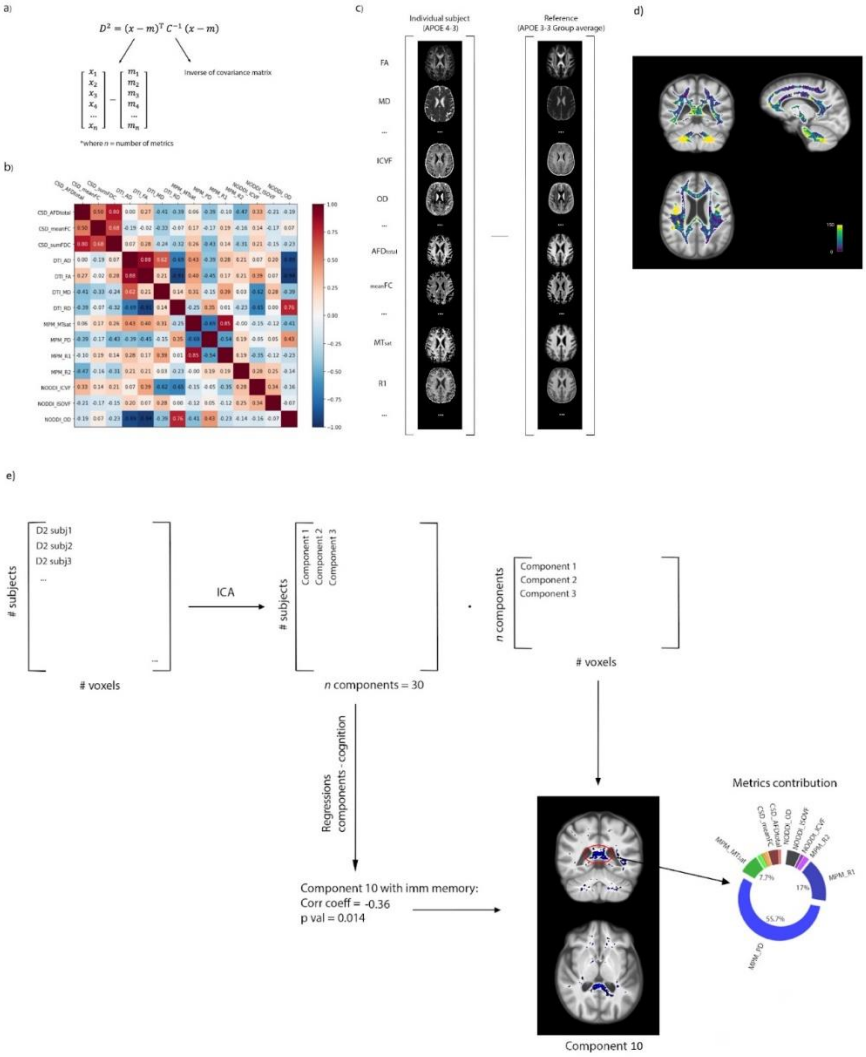
**Abstract:** The etiology of Alzheimer's disease (AD) is known to be complex, developing from a cascade of events that likely vary substantially across individuals. We used a multivariate approach to capture the complexity and heterogeneity of white matter (WM) pathologies in individuals at risk of AD in a more holistic manner. The MRI data of 105 older adults with a family history of AD were analysed. Diffusion-weighted imaging and multi-echo magnetization transfer, proton density and T1-weighted data were used to compute several WM metrics (Fig1b-c). We computed the voxel-wise Mahalanobis distance (D2) in WM between APOE4-3 individuals (greater risk of developing AD; n=46, 30 females, mean age=66.75) and a reference group consisting of individuals with the APOE3-3 genotype (normal risk; n=59, 49 females, mean age=68.33) (Fig1a-d). D2 is a multivariate measure that combines several MRI metrics, accounting for their covariance, and yielding a score indicative of the degree of abnormality at each WM voxel (Fig1d). Associations between WM D2 and cognitive performance were then investigated (Fig1e). Independent component analysis (ICA) was performed on spatial dimensions and linear regression analyses were conducted between D2 in ICA components and scores on items of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). A significant negative association (R=-0.36, p=0.014) was found between D2 in a component corresponding to the splenium of the corpus callosum (CC) and scores on RBANS-

immediate memory, indicating a link between a greater degree of WM abnormality and poorer performance. Proton density (MPM_PD) and longitudinal relaxation rate (MPM_R1), potentially indicative of WM loss, contributed most to D2 in this region. Our findings suggest that microstructural alterations in the posterior part of the CC may play a role in memory deficit in older adults who are at greater risk of developing AD. This is in line with other studies that showed a link between CC degeneration and memory impairments in early AD stages (Qiu et al., 2016).

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

**Support:** NIH Grant U01 AG068057

**Title:** Predicting Future Decline from Mild Cognitive Impairment to Alzheimer’s Disease with Machine Learning and 3D Brain MRI

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**Abstract:** Mild cognitive impairment (MCI) is an intermediate stage between healthy aging and Alzheimer’s disease (AD) - a progressive neurodegenerative disorder that affects around 50 million people worldwide. One key goal of AD research is to predict which individuals with MCI are most likely to progress to AD over a given interval (typically 2 years); if successful, these individuals could be preferentially enrolled in drug trials that aim to slow AD progression. Here we studied a range of MCI-to-AD predictive models including linear regressions and support vector regressions, using predictors from 3D anatomical brain MRI, age, sex, APOE genotype, and regressed baseline standardized test scores including MMSE and ADAS-cog (ADAS-11 and ADAS-13) in 2 years span. A total of 2,448 subjects (1,132 with MCI, 883 healthy controls, and 433 with dementia) from ADNI were included (1,161 females, 1,287 males). We focused on the correlation among gray matter volumes of cortical regions, extracted from T1-weighted MRI scans using FreeSurfer, by employing kernel Principal Component Analysis (kPCA). kPCA generated new features based on the variance in the features provided. The newly extracted features were combined with other predictors including age, sex, and APOE genotype and then fed to the model for the training. The entire dataset was resampled to counteract the issue of class imbalance based on whether the subjects were converters (MCI->AD/HC->MCI) or not. 70% of the newly formed balanced dataset was fed to the model for training and the remaining 30% was used for cross-validation and testing. For predicting the change over time in MMSE, the model achieved a correlation coefficient of 0.726 with r-squared error of 0.768 in MCI->AD converters, correlation coefficient of 0.741 with r-squared error of 0.813 in HC->MCI converters. Whereas, for ADAS-cog, the model achieved correlation coefficient of 0.792 with r-squared error of 0.836 for HC->MCI, correlation coefficient of 0.750 with r-squared error of 0.792 for MCI->AD and 0.84 for non-converters.

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

**Support:** MEITY 4(5)/2019-ITEA

**Title:** Glutascan: a non-invasive imaging platform for longitudinal monitoring and quantification of brain glutathione for clinical trial.

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**Abstract:** **PURPOSE** Glutathione (GSH) is a potent antioxidant against oxidative damage, and its depletion in the hippocampus is directly linked to Alzheimer's disease (AD) severity. To monitor GSH levels in the brain and evaluate various GSH supplements' effects on brain health, a non-invasive method is crucial. In this study, we assess longitudinally a range of GSH supplements independently and also measure GSH levels in a healthy brain. The objective is to develop a method involving magnetic resonance spectroscopy (MRS) for detecting GSH enrichment through supplements in brain non-invasively.
**METHODS** MRS data was obtained utilizing a 3T MR scanner (Prisma, Siemens) equipped with a 64-channel $^{1}$H head coil. The acquisition was done using the MEGA-PRESS sequence, with the parameters: ON = 4.40 ppm, OFF = 5.00 ppm, TE = 120 ms, TR = 2500 ms, voxel size = 25 × 25 × 25 mm, and average= 32. Phantoms labeled A to E each have a different GSH supplement dose dissolved in a 1 L phosphate buffer, with pH adjusted to 7.0. MRS data was collected from the phantoms as well as from cerebellum of a healthy individual over time. GSH data were processed using KALPANA package.
**RESULTS** The GSH peak area (in phantoms) showed a gradual decline over time. In contrast, the GSH peak area in the cerebellum of a healthy individual had no time-dependent change. Prior research has showed significant GSH depletion in AD in certain key regions only. To effectively monitor the effectiveness of GSH supplements in neurodegenerative disorders, an MRS based scheme is proposed. (Fig. 1)
**CONCLUSION** This MRS scheme presents a valuable approach for monitoring the effectiveness of GSH supplements as evident from these two experimental schemes. Moreover, to assess patient-specific functional outcomes, it is recommended to complement MRS measurements with comprehensive neuropsychological assessments. This integrative approach allows for a more holistic understanding of the potential impact of the supplement/s on cognitive functioning and offers valuable insights into its overall GSH enrichment efficacy.
**CORRESPONDENCE** pravat.mandal@gmail.com

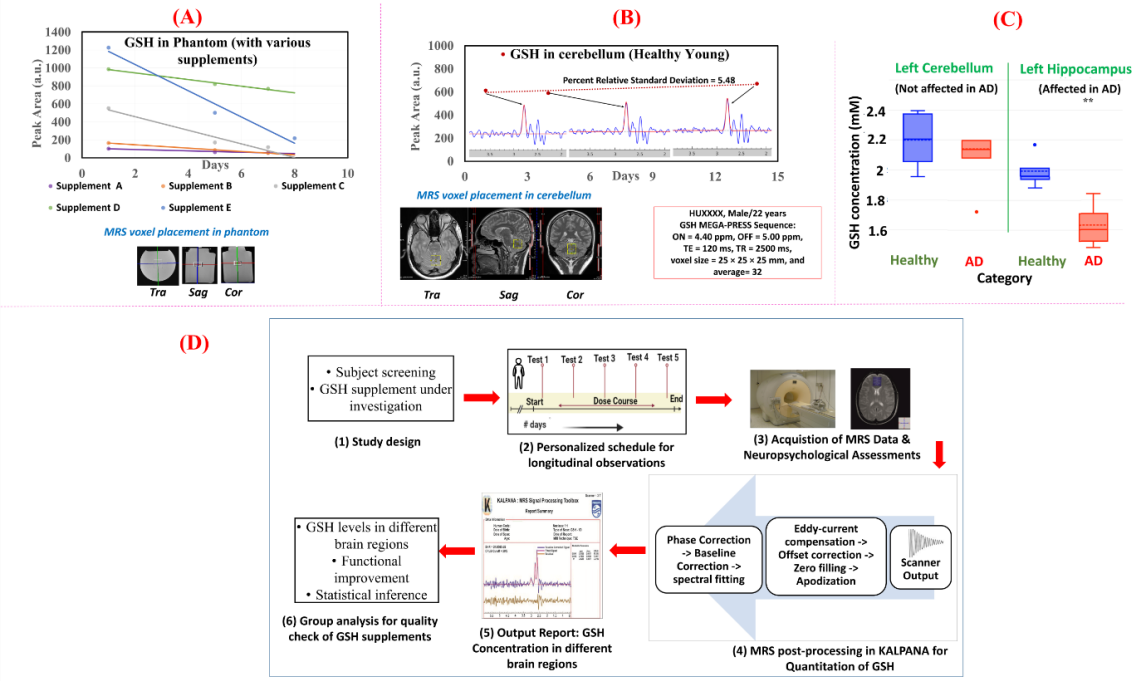


**Figure 1:** Representation of various cases (A) In vitro: Longitudinal GSH levels from 5 supplement samples (B) In vivo: Longitudinal GSH levels from the cerebellum of healthy human subject (C) GSH concentration from left cerebellum and hippocampus for healthy old and AD subjects[1] (D) Proposed non-invasive imaging platform for GSH quantification in human brains along with quality assessment of GSH supplements. *(GSH: Glutathione; AD: Alzheimer's disease; [1]Biological Psychiatry (2015) Volume 78, Issue 10, 15 November 2015, Pages 702-710)*

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

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Rome Technopole PNRR, FP7
D-TAIL IIT Jointlab

**Title:** Nanocage-mediated delivery of tau fluorophores: a promising approach for alzheimer's disease diagnosis in the retina

**Authors: *S. DI ANGELANTONIO**[1,3,4], Y. GIGANTE[3,4], L. MAUTONE[1,4], S. GHIRGA[3,4], L. BAROLO[2], A. SOLOPERTO[5], P. BAIOCCO[2], A. BOFFI[2];
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**Abstract:** Alzheimer's disease (AD) is a devastating neurodegenerative disorder associated with dementia. Despite extensive research, effective therapies and significant progress in symptom management remain elusive. Diagnostic accuracy plays a crucial role in developing therapeutic strategies. Hyperphosphorylated protein units (p-tau) and total-tau monomers (t-tau) have emerged as reliable biomarkers for AD. Promisingly, early detection of neurofibrillary tangles in retinal tissue has become a potential diagnostic tool. A computational model of tau oligomers facilitated the development of highly specific tau fluorophores. By constructing a BODIPY-based probe called BT-1, we successfully created a highly specific tau fluorophore with excellent

photophysical properties and high selectivity. This probe enables in vitro imaging of hyperphosphorylated tau protein filaments with minimal background noise. To deliver BT-1 to living retinal cells derived from induced pluripotent stem cells (iPSC), we identified a class of delivery cargoes known as cell-permeant peptides (CPP), which are natural nanocages capable of encapsulating small molecules and entering human cells. We demonstrated the colocalization of BT-1 labeling with phosphorylated and oligomeric tau in AD retinal slices and iPSC-derived retinal cells. The encapsulation of BT-1 within CPP nanocages provided a unique formulation for efficient delivery of the fluorescent probe to retinal tissue. Our study highlights the potential of CPP nanocages loaded with the fluorescent probe BT-1 as a promising method for specifically identifying NFTs in retinal tissue, aiding in the clinical diagnosis of AD. Furthermore, encapsulating other substrates within CPP nanocages opens up potential applications in neuroscience and nanomedicine.

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**Nanosymposium**

**NANO04: Parkinson's Disease: Cellular and Molecular Mechanisms**

**Location:** WCC 144

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO04.01

**Topic:** C.03. Parkinson’s Disease

**Support:** UGA startup funding

**Title:** Activation of D2Rs ameliorates the dysfunction of microglia in human LRRK2-R1441G transgenic mice

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**Abstract: Background**: An emerging concept in the Parkinson’s disease (PD) research is that the immune system plays a key role in the progressive death of dopamine neurons. Several recent studies highlight the involvement of leucine-rich repeat kinase 2 (LRRK2), the most prevalent genetic cause in both familial and sporadic PD in driving the connection between microglia dysfunction and PD susceptibility. Here, we examined the effects of pathogenic R1441G mutation on microglia functions and contributions to the pathogenesis of PD. **Materials and Methods:** A bacterial artificial chromosome (BAC) transgenic mouse model overexpressing human LRRK2-R1441G has been shown to recapitulate robust motor behavioral, neurochemical and pathological features of PD. We crossed the human LRRK2- R1441G transgenic mice with

the Cx3cr1-EGFP mice and investigated the function and dynamics of microglia with time-lapse two-photon imaging in awake mice in vivo and in acute brain slices ex vivo. **Results**: Our study demonstrates that the R1441G mutation increases the number of activated microglia and microglial polarization in the dorsal striatum. The microglial motility and its response to focal injury is decreased in the LRRK2-R1441G transgenic mice compared to their wild-type controls. Meanwhile, microglial processes retract faster and extend slower in microglia carrying the R1441G mutation. Given the neuroprotective role of dopamine D2 receptors (D2Rs) and aberrant D2R signaling in this mouse model, we administered a D2R agonist, quinpirole, to the R1441G transgenic mice and found that quinpirole ameliorated all the deficits of microglia. **Discussion and Conclusions**: Our results provide the first piece of evidence of the modulation of microglia mobility and response induced by pathogenic LRRK2 mutations. D2R activation may suppress neuroinflammation and neurodegeneration. This work provides important insights of the contribution of microglia to the pathogenesis of PD and opens new avenues for therapeutic intervention by targeting microglia-mediated immune response.

**Disclosures: Y. Chen:** None. **H. Zhang:** None.

**Presentation Number:** NANO04.02

**Topic:** C.03. Parkinson's Disease

**Support:** NIH Grant R01NS115809

**Title:** Understanding the role of secreted astrocytic S100B in altering voltage-gated channel function in dopaminergic neurons during the pathogenesis of Parkinson's disease

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**Abstract:** Parkinson's disease (PD) is a devastating neurodegenerative disorder characterized by the loss of midbrain substantia nigra pars compacta (SNc) dopaminergic (DA) neurons. The dearth of effective neuroprotective treatments for PD stems in part from the fact that we do not fully understand mechanisms that can trigger SNc DA neuron loss. In this regard, we hypothesize that secretable factors from midbrain astrocytes play a crucial role in triggering the loss of DA neurons during early PD. Among the various proteins secreted by astrocytes, S100B is particularly interesting because PD is associated with increased concentrations of S100B within the midbrain and cerebrospinal fluid. In addition, S100B overexpression in mice accelerates the loss of SNc DA neurons, which suggests a role for this protein in the pathogenesis of early PD. In this study, we assessed the extent to which extracellularly secreted S100B abnormally alters the activity of DA neurons. To first assess the extent of astrocytic S100B expression in the mouse SNc, mouse midbrain sections were co-immunolabeled with S100B and tyrosine hydroxylase (TH). We found that in the mouse SNc, S100B labeled astrocytic processes completely envelop the somata of TH expressing DA neurons, suggesting that an increase in S100B secretion by astrocytes within the midbrain is optimally positioned to alter DA function during early PD. We next asked if acute exposure to extracellular S100B alters the activity of identified TH expressing DA neurons in primary mouse midbrain cultures. Acute

exposure to 50 pM S100B specifically inhibited A-type voltage-gated potassium currents in $TH^+$, but not $TH^-$ neurons. This was accompanied by ~2-fold increases in the frequency of both intrinsic firing, as well as L-type voltage-gated calcium channel-mediated calcium fluxes only in $TH^+$ neurons. Further, exposure to 100 µM 4-aminopyridine (4-AP), an A-type voltage-gated potassium channel inhibitor, mimicked the S100B mediated increase in calcium fluxes in $TH^+$ neurons. Taken together, our finding that extracellular S100B specifically alters the activity of native DA neurons via an inhibition of A-type voltage-gated potassium channels has important implications for understanding the pathophysiology of early PD. In ongoing experiments, we are assessing the extent to which S100B secreted from astrocytes accelerates DA neuron loss in a mouse model of PD.

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**Presentation Number:** NANO04.03

**Topic:** C.03. Parkinson's Disease

**Support:** FWF I 3778, ERANET program

**Title:** Interaction of nicotinic acetylcholine receptors and alpha-synuclein in motor behaviour and nigrostriatal dopamine

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**Abstract:** According to numerous epidemiological studies, Parkinson's disease (PD) occurs less frequently in cigarette smokers than in non-smokers. Assuming that nicotinic acetylcholine receptors are periodically active by activation through endogenous acetylcholine even in the absence of nicotine exposure, we tested whether they act against the neurodegenerative effect of alpha-synuclein, a protein relevant in PD. Transgenic mice with a human alpha-synuclein containing two mutations that cause familial PD in humans (SYNhm2) were crossed with mice lacking the nicotinic α7-acetylcholine receptor (nα7). Four groups of mice were obtained: (1) control mice without mutant alpha-synuclein with nα7 (nα7+), (2) mice without mutant alpha-synuclein without nα7 (nα7KO), (3) mice with SYNhm2 with nα7 (SYNhm2-nα7+), and (4) mice with SYNhm2 without nα7 (SYNhm2-nα7KO). Vertical movements determined at 7 and 16 months of age in a photocell-based activity monitor were significantly lower in SYNhm2-nα7KO than SYNhm2-nα7+ mice (Pairwise Multiple Comparison Procedures by Holm-Sidak after Two Way Analysis of Variants for the factors alpha-synuclein and nα7), but did not differ in nα7KO from nα7+ mice. Non-ambulatory movements were significantly lower in mice without nα7 than in mice with nα7 at 7 months, and at 16 months significantly lower in SYNhm2-nα7KO than SYNhm2-nα7+ mice, but did not differ in nα7KO from nα7+ mice. Pole test at 20 months of age did not show significant differences for the factors alpha-synuclein nor nα7. Striatal noradrenaline, serotonin and dopamine tissue levels did not differ between the four groups of mice at 21 months of age however striatal dopamine turnover as determined by the molar ratio of homovanillic acid/dopamine was significantly higher in mice without nα7 than in mice with nα7. Stereological counts of nigral cells positive for tyrosine hydroxylase in the left

and right hemisphere at 21 months revealed that asymmetry, as determined by the absolute value of (cells left minus cells right)/(mean value of cells left and right), was also significantly higher in mice without nα7 than in mice with nα7. In conclusion, up to the age of 20 months there was no obvious PD behaviour in any of the four groups. Absence of the nα7 generally reduced several features of motor behaviour and there was a statistically significant interaction between nα7 and alpha-synuclein in particular motor features at a particular age. Neurochemical and stereological analysis at the age of 21 months did not show signs of PD, however the asymmetry of nigral cell counts and the increased striatal dopamine turnover suggest a stressed nigrostriatal system in mice without nα7.

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**Presentation Number:** NANO04.04

**Topic:** C.03. Parkinson's Disease

**Support:** F31NS129277-02
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Parkinson's Association of Alabama
T32NS095775

**Title:** Heterozygosity of the GBA1 L444P mutation impairs synaptic degeneration through lysosomal dysfunction

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**Abstract:** The most common genetic risk factor of Parkinson's disease is heterozygous mutations in the GBA1 gene which encodes for the lysosomal enzyme, glucocerebrosidase (GCase). Clinically, heterozygosity of the GBA1 L444P mutation ($GBA1^{+/L444P}$) leads to a 5.6-fold increased risk of cognitive impairments, including dementia. Genetic variants in CTSB, the gene which encodes for another lysosomal enzyme, Cathepsin B (CatB), is shown to increase risk for PD in individuals with GBA1 mutations. In this study, we used $GBA1^{+/L444P}$ knock-in mice of both sexes and their wildtype littermates ($GBA1^{+/+}$) as controls, to determine the effects of this severe GBA1 mutation on lysosomal function. As early as three-months of age, expression of the presynaptic excitatory marker, vGLUT1, is reduced by~36%, without changes to the postsynaptic counterpart, Homer1, in the hippocampus of $GBA1^{+/L444P}$ mice compared to $GBA1^{+/+}$ mice. This may contribute to spatial memory deficits observed at later time points, at 9-months of age, evident through the Y maze and Barnes maze behavioral tasks. Hippocampal primary neurons also exhibit a significant reduction in overall lysosomal function as seen through the DQ-BSA assay. In vivo, $GBA1^{+/L444P}$ mice exhibit hippocampal lysosomal dysfunction as early as three-months through the reduction of GCase activity and CatB protein expression. Collectively, lysosomal dysfunction may contribute to the increased memory deficits observed in mice expressing the GBA1L444P mutation. Elucidating the molecular mechanism behind cognitive impairments will likely aid in the development of novel therapies that can slow the progression of Parkinson's disease.

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**Title:** Astrocyte dysfunction at the blood-brain barrier: new insights into Parkinson's disease

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**Abstract:** The blood-brain barrier (BBB) regulates exchanges between the central nervous system and the peripheral circulation to maintain brain homeostasis, and relies on the coordinated action of brain microvascular endothelial cells (dBMECs), pericytes and astrocytes. In Parkinson's disease (PD), a neurodegenerative disorder characterized by neuronal death and astrocyte reactivity, barrier integrity is compromised which may further contribute to disease progression. The OBJECTIVE of this study is to determine how astrocytes with the PD-related mutation LRRK2 G2019S affect the functionality of the BBB. METHODS: We first evaluated the nature of astrocytic biological processes affected by the LRRK2 G2019S mutation by performing a meta-analysis of RNA-sequencing datasets and characterizing the astrocyte inflammatory profile. We then established a novel BBB-on-a-chip model using human induced pluripotent stem cells (iPSCs) with the LRRK2 G2019S mutation or gene corrected controls. This in vitro model reproduces a physiologically-relevant environment and direct cell-cell interactions between dBMECs, astrocytes and pericytes, and enables the formation of a leaktight vessel. To determine if LRRK2 G2019S astrocytes affect the formation of a functional vessel, we exposed control dBMECS to disease astrocytes and monitored vessel formation and barrier integrity using biochemical and imaging techniques. RESULTS: Our results indicate that LRRK2 G2019S astrocytes are pro-inflammatory, and display a transcriptomic profile suggestive of altered angiogenic properties. This mutation impacts the production of trophic factors and upregulates the release of pro-inflammatory molecules via activation of the MEK/ERK pathway.

As a result of this pathological secretome, disease astrocytes prevent the formation of a leaktight BBB and change the morphology of vessel-forming BMECs. These observations may be driven by a dual effect of oversecretion of cytokines combined with a lack of pro-angiogenesis factors. CONCLUSION: The LRRK2 G2019S mutation alters astrocyte paracrine communication and affects the in vitro formation and maintenance of a functional BBB.

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**Title:** Guanylyl cyclase C is a potential therapeutic target to prevent neurodegeneration in Parkinson's disease

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**Abstract:** Parkinson's disease (PD) is the second most common cause of age-related neurodegeneration in the U.S. In PD, mitochondrial dysfunction in dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc) induces oxidative stress and cell death. In turn, this neurodegeneration leads to striatal DA depletion, which underlies the motor dysfunction characteristic of PD. Current PD therapies raise DA levels to relieve motor symptoms, but do not prevent neurodegeneration or slow disease progression. Thus, there is an unmet clinical need to develop novel therapeutic strategies that protect DA neurons from degeneration to prevent and treat PD.
Guanylyl cyclase C (GUCY2C) is an intestinal receptor with a canonical role in fluid and electrolyte balance. In intestine, GUCY2C signaling supports mitochondrial structure and function, and its disruption is central to the pathophysiology of cancer, inflammation, and toxic injury. Beyond the intestine, GUCY2C was recently discovered in two distinct brain regions: the hypothalamus and the nigrostriatal pathway. We and others have demonstrated that GUCY2C expressed by hypothalamic neurons controls leptin signaling to promote satiety and maintain normal body weight. However, GUCY2C is also expressed by DA neurons in the SNpc where its function remains undefined.
Our studies reveal that loss of GUCY2C causes a significant decrease in mitochondrial protein and function, increase in oxidative stress, and loss of DA neurons within the aging SNpc. Furthermore, silencing GUCY2C signaling increases the vulnerability of DA neurons to

degeneration, neuroinflammation, and striatal DA loss induced by MPTP, a mitochondrial toxin that selectively kills DA neurons in the SNpc. Moreover, treating cultured DA neurons with cyclic guanosine monophosphate (cGMP), the downstream product of GUCY2C signaling, protects against loss of mitochondrial integrity, accumulation of oxidative stress, and cell death induced by mitochondrial toxin MPP+.
Our findings emphasize the translational potential of targeting neural GUCY2C with exogenous ligands to slow the rate of neurodegeneration in PD. In that context, GUCY2C ligands linaclotide (*Linzess™*) and plecanatide (*Trulance™*) are already FDA-approved to treat chronic constipation syndromes.

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**Title:** Microtubule interactions in the mediation of LRRK2 activity

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**Abstract:** Pathogenic mutations of LRRK2 increase LRRK2 kinase activity and can cause Parkinson's disease. Recent in situ cryo-electron tomography studies revealed that exogenous LRRK2 oligomerizes as a right-handed double helix around microtubules in transfected cells. This microtubule localization of LRRK2 is enhanced by both pathogenic mutations and some types of ATP-competitive inhibitors, suggesting that LRRK2 interacts with microtubules in a kinase-activity dependent manner. Herein, we find that LRRK2 kinase activity can be altered through interactions with microtubules. Disrupting microtubule polymerization reduces LRRK2 cis-autophosphorylation and trans-Rab phosphorylation activity. In contrast, stabilizing microtubules by inhibiting microtubule disassembly may increase LRRK2 autophosphorylation activity. Studies are ongoing to determine whether trapping LRRK2 on microtubules without the use of LRRK2 inhibitors effects LRRK2 kinase-dependent activities. Overall, emerging results suggest a possible reciprocal regulation between LRRK2 and microtubules, whereby LRRK2 kinase activity might drive the microtubule-localization of LRRK2, and microtubule interactions might in-turn activate LRRK2 kinase activity to phosphorylate downstream Rab substrates.

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**Title:** Deciphering the molecular mechanisms underlying telomere maintenance upon leucine-rich repeat kinase mutation

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**Abstract:** It is well-known that the intricate interplay between aging, genetics, and environment is associated with neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Unlike Alzheimer's disease (AD), which shows a correlation between short telomeres and decreased life expectancy, the evidence that supports the roles of telomere length (TL) in PD remains inconclusive. In this study, we sought to investigate the roles of TL as well as telomerase in PD. 106 PD patients and 28 health donors (HD) were examined. The observations indicated that there was indistinguishable in TL between PD patients and HDs. To explore the detailed mechanisms of how PD causative genes affect TL and telomerase, induced pluripotent stem cells (iPSCs) and SH-SY5Y cells with a PD gene mutation were used. We examined the kinetics of TL maintenance in *LRRK2*$^{G2019S}$ iPSCs and its corrected iPSCs over time. Telomeres in *LRRK2* mutant iPSCs were elongated while the corrected one maintained TLs over the passages, suggesting that *LRRK2*$^{G2019S}$ mutation leads to telomere elongation. Given that telomerase plays a key role in lengthening telomeres, we examined the expression levels of telomerase components, activity, and biogenesis. It was found that factors involved in telomerase biogenesis and trafficking were affected. To identify more candidates, multi-omics approaches, single-cell RNA/ATAC seq as well as proteomic were applied. We found that the expression of most factors was altered at protein levels with minor changes at RNA levels. With this powerful tool, we identified La-related proteins (LARPs) as potential candidates to regulate telomerase and TL. Furthermore, the RNA-FISH analysis indicates that fewer telomerase RNAs (TRs) were recruited to CBs in *LRRK2* mutant cells. These data suggest that protein dyshomeostasis is the major cause of telomere dysfunction due to the dysregulation of telomerase biogenesis. Together, our studies provide additional links between telomere biology and PD caused by a gene mutation and provided more insights into the clarification of the role of telomere and telomerase in the progression of PD.

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**Topic:** C.03. Parkinson's Disease

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**Title:** Impacts of a Parkinson's disease-related UQCRC1 gene mutation on telomere maintenance

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**Abstract:** Mitochondrial damage and telomere dysfunctions are two hallmarks of aging that share intimate links with each other and have vital roles in human neurodegenerative diseases. In clinical studies, telomere shortening in blood cells correlates with decreased life expectancy and increased risk of Alzheimer's disease (AD), but its correlation with Parkinson's disease (PD) remains inconclusive. Here, we focus on PD-related genes and examine the defect in these genes on telomere maintenance. Proteomic analysis is applied in this study to highlight prominent molecules and pathways and identifies several factors involving in regulating TL maintenance as well as other biological processes. Our group, consequently found that there are altered expression levels of telomerase components in these PD-related defective neuroblastoma cell line SH-SY5Y. Our results provide additional links between telomere biology and Parkinson's disease caused by specific gene mutations, which may provide more information assisting in the clarification of their association.

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**Nanosymposium**

**NANO05: Representation of Faces and Bodies**

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**Presentation Number:** NANO05.01

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**Title:** A familiar face and person processing area in the human temporal pole

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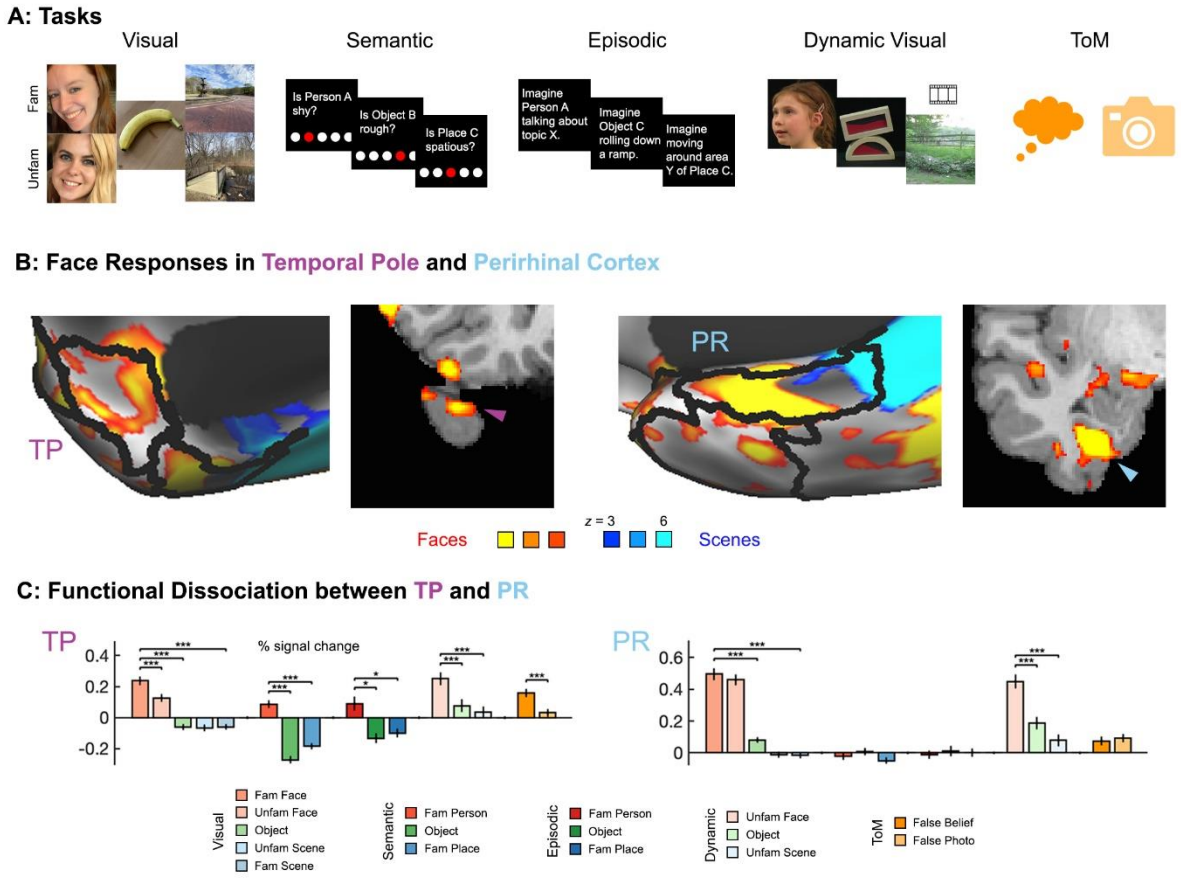
**Abstract:** How does the brain process the faces of familiar people? Neuropsychological studies have argued for an area of the temporal pole (TP) linking faces with person identities, but magnetic susceptibility artifacts in this region have hampered its study with fMRI. We ask this question using data acquisition and analysis methods optimized to overcome this artifact, including multi-echo sequences that substantially boost signal quality in the anterior temporal lobes. To precisely characterize functional organization in individual human brains, we scanned N = 10 participants using fMRI on a range of perceptual and cognitive tasks, across three scan sessions (7.5 hours per participant). Tasks involved visual perception, semantic judgment, and episodic simulation of close familiar people and places, and everyday objects. The resulting data identify a familiar face response in TP, reliably observed across each individual participant. This area responds strongly to visual images of familiar faces over images of unfamiliar faces, objects, and scenes, but also responds to a variety of abstract cognitive tasks that involve

thinking about people, including semantic judgment and episodic simulation. In contrast, a nearby region of perirhinal cortex (PR) - consistent in location with the previously describe "anterior temporal lobe face area" - responds specifically to faces (familiar and unfamiliar), but not to social cognition tasks. This result argues for two separate streams for person and face processing within anterior temporal cortex. Face responses in TP and PR had a similar functional organization to regions our lab has previously observed in macaques, suggesting a possible homology across species. This work identifies a missing link in the human familiar face processing system that is well placed to integrate visual information about faces with higher-order conceptual information about other people.

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**Title:** Reconstructing human brain dynamics during real-world social interactions - one face at a time

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**Abstract:** A fundamental goal of neuroscience is to understand how the brain processes information from the real world. While much has been learned from controlled laboratory experiments, they cannot capture the full richness of real-world environments. This is particularly problematic in the context of social perception, where passive viewing of static, unfamiliar, and isolated faces that are presented briefly on a screen bears little resemblance to rich and dynamic real-world social environments. In this study, we collected intracranial recordings from epilepsy patient-participants who wore eye-tracking glasses to capture everything they saw on a moment-to-moment basis during hours of natural unscripted interactions with friends, family, and experimenters. We used computer vision, machine learning, and artificial intelligence to address the core challenge with real world neuroscience - how to model the uncontrolled variability of the natural world. Computer vision models translated each face the patient-participants saw into a 227-dimensional model that represented distinct pose, shape, texture, and expression information. A bidirectional Canonical Component Analysis (CCA) model was used to reconstruct faces (including face motion) the participant saw at each fixation based on their neural activity alone and reconstruct the dynamics of brain activity based on the face the participant saw alone (d' effect size approximately 1.8 and correlation coefficients exceeding 0.4). Reconstructions were accurate when comparing across different identities (d' approximately 2.47), and also when comparing multiple fixations on faces of the same identity (d' approximately 1.02). Neurally, information about these faces was coded in occipital, temporal, frontal, and parietal regions involved in social visual processing, motion perception, and face processing. Individual Canonical Components of the model enable a more granular breakdown to examine which specific face features in the pose, shape, texture, and expression subspaces are coded by which aspects of neural activity. This approach will be used to assess the representational structure of the neural "face space" for real world face perception and determine how this space is modulated by natural social context. These results demonstrate that studying the brain during real-world social behavior is not only feasible, but also can be done with high fidelity to learn important details about how the brain codes for the natural social environment.

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**Title:** Resolving discrepant conclusions regarding the representation of mirror-symmetric face views in human face-selective areas

**Authors: *F. RAMIREZ**, J. GONZALEZ-CASTILLO, P. A. BANDETTINI;
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**Abstract:** Viewpoint invariant face recognition is a remarkable feat of the primate visual system. Traditional theories hold that viewpoint is coded by view-selective mechanisms at early visual processing stages and representations become gradually tolerant to viewpoint changes in higher-level visual areas. Newer theories, based on single-neuron monkey electrophysiological recordings, suggest a three-step architecture revealing a sharp transition from a view-tuned to a mirror-symmetric representation before achieving viewpoint invariance at the highest level of the hierarchy. Consistent with traditional theories, human studies combining neuroimaging and multivariate pattern analysis have provided evidence of view-selectivity in early visual cortex. However, contradictory results have been reported in higher-level visual areas regarding the existence in humans of a mirror-symmetric processing stage.
We recently proposed a unifying network model for these observations [Revsine, Gonzalez-Castillo, Merriam, Bandettini, and Ramírez, 2023. BioRxiv, DOI: 10.1101/2023.02.08.527219]. The model shows that low-level feature imbalances among images of lateral and frontal face views would lead to artefactual observations of mirror symmetry at levels of the visual hierarchy where neuronal receptive fields are large and span both visual hemifields. These artefacts emerge gradually along the visual hierarchy and manifest when the Euclidean distance is used as measure of dissimilarity among brain patterns, or the correlation distance is used instead but computed on mean-centered data. Mirror symmetry is not observed with the correlation distance if computed on uncentered data. Here, we provide empirical evidence in support of the predictions made by our model. We conducted pattern analyses of functional MRI data from early visual cortex, lateral occipital cortex, and the occipital and fusiform face areas [from study by Ramírez, Cichy, Allefeld and Haynes, 2014. The neural code for face orientation in the human fusiform face area. J Neurosci. 34:12155-67]. As predicted, we found no mirror symmetry when relying on the correlation distance on uncentered data. We also observed a gradual increase of mirror-symmetry as a function of the location of a brain area along the ventral stream when relying on the Euclidean distance or when the correlation distance was computed on mean-centered data. These observations suggest that reports of mirror-symmetry in humans are an artefact due to signal imbalances across conditions, and call attention to the influence of common analysis choices on inferences about neural coding based on pattern analyses of neuroimaging data.

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**Title:** Deep neural networks optimized for both face detection and face discrimination most accurately predict face-selective neurons in macaque inferior temporal cortex

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**Abstract:** Face processing is a multifaceted cognitive process at the core of human social interactions and communication. A rich understanding of this system should include an image-computable computational model that sufficiently explains face-related behaviors and their neural mechanisms. But prior efforts in this direction have encountered a puzzle: artificial neural networks (ANNs) trained on face identification (e.g., VGG-face) excel in predicting human face recognition behavior over those trained on object categorization (e.g., VGG-16, CORnet-Z), but underperform in predicting responses of face-selective neurons in humans and macaques. Why? We hypothesize that face-specific neural populations are tasked with both face detection and identification, implying ANNs trained on both faces and objects should surpass those trained on either task alone in predicting face-selective neurons.
We used three VGG-16 networks trained independently on object categorization (VGG-obj), face identification (VGG-face), and both object and face discrimination (VGG-dual). To test how well these models predict macaque inferior temporal (IT) cortex neurons, we performed large-scale recordings across macaque IT (2 monkeys, 120 sites) while monkeys passively fixated on 200 face images. Consistent with previous results, we found that VGG-obj better predicted IT responses to face images than VGG-face. However, this difference in predictivity reduced significantly as a function of increasing IT face-selectivity. Most importantly, VGG-dual outperformed VGG-obj in predicting IT responses, with its enhanced predictivity increasing as a function of the face selectivity of the recorded neural sites.
What is it about VGG-dual that enables it to outperform VGG-obj? Perhaps networks must be optimized for both face detection and face discrimination to account for neural responses. In that case, a network that maintains separate output categories for each face but assigns all objects to one category (VGG-faceObj) should outperform a network trained on the same stimuli that assign all faces to one category but maintains separate output categories for each object type (VGG-objFace). Indeed, our results support this hypothesis, with improvement in IT predictivity of VGG-faceObj over VGG-objFace increasing with the face selectivity of the neurons.
This finding reconciles a puzzle in the literature, enriches our understanding of the function of face-specific neurons in the brain, and showcases a method for deriving functionally interpretable and computationally precise models of behaviors and their underlying neural circuits.

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**Title:** Position invariant processing of different visual categories in the macaque

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**Abstract:** The visual recognition of specific stimulus categories, such as faces, bodies or scenes, engages partially segregated networks of cortical areas in both humans and monkeys. Recognizing these stimuli requires the ability to withstand significant variations in stimulus appearance, including changes in retinal position, size, color, spatial frequency, and occlusion. Accordingly, several electrophysiology and imaging studies have reported (partial) invariant processing for a variety of objects in the inferior temporal cortex. Yet, it remains unclear whether invariant stimulus processing occurs at different stages along the cortical hierarchy or exhibits distinct neurofunctional characteristics for different stimulus categories. To address this question, we directly compared position invariance across different stimulus categories in two rhesus macaques during a contrast-agent enhanced 3T fMRI experiment. In a 3x4 factorial block-design, two rhesus macaques fixated on a central spot while images (with an average diameter of 15°) of monkey faces, bodies, objects, and their phase-scrambled versions were sequentially presented at three different locations in the visual field (centrally, and peripherally along the diagonals of the lower left and right quadrants at 17° eccentricity). Following the identification of category-selective patches for faces and bodies, irrespective of stimulus location, we tested the impact of stimulus position on each category individually. Caudally, some patches were found to be category-selective only when the respective stimuli appeared in a specific location, as predicted by the retinotopic organization of these areas. However, within the more rostrally located category-selective patches, many voxels were responsive regardless of stimulus position. As predicted, position invariance became stronger in anterior sectors of the ventral stream. In general, position invariance was more pronounced for faces than for bodies, and correspondingly, more for bodies compared to objects, as revealed by position invariance indices. Position invariance for bodies, but not for faces, was more evident on the lateral convexity as compared to the fundus or dorsal bank of the STS. In summary, our study suggests that different stimulus categories may exhibit varying degrees of position invariance, as measured with fMRI. Faces demonstrate the highest degree of invariance, while bodies and objects exhibit more position-variant processing, particularly in more anterior regions of the ventral stream.

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**Topic:** D.06. Vision

**Title:** How do we process faces from different ethnic groups and species?

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**Abstract:** Faces are one of the most important stimulus categories for humans. Own-race bias is defined to indicate that humans process their own-race and other-race faces differently. The research (Meissner and Brigham, 2001; Michel et al., 2006; Tanaka et al., 2004) has shown that humans can recognize their own-race faces better than other-race faces. We aimed to investigate how face perception differs among faces from different ethnicities in two experiments. In the first experiment, we recruited 30 Turkish undergraduate students (M=21.5, SD=2.69) for the recognition task for Black and White faces. The participants were asked to observe each of the 32 faces (16 Black-16 White) for 5 seconds. In the recognition session, these faces were mixed with 32 novel faces; then participants were asked to respond if they had recognized the presented face via keyboard. Repeated measures ANOVA elicited a significant effect of face ethnicity F(1, 29) =5.28, p=0.029, ηp2 =0.154. The results showed that White faces were recognized better than Black faces. In Experiment II, 26 Turkish undergraduate students aged 18-23 years (M=20.2, SD=1.41) participated. Asian and Caucasian human faces were presented. Faces of great apes and scramble images were used as control stimuli. Forty stimuli were selected from each stimulus set. A 500ms fixation dot was followed by a stimulus for 1500ms and a blank screen for 1000ms. The participants indicated if they had perceived a face or not by keyboard pressing. The brain signals were recorded via 64 channel BrainVision Actichamp EEG system. The data was preprocessed and segmented from 200ms before the onset of the stimulus to 1000ms after the onset of the stimulus. P100 (100-160ms) ERP component was analyzed via Brain Vision Analyzer. Three electrode groups were examined: occipital(O1/O2), parieto-occipital(PO3/PO4, PO7/PO8), parietal(P5/P6/P7/P8). Repeated measures ANOVA was conducted to determine the effects of stimulus on ERP signals. Greenhouse-Geisser correction was applied. A significant effect of the stimulus was found on the amplitudes of PO electrodes $F$ (1.56, 38.95)=5.72, $p$ = .01, ηp2=.06 and parietal electrodes $F$(1.62, 40.59)=7.64, $p$=.01. The results of PO electrodes indicated that the amplitudes for Caucasian faces were lower than that for Asian and ape faces however, the results of parietal electrodes indicated that ape faces elicited higher amplitudes in comparison to Asian and Caucasian faces. Our results showed that humans have own-race bias in face recognition and during early visual processing, this bias occurred in the parieto-occipital areas while own-species specific responses occurred in parietal regions.

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**Topic:** D.06. Vision

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**Title:** Face cell-like socially selective visual responses in the northern paper wasp, Polites fuscatus

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**Abstract:** Understanding the fundamental adaptive features of neural circuit design, rather than the idiosyncrasies of a specific model species, requires that we compare behaviors and cognitive abilities that have independently arisen in distant lineages. For example, face recognition is a well-studied example of higher-order visual processing in primates, where neural populations that are highly selective for faces rather than objects in general mediate the process. The selectivity of face neurons has been proposed to allow for efficient discrimination of conspecifics, which tend to share more features than differ. Here we sought to better understand the fundamental and idiosyncratic neural features of visual social partner recognition by studying visual circuits in the northern paper wasp, *Polistes fuscatus.* These paper wasps possess individually distinctive color patterns on their faces, which can be used to recognize individuals on the nest. Behavioral assays demonstrate that individual recognition in this species is mediated by face-specific visual processing, similar to primates. We ask if neural selectivity to forward facing conspecifics is also present in the wasp. Using extracellular 64-channel electrophysiological recordings we identified socially selective visual responses in the brains of these wasps. An abundant subset of visually responsive units, primarily recorded in the mushroom bodies and protocerebrum, show highly selective responses to frontal view images of wasps, indicating the convergent evolution of a face cell-like neuron in an insect. Our recordings identified multiple wasp-selective unit types with stereotyped firing dynamics across multiple animals that are all selective to frontal-view wasp images. Furthermore, we find evidence that one unit type also encodes information about the internal features, i.e. identity, of the wasp faces. Despite having independently evolved vision let alone facial recognition, paper wasp face recognition circuits show remarkable parallels to the face neurons in primates. These findings suggest that (1) a population of neurons that are highly tuned to detect conspecifics and (2) individual neural selectivity within this population that vary along multiple axes of facial diversity space are likely fundamental features of neural circuits performing visual identity discrimination.

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**Title:** Intracranial electrophysiology reveals the neural representation of cononical visual categories across the human lifespan

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**Abstract:** Intracranial electrophysiology reveals the neural representation of canonical visual categories across the human lifespan
Vinitha Rangarajan[1,6], David King-Stephens[2], Kenneth D. Laxer[2], Peter Weber[2], Jack J. Lin[3], Edward F. Chang[4], Kurtis Auguste[4,5], Robert T. Knight[6,7], Danielle S. Bassett[1,8, 9] **Support:** National Science Foundation Graduate Research Fellowship to V.R. (DGE 1106400), University of Pennsylvania Provost Postdoctoral Fellowship to V.R, NINDS NS21135 to R.T.K
Neuroimaging and intracranial studies have shown that visual images (e.g. faces, houses, words, numbers, etc.) are represented in segregated regions of the human brain. These neural responses display a consistent spatial organization with respect to well-established neuro-anatomical landmarks in the brain. While distinct regions show selectivity for a specific visual category, neural responses to the unpreferred categories persist. Numerous studies have investigated the spatial organization of the selected visual categories, but the comprehensive selectivity profile of visual representation (within and between individuals) over development has sparsely been investigated. In the current study, we utilized electrocorticography (ECoG) data from 19 adults and 10 children (ages 6-56) implanted with electrodes for the treatment of refractory epilepsy. Subjects were shown a visual target detection task including faces, houses, numbers, words, and scrambled visual targets. Leveraging the high spatial and temporal resolution of ECoG, we analyzed High Frequency Broadband (HFB: 70-150 Hz) power changes across all categories. Performance across all ages was > 95% of targets detected. We found robust representations of visual stimuli across all participants. Notably, older patients showed distinct selectivity for visual categories in putative “selective” regions (i.e. faces in the fusiform gyrus face area). Younger aged/child subjects showed more overlapping representation profile, with large HFB responses to canonically ‘unpreferred’ visual categories (e.g. houses in the fusiform gyrus where faces are predominant). Altogether, our findings provide intracranial electrophysiological evidence for selective neural representations of visual categories at older ages, and overlapping visual representations in youth. We investigate the profile of visual selectivity by quantifying representational similarity across development.

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**Topic:** D.06. Vision

**Title:** Task-related modulation of visual responses measured with electrocorticography in the human brain

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**Abstract:** Cognitive task demands influence how visual information is processed by our brain. A previous study (Kay & Yeatman, 2017) measured these influences in ventral temporal cortex (VTC) and found that visual functional MRI (fMRI) responses are scaled proportional to cognitive task demands. fMRI, however, does not provide neural signals with high temporal resolution, essential to study temporal dynamics of neural modulation. Electrocorticography (ECoG), on the other hand, records neurophysiological signals at a high temporal resolution, and has been well correlated with fMRI responses. This study investigates the temporal dynamics of cognitive task demands using ECoG data from two human subjects.
Two patients with ECoG electrodes implanted for clinical epilepsy monitoring provided informed consent to participate in the study. They were presented with face and word stimuli, with varying contrast and phase coherence, and a fixation dot at the center which changed color every 600 ms. The subjects were tasked to either press a button whenever the dot turned red (fixation task), or to press one of three buttons based on the stimulus category they perceived (categorization task). Alpha (8-12 Hz) and broadband (70-170 Hz) power changes were extracted from raw ECoG signal, and signal changes with respect to baseline were computed. Electrodes with significant broadband power increases were identified as visually responsive electrodes and were classified based on anatomy into early/intermediate visual cortex (EVC) and VTC. To quantify the effect of cognitive task demands, scaling effects were computed as a ratio of power changes during the categorization over the fixation task.
Broadband power increased with increasing contrast and phase coherence in both tasks. Scaling effects were observed across EVC, and in non-category selective and category selective (face and word) VTC electrodes. These scaling effects showed that broadband responses increased during the categorization task, with larger increases during low contrast stimuli. Moreover, these scaling effects were not a constant across time but had temporal dynamics varying across stimuli. ECoG electrodes also showed significant alpha decreases in EVC and VTC during both the tasks, and some alpha responses scaled with task demands.
In this research, we found that cognitive task demands scale the broadband power as well as lower frequency alpha power in VTC. The broadband changes were comparable to those observed in fMRI responses. These findings, therefore, suggest that cognitive task demands influence multiple neurophysiological signals in VTC that may provide complementary information to fMRI.

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**Title:** Interaction of viewpoint tuning for faces and bodies in the macaque inferior temporal cortex

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**Abstract:** Previous studies have demonstrated that face patch neurons in the macaque inferior temporal (IT) cortex exhibit viewpoint tuning for faces, while body patch neurons show viewpoint tuning for bodies. However, it remains unclear whether viewpoint tuning for faces and bodies interacts at the single-unit level when both the face and body are part of an agent. To address this question, we independently manipulated the viewpoint of a realistic monkey avatar's face and body. The manipulation involved eight viewpoints with 45-degree increments for both the face and body, resulting in 64 face-body viewpoint combinations. Each stimulus had a size of 6 degrees and included sitting and standing poses. The avatar was centered either with respect to the face or the body. We recorded neural activity from single units in the anterior (N = 98) and posterior patches (N = 100) of the ventral bank of the Superior Temporal Sulcus in two male macaques. These patches were selected based on their stronger fMRI activation in response to monkey images compared to object images, as reported in a previous study (Zafirova et al., NeuroImage, 2022). Specifically, we focused on cells that exhibited a stronger response to a monkey, a headless body, or a face compared to objects. Most neurons in both the anterior and posterior patches were tuned to the viewpoint of the avatar's body. This viewpoint selectivity was consistent across different locations, with a stronger location tolerance observed in the anterior patch. Interestingly, the tuning for body viewpoint depended on the viewpoint of the face in approximately half of the neurons, regardless of the pose and centering conditions (significant interaction ($p < 0.05$) of face and body viewpoint (two-way ANOVA)). Notably, a subset of neurons exhibited selective responses to specific combinations of face and body views, resulting in a bell-shaped tuning pattern in the face-body viewpoint space. Overall, our data provide evidence that single neurons in the IT cortex can encode both the viewpoint of faces and bodies. These findings further support the idea that faces and bodies interact at the single-unit level within the IT cortex.

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**Nanosymposium**

**NANO06: Neural Control of Chewing and Swallowing**

**Location:** WCC 152A

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO06.01

**Topic:** E.04. Voluntary Movements

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**Title:** Brainstem circuit operations during swallowing

**Authors: *D. C. BOLSER**[1], T. PITTS[2], L. S. SEGERS[3], S. NUDING[3], P. ALANCAR[3], J. HAYES[1], K. F. MORRIS[3];

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**Abstract:** Dysphagia increases the risk of pulmonary infection due to aspiration, which is the leading cause of death in neuromuscular disease. The pattern generator for the pharyngeal phase of swallow is located in the brainstem and consists of the dorsal swallow group (DSG) in the nucleus tractus solitarius (NTS). The DSG initiates the swallow command, containing the neurons that trigger the swallow motor pattern. The ventral swallow group is located in the ventrolateral medulla and may distribute the swallow command sequentially to cranial and spinal motoneuron pools. The identity and temporal dynamics of brainstem neural circuits that regulate and coordinate oropharyngeal muscle activity during swallow are poorly understood. We speculated that analysis of spike trains from multiple simultaneously recorded pontomedullary neurons during swallowing would enable identification of novel swallow network operations. We also hypothesized that this information would inform a computational model of the swallow network that would enable prediction of swallow motor sequencing with temporal precision. Cats (n=16) were anesthetized, decerebrated, paralyzed and artificially ventilated. Following an occipital craniotomy, arrays of independently mobile electrodes were inserted into the pons, raphe, NTS, ventral respiratory column, and medullary reticular formation. Swallowing was elicited by electrical stimulation of the superior laryngeal nerve or by injection of water into the pharynx. A total of 1,105 neurons were recorded. Swallowing cycle triggered histograms (SCTHs) suggested at least 9 different phenotypes of response patterns of these neurons during this behavior. SCTHs displayed sharp temporal orchestration of activity patterns consistent with demarcation of the swallow burst into phases. Cross correlation analysis yielded 2,869 pairs of neurons with functional correlations consistent with paucisynaptic interactions. Functional interactions were observed between pontine, NTS, raphe and ventrolateral column neurons consistent with a swallow network that is anatomically distributed throughout the brainstem. Complex circuit operations were frequently observed that included limiting functions (e.g. excitation/inhibition) between pairs of neurons. Within the ventrolateral network, functional circuit modules were observed consisting of multiple limiting functions, included at least one laryngeal motoneuron, and were modulated by neurons in the region of the pre-Bötzinger complex. Simulation of these circuits with an integrate and fire model supports the plausibility of a multilayer nested network regulating repetitive swallow.

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**Title:** Cluster Analysis of Optically Recorded Medullary Networks Delineate Functional Anatomical Structures with Distinct Immunohistochemical Profiles

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**Abstract:** Swallow is a behavior that can be initiated at will, but then deploys as a reflex that is labile to both peripheral feedback and phasic modulation by respiratory networks. Some hindbrain constituents of this network have been characterized electrophysiologically in vivo and via retrograde labeling. Command networks for the initiation of swallow are located dorsomedially in the nucleus tractus solitarius (NTS). Previous work suggests that these networks drive propriobulbar networks dorsal to the facial nucleus, which in turn activate laryngeal and esophageal (pre)motoneurons distributed along the compact and loose formation of nucleus ambiguus (cNA, lNA), whose sequential activation mediates the coordinated motor pattern that conveys a food bolus to the gut. The sagittally sectioned neonate mouse brainstem exposes the command neurons for swallow in the NTS at its dorsomedial margin, and propriobulbar and (pre)motor networks mediating both deglutition and respiration near its ventrolateral margin. Interactions between these networks can be characterized in a field of view spanning the rostral margin of the facial nucleus (VIIn) to the caudal pole of the lateral reticular nucleus with cellular resolution. The coordination of evoked swallow and breathing was investigated via mesoscopic-scale (100X) optical recordings (60 Hz) in transgenic mice expressing the genetically-encoded $Ca^{2+}$ indicator GCaMP6F in the germline. Swallows were elicited via bipolar electrode stimulation (20 Hz, 400 ms, 5V- 6V), applied every 10 breaths, triggered off inspiratory onset at fixed delays over long (1,100 s) recording epochs. Regions of interest (ROIs) were extracted using machine vision algorithms to generate time-series of luminance values. To further taxonomize cell populations from which optical recordings were made, the sagittal face of the preparation was isolated in a 400 µm slice and immunoprocessed for choline acetyltransferase (ChAT), the transcription factor phox2b, and somatostatin or neurokinin 1 receptors (SSTR, NK1R). Anatomically overlapping swallow and respiratory networks were consistently parcellated using cluster analysis, and these groups overlapped closely with immunohistochemical expression patterns, particularly between $phox2b^{+}$ neurons and networks implicated in swallow. These findings corroborate the work of others suggesting that networks mediating swallow are specified by developmental modules under the regulation of phox2b.

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**Title:** Increased dorsolateral prefrontal activity during food intake with pleasant and unpleasant emotions

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**Abstract:** The sensory experience of deliciousness has the potential to enhance the overall quality of life. This study investigated cortical activity associated with food intake that evokes pleasant and unpleasant emotions. Twenty-one right-handed healthy individuals (10 males and 11 females, mean age: 28.1±3.7 years) were instructed to consume a spoonful of three types of food: a palatable food, an unpalatable food, and steamed rice for taste and olfactory washout). Participant-specific palatable and unpalatable food options were determined through a questionnaire, and the participants refrained from eating for 3 hours prior to the experiment. Functional near-infrared spectroscopy (fNIRS) was used to measure hemodynamic changes from bilateral frontoparietal regions during the food intake task. A food product (iEat, EN Otsuka Pharmaceutical Co., Hanamaki, Japan) with a soft texture and visual resemblance to ordinary food was used to minimize motion artifacts related to mastication. Emotional valence of each food intake was evaluated using a visual analog scale ranging from 0 to 100 (0: totally unpalatable, 100: most palatable). Raw fNIRS data were processed using a spatial filter method (Zhang, et al., 2016) to remove global systemic effects. A first-level general linear model analysis was conducted to fit the oxygenated hemoglobin signals, and the resultant beta values were interpolated to create a cortical activity map for each participant. A second-level analysis using one-sample t-tests with SPM8 software (Friston et al., 1994) was performed to identify common activity across the participants ($p < 0.005$, uncorrected). Activation of the left dorsolateral prefrontal cortex (DLPFC) significantly correlated with perceived intensity of valence during consumption of both palatable and unpalatable food. Notably, this activity was localized to the dorsal and ventral parts of the left DLPFC during consumption of palatable and unpalatable food, respectively. These findings suggest that emotional states induced by palatable and unpalatable food may elicit distinct processing patterns in the left DLPFC. The activation of the left DLPFC in response to emotional changes associated with food intake suggests its potential impact on higher cognitive functions such as memory and learning through the brain's reward system. The pleasure derived from a delicious meal may potentially influence cognitive function.

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**Topic:** E.04. Voluntary Movements

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**Title:** Opioids alter the laryngeal adductor reflex to superior laryngeal nerve stimulation

**Authors: *J. A. HAYES**[1], M. J. ROSE[1], I. POLIACEK[2], W. WANG[1], K. F. MORRIS[3], L. S. SEGERS[3], S. C. NUDING[3], P. VALAREZO[3], T. Y. SHEN[1], D. C. BOLSER[1];
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**Abstract:** Stimulation of laryngeal afferents results in the laryngeal adductor reflex (LAR), which closes the airway to prevent aspiration of food or liquids. Degradation in this reflex can significantly increase the risk of life-threatening aspiration pneumonia. Furthermore, increased use of opioids, such as codeine and fentanyl, can affect the coordination and response character of LAR. We sought to develop a better understanding of how opioids could impact the coordination of airway muscles and the LAR response in particular.
LAR is a rapid reflex that can be detected in electromyographic (EMG) recordings of airway muscles and can be induced by afferent stimulation of the superior laryngeal nerve (SLN). Electrical stimulation of the SLN will elicit this behavior at latencies of ~10 ms. The low latency of this response suggests that the minimal central neuronal circuit giving rise to this behavior has a short pathway. This circuit has been proposed to include the nucleus tractus solitarius (NTS) neurons receiving afferent signals and nucleus ambiguus (NA) motoneurons driving the muscles recorded in EMGs. However, we hypothesized that there is at least one additional layer of interneurons, likely in the spatially intervening area of the dorsal to ventral medulla. Further, the sensitivity of these elements to opioids is unknown.
We conducted experiments in anesthetized, spontaneously breathing cats. First we investigated the effects of opioids delivered intravenously on unilateral/bilateral LAR with cumulative dose responses to codeine (0.1-10.0 mg/kg) or fentanyl (0.1-10.0 µg/kg). Codeine, but not fentanyl, significantly suppressed the LAR. However, codeine only suppressed the contralateral LAR. In separate experiments, we microinjected codeine (5 mM, ~35 nL) into the ventrolateral medulla in the obex region. These injections had complex effects including depression of the contralateral LAR. In some animals, this depression preferentially reduced the higher frequency components of the EMG power spectra, consistent with an action on circuits driving faster motor units. Next we recorded extracellular activities of several dozen NTS neurons. Approximately 30% of these neurons demonstrated low latency excitatory responses to SLN stimulation. However, after intravenous administration of codeine >80% of recordings showed alterations in basal firing activity compared to vehicle and altered LAR response to SLN stimulation. These results support central actions of some opioids on the LAR. Further, they suggest the participation of complex opioid-sensitive circuits in the recruitment patterns of laryngeal adductor muscle motor units during LAR behavior.

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**Topic:** E.04. Voluntary Movements

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**Title:** Cortical representation of mastication in the primate orofacial sensorimotor cortex

**Authors: Z. LI**[1], *F. I. ARCE-MCSHANE[2,3];
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**Abstract:** Vital and complex oral behaviors, such as eating, require efficient coordination of the tongue and jaw to move and position food inside the mouth. Indeed, swallowing and masticatory dysfunctions, which are prevalent in stroke, Parkinson's Disease, and Alzheimer's Disease, have devastating effects on the quality of life. The orofacial sensorimotor cortex (oSM) has been implicated in the control of feeding, yet little is known about how chewing different food types on either left or right side of the mouth is represented in oSM. Here, we evaluated the cortical representation of chew-side and food-type in the orofacial primary motor cortex (MIo) and primary somatosensory cortex (SIo) and how this was affected by the absence of tactile inputs to the oral cavity. We combined multi-electrode cortical recordings and biplanar video radiography for 3D tracking of the tongue and jaw while rhesus macaques were feeding. Local anesthesia to sensory branches of the trigeminal nerve blocked oral tactile inputs. We used demixed principal components analysis to decompose the dependencies of the population activity to chew-side and food-type. We found that latent activity related to chew-side and food type in both MIo and SIo were clearly separated. In the absence of oral tactile inputs, information accounted for by task parameters decreased in SIo but increased in MIo. Overall, our results show that food type and chew side are represented in the activity of oSM and are dynamically modified in the absence of tactile sensation.

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**Title:** Central processing of olfactory and gustatory signals from the mouth

**Authors:** S. STOCKE, ***C. SAMUELSEN**;
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**Abstract:** The coordinated transmission of sensory information through the various cranial nerves innervating the oral/nasal cavity is essential for integrating olfactory and gustatory signals, which is fundamental for flavor perception. When food enters the mouth, volatile molecules (odors) travel through the oropharynx to activate olfactory receptors in the nasal epithelium, while non-volatile chemicals (tastants) dissolve in saliva to activate taste receptors on the tongue. Due to the different central projection pathways, the gustatory cortex (GC) is considered a principle site for processing multimodal chemosensory signals. Recent studies have shown that cortico-cortical interactions between the gustatory cortex and the posterior piriform cortex (pPC) affect functional and behavioral responses to odors and tastes. However, it remains unclear how GC neurons represent unimodal and multimodal chemosensory signals and whether pPC projections target functionally distinct neuronal populations in GC. Here, we used a virus to express channelrhodopsin-2 (ChR2) in the pPC of rats and implanted a drivable multielectrode

optrode into GC. Next, we recorded single-unit activity in the GC of alert rats during the intraoral delivery of individual odors, individual tastes, and odor-taste mixtures and then photo-activated ChR2-expressing fibers in GC to identify neurons likely influenced by pPC input. Approximately 40% of chemoresponsive neurons in GC were modulated by photostimulation of pPC fibers. Both the laser-responsive and non-laser-responsive populations showed broad tuning and responded to multiple odors, tastes, and odor-taste mixtures. Interestingly, population decoding analyses revealed that both groups accurately represented tastes and odor-taste mixtures, while only the laser-responsive population accurately represented odors. Our preliminary findings suggest a functional role between the chemosensory cortices and support the hypothesis that the gustatory cortex plays a key role in processing multimodal sensory signals originating from the mouth.

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**Title:** A computational model for the central control of swallow's pharyngeal phase

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**Abstract:** Swallow is a highly stereotypical behavior but is also nonstationary, meaning that it is not predictably rhythmic, except under artificial conditions using electrical stimulation. Experimental evidence also demonstrates key features including: 1) swallow and breathing can be decoupled, 2) tonic stimulation produces behavior facilitation, and 3) amplitude and duration of the motor burst are not correlated. Due to its inherent stability, it is appealing to model the swallow pattern generator (SPG) as an oscillator. In that scenario, our model assumes that swallow is under strong tonic inhibition and is then disinhibited by sufficient feedforward afferent drive, like other half-center models (e.g., breathing, cough). In its simplest form, the model is a tri-level oscillator, with each layer experiencing mutual inhibition and each population accommodating to permit oscillation. The first layer allows coordination with breathing and establishes a preference for swallows to occur during expiration. Additionally, when modeling super laryngeal nerve stimulation, breathing is depressed and repeat swallows occur. The second and third layers decouple motor pattern amplitude and duration to produce discrete control over onset and duration while allowing amplitude to independently respond to the strength of the afferent drive. A crucial addition that improved the accuracy of the model was the recruitment of an increasingly larger pool of neurons to stabilize the model during breathing-swallow transitions and under tonic stimulation conditions. The emergent motor patterns replicate many of the key features of *in vivo* swallow, including expiratory phase preference, the classic rostral-caudal delay in sequential muscle recruitment, and amplitude facilitation during tonic stimulation. This

computational model of swallow and its integration with breathing has been iteratively modified to successfully reproduce important experimental characteristics while also corresponding with current theories of the central regulation of the pharyngeal phase of swallow.

**Disclosures: T. Pitts:** None. **K.E. Iceman:** None.

**Presentation Number:** NANO06.08

**Topic:** E.04. Voluntary Movements

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HL126523

**Title:** Potential therapeutics to aid in the rescue of swallow-breathing coordination in a mouse model of Leigh Syndrome

**Authors: *A. HUFF**[1], M. KARLEN-AMARANTE[2], L. M. OLIVEIRA[2], C. MONTGOMERY[3], G. CORTOPASSI[3], S. DUGAR[3], J. M. RAMIREZ[2,4], F. K. KALUME[5];
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**Abstract:** Difficulty swallowing, also known as dysphagia, hinders eating and is among the first clinical symptoms of Leigh Syndrome (LS). LS is a severe neurometabolic disorder and the most common form of mitochondrial disorder in the pediatric population. It has been linked to loss of function mutations in *Ndufs4*, the gene that codes for a subunit of the mitochondrial complex I. Mice lacking *Ndufs4* globally, constitutes an excellent model of LS, mimicking several core symptoms of the disease in humans including seizures, ataxia, hypotonia, rigidity, weight loss, and failure to thrive. Knock out (KO) of *Ndufs4* in mice, specifically in glutamatergic (*Vglut2)* neurons, reproduces motor dysfunctions associated with LS such as respiratory dysfunction and swallow-breathing discoordination. In our previous work we demonstrated that mice exposed to 6 months of Chronic Hypoxia (CH) no longer presented with symptoms such as: ataxia, hunched posture, unbalanced, clasping, weight loss, etc, as seen in mice exposed to room air (RA). CH mice lived significantly longer and weighed significantly more than RA mice. CH mice also had a decrease in neuroinflammation, abnormal swallows, and swallow-breathing coordination was restored. Since CH may not be a translational treatment for swallow-breathing function in humans, we are investigating the use of MYT-109, a drug that improves mitochondrial function under development by Myto Therapeutics, as a potential therapeutic. Using freely breathing urethane anesthetized adult *Vglut2:Ndufs4*KO mice, swallow was evoked by injection of water into the oral cavity. Swallow and respiratory activity was measured via monopolar suction electrodes of the hypoglossal (XII) and vagus (X) nerves as well as bipolar electromyogram (EMG) of the submental, laryngeal complex and costal diaphragm muscles. This protocol was performed in *Vglut2:Ndufs4*KO mice 1) exposed to RA plus treatment of peanut butter for 7 weeks and 2) exposed to RA plus treatment of MYT-109 mixed in peanut butter for 7 weeks,

starting at weaning age. Post-hoc histological analysis of swallow-breathing related brainstem areas using IBA1 to stain for microglia and TMEM119 for macrophage. Similar to the use of CH, we hypothesize that treatment of MYT-109 will maintain body weight, increase life expectancy, decrease neuroinflammation, restore swallow-breathing coordination and decrease the number of abnormal swallows. This study gives first insights into possible mechanisms of LS and treatment for dysphagia in LS.

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**Presentation Number:** NANO06.09

**Topic:** E.04. Voluntary Movements

**Support:** JSPS KAKENHI Grant Number 20H03877

**Title:** Effect of habituating intentional chewing on brain activity in older adults

**Authors: *Y. MIYAZAKI**[1], R. TAKEHARA[1], T. SUZUKI[1], Y. HASEGAWA[2], S. YOSHIMURA[2], M. STA.MARIA[2], K. HORI[2], K. YAMAMURA[3], T. ONO[4], Y. ONO[5]; [1]Electrical Engin. Program, Grad. Sch. of Sci. and Technol., Meiji Univ., Kanagawa, Japan; [2]Div. of Comprehensive Prosthodontics, [3]Div. of Oral Physiol., Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata, Japan; [4]Dept. of Geriatric Dent., Osaka Dent. Col. Sch. of Dent., Osaka, Japan; [5]Dept. of Electronics and Bioinformatics, Sch. of Sci. and Technol., Meiji Univercity, Kanagawa, Japan

**Abstract:** Recent studies have highlighted the significance of maintaining the masticatory function, not only to prevent malnutrition and frailty but also to preserve brain function. However, most studies have focused on the immediate effects of temporal chewing, and little is known about the impact of habituating intentional chewing in daily life on brain activity. Therefore, this study investigated the influence of intentional chewing during meals on food intake behavior and brain activity during chewing and cognitive task in older adults individuals. Fifty participants (25 males, mean age 71.9 years) were randomly assigned to either the intervention or control groups. Participants in the intervention group were encouraged to increase the number of chewing strokes during meals using a wearable device (bitescan, Sharp Corporation) for one month. In contrast, those in the control group maintained their usual dietary habits. The number of chewing strokes during the consumption of 100g rice balls was measured for both groups before and after the intervention period to assess the effectiveness of the intervention. The cortical activity was also measured using functional near-infrared spectroscopy (fNIRS) while chewing a tasteless and odorless gum base. Cognitive functions of memory, spatial awareness, planning, disorientation, and attention were also evaluated using the CogEvo app (Total Brain Care Co.Ltd.). The results demonstrated a significant increase in the number of chewing strokes during consumption of a rice ball in the intervention group, but not in the control group, indicating that the intervention successfully promoted the development of a habit of chewing food more thoroughly. Participants in the intervention group showed a positive correlation between the increased rate of chewing strokes during rice ball consumption and

enhanced cortical activity in the supramarginal gyrus during gum chewing at post-intervention (FWE corrected, $p<0.05$). The memory function score was significantly higher in the intervention group relative to the control group at the end of the intervention while they were comparable at baseline (Two-way ANOVA followed by multiple comparisons, $p<0.05$). The relationship between changes in masticatory behavior and those in cognitive function will be presented on-site.

**Disclosures: Y. Miyazaki:** None. **R. Takehara:** None. **T. Suzuki:** None. **Y. Hasegawa:** None. **S. Yoshimura:** None. **M. Sta.Maria:** None. **K. Hori:** None. **K. Yamamura:** None. **T. Ono:** None. **Y. Ono:** None.

**Presentation Number:** NANO06.10

**Topic:** E.04. Voluntary Movements

**Support:** National Institute of Aging Grant R01AG069227
National Institute of Dental and Craniofacial Research Grant R01DE027236

**Title:** Effects of aging on lingual movements during swallows

**Authors: *S. PUNACHA**, F. ARCE-MCSHANE;
Oral Hlth. Sci., Univ. of Washington, Seattle, WA

**Abstract: Effects of aging on lingual movements during swallows**
Age-related oral health problems affecting the tongue, such as loss of muscle strength and diminished sensation, can significantly impact safe swallowing. While dysphagia is commonly found in both healthy aging individuals and individuals with Alzheimer's Disease (AD), how aging affects the cortical and biomechanical control of tongue movements during swallows remains unclear. Here, we evaluated the presence of stereotypic patterns of tongue movements during swallows, their cortical correlates, and how these were affected by aging and loss of sensation. We employed high-resolution biplanar video-radiography to track three-dimensional tongue movements and large-scale multi-electrode cortical recordings to capture neural signals in the primary motor (MIo) and somatosensory (SIo) areas of the orofacial cortex of rhesus macaques while engaged in natural feeding behavior. To compare the effects of sensory loss and healthy aging, we administered a nerve block to temporarily eliminate oral tactile sensations while preserving motor and proprioceptive signals in young animals. Tongue kinematics and spiking activity of individual neurons were analyzed relative to the minimum gape in each swallow cycle. We found an overall increase in stereotypy of tongue trajectories from the anterior to posterior region of the tongue under all conditions, as shown by antero-posterior decrease in the variance of trajectory length and trajectory end points. The posterior region of the tongue exhibited distinct stereotypy, although the effect is reduced in older animals. To evaluate whether the regional variations in tongue kinematics were reflected in the informational content of the neuronal responses, we computed the time-evolved mutual information (MuI) between the tongue kinematic data and the spiking activity. We found differential patterns of MuI as a function of time relative to swallow and tongue regions; the anterior region of the tongue exhibited peak MuI at the start of the swallow cycle, while the posterior region had peaks at the minimum gape in the young animal. In the absence of sensation, the peak MuI associated with

movements of the posterior tongue occurred after the minimum gape. These patterns were observed for both MIo and SIo. Our results suggest that regional variation in tongue stereotypy and in the dynamics of information in the spiking activity of MIo and SIo neurons during swallows may underlie differing contributions of lingual action for swallowing. This knowledge provides valuable insights into the neuromechanical changes associated with healthy aging.

**Disclosures: S. Punacha:** None. **F. Arce-McShane:** None.

**Nanosymposium**

**NANO07: Neuroethology of the Sensorimotor System**

**Location:** WCC 147A

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO07.01

**Topic:** F.01. Neuroethology

**Support:** NIH Grant 23800-G0001-10012158-101

**Title:** Measuring, modeling, and predicting learned odor-guided navigation in C. elegans

**Authors: *K. CHEN**[1], E. OSBORNE[2], J. W. PILLOW[1], A. LEIFER[1,2];
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**Abstract:** The nematode *C. elegans* changes its preference towards an odor source depending on its learned association with food or starvation. However, the mechanism by which the worm adjusts its navigation strategy in response to olfactory learning remains unknown. Here, we developed a novel odor delivery apparatus to measure learned odor-navigation strategies. We fit a statistical model to detailed locomotion data under precisely measured butanone concentration for naïve worms and for worms that learned to associate butanone with food (appetitive learning) or starvation (aversive learning). We developed a "weathervane and pirouette" model to characterize navigation. This corresponds to a dynamic Mixture of Generalized Linear Models (MoGLMs), where each GLM corresponds to a particular navigation strategy known to exist in worms: (1) weathervaning, in which the head angle depends on the local odor gradient and (2) pirouette, in which the probability of a sharp turn is modulated by the temporal derivative of the odor concentration experienced by the animal. We fit this model to a detailed time series of the worm's locomotion and experienced odor concentration after the animal underwent associative learning. The inferred parameters show that learning bidirectionally modulates the GLM weights on odor signal for both strategies. Specifically, appetitive learning increases the inferred weights while aversive learning decreases it, compared to naïve conditions. We corroborate this finding using optogenetics, confirming that the behavioral response to activation of an olfactory neuron is bidirectionally modulated by learning. We also used our model fits as a way to decode the previous associations experienced by the animal (naive, appetitive or aversive). Our MoGLM framework provides model-based decoding performance above 70%, much higher than chance (33.3%) and significantly higher than previously reported metrics of learning, such as the "chemotaxis index" which achieves only 40% decoding performance. Finally, we discuss

progress towards whole-brain imaging in freely moving worms after learning, with preliminary results showing odor-evoked behavior in our whole brain imaging apparatus. Through the integration of novel models and measurements, our study presents a new paradigm for comprehending learned odor-guided navigation in *C. elegans*.

**Disclosures: K. Chen:** None. **E. Osborne:** None. **J.W. Pillow:** None. **A. Leifer:** None.

**Presentation Number:** NANO07.02

**Topic:** F.01. Neuroethology

**Support:** N0001421-1-2516
N00014-23-1-2083
RGP0042/2019

**Title:** Deciphering spike activity in the brain of freely behaving octopuses

**Authors: *L. ZULLO**[1,2], D. BOLIGIN[3], M. LONDON[3], B. HOCHNER[3];
[1]Inst. Italiano di Tecnologia, GENOVA, Italy; [2]IRCCS Ospedale policlinico San Martino, Genova, Italy; [3]Hebrew Univ., Jerusalem, Israel

**Abstract:** The very large and complex nervous system of *Octopus vulgaris* supports highly complex and flexible behaviors including a variety of hunting and defensive strategies and the ability to learn from experience to adjust motor control strategies to new tasks. From a motor control perspective, it is challenging to understand how these capabilities are achieved in a soft-bodied animal where the number of controlled variables (DOFs) is not restricted, as in skeletal animals, by a fixed number of joints. In this work we aimed at studying the brain neuronal activity underlying octopus behaviors with a particular focus on mapping the higher neural organization of sensory encoding regions. Freely behaving animals were stimulated tactilely and visually while making extracellular multi-units recordings from different areas in the central brain. A computational methodology for spike sorting and clustering analysis was developed to characterize the neuronal units activated by the different simulation modalities and during spontaneous motion. Brain areas recorded were either silent (e.g. vertical lobe) or showed different degrees of multimodalities, but with no topographic representation of body portions. Our clustering analysis interestingly suggests that, although lacking somatotopy, the activity patterns of neuronal clusters recorded at single discrete brain locations are correlated to sensory-motor modalities. We also show that the overall unit's response reflects the connectivity map of the recording sites. Our results suggest that the body of the octopus and its higher motor control centers have co-evolved to allow efficient interfacing of sensory information coming from various body areas and different type of sensory inputs with motor commands in a unique way that better fits control of movements in a soft-bodied animal which make central representation in body parts coordinates impractical. This work represents the first ever achieved recording and 'decoding' of brain activity in a live and freely-behaving Cephalopod, the *Octopus vulgaris* and allow to finally assess the absence of a sensory 'octopunculus' at higher sensory-motor centers and further support their strong integrative function. This well relates with the animal need of integrating a vast amount of information from multiple limbs and senses to produce effective behaviors.

**Disclosures: L. Zullo:** None. **D. Boligin:** None. **M. London:** None. **B. Hochner:** None.

**Presentation Number:** NANO07.03

**Topic:** F.01. Neuroethology

**Support:** McDonnell Foundation 220020516

**Title:** The evolution of motor cortex in mammals: Insights from comparative intracortical microstimulation in rodents, primates, bats, and marsupials

**Authors: *A. C. HALLEY**[1], L. KRUBITZER[2];
[1]Univ. of California Davis, Davis, CA; [2]Univ. of California, Davis, Davis, CA

**Abstract:** Which areas of the neocortex are involved in the generation of movement in different lineages of mammals; what are the organizing principles of regions of the cortex involved in motor control; and how did these areas evolve? To address these question we have utilized long-train intracortical microstimulation techniques to examine the organization of movement representations in primary motor cortex (M1), somatosensory cortex (S1), and posterior parietal cortex (PPC) in a broad range of mammals. This comparison draws on a long-term comparative project in our laboratory that has so far examined rat (*Rattus norvegicus*), galago (*Otolemur crassicaudatus*), cebus monkey (*Sapajus* ssp.), titi monkey (*Callicebus cupreus*), rhesus monkey (*Macaca mulatta*), Egyptian fruit bat (*Rousettus aegyptiacus*), and short-tailed opossum (*Monodelphis domestica*). An important feature of this study is that similar stimulation parameters, histological processing of cortical tissue, and data analysis techniques have been utilized in all of the species studied, allowing us to make direct and accurate comparisons between species. Phenotypes that have been evolutionarily conserved include the involvement of both S1 and M1 in generating movement, distinct roles for each field in the direction of movement, and a patchy somatotopic representation of movement types progressing from hindlimb movements in caudal/medial areas of cortex to forelimb and face movements in rostral/lateral areas. Individual species also exhibit specializations in motor organization related to body morphology and the types of behaviorally relevant movements that require similar muscles synergies. Examples include the cortical magnification of the precision grip representations in some primates, whisker representations in opossums and rats, and tongue representations associated with echolocation as well as overlapping representations of hindlimb and forelimb muscles involved in self-propelled flight in fruit bats. In primates, the extent of cortex involved in motor control is greatly expanded and includes areas in anterior parietal cortex, and multiple fields in posterior parietal cortex involved in motor control of the forelimb and hand.

**Disclosures: A.C. Halley:** None. **L. Krubitzer:** None.

**Presentation Number:** NANO07.04

**Topic:** F.01. Neuroethology

**Support:** Glen de Vries Presidential Fellowship for Biological Sciences
Oracle for Research Grant

**Title:** Naturalistic action encoding across corticostriatal motor axis

**Authors: *A. HSU**[1], M. A. NICHOLAS[1], E. A. YTTRI[2];
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**Abstract:** The overwhelming majority of what is known about motor planning, performance, and action selection is the result of studies employing stereotyped, arbitrary tasks repeated hundreds of times. While animals are capable of executing these tasks, the nature of these tasks may create confounds due to overtraining, reduced behavioral dimensionality, or the artificial nature of these tasks. Thus, our understanding of neuroscience and the rapidly expanding field of neuroethology comes with a huge caveat. More importantly, understanding the mechanisms underlying the rich dynamics of naturalistic behavior may provide a more accurate account of neural function and disease.To gain an accurate and thorough account of the cell and circuit mechanisms of innate action selection and performance, we chronically implanted Neuropixels electrodes to simultaneously record all layers of motor cortex, dorsal and ventral striatum concurrently with 24/7 video. From the extracted three-dimensional pose estimation, we applied our state-of-the-art unsupervised behavioral identification software, B-SOiD (Nat Comms), to discover and quantify the spontaneous behaviors of the mouse. We discovered robust and distinct neural representations of the naturalistic behaviors, their kinematics, and their state transitions.1) Across the areas we recorded, the majority of neurons in corticostriatal circuit modulated their activity at the onset of the ML-derived behaviors. Across brain areas, both positive and negative modulation was observed, typically starting around ~200ms before the onset of a given behavior. This modulation was statistically less diverse for more kinematically similar behaviors, and was conserved across animals. 2) While each area demonstrated different dynamics, we were able to use machine learning to accurately predict any ongoing action with an accuracy over ten times greater than chance. 3) Additionally, we were able to estimate limb kinematics using the activity of a different set of neurons, describing the integrated computational machinery that enables the flexible control of actions and kinematics.

**Disclosures: A. Hsu:** None. **M.A. Nicholas:** None. **E.A. Yttri:** None.

**Presentation Number:** NANO07.05

**Topic:** F.01. Neuroethology

**Support:** HHMI

**Title:** The neural basis of cuttlefish dynamic camouflage

**Authors: *T. MONTAGUE**, E. SHOOK, T. BARLOW, D. GARCIA-ROSALES, L. ABBOTT, R. AXEL;
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**Abstract:** Cuttlefish are coleoid cephalopods that dynamically change the color, pattern and texture of their skin to camouflage with their surroundings. Camouflage is achieved by expanding and contracting pigment-filled saccules in the skin called chromatophores, through the action of motor neurons that project from the brain. Thus, the patterning of the skin is a physical manifestation of neural activity in the brain. We are using this system to understand how the physical properties of the visual world are represented by patterns of neural activity in the brain, and how this representation is transformed into an approximation of the physical world on the skin. We have performed a series of experiments to develop the dwarf cuttlefish, *Sepia*

*bandensis*, as a model to investigate the neural basis of camouflage. We have described the stages of embryonic development, sequenced the genome and neural transcriptome, completed a 3D brain atlas, and developed a visually-evoked camouflage behavioral paradigm. Furthermore, we are generating transgenic cuttlefish that express genetically-encoded calcium indicators and light-activated channels, permitting the live imaging and manipulation of neural activity. These technologies should permit us to simultaneously record neural activity and measure behavior to uncover how visual information is deconstructed in the brain, and then reconstructed into an image of the physical world on the skin.

**Disclosures: T. Montague:** None. **E. Shook:** None. **T. Barlow:** None. **D. Garcia-Rosales:** None. **L. Abbott:** None. **R. Axel:** None.

**Presentation Number:** NANO07.06

**Topic:** F.01. Neuroethology

**Support:** NIH R35GM124883

**Title:** How spiders actively modulate web-vibration sensing during prey localization

**Authors: *H.-Y. HUNG**[1], A. CORVER[1], A. GORDUS[2];
[1]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Johns Hopkins Univ., Baltimore, MD

**Abstract:** Organisms flexibly adjust postures to acquire information from the environment based on real-time sensory feedback. This study aims to investigate how spiders actively engage in a series of sensorimotor actions to manipulate the vibrational sensory field during prey localization. Our hypothesis proposes that **orb-weaving spiders, *Uloborus diversus,* actively adjust leg postures and produce web vibrations to increase sensory gain**.We first used scanning electron microscopy to examine the morphology of the spider's vibration receptors, known as slit sensilla, in the metatarsal joints that are crucial for detecting environmental vibrations. As observed in other spdiers, the metatarsal slits are located on the middle dorsal surface, with their orientation perpendicular to the long axis of the leg. To determine the mechanical properties of the slits, we applied millinewton forces to the spider's tarsus, and observed the metatarsal joint exhibits an exponential force-angle relationship, consistent with *Cupiennius salei*, another spider species. This indicates that the resonant frequency of this joint is a function of joint angle.To simultaneously monitor the sensory input induced by the prey, *Drosophila*, and the behavior of the spiders, we used a high-speed camera to capture web vibrations, while side cameras recorded spider behavior. We found that *Drosophila* produces low frequencies between 5-30 Hz on the web. During prey localization, spiders actively produce vibrations by crouching and pulling threads, inducing multiple harmonics on the web. Additionally, we conducted manual web perturbations using a piezo actuator, simulating artificial prey signals with well-defined frequencies and amplitudes. This enabled us to deceive the spiders and analyze their behavioral responses to defined web frequencies, specifically examining how leg posture is altered as a function of web frequency.By combining these measurements, we aim to infer how spiders modulate their leg vibration sensitivity to effectively detect prey. Importantly, this study will improve our understanding of sensorimotor integration of substrate vibration sensation.

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**Presentation Number:** NANO07.07

**Topic:** F.01. Neuroethology

**Support:** NSF Postdoctoral Research Fellowships in Biology Program under Grant No. 2109747
NIH Grant 2R35GM124883-06

**Title:** A three-dimensional brain atlas of the hackled orb-weaver spider, Uloborus diversus

**Authors: *G. ARTIUSHIN**, A. CORVER, L. POLACK, A. GORDUS;
Johns Hopkins Univ., Baltimore, MD

**Abstract:** Orb-web building in spiders is a striking example of the phenomenon of animal construction. This innate behavior is a complex, multi-hour sequence, performed by an organism with a nervous system comparable in size to traditional invertebrate models, such as the fruit fly. The web is reducible to analysis as a two-dimensional representation, composed of simple geometric shapes. As such, orb-web building is a tractable model of animal construction, but little is known regarding the neurobiological underpinnings of this behavior. Atlases of brain morphology, including neuronal sub-type and genetic expression pattern maps, are essential tools for understanding behavior at the circuit, cellular and molecular levels in model species. Staining in slices has revealed neurotransmitter expression and general morphology in the wandering spider, *C. salei*, serving as the reference for spider brain anatomy at large. Nevertheless, the brains of web-building spider species have not been well-described, and a complete three-dimensional standard brain including expression patterns for the major neurotransmitter and neuromodulator-expressing populations does not exist for any spider species. We present a three-dimensional immunostained volume of whole-mounted *Uloborus diversus* synganglia. Using synapsin staining as a common neuropil reference channel, we have aligned image volumes of neurotransmitter/neuromodulator (serotonin, acetylcholine, octopamine/tyramine, etc.) and neuropeptide (CCAP, FMRFamide, proctolin, etc.) expressing populations to a standard *U. diversus brain* using the *elastix* image registration toolkit. This assembly enables analysis of expression patterns across the complete synganglia, highlighting many features - such as extensive subesophagael neuropeptide expression. Relative to studied species, *U. diversus* has diminished optic neuropils, but robustly present corpora pedunculata (mushroom bodies). Also detailed are a minimum of two previously indiscernible sub-layers in each arcuate body layer, revealed by differential patterns in neuropeptide and serotonergic presence. This atlas provides a foundation for future studies of neurocircuitry governing web-building, and an orb-weaving reference for neuroethological comparisons of spider brain anatomy.

**Disclosures: G. Artiushin:** None. **A. Corver:** None. **L. Polack:** None. **A. Gordus:** None.

**Presentation Number:** NANO07.08

**Topic:** F.01. Neuroethology

**Support:** NSF (CRCNS 1822550; 2203119)
Vannevar Bush Faculty Award (ONR N000142012828)

**Title:** Neuropeptides generate somersaulting in Hydra vulgaris

**Authors: *W. YAMAMOTO**, R. YUSTE;
Columbia Univ., New York, NY

**Abstract:** *Hydra vulgaris* is a freshwater cnidarian that locomotes by somersaulting: an acrobatic maneuver performed by attaching the tentacles to the substrate and swinging the foot over the head to stand in a new position. Although *Hydra's* somersaulting was already described as early as the 18th century, how a distributed nervous system of a few hundred neurons can exercise sensory-motor coordination to achieve such sophisticated behavior still remains a mystery.To understand the neuronal mechanisms of somersaulting, we used *Hydra* expressing the calcium indicator GCaMP6s, to image the entire neuronal and muscle activity during somersaulting. At the start of somersaulting, we found that the activity of the rhythmic potential 1 (RP1) circuit, an ensemble of synchronously firing neurons distributed throughout the body, significantly increased (RP1 burst: >0.5 Hz). During RP1 bursts, the activity of basal disc muscles and nematocytes on the tentacles also increased, which corresponds to the tentacle attachment and foot detachment of the somersaulting steps.To elucidate the causal relationship between the RP1 circuit and somersaulting, we altered RP1 activity. Firstly, increasing medium osmolarity decreased RP1 activity (<0.5 Hz) and suppressed somersaulting. Secondly, targeted optical ablation of RP1 neurons using a two-photon laser also led to the suppression of somersault behavior. Finally, Hym-248, a neuropeptide synthesized by RP1 neurons, evoked somersaulting and regulated the activity of different cellular targets of the body at different times. These results reveal the role of neuropeptides in cnidarian behavior and expand our understanding of the functions of an evolutionary ancient nervous system.

**Disclosures: W. Yamamoto:** None. **R. Yuste:** None.

**Nanosymposium**

**NANO08: Physiology and Pharmacology of Serotonin and Hallucinogens**

**Location:** WCC 152B

**Time:** Saturday, November 11, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO08.01

**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** NIH Grant R01NS121014
Owens family foundation
Alzheimer's Association Resaerch Fellowship AARF-19-619387
UVA Brain Institute

**Title:** Dopamine transporters gate multiple dopaminergic transmission modes via a perisynaptic mechanism

**Authors: *L. HUANG**[1], Y. CHANG[2], W. LYNCH[3], J. VENTON[2], J. ZHU[1];
[1]Univ. of Virgina, Charlottesville, VA; [2]Departments of Chem., Univ. of Virginia, Charlottesville, VA; [3]Departments of Psychiatry, Univ. VA Sch. Med., Charlottesville, VA

**Abstract:** Midbrain dopamine neurons participate in a diverse range of behaviors, and their heterogeneous firing patterns appear to influence the dynamics of dopaminergic transmission at synapses during various behaviors. However, the precise regulatory mechanisms of dopaminergic transmission remains unclear. Here, we report the development of a multiplexed genetic encoded sensor-based imaging and fast-scan cyclic voltammetry (FSCV) method that enables the first simultaneous recordings of synaptic, perisynaptic, proximate and distal extrasynaptic dopaminergic transmission. Nanoscopic delineation of dopamine spatial diffusion and release properties with genetically-encoded sensor-based image analaysis program (GESIAP) in the mouse ventral striatum and prefrontal cortex reveals that dopamine transporters reuptake released dopamine selectively at perisynaptic sites without affecting dopamine release. This reuptake mechanism effectivley confines dopamine within synaptic clefts under both tonic (continuous) and phasic (burst-like) activities. Under certain conditions, such as phasic activities resulting from co-activated dopaminergic neurons, cocaine exposure, and the presence of a selective transporter inhibitor GBR12935, dopamine may escape out of synaptic clefts via a few outlets to mediate extrasynaptic transmission. Our study discloses that perisynaptic dopamine transporters play a crucial role in governing the activity pattern-dependent modes of dopaminergic transmission.

**Disclosures: L. Huang:** None. **Y. Chang:** None. **W. Lynch:** None. **J. Venton:** None. **J. Zhu:** None.

**Presentation Number:** NANO08.02

**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** NIH R01NS121014
the Owens family foundation
AARF-19-619387
UVA Brain Institute

**Title:** A new perisynaptic mechanism governing synaptic serotonergic transmission

**Authors: *Y. ZHANG**, P. ZHANG, M. SHIN, Y. CHANG, J. VENTON, J. ZHU;
Univ. of Virginia, Charlottesville, VA

**Abstract:** The serotonin-mediated intercellular communication has been implicated in myriad human behaviors and diseases, yet how serotonin communicates and how the communication is regulated remain unclear due to limitations of available monitoring tools. Here, we report the development of a multiplexed genetic encoded sensor-based imaging and fast-scan cyclic voltammetry (FSCV) method that enables the first simultaneous recordings of synaptic, perisynaptic, proximate and distal extrasynaptic transmission. Nanoscopic delineation of serotonin spatial diffusion and release properties with genetically-encoded sensor-based image analaysis program (GESIAP) in the mouse dorsal raphe nucleus and amygdala discloses that serotonin transporters effectivley reuptake released serotonin selectively at perisynaptic sites, which restricts serotonin within synaptic clefts to mediate synaptic transmission evoked by physiological stimuli. Stronger stimuli and/or inhibiting serotonin reuptake do not alter serotonin release properties at synapses, but drive serotonin out of synaptic clefts via a few outlets to mediate extrasynaptic transmission. Our analysis unveils the multiple modes of serotonergic

transmission, defines the specific function of transporters in governing serotonergic transmission, and validates the potential applicability of multiplexed GESIAP-based imaging and FSCV method in causally linking neurmodulation with physiological actions, clinical symptoms in diseases, and therapeutic benefits.

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**Presentation Number:** NANO08.03

**Topic:** G.09. Drugs of Abuse and Addiction

**Title:** Gesiap: a versatile genetically encoded sensor-based image analysis program

**Authors:** W. ZHENG[1], Y. ZHANG[1], R. E. ZHU[2], P. ZHANG[1], S. GUPTA[1], L. HUANG[1], Z. YANG[1], D. SAHOO[1], K. GUO[3], M. E. GLOVER[4], K. C. VADODARIA[5], M. LI[6], T. QIAN[6], M. JING[6], J. FENG[6], J. WAN[6], P. M. BORDEN[7], F. ALI[8], A. C. KWAN[8], L. GAN[9], L. LIN[3], F. H. GAGE[5], J. VENTON[10], J. S. MARVIN[7], K. PODGORSKI[7], S. M. CLINTON[4], M. ZHANG[11], L. L. LOOGER[7], Y. LI[6], ***J. ZHU**[1];
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**Abstract:** Intercellular communication mediated by a large number of neuromodulators diversifies physiological actions, yet neuromodulation remains poorly understood despite the recent upsurge of genetically encoded transmitter sensors. Here, we report the development of a versatile genetically encoded sensor-based image analysis program (GESIAP) that utilizes MATLAB-based algorithms to achieve high-throughput, high-resolution processing of sensor-based functional imaging data. GESIAP enables delineation of fundamental properties (e.g., transmitter spatial diffusion extent, quantal size, quantal content, release probability, pool size, and refilling rate at single release sites) of transmission mediated by various transmitters (i.e., monoamines, acetylcholine, neuropeptides, and glutamate) at various cell types (i.e., neurons, astrocytes, and other non-neuronal cells) of various animal species (i.e., mouse, rat, and human). Our analysis appraises a dozen of newly developed transmitter sensors, validates a conserved model of restricted non-volume neuromodulatory synaptic transmission, and accentuates a broad spectrum of presynaptic release properties that variegate neuromodulation.

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**Title:** Pattern Dynamics of Brain Rhythms in LSD and Sleep

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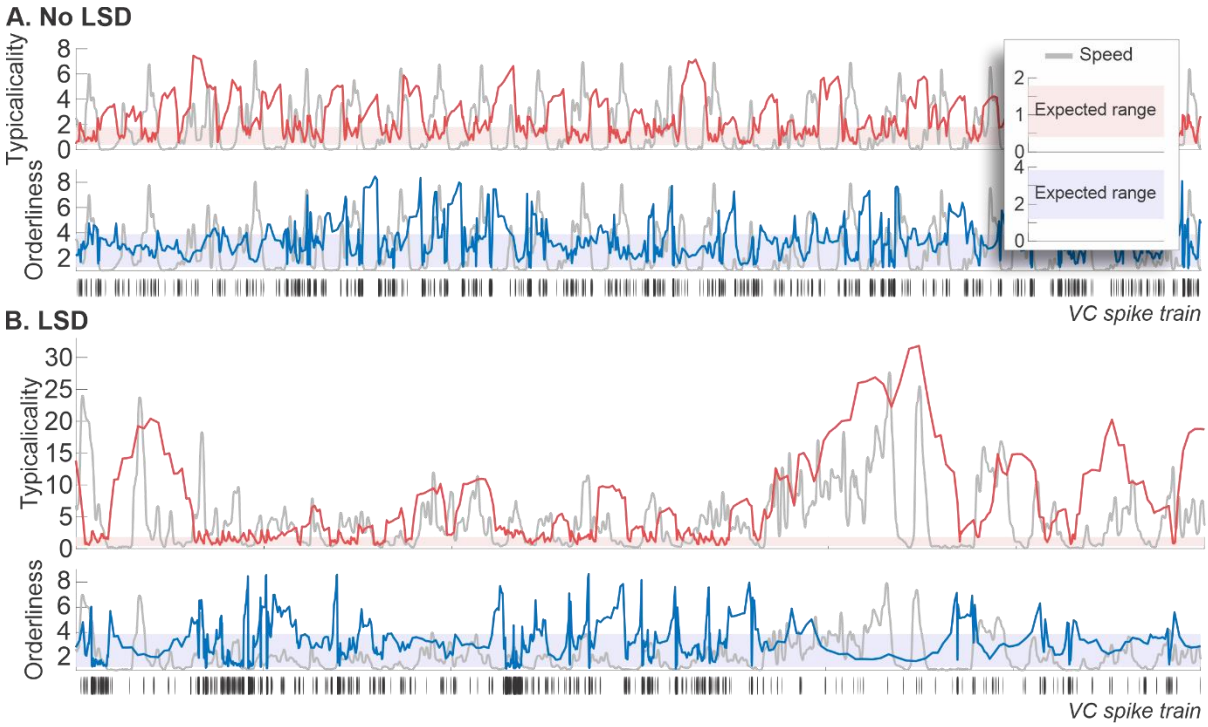
**Abstract:** Psychedelic drugs, such as lysergic acid diethylamide (LSD), have been shown to be powerful modulators of a brain state, causing vivid visual hallucinations, auditory distortions, and altered perception of time and space. These effects can be used for therapeutic purposes, as an effective treatment of many psychiatric disorders. In our study, we seek to unravel the effects of LSD in neurocircuits by studying patterns of spike trains and brain rhythms recorded from the CA1 area of the hippocampus (HP) and visual cortex (VC) of male and female rats. We use two complementary "stochasticity scores" for quantifying pattern dynamics, first in sober rats during sleep and track running, then in the same rats after being given a dose of LSD. The first score measures the pattern's consistency with a long-period trend, and the second assesses how "structured" or "orderly" (e.g., periodic-like or time-clustered) the pattern is. Our previous studies in mice revealed conspicuous coupling between hippocampal θ-, γ-, and ripple waveforms with the animal's acceleration, speed, and location (layouts reminiscent of HP place fields). In this study, each rat had four sessions: 1) a control sleep; 2) control runs over a U-shaped track with food wells on either end; 3) LSD sleep and 4) LSD track running. During the former two sessions, sober rats demonstrate spatial and lap-specific patterning of spike trains and LFP rhythmicity, e.g., their VC spike trains are coupled to the rat's speed, increasing in number during periods of quiescence (Fig. A). During the LSD sessions, the rats exhibit clear alterations in circuit activity, e.g., the spike train vs. speed coupling is inverted, increasing during period of active movement (Fig. B). Additionally, we found a variegated changes of sleep patterning in both VC and HP spiking and LFP, between LSD and no LSD rats. These differences in brain wave and spike dynamics offer a novel perspective on understanding the circuit-level effects of psychedelics and their influence on the information exchange between brain regions, in wakefulness and in sleep.

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**Title:** The effects of psychedelic hallucinogens on claustrum cortical projection neurons and a characterization of claustrum serotonin receptors after cocaine self-administration

**Authors: *T. L. ANDERSON**, J. KEADY, J. SONGRADY, J. R. TURNER, P. I. ORTINSKI; Univ. of Kentucky, Lexington, KY

**Abstract:** Psychedelic hallucinogens have attracted attention for their promising therapeutic potential to treat psychiatric diseases such as substance use disorders. Though early clinical results regarding psychedelic drugs, which act as agonists or partial agonists of the serotonin 2A receptor (5-HT2AR), are promising, the mechanisms by which psychedelics achieve long-term therapeutic effects and alter brain neurocircuitry are largely unknown. The claustrum (CLA), a subcortical nucleus, has the highest density of the serotonin 2A receptor (5HT2AR) in the brain with extensive connections to other brain areas, most prominently the anterior cingulate cortex (ACC) that is involved in both cognitive flexibility and drug-seeking behaviors. We propose that cocaine induces alterations at CLA-ACC glutamatergic synapses, and that psychedelic drugs act at CLA 5-HT receptors to influence this plasticity. We utilized whole cell recordings to characterize effects of 5-HT on CLA neurons that project to the ACC. 5-HT caused dramatic inhibition of glutamatergic synaptic activity. Significant decreases in sEPSC frequency and amplitude were observed, as well as decreases in action potential firing rate and

hyperpolarization of the resting membrane potential. Next, we used qPCR to observe the relative abundance of 13 different 5-HT receptor subtypes within the CLA, finding elevated levels of 5-HT1A, 2A, 2B, and 2C receptors. CLA-ACC neurons were then recorded in the presence of 5-HT and antagonists of each of these receptors to observe their contributions to the 5-HT effects in both cocaine and saline-yoked animals. Blockade of the 5HT2AR with ketanserin eliminated synaptic effects of 5HT, indicating a regulatory role of the 5HT2AR in claustrocortical signaling. Conversely, recordings performed in the presence of the psychedelic 5-HT2AR agonist, DOI, caused increases in sEPSC frequency and amplitude. Antagonism of the 5-HT1A receptor also attenuated the 5-HT effects on RMP in saline rats, an effect that was absent in cocaine rats. Next, we observed spike-timing dependent plasticity (STDP) in CLA-ACC neurons, revealing anti-hebbian long-term depression. DOI reversed this LTD into a robust long-term potentiation. Finally, RNA scope combined with confocal imaging was performed to interrogate the colocalization of each of the 5-HT receptor subtypes in the claustrum. These findings provide the first evidence that the large population of CLA-ACC neurons are under inhibitory control from 5-HT, and suggest that 5-HT1 and 5-HT2 receptors are separately involved in serotonin regulation of intrinsic membrane properties and excitatory synaptic plasticity, respectively.

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**Topic:** G.09. Drugs of Abuse and Addiction

**Title:** Pharmacological and behavioral effects of GM-2040 and other putative non-hallucinogenic 5-HT2A agonists

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**Abstract:** Rationale: Psychedelic drugs like psilocybin have shown potential in the treatment of several psychiatric disorders, including depression, substance abuse, and post-traumatic stress disorder. Recently, there has been interest in the development of non-hallucinogenic 5-HT2A agonists, which are purported to recapitulate the therapeutic efficacy of psychedelic drugs without inducing hallucinogenic effects. The rodent HTR has been used as a proxy for hallucinogenic activity in humans and shows a strong correlation with the known potency in humans of a diverse set of psychedelic drugs.
Objective: To compare GM-2040, a novel, putative non-hallucinogenic 5-HT2A agonist with other non-hallucinogenic and hallucinogenic 5-HT2A agonists reported in the literature.
Methods: GM-2040 was tested along with both hallucinogenic/non-hallucinogenic comparators for in vitro activity (FLIPR) at 5-HT receptors. These compounds were tested in the rodent Head Twitch Response (HTR) assay. GM-2040 was additionally tested for antidepressant-like efficacy in the Forced Swim Test (FST) and Chronic Mild Stress (CMS). Finally, ex vivo receptor occupancy was measured to demonstrate target engagement.
Results: GM-2040 is a potent and selective 5-HT2A agonist in vitro that does not cause a significant increase in the HTR, despite reaching high levels of receptor occupancy. Most other

non-hallucinogenic compounds showed a similar lack of the HTR, however these results are complicated by the pharmacology of these compounds. In both the FST and CMS, GM-2040 showed robust antidepressant-like efficacy.
Conclusions: We demonstrate that there is a risk of erroneously characterizing compounds as non-hallucinogenic 5-HT2A agonists in the absence of careful PK/PD measurements and target engagement. This may be due to very low affinity/efficacy at 5-HT2A, off-target pharmacology masking the HTR, or the short duration of hallucinogenic-like effects. Careful interpretation of both the in vitro pharmacology and the rodent HTR assay is necessary to avoid "false positives" in the search for non-hallucinogenic 5-HT2A agonists.

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**Presentation Number:** NANO08.07

**Topic:** G.09. Drugs of Abuse and Addiction

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**Title:** Psychedelics, reward, and drug-related plasticity in ventral tegmental area in mice

**Authors: *E. R. KORPI**, L. ELSILÄ, J.-P. LUUKKONEN, A.-M. LINDEN, E. I. NAGAEVA; Dept Pharmacol, Univ. of Helsinki, Helsinki, Finland

**Abstract:** Research on the clinical applications of serotonin 2A receptor ($5HT_{2A}R$) agonists, often referred to as psychedelics, is reviving after several decades of being banned. These drugs have promising antidepressant effects in animal models and in humans (Hesselgrave et al. 2021; doi/pdf/10.1073/pnas.2022489118; Carhart-Harris et al. 2021; DOI: 10.1056/NEJMoa2032994). Other possible applications of psychedelics include the treatments of anxiety related to life-threatening illness, post-traumatic stress disorder and drug addictions. However, the basic mechanisms of how these drugs influence neuronal circuits are still poorly known, with some of them even acting directly on brain-derived neurotrophic factor receptor TrkB (Moliner et al. 2023; DOI: 10.1038/s41593-023-01316-5). It is especially important to confirm that these drugs do not have addictive potential themselves. Here, we addressed this question, using behavioral conditioned place preference (CPP) method and ex-vivo electrophysiological approach in mice. We found that mice did not show any clear preference for the cage compartment where they previously had received either 0.1 mg/kg of lysergic acid diethylamide (LSD) or 3 mg/kg of a selective $5HT_{2A}R$ agonist 25CN-NBOH (Jensen et al. 2017; DOI: 10.1124/jpet.117.239905). Drugs of abuse, such as morphine, cocaine and benzodiazepines are known to produce glutamate synaptic plasticity in dopamine (DA) neurons of the ventral tegmental area (VTA) after a single injection. This plasticity can be measured as an increase in the ratio between evoked currents of AMPA and NMDA receptors. We did not observe any increases in this ratio 24 h after a single injection of the above-mentioned doses of either LSD or 25CN-NBOH, as compared to saline. In contrast, 10 mg/kg of morphine both induced a significant CPP in mice and increased the

AMPA/NMDA ratio in VTA DA cells. Our results suggest that the psychedelic drugs tested here do not have addictive potential in mice.

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**Nanosymposium**

**NANO09: Mechanisms of Attention: Human Studies**

**Location:** WCC 201

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO09.01

**Topic:** H.01. Attention

**Support:** UO1 Grant NS128921

**Title:** Intracranial neural dynamics of attention in rapid visual recognition

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**Abstract:** Attention is theorized to be a rhythmic process, by which limited attentional resources are allocated to behaviorally relevant stimuli. Visual categorization of words and other stimuli is known to involve activation in category-selective regions of the ventral occipitotemporal cortex (vOTC) as well as interaction with broader language and attentional networks. Recent evidence suggests that the boundaries of category-selective regions in vOTC may shift based on selective attention, implying a task driven recruitment of amodal substrates. A proposed neural substrate of attention is oscillatory coupling between task-relevant brain regions, indexed by activity in distinct frequency bands. However, direct evidence for these theories in humans remains lacking. We utilize the high spatiotemporal resolution of intracranial EEG recordings during a task where visual attention is directly modulated.
Patients (n=25; 2500+ electrodes) undergoing electrode implantation for seizure localization in intractable epilepsy performed a rapid visual recognition task in which visual stimuli of different categories (Faces, Words, Scenes, Animals) were presented. For the same stimulus set, patients tracked and responded to one specific feature: a color change of a central fixation point, repetition of a stimulus (i.e., a one-back task), or stimuli category membership (e.g., the category "fruits" for which an exemplar is "apple"). This task design enables the isolation of attentional dynamics when the same stimuli are presented but task demands shift.
We compared broadband gamma activity (BGA, 70-150 Hz) following stimulus onset across categories and attentional conditions. Through implementing a d-prime selectivity analysis, we identified clusters of category-selective electrodes across vOTC and inferior frontal gyrus (IFG). We found significant BGA effects of stimulus category and attentional condition within vOTC and frontal regions. In the 17 participants who had concurrent electrode coverage in vOTC and

frontal regions, we identified pairs of electrodes with significant generalized phase coherence during the stimulus presentation across a broad low frequency (3-40 Hz). We discovered that BGA and low-frequency phase dynamics significantly varied across stimulus category and attentional condition, pointing to a plausible mechanism by which frontal networks modulate activity within vOTC based on task demands. This provides evidence that inter-areal oscillatory coupling between vOTC and frontal regions indexes the process of rapid visual recognition.

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**Topic:** H.01. Attention

**Support:** NIH/NIAAA Y1AA3009

**Title:** Methylphenidate-induced reduction in central-autonomic coupling is inversely related to D2 receptor availability

**Authors: *E. SHOKRI KOJORI**, P. MANZA, D. TOMASI, G.-J. WANG, N. D. VOLKOW; NIH, Bethesda, MD

**Abstract:** Selective attention leads to a desynchronized state of brain activity, a reduction in low frequency power of local field potentials, and a suppression of internally generated activity patterns. Methylphenidate (MP) is an attention enhancing medication used to treat attention deficit hyperactivity disorder that modulates dopaminergic (and noradrenergic) systems by blocking the associated transporters. Several fMRI-based studies have shown that MP leads to reduced low-frequency (LF < 0.1 Hz) activity and connectivity in sensorimotor and visual regions, yet the underpinnings of these effects remain unclear. Recently we showed that similar brain regions had consistent phase coupling with LF pulse changes (regulated by sympathetic innervation), that were labeled as an autonomic network (AN). Here, we hypothesized that reduced LF synchronization in AN with a MP challenge is associated with altered coupling between brain and autonomic function, an effect mediated by cortical dopamine signaling. In a group of 26 healthy individuals (9 females, 22-64 years old), we measured brain resting-state activity with fMRI (7.7 min) and concurrently recorded pulse and respiratory signals once after administration of oral MP (60 mg) and once after placebo (PL) in a random order. Baseline D1 and D2 receptor (D1R and D2R) availability were measured with [11C]NNC112-PET and [11C]raclopride-PET, respectively. Consistent with prior studies, we found MP decreased fractional amplitude of LF fluctuations (fALFF) primarily in the visual and sensorimotor regions of AN ($pFWE < 0.05$) and concurrently reduced LF cortical synchrony as indexed by decreases in LF power of the average signal within these regions ($p < 0.001$). MP did not significantly affect LF power of pulse and respiratory signals ($p > 0.15$), yet it diminished LF phase between pulse (but not respiratory) signal and brain in the AN, thalamus, medial orbitofrontal regions, insula, and pons ($pFWE < 0.05$). More reduction in cortical LF power was associated with more reduction in LF phase coupling between brain and pulse ($r(24) = 0.62$, $p < 0.001$). In addition, more phase uncoupling between pulse and brain was associated with lower baseline D2R (but not D1R) availability in the same regions ($r(23) = -0.58$, $p = 0.002$, partial correlation). D1R and D2R availability were not associated with MP-induced reduction in cortical LF synchronization

(though striatal D2R was associated with reductions in cortical fALFF). Findings implicate D2R in MP-induced reduction in central-autonomic coupling and highlight the contribution of this uncoupling to modulated cortical synchronization when attention is pharmacologically enhanced.

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**Presentation Number:** NANO09.03

**Topic:** H.01. Attention

**Support:** DFG SFB 1436 B05

**Title:** Bilateral field advantage during multiple object tracking explained by representational hemispherical biases

**Authors: *C. MERKEL**, A.-M. FELßBERG, N. SCHÖNEMANN, J.-M. HOPF, A. SCHÖNFELD;
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**Abstract:** Keeping track of multiple visually identical and independently moving objects is a remarkable feature of the human visual system. Theoretical accounts for this mechanism focus on resource-based models that describe parametric decreases of performance with increasing demands during the task (i.e. more relevant items, closer distances, higher speed). Additionally, the presence of two central tracking resources, one within each hemisphere, has been proposed, allowing for an independent maintenance of moving targets within each visual hemifield. Behavioral evidence in favor of such a model shows that subjects are able to track almost twice as many targets across both hemifields compared to within one hemifield. A number of recent publications argue for two separate and parallel tracking mechanisms during standard object-tracking tasks that allow for the maintenance of the relevant information in a location-based and object-based manner. Unique electrophysiological correlates for each of those processes have been identified. The current study shows, that these electrophysiological components are differentially present during tracking within either the left or right hemifield. The present results suggest, that targets are mostly maintained as an object-based representation during right hemifield tracking while location-based resources are preferentially engaged during left hemifield tracking. Interestingly, the manner of representation does not seem to have an impact on behavioral performance within the subjects, while the electrophysiological component indicating object-based tracking does correlate with performance between subjects. We propose that hemifield-independence during multiple object tracking may be an indication of the underlying hemispheric bias for parallel location-based and object-based tracking mechanisms.

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**Title:** Dissociable neural processes during attentional selection within working memory and long-term memory

**Authors: *D. GONG**, D. DRASCHKOW, A. C. NOBRE;
Univ. of Oxford, Oxford, United Kingdom

**Abstract:** Research has increasingly emphasized the ability to orient attention selectively to prioritise internal contents for retrieval - in both working memory (WM) and long-term memory (LTM). However, little is known about the potential degree of overlap between mechanisms for internal attention in WM and LTM. We developed a task for comparing shifts of internal attention in WM and LTM for retrieving equivalent stimulus attributes. Eye tracking and EEG recordings from human participants (N = 30) tracked oculomotor and neural signals triggered by retrospective attention cues (retrocues) that prioritised object features of WM or LTM items on a trial-by-trial basis. The eye-tracking results confirmed our recent observation that gaze biases toward the attended item were more pronounced in WM than LTM. The EEG data revealed striking differences in neural processes following retrocues prioritising WM and LTM items. Replicating previous findings, transient lateralization of alpha power (8-12 Hz) at posterior sites occurred for shifts of attention in WM, but no such effects occurred for attention within LTM. Instead, frontal modulation of theta power (4-8 Hz) occurred during shifts of attention in LTM but not in WM. Further dissociable markers of internal shifts of attention in WM and LTM were observed in the event-related potentials. Our findings show that the robust consequences of orienting selective attention in WM and LTM occur through different routes, thus also providing valuable insights into the long-debated relationship between these two memory systems.

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**Presentation Number:** NANO09.05

**Topic:** H.01. Attention

**Support:** T32MH065214

**Title:** Predictively Modeling the Attention of Others

**Authors: *K. ZIMAN**, S. C. KIMMEL, K. T. FARRELL, M. S. GRAZIANO;
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**Abstract:** As social animals, people are highly sensitive to the attention of others. Seeing someone else gaze at an object automatically draws one’s own attention to that object. Monitoring the attention of others aids in reconstructing their emotions, beliefs, and intentions, and may play a crucial role in social alignment. Recently, however, it has been suggested that the human brain constructs a predictive model of other people’s attention that is far more involved

than a moment-by-moment monitoring of gaze direction. The hypothesized model learns the statistical patterns in other people's attention and extrapolates how attention is likely to move. Here we tested the hypothesis of a predictive model of attention. Subjects saw movies of attention displayed as a bright spot shifting around a scene. Subjects were able to correctly distinguish natural attention sequences (based on eye tracking of prior participants) from altered sequences (e.g. played backward or in a scrambled order). Even when the attention spot moved around a blank background, subjects could distinguish natural from scrambled sequences, suggesting a sensitivity to the spatial-temporal statistics of attention. Subjects also showed an ability to recognize the attention patterns of different individuals. These results suggest that people possess a sophisticated model of the normal statistics of attention and can identify deviations from the model. Monitoring attention is therefore more than simply registering where someone else's eyes are pointing. It involves predictive modeling, which may contribute to our remarkable social ability to predict the mind states and behavior of others.

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**Presentation Number:** NANO09.06

**Topic:** H.01. Attention

**Support:** R01MH128187

**Title:** Intracranial Reward Positivity Underlies Impulsive Choice

**Authors: *R. L. COWAN**[1], T. S. DAVIS[1], B. KUNDU[3], S. RAHIMPOUR[2], J. D. ROLSTON[4], E. H. SMITH[1];
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**Abstract:** The reward positivity (RewP) is an ERP component occurring around 250 ms after a reward outcome. Its amplitude is dependent on the reward outcome feedback, including the expected value i.e., reward estimate based on the probability previous experience) and prediction error related to the reward outcome (i.e., the difference between expected and actual outcome). RewP has previously been linked to basal ganglia and cortical structures such as anterior cingulate, posterior cingulate, and orbital frontal regions, though RewP is primarily studied using scalp EEG which lacks precise spatial resolution. Further, RewP has previously discriminated between impulsivity levels, with high impulsive choosers eliciting greater reward sensitivity, shown via greater amplitudes to unexpectedly larger rewards and smaller amplitudes when an expected reward was not received. However, to date, limited neural recordings have stifled interpretations of the RewP component regarding impulsivity. Here, we sought to understand the role of the RewP signal in impulsive choice behavior by examining intracranial EEG and behavior in 36 patients with drug-resistant epilepsy while they performed the Balloon Analog Risk Task. Using the Kullback-Leibler divergence between active and passive balloon inflation times as a measure of impulsive choice, we categorized subjects into more- or less-impulsive choosers, to scrutinize group-level differences between RewP characteristics in precise brain regions. We regressed spectral power in the theta and delta bands against point totals for each successful trial to characterize the RewP from intracranial recordings, evaluating an average of

73.41 linear models per subject. We found 5.86% of contacts encoded RewP in theta and 10.29% encoded RewP in delta power. Of the 36 subjects, 17 were classified as impulsive based on the BART task. Proportion tests revealed significantly more parametric encoding of RewP in more impulsive choosers for theta (more impulsive: 87/1275, less impulsive: 68/1368), ($X2(1) = 4.1035$, $p = .0428$, and delta (more impulsive: 159/1275, less impulsive: 113/1368), ($X2(1) = 12.6716$, $p = .00037$) frequency bands. Our findings suggest that impulsive choosing is characterized by increased reward sensitivity in the brain. Our next analysis step will detail anatomical locations for the elicited RewP component to elucidate the neural pathways associated with reward. Future work will further scrutinize the neural underpinnings of reward processing, by comparing the extracranial definitions of ERPs to the spectral representations of LFPs, bridging the gap between invasive and non-invasive neurophysiological techniques.

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**Presentation Number:** NANO09.07

**Topic:** H.01. Attention

**Support:** European Research Council (ERC) Starting Grant. Project ID: 950313, Project acronym: AWAR

**Title:** Automatic processing of potentially threatening human coalitions. Evidence from dot-probe, surprise-recall, and enumeration experiments

**Authors: *S. AMINIHAJIBASHI**, S. GOETZ, T. HAGEN, H. BARTUSEVIčIUS;
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**Abstract:** A large body of cognitive neuroscience research has shown that humans automatically extract threatening information, e.g., facial affect, physical formidability, and group affiliations, using basic visual cues, e.g., static facial structure, over-body masculinity, and tattoos. Here, we propose analogous computational mechanisms for *coalitional* formidability assessments and hypothesize that people automatically (i.e., unintentionally and efficiently) attend outgroup male coalitions (H1) and determine their size (H2). To test these hypotheses, we created visual stimuli and adapted dot-probe, surprise-recall, and dot-enumeration paradigms along with rating surveys for online administration using the Inquisit Web and Prolific platforms. Studies 1–9 ($N \approx 1000$ healthy American adults, mean age = 40, females = 40%) revealed supporting evidence for H1 and H2. We found a significant attentional bias toward coordinated male coalitions (vs. non-coordinated males and coordinated female coalitions) elicited and replicated only at 100ms exposures indicating that an initial attentional shift is involved. Also, the group size of coordinated male coalitions was more accurately estimated than other categories. However, the effect sizes are small which was expected given the low ecological validity of experimental settings, and we did not find moderation effects of participant gender or other individual differences. Currently, we are conducting additional studies (to be presented at the SfN Meeting), to replicate all results while also testing changes in the amount of attentional bias when manipulating the perceived coalitional level of visual stimuli in the dot-probe tasks and examining the effect of cognitive load on categorical advantages when performing different variants of the enumeration tasks. These findings contribute to a broader interdisciplinary debate

on human nature and its relation to warfare by providing preliminary evidence for potential cognitive adaptations to coalitional aggression.

**Disclosures: S. Aminihajibashi:** None. **S. Goetz:** None. **T. Hagen:** None. **H. Bartusevičius:** None.

**Presentation Number:** NANO09.08

**Topic:** H.01. Attention

**Title:** Brain vital signs track cognitive processing after sleep deprivation: metrics and potential interventions

**Authors: T. FRIZZELL**, V. J. MICKELSON, S. FICKLING, *R. C. N. D'ARCY;
Ctr. for Neurol. Studies, HealthTech Connex, Surrey, BC, Canada

**Abstract: Introduction:** Brain vital signs are a sensitive, objective neurophysiological evaluation of cognitive function. The NeuroCatch Platform is a medical device for rapid evaluation and monitoring of brain vital signs from event-related potentials. In the study, NeuroCatch was used to characterize the impacts of sleep deprivation on cognitive processing. **Methods:** Participants (n=30) were healthy adults with typical sleep for the previous 2 weeks. Participants were randomly assigned to groups, Sleep Deprivation or Control, and all completed two morning assessments with NeuroCatch approximately 24 hours apart. NeuroCatch scans included vital signs for auditory sensation (N100), basic attention (P300), and cognitive processing (N400). Participants in the control group left the lab, followed their regular sleep routine, and returned the following morning for the follow-up assessment. Participants in the sleep deprivation group remained at the lab and were kept awake throughout the night. **Results:** A repeated-measures analysis of variance showed significant differential effects of time in brain vital signs responses between the two groups. The sleep deprivation group showed significantly reduced basic attention, as indexed by the P300 amplitude, relative to controls. There was also a reduction in cognitive processing in the sleep deprivation group, as measured by the N400 amplitude, although this was not significant after correcting for multiple comparisons. **Conclusion:** Brain vital signs were sensitive to the effects of sleep deprivation on cognition in healthy adult participants. Future research will replicate this model to determine if interventions like dietary supplements or non-invasive neuromodulation devices can be used to reverse these effects, as both have been shown to optimize cognitive performance in healthy controls and elite athletes.

**Disclosures: T. Frizzell:** A. Employment/Salary (full or part-time):; HTC employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **V.J. Mickelson:** A. Employment/Salary (full or part-time):; HTC employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **S. Fickling:** A. Employment/Salary (full or part-time):; HTC employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options. **R.C.N. D'Arcy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

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**Nanosymposium**

**NANO10: Mapping and Probing Cell Types Across Scales**

**Location:** WCC 147B

**Time:** Saturday, November 11, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO10.01

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH, New Innovator Award DP2NS116768
Simons Foundation SCGB 543003

**Title:** New transgenic lines for measuring functional connectome of Caenorhabditis elegans

**Authors: *A. SHARMA**[1], F. RANDI[1], S. KUMAR[2], S. DVALI[1], A. LEIFER[1,2];
[1]Dept. of Physics, [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

**Abstract:** We present a collection of transgenic lines to enable optical functional investigations of how the nervous system responds to activation at brain scale and cellular resolution in the nematode *C. elegans*. Strains were designed to express an optical actuator and indicator that avoided optical crosstalk, with strong expression and minimal toxicity, in a genetic background that enabled neural identification. We generated transgenic animals that express in every neuron a calcium indicator GCaMP6s, localized to the nucleus, and a purple-shifted light-sensitive actuator, the gustatory receptor homolog system GUR-3+PRDX-2 [Bhatla et al., 2015]. This combination of indicator and activator allows for 2-photon targeted optogenetic stimulation during simultaneous 1-photon calcium imaging with minimal optical cross-talk. To achieve high expression levels while avoiding toxicity, we used a dexamethasone dependent drug-inducible system, QF+GR > QUAS, to turn on gene expression only in adulthood [Monsalve et al., 2019]. These optogenetic tools were expressed in a NeuroPAL background to allow each neuron to be uniquely identified [Yemini et al., 2021]. We then mapped out functional connections in the brain by stimulating individual neuron and measuring the network's calcium response. To investigate the relative contribution of different signaling mechanisms in the brain, we made a library of strains with defects in several modes of neural communication. Specifically, we made strains defective for dense core vesicle release, synaptic vesicle release, and gap junction signaling. Using these strains, we have found that peptidergic signaling contributes significantly to neural dynamics [Randi et al., 2023]. This transgenic toolkit will be a resource for large scale investigations of functional connectivity in the brain.

**Disclosures: A. Sharma:** None. **F. Randi:** None. **S. Kumar:** None. **S. Dvali:** None. **A. Leifer:** None.

**Presentation Number:** NANO10.02

**Topic:** I.02. Systems Biology and Bioinformatics

**Support:** NIH U19MH114830
NIH RF1MH121274

**Title:** A suite of transgenic and enhancer AAV tools for targeting neuronal cell types in the cortex

**Authors: *B. TASIC**[1], J. T. TING[2], Y. BEN-SIMON[1], B. P. LEVI[2], T. L. DAIGLE[2], D. STAFFORD[4], M. HOOPER[1], N. JOHANSEN[3], M. WIRTHLIN[3], T. BAKKEN[3], J. MICH[3], E. MORIN[3], J. BENDRICK[3], L. SIVERTS[3], S. NARAYAN[1], R. MARTINEZ[3], M. TAORMINA[3], J. ARIZA[3], L. POTEKHINA[3], M. REDING[3], K. RONELLENFITCH[3], S. W. WAY[1], A. OSTER[1], R. SANCHEZ[3], B. THYAGARAJAN[3], K. SMITH[3], L. ESPOSITO[3], J. NGAI[4], E. S. LEIN[5], H. ZENG[5];
[1]Mol. Genet., [2]Human Cell Types, [3]Allen Inst. For Brain Sci., Seattle, WA; [4]Univ. of California, Berkeley, Berkeley, CA; [5]Allen Inst. for Brain Sci., Seattle, WA

**Abstract:** Single cell genomics has transformed the way we define cell types and our approaches for generating genetic tools for experimental cell type access. We used single-cell transcriptomics and epigenomics to define marker genes and enhancer elements for the generation of genetic tools to access cortical neuronal types. We present a large suite of transgenic and viral tools for access to most cell classes, subclasses, and select types of cortical neurons. These tools can be used individually or together to access, interrogate, and manipulate cortical neuronal types with unprecedented precision and ease.

**Disclosures: B. Tasic:** None. **J.T. Ting:** None. **Y. Ben-Simon:** None. **B.P. Levi:** None. **T.L. Daigle:** None. **D. Stafford:** None. **M. Hooper:** None. **N. Johansen:** None. **M. Wirthlin:** None. **T. Bakken:** None. **J. Mich:** None. **E. Morin:** None. **J. Bendrick:** None. **L. Siverts:** None. **S. Narayan:** None. **R. Martinez:** None. **M. Taormina:** None. **J. Ariza:** None. **L. Potekhina:** None. **M. Reding:** None. **K. Ronellenfitch:** None. **S.W. Way:** None. **A. Oster:** None. **R. Sanchez:** None. **B. Thyagarajan:** None. **K. Smith:** None. **L. Esposito:** None. **J. Ngai:** None. **E.S. Lein:** None. **H. Zeng:** None.

**Presentation Number:** NANO10.03

**Topic:** I.02. Systems Biology and Bioinformatics

**Support:** National Science and Technology Innovation 2030 Major Program (STI2030-2021ZD0200100)

**Title:** Single-cell spatial transcriptomic analysis reveals global organization of diverse cell types in the macaque cortex

**Authors: *Y. SUN**[1], J. MENG[2], L. FENG[2], C. T. LI[3];
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**Abstract:** The neocortex is greatly expanded in primates compared with rodents, especially the prefrontal cortex. Understanding its brain organization at the cellular level holds the key to deciphering neural circuit functions of the primate brain and to developing treatments for brain

disorders. To systematically elucidate the cellular diversity in primates at single-cell and spatial resolution, here we performed large-scale single-nucleus RNA-seq and spatial transcriptome analysis for millions of cells covering 143 cortical regions of cynomolgus monkeys. We derived a comprehensive cell-type taxonomy of neuronal and non-neuronal cells in the macaque cortex. Spatial mapping revealed laminar and regional preferences of various cell types and regional differences in cell-type composition and neighborhood complexity. Further comparative analysis revealed glutamatergic neuron types in the cortex of primates with layer-specific distribution and high expression of functionally important genes. Together, these results demonstrate the increased complexity of glutamatergic neuron subtypes in primates, and provide spatial underpinning for molecular understanding of organizing principles of the primate brain.

**Disclosures: Y. Sun:** None. **J. Meng:** None. **C.T. Li:** None.

**Presentation Number:** NANO10.04

**Topic:** I.02. Systems Biology and Bioinformatics

**Support:** NIGMS grant 1R01GM135247-01
Brain Initiative grant 1RF1MH121267-01
NIDA grant U01 DA053625-01
Feil Family Foundation
NSF GRFP No. NSF 2139291
NIDA grant 2T32DA039080

**Title:** Scisor-atac reveals convergent and divergent splicing and chromatin specificities between cell types across cortical regions and in Alzheimer's disease.

**Authors: *W. HU**[1,3,4], C. FOORD[2,4], J. HSU[2,4], L. FAN[3,5], M. CORLEY[6,3], T. N. BHATIA[7], S. XU[8], N. BELCHIKOV[3,4], Y. HE[3,4], A. PANG[6,3], S. N. LANJEWAR[7], J. JARROUX[3,4], A. JOGLEKAR[3,4], T. A. MILNER[3], L. C. NDHLOVU[3,6], J. ZHANG[8], E. BUTELMAN[9], S. A. SLOAN[7], L. GAN[3,5], H. U. TILGNER[3,4];
[1]Weill Cornell Medicine, Feil Family Brain & Mind Res. Inst., NEW YORK, NY; [2]Weill Cornell Medicine, Feil Family Brain & Mind Res. Inst., New York, NY; [3]Weill Cornell Med. Feil Family Brain & Mind Res. Inst., New York, NY; [4]Ctr. for Neurogenetics, Weill Cornell Med., New York, NY; [5]Helen and Robert Appel Alzheimer's Dis. Res. Inst., New York, NY; [6]Dept. of Med., Div. of Infectious Dis., New York, NY; [7]Dept. of Human Genet., Emory Univ. Sch. of Med., Atlanta, GA; [8]Dept. of Computer Sci., Univ. of California, Irvine, CA, USA, Irvine, CA; [9]Neuropsychoimaging of Addiction and Related Conditions Res. Program, Dept. of Psychiatry., Icahn Sch. of Med. at Mount Sinai, New York, NY

**Abstract:** Multimodal measurements have become widespread in genomics, however measuring chromatin accessibility and splicing simultaneously in frozen brain tissues remains unconquered. To this effect, we have devised Single-Cell-ISOform-RNA sequencing coupled with the Assay-for-Transposase-Accessible-Chromatin (ScISOr-ATAC). First, we applied ScISOr-ATAC to Rhesus macaque (*Macaca mulatta*) prefrontal (PFC) and visual cortex (VIS) to compare splicing and chromatin accessibility across brain regions and investigate how they correlate. We identified 3 distinct subtypes of excitatory neurons (ExN) characterized by layer-specific markers; *RORB* marks L3-L5; *CUX2* marks L2/3 and L6; both *CUX2 and RORB* mark L2-L4.

We profiled splicing and chromatin accessibility patterns in each ExN subtype between regions and showed that splicing is highly brain-region specific for RORB ExN, moderately specific in CUX2.RORB ExN and unspecific in CUX2 ExN. At the chromatin level, however, CUX2.RORB ExN show the highest brain-region specificity compared to other subtypes. These results indicate that some excitatory subtypes exhibit variable transcriptomic and chromatin accessibility patterns across tissues, while others do not. This suggests that splicing and chromatin can identify differences in excitatory subtypes within and across multiple regions. As an application to case-control study, we performed ScISOr-ATAC on human PFC samples of Alzheimer's Disease and control individuals to identify disease specific chromatin accessibility and splicing patterns. To exclude the bias introduced by cell type composition difference between cases and controls, we developed a down-sampling approach which randomly samples from an equal number of features and reads or cells across RNA and ATAC data. With this method, we found that oligodendrocytes are most affected by changes in splicing patterns and chromatin accessibility, suggesting that myelination may play an important role in AD. Among oligodendrocyte subtypes labeled by ENPP6 or LAMA2, we observed subtype specific differential accessible regions locate
in genes which have been reported to associate with brain diseases. Also, we found an enrichment in differentially spliced genes related to neurotransmitter receptors and transmission across chemical synapses. Altogether, these results indicate that the ScISOr-ATAC method can be applied to show convergent or divergent dynamics depending on specific cases and justify the need for multimodal measurement to investigate complex systems and disease states.

**Disclosures: W. Hu:** None. **C. Foord:** None. **J. Hsu:** None. **L. Fan:** None. **M. Corley:** None. **T.N. Bhatia:** None. **S. Xu:** None. **N. Belchikov:** None. **Y. He:** None. **A. Pang:** None. **S.N. Lanjewar:** None. **J. Jarroux:** None. **A. Joglekar:** None. **T.A. Milner:** None. **L.C. Ndhlovu:** None. **J. Zhang:** None. **E. Butelman:** None. **S.A. Sloan:** None. **L. Gan:** None. **H.U. Tilgner:** None.

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**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** National Key R&D Program of China 2019YFA0111000
Natural Science Foundation of Shanghai 20ZR1403100
Shanghai Municipal Science and Technology 20JC1419500
NSFC Grant #32000726

**Title:** Whole-brain in vivo base editing reverses autistic-like behaviors in mice

**Authors: *T.-L. CHENG**[1,2], S. ZHANG[1], W. LI[3], Z. QIU[4], W. PENG[5], Y. SHI[6], B. YUAN[7], S. ZHU[3], Y. TAI[8];
[1]Fudan Univ., Shanghai, China; [2]Inst. for Translational Brain Res., Shanghai, China; [3]Inst. of Neurosci., Shanghai, China; [4]Songjiang Res. Institute, Songjiang Hospital, Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China; [5]Inst. of Neuroscience, CAS, Shanghai, China; [6]Univ. of California San Diego, La Jolla, CA; [7]Inst. of Neuroscience, SIBS, CAS, Shanghai City, China; [8]Inst. of Brain Science, State Key Lab. of Med. Neurobiology, MOE Frontiers Ctr. for Brain Science, Fudan Univ., Shanghai, China

**Abstract:** Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorder characterized by deficits in social communication and stereotypical behaviors. However, whole-brain genome editing has not yet been achieved to correct single-base mutations and alleviate autistic-like behaviors in animal models. In this study, we developed an APOBEC-embedded cytosine base editor (AeCBE) system for converting C·G to T·A base pairs. We demonstrate its effectiveness by targeting AeCBE to an ASD-associated mutation of the *MEF2C* gene (c.104T>C, p.L35P) *in vivo*. We first constructed a *Mef2c* L35P heterozygous mouse that exhibited autistic-like behavioral deficits. We then programmed AeCBE to edit the mutated C·G base pairs of *Mef2c* in the mouse brain through the intravenous injection of blood-brain barrier (BBB)-crossing AAV. This treatment successfully restored MEF2C protein levels in various brain regions and reversed impairments in social interactions and repetitive behaviors in *Mef2c* mutant mice. Our work presents an in vivo base editing paradigm that could potentially correct a single-base genetic mutation in the brain.

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**Presentation Number:** NANO10.06

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** Autism Brain Net Analysis Award #953759

**Title:** Identification and impact of somatic mutations in regulatory regions in autism brain

**Authors:** A. MO[1], A. J. KRIZ[1], V. VISWANADHAM[2], ***S. E. GILL**[3,4], P. J. PARK[2], C. A. WALSH[1];
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**Abstract:** Autism spectrum disorder (ASD) has a large genetic component, but its causes are still not identified in most affected individuals. We previously found that, although ASD and control brains have similar rates of somatic single nucleotide variants (sSNVs) overall, sSNVs in ASD brains are enriched in enhancers that are active in the brain. To accurately identify somatic variants in enhancers and in other putative gene regulatory regions, we hypothesize that deep sequencing using the Assay for Transposase-Accessible Chromatin (ATAC-seq) is an efficient approach. To address this hypothesis, we performed flow cytometry to isolate neurons from postmortem human brains of ASD (n=55) and control (n=30) individuals. Control brains were selected to be age-, sex-, postmortem interval-, and RIN-matched to ASD brains to remove any potential confounding variables. Taking one of the brain samples as an example, its ATAC-seq profile showed a mean coverage of 165X at ATAC-seq peak summits, with 11% of peaks having >319X coverage and 5% of peaks having >500X coverage. Using a machine learning algorithm MosaicForecast, we detected 43 candidate sSNVs in this sample. Most of the variants (63%) had alternate alleles that were also detected in 250X whole genome sequencing for the sample, suggesting that these are real variants. The identification, characterization, and functional analysis of noncoding variants via ATAC-seq will help elucidate the contribution of somatic mutations in the pathogenesis of ASD as well as implicate potential mechanistic processes.

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**Presentation Number:** NANO10.07

**Topic:** I.02. Systems Biology and Bioinformatics

**Support:** NSF 2207891
NIH RF1MH125933

**Title:** Transcriptomic associations of brain organization across large-scale neuroimaging and clinical biobanks.

**Authors: *N. HOANG**[1], N. SARDARIPOUR[1], G. RAMEY[2], X. BLEDSOE[1], E. LIAO[1], Y. CHEN[1], J. PARK[1], B. A. LANDMAN[1], E. R. GAMAZON[3,4], K. G. SCHILLING[1], M. BENTON[5], J. A. CAPRA[2], M. RUBINOV[1];
[1]Vanderbilt Univ., Nashville, TN; [2]Univ. of California San Francisco, San Francisco, CA; [3]Vanderbilt Univ. Med. Sch., Nashville, TN; [4]Univ. of Cambridge, Cambridge, United Kingdom; [5]Baylor Univ., Waco, TX

**Abstract:** Analyses of large-scale neuroimaging datasets have shown substantial variation in neuroimaging phenotypes at the individual level. Separately, analyses of the Allen Human Brain Atlas dataset have shown associations between these phenotypes and gene expression at the group level. However, few analyses have considered the relationship between neuroimaging phenotypes and gene expression at the individual level. This analysis gap has ultimately limited our understanding of the transcriptomic basis of human brain individuality.
Here, we addressed this gap by inferring gene expression at the individual level in large-scale neuroimaging-genomic biobanks. Specifically, we used Joint Tissue Imputation to infer genetically regulated gene expression (gr-expression) for hundreds of genes across eight brain regions for 39,565 individuals in the UK Biobank (UKB; 64.43 ± 7.69 mean age, 52% female) and 772 individuals in Human Connectome Project (HCP; 28.97 ± 3.58, 52% female). We then associated the subject-level, regional gr-expression in these data with corresponding regional measures of structure and activity. We finally integrated these results with electronic health records (EHRs) from BioVU, the Vanderbilt University clinical biobank.
We identified 96 unique genes associated with regional volumes in the UKB cohort ($p \leq 0.05$, FDR) [fig. 1A]. We validated these associations through direct comparison with results from genome-wide association studies. This comparison showed strong correlations between the two approaches ($r = 0.67$, $p < 0.001$), but considerably higher absolute effect sizes in the transcriptomic associations [fig. 1B]. Separately, we leveraged the high-quality functional MRI data in the HCP cohort to discover 321 unique genes associated with regional homogeneity ($p \leq 0.05$, nominal) [fig. 1C]. Finally, we linked all our discovered genes with multiple neurological and psychiatric phenotypes in BioVU [fig. 1D]. Collectively, our integrative analysis establishes a new direction for transcriptomic discovery of human brain individuality.

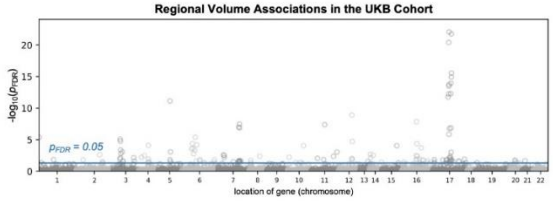


**Fig. 1A:** Manhattan plot depicting gene associations with regional volumes in the UKB cohort. Points represent associations and the y-axis refers to significance, with the blue line marking $p_{FDR}$ = 0.05.

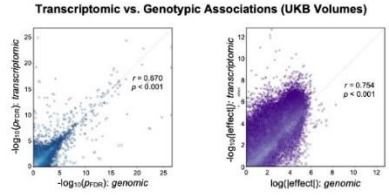


**Fig. 1B:** Scatter plots comparing regional volume associations with genes (transcriptomic) and genetic variants (genotypic) in the UKB. Left plot shows association significance and right plot shows absolute effect size. In both plots, lighter colors and larger circles denote higher variant weights in the corresponding gene's gr-expression inference model.

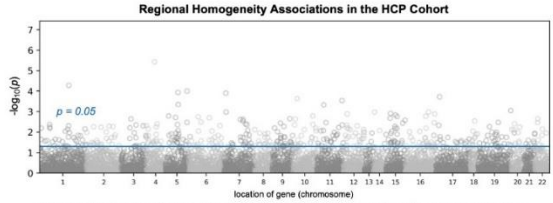


**Fig. 1C:** Manhattan plot depicting gene associations with regional homogeneity in the HCP cohort. Points represent associations and the y-axis refers to significance, with the blue line marking $p$ = 0.05 (nominal).

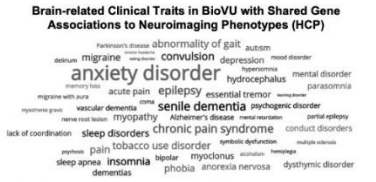


**Fig. 1D:** Word bubble showing BioVU clinical traits associated with genes whose gr-expression was linked to neuroimaging phenotypes in the HCP cohort. Size of the individual words reflects the relative frequency in the number of shared genes between the corresponding traits and neuroimaging phenotypes.

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**Presentation Number:** NANO10.08

**Topic:** I.02. Systems Biology and Bioinformatics

**Support:** NIH RF1MH125933

**Title:** Local-global neuroimaging analysis associates genetically regulated gene expression with brain network phenotypes

**Authors: *N. SARDARIPOUR**[1], N. HOANG[2], K. SCHILLING[3], M. BENTON[4], J. CAPRA[5], M. RUBINOV[1];
[1]Biomed. Engin., [2]Computer Sci., [3]Dept. of Biomed. Engin., Vanderbilt Univ., Nashville, TN; [4]Computer Sci., Baylor Univ., Waco, TX; [5]Epidemiology & Biostatistics, Univ. of California, San Francisco, San Francisco, CA

**Abstract:** Human brain networks facilitate sensory, motor, and cognitive function. The organization of these networks is largely heritable, but the specific genes that underpin their formation and plasticity remain elusive. Here, we built a machine-learning model to discover these genes through integration of neuroimaging, genomic, and transcriptomic datasets.
We first defined brain networks using an established network template and, separately, using independent-component analysis of resting-state fMRI data. We then combined regularized regression with 5-fold cross-validation to predict the average correlation of these networks using structural, diffusion, and functional phenotypes of 10 cortical and subcortical regions. We finally leveraged joint-tissue transcriptome analysis to infer the genetically regulated gene expression across these regions and integrated these results to associate regional gene expression with brain-network phenotypes in UK Biobank and Human Connectome Project datasets. Separately, we validated our findings on directly assayed gene expression data from the Allen Human Brain Atlas (Fig. 1A).

Our model predicted network correlations from regional phenotypes with considerable accuracy (r mean ± SD : 0.56 ± 0.08 for the test sets). We used this model to find significant links ($p<0.01$) between network correlations and genetically regulated gene expression that associates with the volume of the putamen. Specifically, we linked a group of genes (e.g., *SLC5A9*, *ALLC* and *CLEC12B*) with the default and dorsal attention networks. We linked another group of genes, (e.g., *STAP1*, and *PROZ*) with control and limbic networks. Separately, we found that these genes showed increased (>3 SD) expression in corresponding regions in the Allen Human Brain Atlas data.
Our integrative analysis enables the discovery of the transcriptomic basis of variation in brain network organization. Future studies can leverage these analyses to study alterations of this network organization across healthy and diseased states.

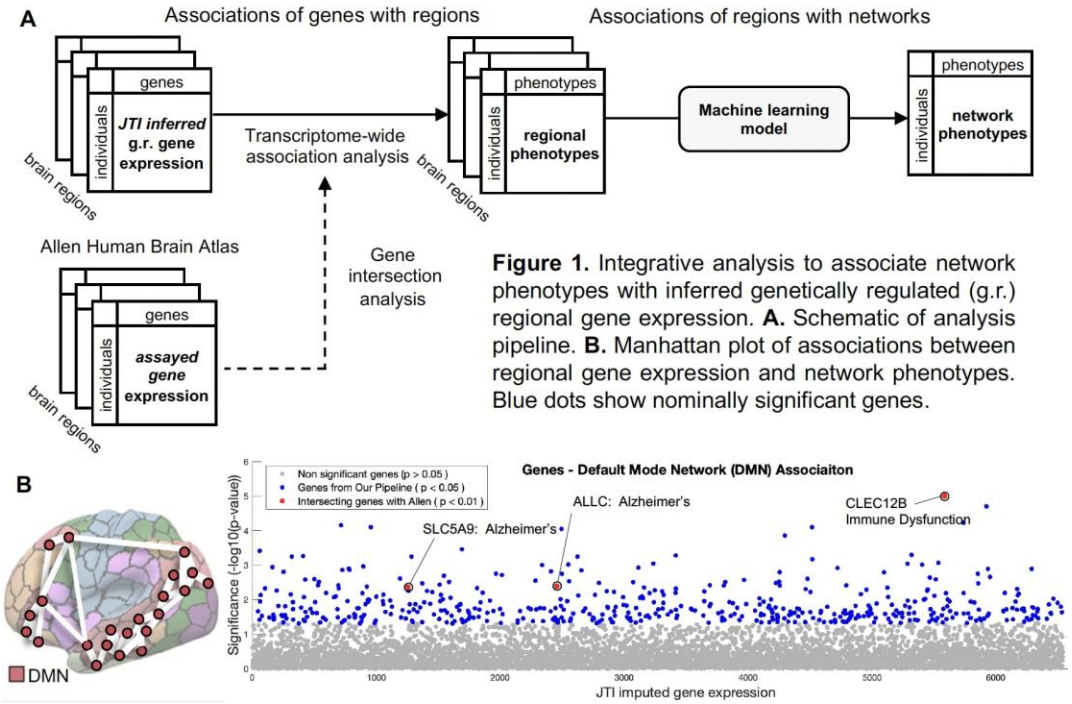


**Figure 1.** Integrative analysis to associate network phenotypes with inferred genetically regulated (g.r.) regional gene expression. **A.** Schematic of analysis pipeline. **B.** Manhattan plot of associations between regional gene expression and network phenotypes. Blue dots show nominally significant genes.

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**Presentation Number:** NANO10.09

**Topic:** I.02. Systems Biology and Bioinformatics

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**Title:** Precise mapping of Allen Human Brain Atlas sample coordinates to MNI space

**Authors: *Y. YEE**[1,2], G. A. DEVENYI[2,1], Y. ZEIGHAMI[1,2], M. CHAKRAVARTY[1,2];
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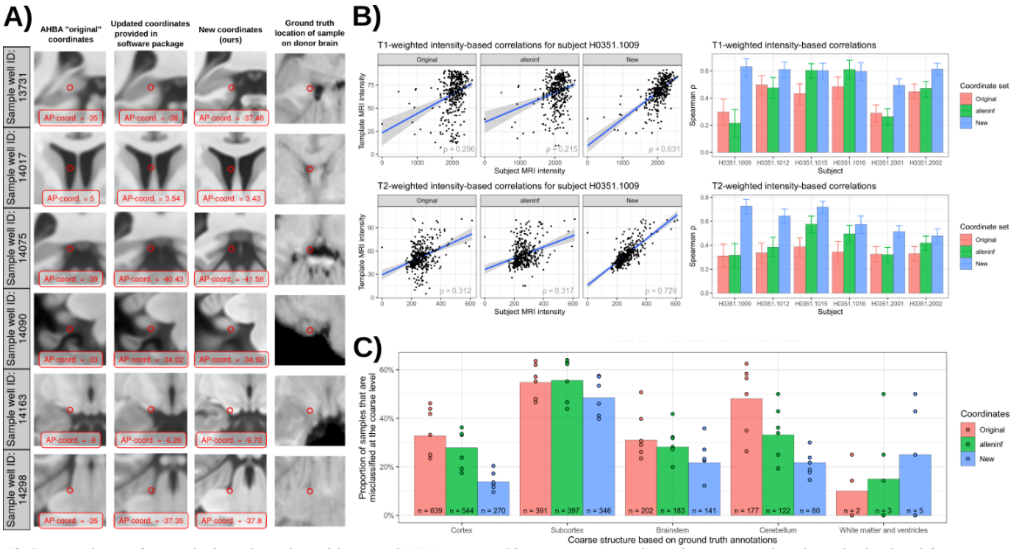
**Abstract:** The Allen Human Brain Atlas (AHBA) consists of microarray-based gene expression data (29131 genes), sampled from tissue at 3702 points across six donor brains. The AHBA has been extensively used to study the transcriptomic architecture of healthy and diseased brains, and its wide use in neuroimaging follows from tissue sample coordinates being reported in Montreal Neurological Institute (MNI) space, the most common coordinate framework in human neuroimaging. Sample MNI coordinates were originally obtained by determining tissue sample locations within donor brains scanned using magnetic resonance imaging (MRI), and subsequently warping these donor brain images (and associated tissue sample coordinates) to an MNI space brain template. An updated set of coordinates are also provided within the alleninf software package through an alternate set of image alignments. Both versions are widely used in neuroimaging.

Here, we show that there is substantial error within these two previously-reported sets of coordinates. We derive an alternate set of new MNI coordinates for AHBA tissue samples by careful alignment of donor brain images to a newer MNI space template (ICBM152 nonlinear 2009c symmetric) using Advanced Normalization Tools. Importantly, our procedure aligns images by simultaneously considering T1-weighted and T2-weighted MRI contrast, resulting in a more precise set of tissue sample coordinates relative to the reference brain. We validate that our MNI coordinates are more representative of true locations within donor brains by visual inspection (Fig. A), spatial correlation of donor MRI and template intensities (Fig. B), and comparison of structure assignment (based on coordinate location) to expert annotation at time of tissue dissection (Fig. C). Together, these results suggest that previously-reported coordinates of AHBA tissue samples are imprecise, may provide incorrect information regarding neuroanatomical location of samples, and that the new coordinates are a major improvement. We make these new coordinates openly available to the neuroscience community.




**A)** Comparison of sample locations in subject and MNI space. Shown are 4 random tissue samples (rows) obtained from one donor brain. Samples are plotted in MNI space using coordinates provided by each of the three coordinate sets (original, alleninf, ours; first three columns). The final column displays samples in relation to the subject brain image (which we take as ground truth). **B)** Spearman correlations between the image intensities of subject scans and template images at reported sample locations for one donor (left). Each point is a sample; higher (Spearman) correlations imply stronger monotonic relationships that points of similar intensities are aligned together, and therefore better image alignment quality. Correlations are also shown for all other donors (right); error bars denote the 95% range via bootstrapping. **C)** mislabeling of coordinates when overlaying a neuroanatomical atlas in MNI space and comparing to expert annotations, as quantified as the false negative rate (FNR). FNR is lowest for the new coordinates in all major structures except "white matter and ventricles", which truly consist of vastly fewer samples compared to the other four major structures. Each point is a subject, and bars show FNR pooled across all subjects.

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**Topic:** A.08. Development of Neural Systems

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**Title:** High-throughput spatial mapping of clonal lineages in mouse brain.

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**Abstract:** Delineating the spatially resolved single-cell lineages in the mammalian nervous system has been a fundamental interest in developmental neuroscience. Traditionally, sparse labeling and whole brain imaging have been applied to map individual clones in the brain. However, such experiments are extremely low-throughput and require a large amount of labor and time. Moreover, the precise cell identity cannot be determined as only a few antibodies or probes can be used when performing immunohistochemistry staining. Single-cell RNA sequencing (scRNA-seq) enables simultaneous acquisition of lineages and transcriptomes of individual cells when combined with the various cell barcoding techniques, which allow unique labeling of progenitor cells with highly diverse DNA sequences. However, the spatial information is lost during sample preparation and clonal information are incomplete as only partial cells can be captured during dissociation and scRNA-seq. Recently, spatial transcriptome techniques have been combined with viral barcoding to delineate the lineages with transcriptome and spatial information in the mouse forebrain. A major caveat of viral barcoding is that it can only label partial lineages due to the limited accessibility of barcoded virus to the target progenitor cell population. The infection of virus in early developing brain is also a technical challenge. We have recently developed an unbiased and universal tool, CREST (CRISPR editing-based lineage specific tracing), to allow single-cell lineage tracing in any cell population of interest. CREST also holds the potential for spatially resolved lineage tracing. In this work, we combined CREST with 10X Visium spatial transcriptome technique to simultaneously acquire the lineage, transcriptome, and spatial information in the embryonic mouse midbrain with high

throughput. We found that (1) transcriptionally defined midbrain subregions differ from those defined anatomically; (2) spatial transcriptome reveals a distribution map of various cell types in the embryonic mouse midbrain; (3) CREST barcodes can be retrieved from the spatial transcriptome datasets for spatial lineage analysis; (4) the lineages between neighboring midbrain subregions are highly correlated; (5) individual midbrain clones showed restricted and graded distribution along the dorsal-ventral axis. Together, we have delineated a spatially resolved transcriptome and lineage map in the embryonic mouse midbrain. The results further demonstrate the great power of our CREST method in deciphering the clonal lineage landscape of developing brains when combined with the spatial transcriptome techniques.

**Disclosures: L. Xie:** None. **H. Liu:** None. **X. Zhang:** None. **W. Wei:** None. **Y. Chen:** None.

**Nanosymposium**

**NANO11: Neural Circuit Development and Plasticity: Novel Mechanisms and Approaches**

**Location:** WCC 147B

**Time:** Sunday, November 12, 2023, 8:00 AM - 11:15 AM

**Presentation Number:** NANO11.01

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** 1ZIANS003140-08

**Title:** Trio and CRMP2 suppress axon branching

**Authors: *E. FINGLETON**[1], K. W. ROCHE[2], A. LOMBARDO[4], Y. LI[3];
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**Abstract:** Trio is a neuronal Rho Guanosine nucleotide Exchange Factor (GEF) that mediates aspects of axon patterning, a critical neurodevelopmental process through which axons elongate, branch, and are guided to innervate their correct anatomical targets. Neuronal knock-out of TRIO in mice results in axon guidance defects, and in humans, mutations in Trio's GEF domains are associated with profound neurodevelopmental disorders. To identify downstream signaling pathways, we used co-immunoprecipitation (co-IP) and mass spectrometry to identify Trio's interactome. Using this approach, we identified Collapsin Response Mediator Protein 2 (CRMP2), a well-known regulator of axon guidance, as a Trio interactor. Through co-IP and western blotting, we observed that Trio preferentially interacts with phosphorylated CRMP2 (pCRMP2), which mediates growth cone repulsion and branch suppression, whereas non-phosphorylated CRMP2 promotes axon elongation and branching. To assess whether Trio suppresses axon branching downstream of pCRMP2, we expressed phospho-null (S522A) and phosphomimetic (S522D) CRMP2 and knocked-down Trio. We find that Trio is required for CRMP2 S522D-mediated branch suppression and not for CRMP2 S522A-mediated branching, consistent with our findings that Trio preferentially interacts with pCRMP2. When co-expressed in COS7 cells, Trio and CRMP2 S522D colocalize. Our working model is that pCRMP2 localizes Trio to F-actin and activates Trio's GEF function to suppress actin polymerization.

Conversely, in the absence of Trio, pCRMP2 localizes to F-actin and catalyzes actin polymerization, which leads to filopodia extension and axon branching. Future investigation of the role of Trio and CRMP2 in axon patterning will both contribute to our understanding of how extracellular signals are converted into cytoskeletal phenotypes and may yield new targets for clinical intervention for individuals affected by pathogenic TRIO variants. This work is funded by the NIH Intramural Research Program under grant 1ZIANS003140-08

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**Presentation Number:** NANO11.02

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Title:** Identification of novel cis interaction between trans-synaptic protein kinase EphB2 and ligand ephrin-B3 and examining its role in synaptic target selection

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**Abstract:** Synaptic target selection is a critical process during development, is essential for the establishment of specific synaptic connections between neurons and is required for proper circuit formation. One important mechanism governing synaptic target recognition is transsynaptic signaling by the receptor tyrosine kinase EphB2, which also regulates the motility of dendritic filopodia and synapse formation. During this process, EphB2 is localized to the tips of filopodia where it acts as a decision-maker to determine whether a contact stabilizes or retracts. This decision is governed by the rate of EphB2 kinase activation, where fast signaling (<1 min rise) leads to retraction, while slower rates of activation (>5 min) result in stabilization.
Here we test the hypothesis that the ability of EphB2 to regulate filopodia motility and accept or reject putative synaptic contacts is due to a *cis* interaction with postsynaptic ephrin-B3. Using a combination of biochemistry, immunocytochemistry and proximity ligation assays (PLA) we demonstrate that ephrin-B3 interacts with EphB2 at a single amino acid residue located within the fibronectin type III (FNIII) domain. Disruption of the *cis* interaction in rat cortical neurons by expressing mutant EphB2 proteins that cannot interact with ephrin-B3 in *cis* significantly increases filopodia motility. To then test whether inducing the EphB2-ephrin-B3 interaction might decrease motility, we generated a photo-activatable EphB2 and ephrin-B3 using the CRY2-CIBN optogenetic system. Preliminary results suggest that light exposure of HEK-293T cells or neurons transfected with EphB2-CRY2 and ephrin-B3-CIBN induces clustering of EphB2 and ephrin-B3 in cis and results in reduced motility of photo-activated filopodia, suggesting that the *cis* interaction between EphB2 and ephrin-B3 regulates filopodial motility. EphB-dependent stabilization of filopodia relies on a slow rate of kinase activation (>5 min). To test whether the EphB2-ephrin-B3 interaction might reduce the rate of EphB2 activation, we measured EphB2 kinase kinetics with the GPhosEphB2 tyrosine kinase reporter. HEK-293T cells were co-transfected with GPhosEphB2, EphB2-CRY2 and ephrin-B3. Preliminary data suggests that photoactivation of EphB2-CRY2 in cells co-expressing ephrin-B3 may have a lower rate of kinase activation than when ephrin-B3 is not co-expressed. All together, these data suggest a model where FNIII-mediated EphB2-ephrinB3 *cis*-interaction can modulate EphB2 kinase signaling to generate different functional outcomes in cellular behavior.

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**Title:** Rac1 and Nectin3 are effectors of planar cell polarity signaling essential for spiral ganglion neuron afferent pathfinding in the peripheral auditory system

**Authors: *S. CLANCY**, A. HOGAN, Y. ZHENG, M. SMITH, E. FATEH, T. ELUVATHINGAL MUTTIKKAL, X. LU;
Cell Biol., Univ. of Virgina, Charlottesville, VA

**Abstract:** Our sense of hearing is critically dependent on the formation of functional neural circuits. The spiral ganglion neurons (SGNs) extend axons that must navigate complex tissue environments, integrating chemical and mechanical guidance cues to reach their targets. The SGNs connect the sound receptors in the organ of Corti (OC) to the cochlear nuclei of the hindbrain. Type I SGNs innervate inner hair cells (IHCs) to transmit sound signals, while type II SGNs (SGNIIs) innervate outer hair cells (OHCs) to detect acoustic trauma. Despite their essential functions in hearing, our understanding of the molecular mechanisms that mediate wiring of the auditory periphery is still fragmentary. It has been shown recently that guidance of SGNII peripheral projections is regulated by the Planar Cell Polarity (PCP) pathway. Intercellular PCP signaling mediates polarized cell behaviors within the plane of a tissue in a plethora of developmental processes. In the wild-type OC, SGNII afferents make a characteristic 90-degree turn toward the base of the cochlea and innervate multiple OHCs. In several PCP mutants, SGNII afferents turn randomly towards either the cochlear base or the apex. Although it has been shown that PCP proteins localize asymmetrically to supporting cell (SC)-SC junctions and act in the cochlear epithelium to guide SGNII afferents, the underlying mechanisms are currently unknown. We hypothesize that PCP signaling regulates multiple downstream effectors including adhesion molecules and Rho GTPases to influence cell adhesion and the cytoskeleton in SCs, which serve as intermediate targets of SGNII afferents. We have found that core PCP gene Vangl2 regulates localization of the active form of the small GTPase Rac1 and the cell adhesion molecule Nectin3. Via conditional knockout mouse lines and immunofluorescence staining we found that the small GTPase Rac1, but not Rac3, is necessary for proper SGNII afferent innervation. However, the constitutive activity of Rac1 was insufficient to direct SGNII afferents when PCP signaling was disrupted. Additionally, we generated two *Nectin3* knockout mouse lines via CRISPR-Cas9 and have characterized an SGNII afferent misturning phenotype in both mutant lines. Together these experiments indicate that Nectin3 and Rac1 are both necessary for proper SGNII afferent innervation, and continuing work will further investigate the relationship of these genes to the PCP pathway and the mechanisms through which they affect SGNII afferent guidance.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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**Title:** Investigating the role of the F-BAR proteins CIP4 and FBP17 in regulating neurite dynamics during cortical neuron migration

**Authors: *L. A. ENGLISH**[1], R. J. TAYLOR[2], C. CAMERON[2], E. W. DENT[2];
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**Abstract:** Neurite initiation from newly born neurons is a critical step in neuronal differentiation and migration. Additionally, neuronal migration in the developing cortex is accompanied by dynamic extension and retraction of neurites as neurons progress through bipolar and multipolar states. However, there is a relative lack of understanding regarding how the dynamic extension and retraction of neurites is regulated during neuronal migration. In recent work, we have shown that CIP4, a member of the F-BAR family of membrane-bending proteins, inhibits cortical neurite formation in culture, while family member FBP17 induces premature neurite outgrowth. CIP4 overexpression results in protrusion of lamellipodia-like veils, which inhibit filopodia formation and neurite initiation, while knockdown of CIP4 may inhibit neurite retraction. These results beg the question of how CIP4 and FBP17 function in radial neuron migration and differentiation *in vivo*, including the timing and manner of neurite repression. To examine the effects of modulating expression of CIP4 and FBP17 *in vivo*, we used *in utero* electroporation, in combination with our published Double UP technique, to allow for comparison of experimental (knockdown or overexpression) and control cells within the same tissue. We show that either knockdown or overexpression of CIP4 and FBP17 results in the marked disruption of radial neuron migration by modulating neurite outgrowth. Additionally, we show that these disruptions in migration are sustained into maturity and result in dramatic changes in axon outgrowth and targeting. Further experiments will determine the mechanism CIP4 and FBP17 utilize to inhibit and promote neurite initiation respectively. Together these results provide insight into the function of F-BAR proteins in cortical neuronal migration and axon outgrowth in the developing brain.

**Disclosures: L.A. English:** None. **R.J. Taylor:** None. **C. Cameron:** None. **E.W. Dent:** None.

**Presentation Number:** NANO11.05

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH Grant NS089578
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**Title:** Loss of the polarity protein Par3 promotes dendritic spine neoteny and enhances learning and memory

**Authors: M. VOGLEWEDE**[1], E. N. OZSEN[1], N. IVAK[1], M. BERNABUCCI[2], M. SUN[1], Z. P. PANG[4], *H. ZHANG[3];
[1]Neurosci. and Cell Biol., Rutgers University, Robert Wood Johnson Med. Sch., Piscataway, NJ; [2]Child Hlth. Inst. of New Jersey, Rutgers Univ., New Brunswick, NJ; [3]Rutgers Univ., Piscataway, NJ; [4]Neurosci. and Cell Biol., Child Hlth. Inst. of NJ, Rutgers-Rwjms, New Brunswick, NJ

**Abstract:** Partitioning defective 3 (Par3) is critical for subcellular compartmentalization in different developmental processes. In addition, several single nucleotide polymorphisms (SNPs) and copy number variation (CNV) of *Pard3*, which encodes Par3, are associated with intelligence, schizophrenia, and autism spectrum disorder (ASD). However, the role of Par3 in glutamatergic synapse formation and cognitive functions *in vivo* remains completely unknown. Here we show that postnatal forebrain conditional knockout of Par3 leads to an increase in the density of dendritic spines, which are the main sites of glutamatergic synapse formation, in hippocampal CA1 pyramidal neurons *in vivo*. Specifically, loss of Par3 leads to an increase in long, thin dendritic spines without significantly impacting mushroom spine formation. Consistent with the morphological changes, we observed a decrease in the amplitude of miniature excitatory postsynaptic currents (mEPSC). Surprisingly, we found loss of Par3 *in vivo* enhances hippocampal-dependent spatial learning. Phosphoproteomic analysis revealed that proteins regulating cytoskeletal dynamics are significantly dysregulated downstream of Par3. Mechanistically, we found loss of Par3 causes increased activation of the Rac1 GTPase pathway, which is a key regulator of cytoskeletal dynamics. Together, our data reveal an unexpected role for Par3 in limiting the pool of dendritic spine with immature morphology and negatively regulating learning and memory *in vivo*.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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NIH R01- 5R01NS113314-04

**Title:** Role of ErbB-receptor signaling in the formation and function of invadosomes in pioneering sensory and motor neurons

**Authors: *T. LOUGHERY**, C. WARLICK-SHORT, R. NAGARIMADUGU, E. KEEFNER, T. DAVIG HUESMANN, T. GOMEZ;
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**Abstract:** In the developing central nervous system (CNS), axons must navigate to their correct targets to form functional circuits. While most CNS axons remain within the CNS throughout their development, axons of pioneering sensory and motor neurons must exit the CNS and traverse diverse tissue environments to reach their peripheral targets. Sensory motile structures at the tips of growing axons, called growth cones, respond to environmental cues and guide axon outgrowth. Our lab showed that growth cones form orthogonal filopodia-like protrusions from

their apical and basal surfaces and along axons that may facilitate guidance across tissue barriers within a 3-dimensional *in vivo* environment. These F-actin rich protrusions, known as invadosomes, were first characterized in metastatic cancer and immune cells. Through the recruitment and release of matrix metalloproteases (MMPs), initiated by various actin-binding and scaffolding proteins, invadosomes remodel the extracellular matrix (ECM) allowing these invasive cells to penetrate surrounding tissue, including the dense basal lamina surrounding the spinal cord. In cancer cells, several growth factors have been shown to induce invadosome formation and maturation, but it is unknown what growth factors serve this role in developing axons. Here we report that epidermal growth factor (EGF) receptor ligands (EGF and neuregulin1) induce invadosome formation in spinal neurons from *Xenopus laevis* via kinase and phospholipid signaling. Several *in vitro* assays have confirmed the activation of downstream signals including F-actin remodeling, Src and cortactin activation, as well as increased gelatin degradation indicative of increased MMP activity in response to EGF stimulation. Using HCR RNA-FISH, immunohistochemistry, and whole-mount confocal imaging, we are characterizing EGF receptor expression in the spinal cord during developmental stages of axon exiting. We are also conducting both gain and loss of function studies. Interestingly, over-expression of ErbB4-GFP, a member of the EGF receptor family, promotes cell autonomous ectopic exiting of spinal neuron cell bodies. Ongoing CRISPR/Cas9 gene editing experiments will further examine which EGF receptors are necessary for sensory and motor neuron invadosome formation and peripheral axon exiting. Understanding how specific neuron sub-types project their axons across basal lamina barriers to enter or exit tissues is crucial for facilitating therapeutic regeneration following injury or illness-related axonal loss.

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**Title:** Extracellular vesicles derived from sympathetic neurons promote survival of cultured spinal cord neurons

**Authors: *J. SEWELL**, A. MASON, A. KEELER, B. WINCKLER, C. DEPPMANN; Univ. of Virgina, Charlottesville, VA

**Abstract:** In the developing nervous system, neurons are overproduced and subsequently pared down through cell death to ensure the proper connections are supported and stabilized. In the developing sympathetic circuit of the autonomic nervous system, this process is regulated by limiting amounts of nerve growth factor (NGF) secreted by a target organ (e.g. eye or salivary gland) and binds its receptor TrkA at the distal axon of innervating sympathetic neurons. Thus, sympathetic neuron survival is set by the level of NGF-TrkA signaling. Sympathetic neurons are innervated by cholinergic neurons from the spinal cord. Survival and proper development of this

neuron population is also NGF-dependent, despite lacking TrkA or access to soluble NGF. How these neurons are developmentally regulated without access to soluble NGF is unknown. We hypothesize that TrkA-positive extracellular vesicles (EVs) secreted from sympathetic neurons provide this neurotrophic support.
Using cultured sympathetic neurons from mouse superior cervical ganglia, we isolated EVs from serum-free, conditioned media by differential centrifugation and characterized them using nanoparticle tracking analysis, immunoblot assays, and cryo-electron microscopy. The sizing distribution of isolated EVs show a predominant peak at 45-75nm with a shoulder of larger sizes, while protein analysis shows an enrichment of EV markers (Alix, CD63, CD81) and absence of intracellular markers (Cytochrome C, calreticulin). We identify TrkA as an EV cargo, which can be present in its active, phosphorylated state. Using a compartmentalized culture system, we show that TrkA originating from distal axons can be packaged in EVs secreted from the somatodendritic domain. To assess neurotrophic capacity, mouse spinal cord neurons from choline acetyltransferase (ChAT)-GFP mice were cultured with sympathetic EVs and cell death in ChAT-GFP-positive neurons was assessed by Annexin V staining. Indeed, sympathetic EVs promote ChAT-GFP-positive neuron survival, suggesting they provide neurotrophic support.
In summary, we have rigorously characterized EVs from sympathetic cultures and shown that retrogradely trafficked and phosphorylated TrkA can be a cargo. These EVs are capable of supporting recipient cholinergic neuron survival, suggesting a novel mechanism in sympathetic circuit development.

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**Title:** Visualization of liquid-liquid phase separation of postsynaptic proteins at the nanoscale in living neurons

**Authors: *R. E. CAIN**[1], M. HRUSKA[2], M. B. DALVA[3];
[1]Thomas Jefferson Univ. Grad. Neurosci. Program, Philadelphia, PA; [2]Dept. of Neurosci., West Virginia Univ., Morgantown, WV; [3]Thomas Jefferson Univ., Philadelphia, PA

**Abstract:** Super resolution imaging, including Stimulated Emission Depletion (STED) microscopy, has revealed that synapses contain discrete nanoscale clusters of pre- and post-synaptic proteins that scale in number with spine size, termed nanodomains or nanomodules. It is unknown how these nanomodules are formed or regulated. Liquid-liquid phase separation (LLPS) is an attractive mechanism for organizing synaptic components due to its ability to segregate proteins without membrane barriers. Cell-free assays have illustrated the ability for

synaptic proteins such as PSD95 and glutamate receptor subunits to undergo LLPS. However, it is unknown whether these proteins are in LLPS in living neuronal synapses. Given the relationship between nanomodules and synaptic plasticity and function, we tested whether LLPS may serve as a mechanism underlying the compartmentalization of these key nanoscale structures. To test this, we developed an approach to visualize endogenous synaptic proteins on the nanoscale in dendritic spines in live neurons using high-speed tau-STED (τSTED) imaging and CRISPR Cas9. Overexpression of proteins can alter their function and impact synaptic organization; therefore, to visualize endogenous synaptic proteins we optimized a CRISPR knock-in library, an Open Resource for the Application of Neuronal Genome Editing (ORANGE), to tag proteins with EGFP with 20-40% knock-in efficiency. To visualize endogenously tagged proteins, we use τSTED, which improves the temporal and spatial resolution of conventional STED while also reducing photo bleaching and toxicity. Combining fast τSTED live-cell imaging and endogenously tagged PSD95 and glutamate receptors, we tested whether nanomodules are in LLPS in living neurons. Using an aliphatic alcohol, 1-6 Hexenadiol, known to disrupt LLPS in living cells, we tested whether nanoscale synaptic clusters in dendritic spines dissolve in response to LLPS disruption using live-cell τSTED. Our results suggest that nanomodules of specific postsynaptic proteins may be in LLPS and this state may change as synapses mature. Interestingly, the response to LLPS disruption varies between synaptic proteins, suggesting LLPS may play differential roles in organization of proteins at the nanoscale. These results provide a demonstration of the nanoscale impact of LLPS and provide insights into the role of LLPS during synapse development.

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**Title:** Brain-wide mapping of fear memory circuits throughout development

**Authors: *B. JIN**[1], L. A. DENARDO[2];
[2]UCLA, [1]UCLA, Los Angeles, CA

**Abstract:** Infantile amnesia describes a process wherein episodic memories formed early in life are rapidly forgotten. On the other hand, memories formed in older animals can last a lifetime. Although the neural substrates of memory have been well studied in adults, how memories are stored and retrieved during development remains poorly understood. Here, we performed a brain-wide screen to identify developmental changes in memory networks. We used TRAP2 mice (Targeted Recombination in Active Populations) in combination with brain clearing and light sheet fluorescence microscopy to compare neuronal populations and networks activated by recent (1 day) fear memory retrieval at infant (P17), juvenile (P25) or adult (P60) stages. While adults had more activated neurons in the prefrontal cortex, and anterior thalamic nuclei, juveniles had more activated cells in the hypothalamus and midbrain during fear memory retrieval.

Network analyses revealed that the functional organization of memory networks was also developmentally regulated. Adult memory networks included a highly interconnected set of cortical regions. Of these, the retrosplenial cortex (RSP), a key memory center, was absent from juvenile networks. To further understand how activity in these regions related to memory retrieval, we examined correlations between TRAPed cell numbers and freezing levels of individual animals. Regions that were highly correlated with freezing differed by age. Notably, RSP became increasingly salient for freezing with developmental age. To further examine how RSP memory functions change with age, we looked at the extent to which neurons activated during recent memory retrieval (1d) are reactivated during later memory retrieval (7d) at different ages. Adults had higher reactivation rates in RSP compared to younger groups. To determine whether we could enhance memory retrieval, we injected cre-dependent excitatory DREADDs to label and manipulate TRAPed RSC ensembles between 1d and 7d retrieval sessions. We found an age-dependent effect: chemogenetic reactivation increased freezing in adults, but not in infants, and had an intermediate effect in P25 mice. Together these data reveal specific changes in the activity of brain regions that coincide with the developmental transition from amnesic to persistent memories. RSP, which has not been studied in the context of infantile amnesia, may be key for this switch. In ongoing work, we are tracing developing RSC connections to identify circuit changes that could underlie RSP's role in the maturation of memory persistence across development.

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**Presentation Number:** NANO11.10

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** ISF 1384/21
ARI

**Title:** An ultrasensitive biosensor for in vivo imaging of cell-specific PTEN dynamics during cortical development

**Authors: *T. LAVIV**, T. KAGAN, M. GABAY, A. NITZAN, M. GAL, R. ZAIDEL-BAR; Tel Aviv Univ., Tel Aviv, Israel

**Abstract:** Autism Spectrum Disorders (ASD) are closely associated with genes that regulate synaptic structure and function during early brain development. One prominent example is the well-characterized tumor suppressor gene, Phosphatase and Tensin homolog (PTEN). Germline mutations in PTEN are frequent in individuals diagnosed with ASD and macrocephaly. Current methodologies to study PTEN, mainly genetic manipulations and biochemistry, do not allow to monitor PTEN activity dynamics during cortical development. To monitor the activity state of PTEN in living cells, we have developed a novel FRET based PTEN sensor, optimized for two-photon fluorescent lifetime imaging (2pFLIM). Our biosensor is composed of PTEN flanked by a FRET donor and acceptor, allowing dynamic monitoring of changes in PTEN conformation as a proxy of its activation. We have introduced a point mutation in the biosensor that suppresses exogenous PTEN activity, limiting its effect on endogenous PTEN signaling. We further show that our approach allows sensitive detection of abnormal PTEN conformation due to multiple known pathogenic point mutations. We developed spectral variants of the PTEN sensors, for

simultaneous imaging of PTEN in multiple cell types. We are currently using this approach to monitor in vivo PTEN activity in excitatory and inhibitory neurons, as well as astrocytes during the first weeks of mouse cortical development, unraveling both cell-type specificity and temporal dynamics. Overall, our novel experimental approach will be fundamental to unravel the role of PTEN dynamics during development and shed light on the mechanisms leading to neuronal dysfunction in ASD.

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**Presentation Number:** NANO11.11

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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**Title:** Spatiotemporal mapping and molecular basis of brain circuit maturation

**Authors: *J. XUE**[1], J. R. THOMPSON[1], A. T. BRAWNER[1], T. D. YELHEKAR[1], K. T. NEWMASTER[2], Q. QIU[3], Y. A. COOPER[4], R. YU[5], Y. H. AHMED-BRAIMAH[6], Y. KIM[7], Y. LIN[1];
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**Abstract:** Brain development is highly dynamic and asynchronous, marked by the sequential maturation of functional circuits across the brain. This process is highly influenced by neuronal activity. However, the timing and mechanisms driving circuit maturation remain elusive due to an inability to identify and map neuronal populations as they mature. Importantly, circuit maturation is believed to be impaired in many neurodevelopmental disorders (NDDs), but it has been challenging to pinpoint when and where circuit maturation is affected by the genetic and environmental factors in NDDs. To meet these challenges, we have created a reporter system, DevATLAS (Developmental Activation Timing-based Longitudinal Acquisition System), which is based on the activation of an immediate early gene (IEG), *Npas4*, to capture neurons undergoing activity-dependent circuit maturation. This is because Npas4 is known to be selectively and robustly induced by neuronal activity and functionally important for activity-dependent synaptic development. Neurons activated to express Npas4 during early development, to initiate activity-dependent synaptic development, are permanently labeled by tdTomato. With our newly established whole-brain imaging and analysis pipeline, we have constructed the first longitudinal, spatiotemporal map of whole-brain circuit maturation. Using this resource, we have

uncovered dramatic impairments in the deep cortical layers in an autism mouse model, demonstrating how DevATLAS can be used to pinpoint when and where circuit maturation is disrupted in NDDs. We also revealed that the early experiences (via environmental enrichment) accelerate the development of hippocampus-dependent learning by increasing the synaptically mature population in the dentate gyrus. Finally, DevATLAS has facilitated the discovery of converging molecular mechanisms driving activity-dependent circuit maturation across different brain regions and cell types. In summary, DevATLAS will have a wide impact on the way we study neural circuit maturation during early brain development as it: (1) provides a systematic blueprint of whole-brain circuit development; (2) aids in the identification of disease etiologies has proven to be particularly powerful to identify targeting regions; (3) facilitates the study of genetic pathways impacted by developmental interventions and address fundamental questions, such as the circuit maturation within the context of manipulated critical pathways.

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**Presentation Number:** NANO11.12

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** ERC HOPE 951330
Fondation Roger de Spoelberch

**Title:** Emergent activity patterns in the developing mouse somatosensory cortex

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**Abstract:** Synchronous neuronal activity plays a pivotal role in the developing brain, building the architecture of cortical circuits, and thus coordinating the formation of functional sensory maps.In early developmental periods, neuronal firing undergoes substantial successive changes in its spatiotemporal features. Activity is initially mediated by asynchronous single-neuron firing in embryonic stages and switches to correlated activations by birth. In vitro studies support the hypothesis that early correlated activities are mediated by electrical synapses or gap-junctions and sequentially by chemical synapses. These changes occur during a critical time-window spanning the first postnatal week in the mouse.In order to understand how functional sensory circuits form during development, we focus on uncovering the activity-dependent mechanisms underlying network formation during the first postnatal week (P0-P7).To do so, we performed longitudinal in vivo two-photon calcium imaging of the developing barrel cortex in un-anesthetized Emx1-Cre GCaMP6s and Gad-Cre GCaMP6s mouse pups, so as to label excitatory and inhibitory neurons respectively.For Emx1-Cre GCaMP6s mice, we observe the emergence of patches of correlated neuronal activations already at birth (P0). The spatiotemporal characteristics of these patches change over the first postnatal days, as the circuit gets refined. While correlated activities could be observed already at P0 in Gad-Cre GCaMP6s mice, their spatial characteristics differed and patches of correlated activity only prevailed byP4.Based on studies that have demonstrated that clonal origin influences the development of functional

networks with clonally related neurons preferentially connecting, we are testing the implication of clonally related excitatory neurons in the immature functional circuit organization.

**Disclosures: S. Zangila:** None. **J. Majnik:** None. **R. Cossart:** None. **J. Platel:** None.

**Presentation Number:** NANO11.13

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH grant K99NS124993
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HHMI

**Title:** Activity-dependent structural and molecular maturation of touch sensory neurons

**Authors: *C. SANTIAGO**[1], N. SHARMA[2], N. AFRICAWALA[1], J. SIEGRIST[1], A. HANDLER[1], A. TASNIM[1], R. ANJUM[3], J. TURECEK[1], B. P. LEHNERT[1], S. RENAULD[1], A. MAGEE[1], S. PARADIS[4], D. D. GINTY[1];
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**Abstract:** Experience-dependent activity plays an essential role in the formation of the neural circuits that process sensory stimuli. Here, we explore a role for mechanically evoked activity in the maturation of the primary sensory neurons that detect light touch, the low threshold mechanoreceptors (LTMRs). Genetic deletion of the mechanically activated ion channel Piezo2 causes structural changes in mechanosensory end organs in peripheral tissues. These defects emerge over development, and are observed when Piezo2 is deleted from sensory neurons, and to a lesser extent from peripheral glial cells, depending on the end organ type. Piezo2 could be contributing to mechanosensory end organ formation through its role in generating mechanically evoked spiking, or through another mechanism. To manipulate neural activity by an alternate method, we genetically deleted the voltage-gated sodium channel $Na_V1.6$ from somatosensory neurons. Patterns of mechanically evoked activity and the structures of peripheral mechanosensory end organs are also disrupted in these mutants, although to a lesser extent than in the absence of Piezo2. Finally, single cell RNA sequencing of dorsal root ganglia (DRG) reveals changes in the molecular differentiation of LTMRs in *Piezo2* mutants, whereas the sensory neurons that detect temperature or noxious stimuli are largely unaffected. Together, these results suggest that Piezo2-dependent activity allows mechanosensory neurons to refine their distinct peripheral morphologies and gene expression profiles during development. Tactile experiences in early life may therefore influence somatosensory circuit formation by acting on the development of peripheral touch receptors.

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**Nanosymposium**

**NANO12: Neuropeptides, Cytokines, Growth Factors, and Other Signaling Molecules**

**Location:** WCC 152A

**Time:** Sunday, November 12, 2023, 8:00 AM - 10:00 AM

**Presentation Number:** NANO12.01

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

**Support:** NIH Grant NS114478 and NS107342

**Title:** Sympathetic NPY controls glucose homeostasis, cold tolerance, and cardiovascular functions in mice.

**Authors: *R. KUMARI**[1], R. PASCALAU[1], H. WANG[2], S. BAJPAYI[3], M. YURGEL[2], K. QUANSAH[1,3], S. HATTAR[2], E. TAMPAKAKIS[3], R. KURUVILLA[1];
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**Abstract:** Neuropeptide Y (NPY) is an abundant neuropeptide that is best known for its powerful effects in the brain as an orexigenic and anxiolytic agent and in reducing energy expenditure. However, NPY actions in peripheral sympathetic neurons where it is co-expressed with the classical neurotransmitter, Norepinephrine (NE), are under-appreciated. Here, we define the distribution of NPY-expressing neurons in discrete sympathetic ganglia, characterize projections of NPY-expressing sympathetic axons, and show that sympathetic-derived NPY is necessary for metabolic and cardiovascular functions in mice. We found that the NPY expression in noradrenergic sympathetic neurons is dependent on their location in the sympathetic chain, with only 43% of mature para-vertebral neurons co-expressing NPY and NE, compared to 93% of pre-vertebral neurons. Axons of NPY-expressing sympathetic neurons primarily innervate blood vessels in peripheral organs. Sympathetic-specific loss of NPY results in pronounced metabolic and cardiovascular defects in mice, including elevated circulating norepinephrine levels, reduced insulin secretion, attenuated glucose and cold tolerance, reduced pupil size, and elevated heart rate, while basal blood pressure was unaffected. Together, these findings provide new knowledge about tissue-specific functions of NPY and implicate peripheral NPY in the development of metabolic and cardiovascular diseases.

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**Presentation Number:** NANO12.02

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

**Support:** NIH IRP 1ZIANS003137

**Title:** Synaptotagmin beta regulates neuropeptide release and circadian output in Drosophila

**Authors: *H.-L. CHEN**, Y. CHEN, J. YIN, A. DERMADY, J. LEI, J. SHORT, C. LONG, Q. YUAN;
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**Abstract:** Neuropeptide signaling plays an important role in regulating a myriad of developmental, physiological, and behavioral functions throughout an animal's life cycle. Although much progress has been made in identifying the functional roles of a variety of neuropeptides, the molecular mechanisms controlling neuropeptide release remain largely elusive. The *Drosophila* lateral ventral neurons (LNvs) serve as a suitable system to study this fundamental question. LNvs produce pigment-dispersing factor (PDF), a neuropeptide well-known for its function in synchronizing of the central clock system and regulating the circadian output, such as the locomotor activity and sleep. Notably, despite the high level of *pdf* transcripts produced throughout the day, the amount of PDF peptides at the axonal terminal region of LNvs shows clear circadian oscillations, suggesting a mechanism that tightly controls the trafficking and release of PDF. Using cell-type-specific transcriptome analyses and genetic studies, we first examined a group of LNv enriched genes associated with vesicle trafficking and release. Our behavioral and anatomical data suggest that the *Drosophila* Synaptotagmin β (Sytβ), an atypical synaptotagmin enriched in peptidergic neurons, acts as a part of the control mechanism that regulates PDF release in LNvs. Similar as the *pdf* null mutants, *sytβ* knock out flies show specific circadian behavior deficits, as well as abnormal distribution of PDF peptides. In addition, using the transgenic expression of chimeric proteins of Syt1 and Sytβ, we demonstrate that the C2B domain of Sytβ mediates its inhibitory activity on PDF release and provide evidence for a role of disinhibition in the temporal control of neuropeptide release. Taken together, our genetic studies in the *Drosophila* system help us identify an inhibitory synaptotagmin regulating neuropeptide release. This finding is potentially generalizable to other circuits and organisms and could help improve our understanding of the fundamental mechanisms underlying neuromodulation of behaviors and brain states.

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**Presentation Number:** NANO12.03

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

**Support:** This work was funded by a TIFR intramural grant to V.A.V. and a SERB-POWER grant to S.F. and V.A.V.

**Title:** Withania somnifera evokes mitochondrial and neuroprotective effects in rat cortical neurons via the BDNF-SIRT1 axis

**Authors: *S. E. FANIBUNDA**[1,2,3], K. KUKKEMANE[2], U. GHAI[2], U. KOLTHUR[2], L. HINGORANI[4], A. D. B. VAIDYA[3], V. A. VAIDYA[2];
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Mumbai, India; [3]Kasturba Hlth. Society-MRC, Mumbai, India; [4]Pharmanza Herbal, Gujarat, India

**Abstract:** *Withania somnifera* (Ashwagandha) is a potent psychoactive plant used in Ayurveda as a neuroprotective agent, antidepressant and anxiolytic. We hypothesize that the pleiotropic and neuroprotective effects of *Withania somnifera* may involve the targeting of mitochondria. Mitochondria have emerged at the epicenter of contributing to adaptive mechanisms that promote neuronal viability and stress adaptation. Here we sought to address the direct effects of *Withania somnifera* and its constituents on mitochondrial biogenesis and function in rat cortical neurons in particular in the context of neuronal survival and stress coping. Using a hydroalcoholic root extract (RE) (1.5% withanolides and withanosides) of *Withania somnifera* and an enriched withanolide - withanoside rich fraction (WLS) (15% withanolides and withanosides), prepared at Pharmanza Herbal, India, we assessed for mitochondrial effects, in rat cortical neurons *in vitro* and in the neocortex of Sprague Dawley rats *in vivo.* WLS was found to enhance mitochondrial biogenesis as reflected by an increase in mitochondrial DNA levels and expression of mitochondrial components. RE and WLS enhanced mitochondrial function as evidenced by an increase in OxPhos efficiency and a consequential increase in cellular ATP levels. RE and WLS enhanced the expression of the trophic factor BDNF and its release, and enhanced BDNF signaling via increasing TrkB-mediated Akt signaling. SIRT1 expression was enhanced by RE and WLS, and abolished in the presence of inhibitors of TrkB signaling. Further, the mitochondrial effects of RE and WLS were abolished in the presence of inhibitors of BDNF signaling and SIRT1. These findings demonstrate that the BDNF-SIRT1 axis is critical to the mitochondrial effects of *Withania somnifera* (RE and WLS). WLS was found to protect against corticosterone-induced cell death, a neuroprotective effect that required BDNF and SIRT1. Withanolide A (WLA) and Withanoside IV (WSIV) were identified as the active constituents of the WLS fraction, enhancing mitochondrial biogenesis and function and protecting against corticosterone-induced death in a BDNF-SIRT1 dependent manner. These findings identify the RE, WLS extracts of *Withania somnifera* and their constituents WLA and WSIV as regulators of mitochondrial function and implicate their mitochondrial effects in facilitating stress adaptation via the BDNF-SIRT1 axis.

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**Presentation Number:** NANO12.04

**Topic:** B.04. Synaptic Transmission

**Support:** Presbyterian Health Foundation Team Science Grant
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**Title:** Synaptic defects in the hippocampus of a mouse model of very long chain-saturated fatty acid deficiency

**Authors:** R. YELAHANKA NAGARAJA[1], M.-P. AGBAGA[2], D. M. SHERRY[2], ***M. AHMAD**[3];
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**Abstract:** The enzyme Elongation of Very Long Fatty Acids-4 (ELOVL4) catalyzes the rate-limiting step in the biosynthesis of Very Long Chain Saturated and Polyunsaturated Fatty Acids (VLC-SFA and VLC-PUFA, respectively, ≥28 carbons). ELOVL4 and its VLC-FA products are critical to the function of the central nervous system. Several mutations in the human ELOVL4 gene that cause neurological and skin disorders have been identified. Homozygous inheritance of ELOVL4 loss-of-function mutations causes a devastating neurological disorder characterized by seizures, intellectual disability, spastic quadriplegia, ichthyosis, and pre-mature death. Homozygous expression of these mutations in *Elovl4* or global deletion of *Elovl4* in mice leads to their death within hours of birth due to dehydration. To overcome this neonatal lethality, we had previously generated skin-rescued ($S^+$) mice that express two nonfunctional copies of *Elovl4* containing the 5-bp deletion found in Stargardt-like macular dystrophy (STGD3) patients. These mice ($S^+Elovl4^{mut/mut}$) survived, but starting at post-natal day 19 (P19), developed a severe, progressive seizure phenotype that resulted in death by P21. Our previous studies showed that VLC-SFA are absent in the brain of homozygous mutant mice. In order to decipher the synaptic defects that are caused by the absence of VLC-SFA, we have performed patch-clamp electrophysiological investigations on acute hippocampal slices derived from the skin rescued $S^+Elovl4^{mut/mut}$ mice (MUT). Our results show that miniature Excitatory Postsynaptic Current (mEPSC) frequency is reduced in MUT mice with no change in mEPSC amplitude compared to wild-type (WT), suggesting a presynaptic defect of excitatory synaptic transmission on hippocampal pyramidal neurons. Paired-pulse ratio of evoked EPSC is increased at the Schaffer collateral-CA1 synapses in MUT mice, suggesting a decrease in release probability. We also found alterations in inhibitory synaptic transmission as miniature Inhibitory Postsynaptic Current (mIPSC) frequency and amplitude are decreased in MUT hippocampal pyramidal neurons. In addition, we identified that the basal expression level of immediate early gene *c-fos* is increased in cortex and hippocampus of the heterozygous (HET) mice. These results reveal the action of VLC-SFA in maintaining hippocampal synaptic transmission at excitatory and inhibitory synapses, and clarify the synaptic and circuit dysfunction underlying the seizure activity in the mutant animals.

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**Topic:** B.04. Synaptic Transmission

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CAPES

**Title:** Enhancement of the excitatory transmission in NTS neurons in response to sustained hypoxia is abolished in $A_{2A}$ receptors knockout mice

**Authors: *J. REIS SOUZA**, L. LIMA-SILVEIRA, D. ACCORSI-MENDONÇA, B. H. MACHADO;
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**Abstract:** The Nucleus Tractus Solitarius (NTS) is an integrating center for several sensory systems, including the synaptic processing of chemoreflex afferents. Current study in our laboratory shows that exposure of mice to sustained hypoxia (SH - 24h, $FiO_2$ 0.1) facilitates the glutamatergic transmission in NTS. Adenosine is an important neuromodulator of synaptic transmission and hypoxia contribute to increase its extracellular concentration. Studies indicate the $A_{2A}$ receptors play a key role in the modulation of the neuronal networks in the NTS. In this context, we evaluated whether the $A_{2A}$ adenosine receptors are important to changes in NTS synaptic transmission of mice submitted to SH. For this, we used male knockout (KO) mice for adenosine $A_{2A}$ subtype receptors, and male Balb/c wild-type (WT) mice. These animals were divided into two groups: 1) mice submitted to SH, and 2) mice maintained under normoxia (control). At the end of these protocols, mice were deeply anesthetized with Isoflurane and then decapitated. The eletrophysiological activity of NTS neurons were recorded using the whole-cell patch-clamp (Institutional ethical committee #029/2021). No statistical differences were found between groups in relation to resting membrane potential [RMP; Balb/c WT normoxia (n=29) vs SH (n=18): -65 ± 3 vs -64 ± 2 mV, P>0,9999, $A_{2A}$KO normoxia (n=15) vs SH (n=20): -65 ± 1 vs -66 ± 1 mV, P>0,9999] and input resistance [Balb/c WT normoxia (n=12) vs SH (n=11): 0,58 ± 0,05 vs 0,65 ± 0,07 GΩ, P>0,9999, $A_{2A}$KO normoxia (n=17) vs SH (n=19): 0,57 ± 0,06 vs 0,55 ± 0,07 GΩ, P>0,9999] or in relation to the firing frequency of spontaneous action potentials between groups [Balb/c WT normoxia (n=14) vs SH (n=9): 6 ± 1 vs 6 ± 1 Hz, P > 0,9999, $A_{2A}$KO normoxia (n=8) vs SH (n=12): 9 ± 1 vs 5 ± 1 Hz, P=0,3123]. Under normoxia condition the amplitude of evoked glutamatergic currents were similar between WT (n=11) and $A_{2A}$KO (n=14) mice (-307 ± -37 vs -210 ± 32 pA, P=0,4061). Exposure to SH (n=10) enhances the amplitudes of evoked glutamatergic currents in NTS neurons of WT Balb/c mice (-429 ± 87 vs -210 ± 32 pA, P = 0,0203) but not from $A_{2A}$KO SH (n=12) mice (-268 ± 61 vs -307 ± 37 pA, P>0,9999). The overall data indicates that adenosine $A_{2A}$ receptors do not modulate the glutamatergic transmission in the NTS neurons of control mice, but they are required for the enhancement of glutamatergic transmission observed in the NTS neurons of mice submitted to SH.

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**Presentation Number:** NANO12.06

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

**Support:** NIH R01NS123084
2T32-AI052077-19
Marcus Foundation

**Title:** The role of interferon-gamma and STAT1 signaling in neuronal excitability and behavior.

**Authors: *D. N. CLARK**[1], S. M. O'NEIL[2], S. V. BROWN[3], L. XU[3], J. T. STEPPE[3], J. D. MILNER[5], A. J. FILIANO[4];

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**Abstract:** Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects 1 in 54 children, yet there is no known cause or treatments. Recently, the immune system has been implicated in ASD pathophysiology, with the interferon (IFN) pathway being the most enriched pathway in brains of individuals with ASD. Children born to mothers who are hospitalized for infection during pregnancy are at a higher risk of developing ASD, and mouse models demonstrate that elevated cytokines in the brain during neurodevelopment cause ASD-like phenotypes. While microglia are thought to be the major targets of IFNs in the brain, neurons can respond to IFNs and require physiological levels of IFN-γ for proper function. Here, we demonstrate that developing neurons respond to IFN-γ, and high pathological levels of IFN-γ cause robust and prolonged activation of STAT1, a key transcription factor in the IFN-γ pathway, in neurons but not microglia. In neurons, continuous janus kinase (JAK) activity enabled the prolonged activation of STAT1, even in the absence of IFN-γ. While initially inducing a typical IFN-γ transcriptional response, high levels of IFN-γ led to persistent alterations in synaptic pathway transcripts. To determine the effects of prolonged STAT1 activation *in vivo*, we developed a novel mouse model in which STAT1 is constitutively activated in neurons. These mice display hyperactive behavior which is a common comorbidity of ASD. Moreover, we demonstrate that this phenotype is neuron specific, as mice with prolonged STAT1 activation in microglia do not have behavior deficits or neuronal hyperexcitability. Our findings suggest that the IFN-γ/STAT1 pathway is critical for normal neurodevelopment and neuronal function in adulthood and provides new insight into a neuron specific neuroimmune mechanism which may contribute to ASD pathophysiology.

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

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**Title:** Peripheral inflammation limits 5-HT neuron signaling capacity via serotonergic IL-1R1 to reduce 5-HT neuron excitability and enhance 5-HT clearance

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**Abstract:** Neuropsychiatric disorders have been associated with dysfunction in the serotonin (5-HT) system. Associations may stem from changes in intrinsic 5-HT neuronal properties and/or alterations in downstream 5-HT responsive circuits. Studies report elevated levels of inflammatory cytokines in association with mood disorders and chronic inflammatory conditions

are a known risk factor in the development of depression. Depression, anxiety, OCD, autism, and schizophrenia, among others, are commonly treated with medications that inhibit the presynaptic 5-HT transporter (SERT), drawing our attention to the transporter as a factor of 5-HT signaling that may be overactive during inflammation. In this regard, we have shown that inflammatory cytokines alone or activation of the peripheral innate immune system can increase activity of SERT. Although inflammatory cytokines are known to impact multiple aspects of neural signaling, neuronal expression and function of specific cytokine receptors remains to be fully characterized. We and others have found that midbrain raphe 5-HT neurons express high levels of IL-1R1, the receptor for the inflammatory cytokine IL-1β, with sex-specific differences evident that may contribute to sex-dependent differences observed in incidence or presentation of neuropsychiatric disorders. Interestingly, our *in situ* studies reveal non-uniform expression of IL-1R1 across raphe subregions, suggesting that specific IL-1R1 expressing 5-HT projections support the behavioral actions of CNS IL-1β. Here we examine the actions of serotonergic IL-1R1 on SERT function and examine the circuit-specific sensitivity to serotonergic IL-1R1 activation. High-speed chronoamperometry reveals locally applied IL-1β increases the rate of 5-HT clearance. Elimination of serotonergic IL-1R1 results in an inability of peripheral LPS to stimulate SERT and reveals that the receptor contributes to the ability of peripheral immune activation to modulate the activity of distinct brain circuits innervated by 5-HT neurons. Since peripheral LPS and direct application of IL-1β inhibit 5-HT neuron activity, as measured by cFos labeling, slice physiology, and *in vivo* fiber photometry, we propose that peripheral innate immune activation, acting via IL-1R1, limits 5-HT signaling by reducing 5-HT release and enhancing clearance. Our findings are consistent with a model whereby serotonergic IL-1R1, translates peripheral innate immune activation into changes in 5-HT signaling capacity to shape behavior so as to deal with environmental threats. Neuropsychiatric disorders may arise from excessive or inappropriate use of this normally protective process.

**Disclosures: P.A. Gajewski-Kurdziel:** None. **H. Iwamoto:** None. **A. Tillman:** None. **N.L. Baganz:** None. **M.J. Robson:** None. **N. Quan:** None. **R.D. Blakely:** None.

**Presentation Number:** NANO12.08

**Topic:** I.04. Physiological Methods

**Support:** NIH Grant 008914

**Title:** Evolving chemogenetic and optogenetic tools for multiplexed control of peptide functions

**Authors: *L. A. VAZQUEZ RIVERA**[1], J. SHEN[2], G. ZHOU[2], G. SHINGLES[2], W. WANG[2]; [1]Program in Chem. Biol., Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI

**Abstract:** The study of opioid receptors is essential to gain insight into the mechanisms underlying the effects of opioid medications such as pain relief and addiction. The mu opioid receptor (MOR) is particularly important in pain modulation, with activation reducing pain sensation as well as inducing euphoria and relaxation. Understanding the molecular and cellular interactions of opioid receptors has the potential to inform the development of safer pain management strategies, novel opioid medications, and interventions to address the harmful addictive properties. Protein tools have played an important role in understanding opioid receptor

function and developing selective opioid receptor modulators. Chemogenetics tools have emerged as powerful tools in neuroscience research to manipulate and study specific neural circuits with high temporal and spatial precision. Here, we developed a platform to improve peptide caging of chemogenetic and optogenetic tools, specifically Chemical Activated Protein (CAP) to control opioid peptides. We successfully evolved CapC and CapN libraries and demonstrated that it cages the peptide more efficiently, resulting in less leaky binding in the absence of shield-1.

**Disclosures: L.A. Vazquez Rivera:** None. **J. Shen:** None. **G. Zhou:** None. **G. Shingles:** None. **W. Wang:** None.

**Nanosymposium**

**NANO13: Molecular, Structural, and Network Changes Across the Lifespan**

**Location:** WCC 144

**Time:** Sunday, November 12, 2023, 8:00 AM - 9:45 AM

**Presentation Number:** NANO13.01

**Topic:** C.01. Brain Wellness and Aging

**Title:** Connecting the voxels: integrating atrophy, microstructure, and cognition in age-related network-like patterns across the adult lifespan

**Authors: *S. TULLO**[1], J. DUFFY[1], R. PATEL[1], G. A. DEVENYI[1], A. SALACIAK[1], S. A. BEDFORD[1], S. FARZIN[1], T. AGYEKUM[1], C. GARCIA GARCIA[1], S. VILLENEUVE[1], J. POIRIER[1], C. TARDIF[2], M. CHAKRAVARTY[1];
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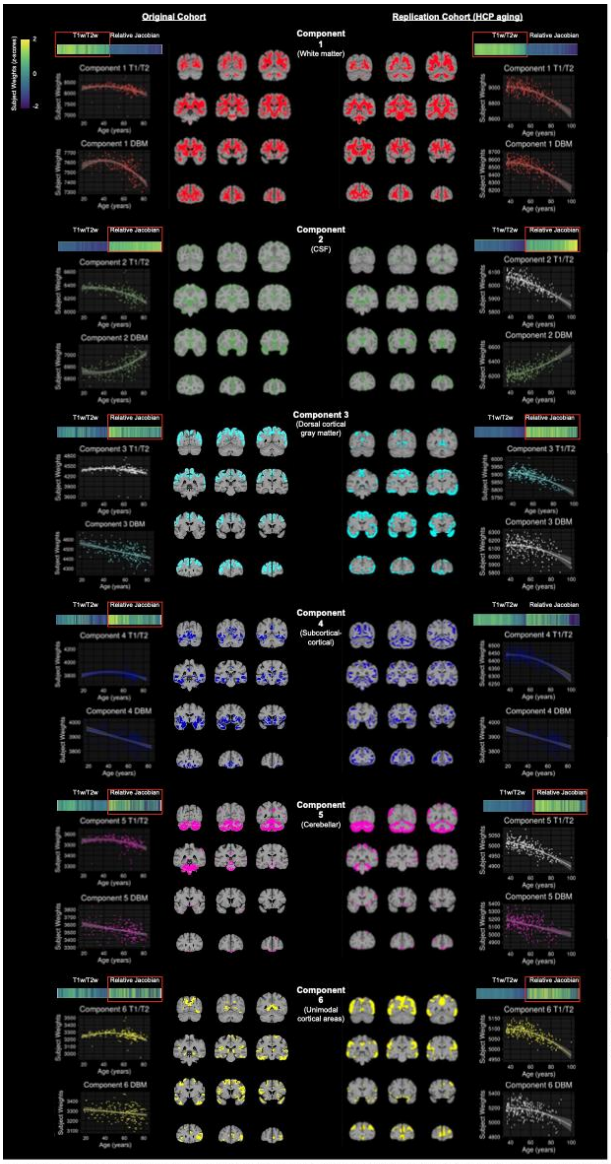
**Abstract:** Neuroimaging studies on aging usually focus on a single dimension of brain architecture and its relationship with age. However, combining measures can offer a complementary understanding of brain aging. Here, we investigate variations in brain morphology and microstructure across adulthood.
We acquired voxel-wise metrics of brain morphology (relative Jacobians [JAC] from deformation-based morphometry of T1w; 1 $mm^3$) and microstructure (T1/T2 ratio) in 189 subjects (ages 18-83; 112 F). The HCP aging cohort was used to examine generalizability of the derived age patterns (n=243 (152 F); ages 36-100; 0.8 $mm^3$). JAC and ratio values were combined into a single matrix, z-scored, and subjected to orthogonal projective non-negative matrix factorization (NMF) for age-related variance decomposition. NMF outputs positively valued, sparse, and orthogonal brain components indicating brain patterns with voxel and subject weights. The relationship between age and subject weights for each component and metric were assessed using Bonferroni-corrected general linear models, and best polynomial fits with Akaike information criterion. Partial least squares (PLS) analysis identified covariance signatures of NMF weights with cognition, age, sex, and education in the original sample.
Our results suggest significant changes across the brain with general monotonic decreases in JAC and quadratic ratio trajectories peaking around middle age in both samples (Fig 1A). PLS identified two significant LVs ($ps < 0.05$) (Fig 1B-C). LV1 (86% covariance explained)

associated older age, lower education, and reduced visuospatial scores with lower JAC and ratio for all comps except comp 2 JAC. LV2 (7% covariance) associated women, lower memory scores, and higher language scores with lower ratio values for comps 4 & 5 and increased JAC for comp 3.

We identified replicable aging patterns linked to cognitive decline. This data-driven approach confirms previous findings of decreased functioning in default mode and memory networks with age, as observed in neurodegenerative diseases like Alzheimer's.



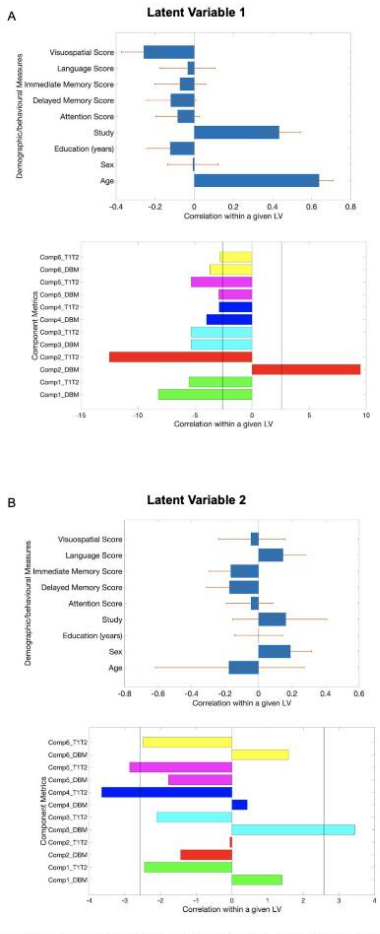




**Figure 2** [A] PLS analysis revealed two statistically significant LVs (ps < 0.05). LV1 accounted for ~86% of covariance explained and was associated with older age, less years of education, and lower visuospatial scores. The correlating brain features included decreased Jac and ratio for all comps, except comp 2 Jac. [B] LV2 accounted for ~7% of covariance and was associated with females, lower delayed and immediate memory and higher language scores. The correlating microstructural features included decreased ratio values for comps 4 & 5 and increased Jac for comp 3.

**Figure 1.** We observed 6 distinct spatial comps resembling well-known brain networks or neuroanatomical subcompartments (as determined by stability analyses detailed in Patel et al., 2020) in two independent cohorts of similar age spans, with similar characterization by voxel-wise anatomical and microstructural metrics. The spatial pattern for comp 1, characterized by higher ratio weights, encapsulates the white matter of the brain. A significant inverted U shaped age relationship was observed for ratio (p=6.04e-8) and Jac subject comp weights (p=8.06e-3). Comp 2 is characterized by higher Jac weights, encapsulating CSF-filled voxels, with a quadratic increasing trend for Jac subject comp weights with age (p=1.38e-3) and a decreasing trend for ratio (p=2.35e-2). Comp 3 consists of dorsal cortical gray matter with linearly declining age related to Jac (p=1.83e-11). Comp 4 was characterized by higher Jac weights with a linear declining trend with age (p=6.27e-17) for ventral cortical and subcortical gray matter voxels; and an inverted U shaped age relationship was observed for ratio (p=4.47e-3). Comp 5 was also characterized by Jac, where a linear declining trend with age was observed (p=1.71e-6); and an inverted U shaped age relationship was observed for ratio (p=0.04). Finally, comp 6 was characterized by higher Jac weights, with a linear decline over age (p=0.049), consisting of voxels in primary sensory cortical areas and an inverted U shape relationship with age for ratio weights (p=2.37e-5), with peaks occurring at ~50 years old (Grydeland et al., 2019; Tullo et al., 2019). For the replication cohort, similar spatial patterning and metric weightings weres obtained. For comp 1, 3, 4 and 6, we observed a trending decrease in ratio values across the age spans (p = 2.55e-04, p = 1.53e-03, p = 8.06e-27, p = 8.15e-06, respectively) and for comps 1, 4, and 5, we observed a trending decrease in Jac values across the age spans (p=1.01e-03, p=2.74e-29, p=3.05e-11, respectively). We also observed a trending increase in Jac value across the age span for comp 2 (p=1.49e-02), reflecting the increase in ventricular volumes observed with age. Colours data points on plot describe significant relationships between age and subject weights (p<0.05) after Bonferroni correction.

**Disclosures: S. Tullo:** None. **J. Duffy:** None. **R. Patel:** None. **G.A. Devenyi:** A. Employment/Salary (full or part-time):; Douglas Research Center. **A. Salaciak:** A.

Employment/Salary (full or part-time):; Douglas Research Center. **S.A. Bedford:** None. **S. Farzin:** A. Employment/Salary (full or part-time):; Douglas Research Center. **T. Agyekum:** None. **C. Garcia Garcia:** None. **S. Villeneuve:** A. Employment/Salary (full or part-time):; Douglas Research Center. **J. Poirier:** A. Employment/Salary (full or part-time):; Douglas Research Center. **C. Tardif:** A. Employment/Salary (full or part-time):; Montreal Neurological Institute. **M. Chakravarty:** A. Employment/Salary (full or part-time):; Douglas Research Center.

**Presentation Number:** NANO13.02

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIA/NIH P30AG072977
NIA/NIH R01AG067781
NIA/NIH U19AG073153

**Title:** Integrity of myelin in cortical white matter in cognitive SuperAgers

**Authors: *A. ZOURIDAKIS**, I. AYALA, A. TZIORTZIS, R. CASTELLANI, E. ROGALSKI, T. GEFEN, C. GEULA;
Mesulam Ctr. for Cognitive Neurol. and Alzheimer's Dis., Northwestern Univ., Chicago, IL

**Abstract: Introduction:** SuperAgers are individuals over the age of 80 with superior episodic memory equal to that of individuals 20 years their junior, and who exhibit resistance to age-related neurofibrillary degeneration. We have observed significantly lower density of activated microglia in the white matter of SuperAgers compared to their cognitively normal peers. In this study we utilized immunohistochemistry and western blot analysis to test the hypothesis that integrity of white matter myelin is enhanced in SuperAgers when compared with age-matched cognitively average peers. **Methods**: Whole-hemisphere tissue taken from 3 SuperAgers and 3 age-matched cognitively normal controls was fixed in 4% paraformaldehyde for 30-36 hours at 4º C and taken through sucrose gradients for cryoprotection. Blocks were sectioned at a thickness of 40 µm on a freezing microtome and 1 in 24 series of sections were stored in 0.1 M phosphate buffer until use. Paraffin-embedded blocks were also prepared from 3 SuperAgers and 3 controls and sectioned at 5 µm. Slides from frontal, temporal, and occipital regions were stained for myelin basic protein (MBP) to examine integrity of myelination in each group. Of the whole-hemisphere slides, 2 SuperAger and 2 control cases stained for MBP were quantified for optical density using ImageJ. Western blot analysis was performed to detect MBP levels in 4 SuperAgers and 3 controls using frozen postmortem human tissue from frontal cortex. **Results:** MBP immunorectivity revealed observable differences in the whole hemisphere and paraffin-embedded sections. Generally, SuperAger white matter appeared to have denser, darker staining. The ImageJ analysis in the MBP-stained slides revealed significant across-group differences, where the white matter in SuperAgers was more densely stained in every region ($P<0.001$). Western Blot analysis confirmed a trend towards higher levels of MBP in cortical white matter in SuperAgers.**Conclusion:** Together, these preliminary results indicate that there is increased myelin integrity in white matter of SuperAgers compared to controls, warranting further quantitative analysis with additional specimens, different white matter regions, and using markers of axons as well as meylin.

**Disclosures: A. Zouridakis:** None. **I. Ayala:** None. **A. Tziortzis:** None. **R. Castellani:** None. **E. Rogalski:** None. **T. Gefen:** None. **C. Geula:** None.

**Presentation Number:** NANO13.03

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIH AG054748

**Title:** Functional disruptions in aging human neurons revealed by single-cell transcriptomics

**Authors: *A. M. JEFFRIES**[1], T. YU[2], J. S. ZIEGENFUSS[1], A. K. TOLLES[1], Z. WENG[2], M. A. LODATO[1];
[1]Dept. of Molecular, Cellular, and Cancer Biol., [2]Dept. of Bioinformatics and Integrative Biol., UMass Chan Med. Sch., Worcester, MA

**Abstract:** Aging in the brain is a universal process characterized by functional decline, the mechanisms of which remain poorly understood. The advent of droplet based single-nucleus RNA-sequencing revolutionized the kind of data we can get from post-mortem human brain tissue, but this technology has yet to be applied to aging. In this study, a cohort of pre-frontal cortex from 13 donors ranging in age from less than one year old to 104 years old, revealed both infant-specific transcriptomic signatures as well as global down-regulation of cell maintenance processes in aging. Initial clustering revealed L2/3 neuron, L4 neuron, and astrocyte clusters where >97% of the cells came from infant donors. Differential expression and subsequent gene ontology analysis revealed an up-regulation of developmental processes as the driver of infant-specific clustering in these three cell types. Differential expression analysis between elderly and adult brains revealed commonalities across cell types in down-regulation but cell-type-specific and somewhat random up-regulation in the transcriptome of elderly brains. Across all cell types, expression of ribosomal protein genes, as well as genes encoding four of the five electron transport chain complexes, was reduced. Gene ontology of the down-regulated genes is dominated by terms relating to transport, homeostasis, translation, localization, and metabolism, especially in neurons. This points to the possibility of reduced activity in aging neurons. Concordantly, expression of immediate early genes ARC, BDNF, EGR1, and NPAS4 all have statistically significant negative correlations with age. Together, these findings support a hypothesis that during aging, expression of genes integral to neuron identity are preserved but basic cell functions are compromised leading to a reduction in neuronal activity.

**Disclosures: A.M. Jeffries:** None. **T. Yu:** None. **J.S. Ziegenfuss:** None. **A.K. Tolles:** None. **Z. Weng:** None. **M.A. Lodato:** None.

**Presentation Number:** NANO13.04

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIH grant U01 MH122592

**Title:** Epigenetic regulation of transposable element expression throughout the lifespan in the human brain

**Authors: *D. R. W. BURROWS**[1], M. CUOCO[2], J.-F. CHIEN[3], R. VADUKAPURAM[4], A. KOZLENKOV[4], S. DRACHEVA[4], E. A. MUKAMEL[1];
[1]Cognitive Sci., [2]Bioinformatics and Systems Biol., [3]Dept. of Physics, UCSD, San Diego, CA; [4]Dept. of Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

**Abstract:** Aging is associated with accumulation of DNA damage and reorganization of epigenetic marks. Epigenetic dysregulation could disinhibit transcription of transposable elements (TE) sequences that can change position within a genome, thus creating mutations. Notably, elevated TE RNA expression has been reported in aged mammalian tissues. TEs are highly active in neurons. Therefore, the brain may provide fertile ground for the emergence of TE-driven DNA mutagenesis in aging. However, it remains unclear whether TE transcription increases with age in the human brain, and whether such changes are driven by alterations of epigenetic regulation. To address this, we used whole genome bisulfite, oxidative bisulfite, and RNA sequencing to profile the transcriptome and epigenome of glutamatergic and GABAergic cells. The nuclei from these neuronal subtypes were obtained from the dorsolateral prefrontal cortex of 99 donors across the lifespan (0-80 years old) using fluorescence activated nuclear sorting. This dataset provides insight into the lifelong dynamics of two major epigenetic modifications, methylcytosine and hydroxymethylcytosine, alongside RNA expression in major neuronal subtypes in the human brain. To study whether TE transcription increases with age in human neurons, we developed putatively Active Transposable Element Mapping (ATEM), a novel computational procedure that estimates the number of RNAs expressed from active TE insertions. ATEM distinguishes RNA fragments arising from passive transcription of TEs located within introns of active genes, from RNA fragments arising from bona fide transcription driven by a TE promoter. To do this, we identify RNA reads that align to the promoter of non-truncated TEs. We found that expression of several TEs, including evolutionarily younger LINE1 elements (L1PA8, L1PA5, L1PA6) significantly increased in neuronal cells with aging. Next, to study the epigenetic marks that may give rise to such changes, we compared the extent of methylation and hydroxymethylation around the TE insertions and upstream cis-regulatory elements across the lifespan. We uncovered novel, cell-type-specific epigenetic patterns linked to TE RNA expression changes in aging.
This study represents the first comprehensive analysis of the transcriptomic and epigenomic signals that comprise TE activity across the lifespan in the human brain. We demonstrate that RNA expression of several active TEs increases with age, alongside alterations to key epigenetic marks in the vicinity of these TEs, which provides a potential pathway for the accumulation of DNA damage in aging brain tissue.

**Disclosures: D.R.W. Burrows:** None. **M. Cuoco:** None. **J. Chien:** None. **R. Vadukapuram:** None. **A. Kozlenkov:** None. **S. Dracheva:** None. **E.A. Mukamel:** None.

**Presentation Number:** NANO13.05

**Topic:** C.01. Brain Wellness and Aging

**Support:** Stratneuro 2020
kempe Stiftelsen 2019

**Title:** Network reorganization in the aging mouse brain is shaped by sex

**Authors: *F. SULTAN**[1], Ö. ÖZALAY[2], T. MEDIAVILLA[3], B. LIMA GIACOBBO[5], A. RIECKMANN[4], R. PEDERSEN[3];
[1]Integrative medical biology, Umea Univ., Umeå, Sweden; [2]Umea Univ., Umea, Sweden; [4]Radiation Sci., [3]Umeå Univ., Umea, Sweden; [5]Univ. Gronningen, Gronningen, Netherlands

**Abstract:** The human aging brain is characterized by changes in network efficiency that are best captured through longitudinal resting-state functional MRI (rs-fMRI) studies. These longitudinal studies are challenging due to the long human lifespan. Nevertheless, currently no studies of animals with shorter lifespan are available. Here we show that the mouse animal model allows us to follow the functional network organization over most of the animal's adult lifetime up till old age. We used a longitudinal study of the functional connectivity of different brain regions with rs-fMRI under anesthesia. Our analysis uncovers network communities/modules at 12, 18 and 24 months old C57Bl/6J mice, finding similar networks to those previously reported in younger mice and in other mammals including humans (i.e., prefrontal/default mode network (DMN), somatomotor and somatosensory networks). Longitudinal statistical modeling of changes in network coupling reveals opposing sex effects. Males showed a pronounced increase in connectivity of the somatomotor cortical areas to the Nucleus accumbens, together with a weakening of connectivity in secondary somatosensory cortex. In contrast, females showed reorganization more akin to human aging, with de-differentiation of the connectome, mainly due to increases in connectivity of the prelimbic cortex (in addition to other regions of prefrontal/DMN network) to somatomotor networks. Our analysis also showed that these changes were accompanied by attenuated network modularity in females, while males showed an increase in modularity. In summary, in line with human work, our analyses in mice support the concept of de-differentiation in the aging mammalian brain and also reveal striking differences in aging mice due to sex.

**Disclosures: F. Sultan:** None. **Ö. Özalay:** None. **T. Mediavilla:** None. **B. Lima Giacobbo:** None. **A. Rieckmann:** None. **R. Pedersen:** None.

**Presentation Number:** NANO13.06

**Topic:** C.01. Brain Wellness and Aging

**Support:** Keck Foundation
NIH RO1NS072497

**Title:** Age related changes in sodium concentrations in cerebrospinal fluid, plasma, and brain regions: implications for brain function and hypertension risk

**Authors: *C. XIA**[1], W. DAI[2], X. WU[3], N. F. DALLESKA[4], N. ASTRAEA[3], A. N. FONTEH[1], A. VASUDEVAN[1], R. A. KLONER[2], X. ARAKAKI[1];
[1]Neurosci. Dept., [2]Cardiovasular Dept., [3]Analytical Biochem. Core, Huntington Med. Res. Inst., PASADENA, CA; [4]Water and Envrn. Lab., Caltech, Pasadena, CA

**Abstract:** Sodium homeostasis plays a critical role in maintaining normal brain function, and disruptions in sodium levels can have profound effects on physiological processes. However, changes of sodium with age and sex remain poorly understood. We aimed to investigate alterations in sodium concentrations in cerebrospinal fluid (CSF), plasma, and brain regions (hippocampus, cerebellum, and rest of the brain) between young (2-3 months old) and aged (18

months old) Sprague-Dawley rats using ion chromatography. Statistical analyses were performed using unpaired two-tailed t-tests, with p-values < 0.05 considered statistically significant. Our findings demonstrate that sodium concentrations in both CSF and plasma were significantly higher in aged rats compared to young rats. Specifically, aged female rats exhibited higher sodium in CSF (159.3 ± 2.04 mM) and plasma (155.0 ± 3.53 mM), compared to young females with CSF sodium of 141.2 ± 2.03 mM and plasma sodium of 128.4 ± 6.66 mM. Males show the similar changes as females (Fig. 1). Furthermore, distinct regional differences were observed in the brains of aged females and males. For example, aged female rats exhibited elevated sodium levels in the hippocampus compared to their young counterparts (Fig. 1). The age-related change was not observed in males. These findings suggest a potential link between the increased sodium concentrations observed in CSF, plasma, and brain tissues of aged rats and the heightened risk of hypertension in older individuals. Additionally, the observed elevation of sodium levels specifically in the aged female hippocampus, but not in males, may contribute to the previously observed gender disparities in spatial learning and memory performance. It is important to note that further investigations are required to determine the translational implications of these findings to human physiology. Nonetheless, this study provides valuable insights into age-related changes in sodium concentrations and their potential impact on brain function and the development of hypertension.

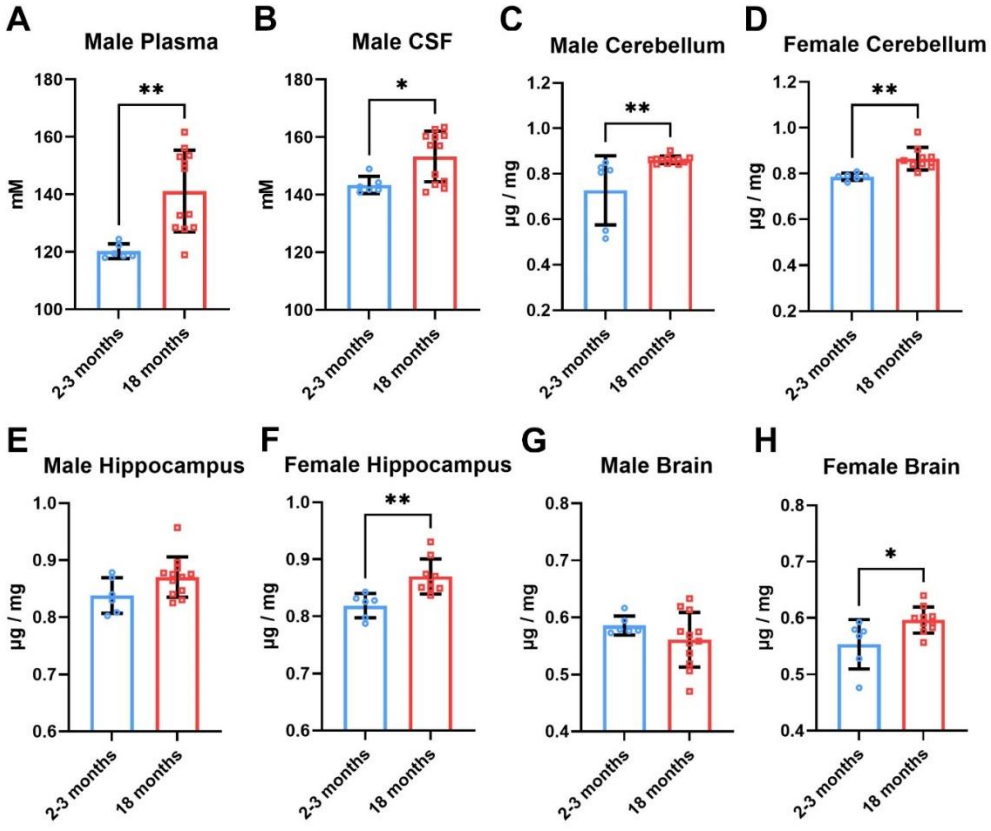



**Fig. 1 Increased sodium in plasma, CSF, and brain regions of aged rats.**

Sodium increased in 18 months male vs. 2-3 month male for plasma (A), CSF (B), and cerebellum (C); and in 18 months female vs. 2-3 month female for cerebellum (D); E) No significant changes were detected in 18 months male vs. 2-3 month male for hippocampus; F) Sodium increased in 18 months female vs. 2-3 month female for hippocampus; G) No significant changes were detected in 18 months male vs. 2-3 month male for brain (rest of the brain); H) Sodium increased in 18 months female vs. 2-3 month female for brain. n=6 for 2-3 months group, n=9-12 for 18 months group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; unpaired t test; mean ± sd.

**Disclosures: C. Xia:** None. **W. Dai:** None. **X. Wu:** None. **N.F. Dalleska:** None. **N. Astraea:** None. **A.N. Fonteh:** None. **A. Vasudevan:** None. **R.A. Kloner:** None. **X. Arakaki:** None.

**Presentation Number:** NANO13.07

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIH R01AG061382
NIH RF1AG072300
NIH T32AG049688
NIH R01AG061382-02S1

**Title:** Neuronal tissue inhibitor of metalloproteinases 2 (TIMP2) regulates hippocampus-dependent plasticity through changes in extracellular matrix complexity

**Authors:** A. FERREIRA, B. M. HEMMER, S. M. PHILIPPI, A. GRAU-PERALES, J. ROSENSTADT, M. VARGHESE, P. R. HOF, ***J. CASTELLANO**;
Icahn Sch. of Med. At Mount Sinai, New York, NY

**Abstract:** The aging process ultimately leads to compromised brain function, the decline of which is a prominent feature of many neurological disorders, including those for which it serves as the major risk factor. A growing body of evidence now supports a model in which processes perturbed in aging and Alzheimer's disease (AD), including adult neurogenesis and synaptic plasticity, are regulated by factors changing across lifespan in the systemic compartment. We have shown that tissue inhibitor of metalloproteinases 2 (TIMP2) is a youth-associated protein in blood that revitalizes hippocampal function in aged mice, but the mechanism by which various pools of TIMP2 regulate hippocampal function is unknown. Here, we hypothesize that TIMP2 plays unappreciated roles in governing synaptic plasticity processes in the healthy hippocampus that are relevant to pathological processes in aging and AD. Using a combination of approaches that include behavioral assessments, RNA-seq, and confocal microscopy, we find that the pool of TIMP2 present within hippocampal neurons declines with age, and global ablation impairs both spatial memory and various stages of adult neurogenesis. TIMP2 deletion also markedly reduces dendritic spine complexity, including a decrease in mushroom spines linked to long-term memory, as assessed by neuronal iontophoretic dye-filling. We developed a $TIMP2^{fl/fl}$ model to conditionally delete TIMP2 in neurons, revealing that phenotypes from global TIMP2 knockout mice are recapitulated when neuronal TIMP2 is targeted. We find several of these changes are consistent with TIMP2's role in maintaining extracellular matrix homeostasis. In either aged mice or mice lacking TIMP2, we find an accumulation of extracellular matrix in the hippocampus; intriguingly, removing this accumulation appears to relieve the migration impairment of cells involved in adult neurogenesis, suggesting that TIMP2 regulates hippocampal plasticity through changes in the extracellular matrix. We also extend these findings in other mouse models to explore the impact of TIMP2 on AD pathology. We find that TIMP2 regulates a diverse set of phenotypes across both normal hippocampal physiology and in the context of AD pathology, including in processes that depending on flexibility for removal of pathological debris. Together our results argue that age-relevant factors in the systemic environment have long-range roles in shaping hippocampal function, including processes that depend on the dynamics of extracellular matrix homeostasis. Overall, these processes may

represent targets of high translational value for development of novel neurodegenerative disease therapies.

**Disclosures: A. Ferreira:** None. **B.M. Hemmer:** None. **S.M. Philippi:** None. **A. Grau-Perales:** None. **J. Rosenstadt:** None. **M. Varghese:** None. **P.R. Hof:** None. **J. Castellano:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.M.C. is listed as a co-inventor on patents related to use of young plasma and TIMP2 for treatment of age-associated conditions.

**Nanosymposium**

**NANO14: Synaptic and Circuit Mechanisms Underlying Alzheimer's Disease**

**Location:** WCC 143

**Time:** Sunday, November 12, 2023, 8:00 AM - 11:30 AM

**Presentation Number:** NANO14.01

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

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Sheila and Denis Cohen Charitable Trust
Rosetrees Trust of the UK
Israel Council for Higher Education,- a PhD fellowship of excellence

**Title:** Thalamic Nucleus Reuniens Regulates Resilience to Synaptic and Cognitive Failures in Alzheimer’s Model

**Authors: *S. SHOOB**, N. BUCHBINDER, O. SHINIKAMIN, I. SLUTSKY;
Physiol. and Pharmacol., Tel Aviv Univ., Tel Aviv, Israel

**Abstract:** What mechanisms confer cognitive resilience to Alzheimer’s Disease (AD)-related pathology? Resilience is principally expressed as a lack of dementia throughout a lifespan in a subset of people with AD pathology, and as a long presymptomatic phase in AD patients. Our recent work has shown that general anesthesia exposes early dysfunctions in the hippocampus of familial AD (fAD) model mice using in vivo electrophysiology and large-scale calcium imaging (Zarhin et al., Cell Reports 2022). Here, we describe a neural circuit mechanism by which anesthesia dampens resilience to cognitive decline in AD.
We detected abnormal epileptiform spikes (ES) in the hippocampal CA1 of 4-5 months old fAD

model mice during anesthesia. We then used the delta-maze alternation test to quantity spatial working memory of young WT and fAD mice. Both groups showed similar memory performance during baseline but a single session of anesthesia (1.5% isoflurane) led to transient impairments of working memory in fAD, but not WT mice. The degree of memory impairments following anesthesia correlated to the frequency of CA1-ES during the anesthesia. Since deactivation of the thalamus is a well-known feature of low-arousal brain states, and as short-term synaptic facilitation is proposed to maintain working memory, we hypothesized that anesthesia might impair short-term synaptic plasticity at the thalamic nucleus reuniens (nRE), the principle thalamic input to the hippocampal CA1. To test that, we measured short-term plasticity in response to high-frequency burst stimulation at the nRE-CA1 synapse of awake mice before and after anesthesia. WT and fAD mice displayed a similar synaptic facilitation during baseline. A single session of anesthesia, however, caused ~40% reduction in synaptic facilitation in fAD, but not WT mice. Using pharmacological and chemogenetic methods, we found that inactivation of the nRE reduced the frequency of CA1-ES during GA by 39%-77%. Moreover, phasic and tonic stimulation of the nRE can bi-directionally modulate CA1 excitability: Tonic deep brain stimulation of the nRE (tDBS-nRE) caused a ~60% decrease in the rate of CA1 ES, restored state-dependent CA1 firing rate homeostasis and excitability and prevented nRE-CA1 synaptic facilitation and spatial working memory impairments in response to anesthesia. Furthermore, tDBS-nRE during prodromal disease stage prevented age-dependent decline of working memory. These findings highlight the nRE as a central node of functional resilience and emphasize the clinical promise of DBS in conferring resilience to AD-pathology by restoration of circuit-level homeostasis.

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

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**Title:** Sex differences in impaired cognition after sleep disruption in a subclinical Alzheimer's disease model

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**Abstract:** Alzheimer’s disease (AD) is the most common cause of senile dementia worldwide. Plaques and neurofibrillary tangles form the neuropathological hallmark of this disease. Plaques, mostly formed by A-β, are insoluble extracellular protein aggregates. As AD progresses, brain regions critical for cognition and memory are affected, resulting in a complex cognitive decline. Sleep disruption is persistent in patients suffering from AD. Disrupted sleep often becomes evident prior to clinical and cognitive manifestations, and sleep symptoms worsen as the disease progresses. The causal connection between these two phenomena is not clearly understood. To determine whether chronic sleep fragmentation accelerates the onset of cognitive decline, or alters subsequent pathology caused by induction of mutant APP in AD preclinical subjects, we

used an inducible APP mouse model (tTA/APPsi). This model enables us to exert temporal control over the expression of APP in brain, thereby allowing us to separate the effects of chronic sleep fragmentation on behavior from those of the underlying pathology. For this study, sleep of adult male and female mice was fragmented (SF) for 2 months and expression of APP was induced for three months (subclinical expression). The order of these manipulations was alternated and temporarily independent (APP expression and SF). Subsequently, behavior of experimental animals and their respective controls was evaluated with open field arena (OFA), elevated plus maze (EPM), fear conditioning tests (FC) and correlated with synaptic function (L-LTP). The FC test revealed a sex-specific effect on cued long-term memory (LTM) along with sex-unspecific impaired synaptic plasticity. LTM impairment was potentiated in female mice (APP ON + SF) compared to all other experimental groups. These results demonstrate differential sensitivities of cognition following the combination of subclinical APP expression and sleep fragmentation in mice. Furthermore, our data suggest utility of this inducible mouse model to elucidate mechanisms linking these two phenomena.

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**Title:** Sex-dependent impairments of parvalbumin expressing neurons in the retrosplenial cortex in Alzheimer’s disease

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**Abstract:** Alzheimer’s disease (AD) is the most common form of dementia and both the incidence of this disease and its associated cognitive decline disproportionally effect women. The retrosplenial cortex (RSC) is of particular interest in the study of AD as, in humans, disrupted metabolic activity of the RSC correlates with the progression of cognitive impairment in AD. Given sex differences in onset and transition to AD, we were interested in examining potential sex differences in RSC impairments in the 5xFAD mouse model of AD. Early hypometabolic and hyperexcitable RSC phenotypes associated with clinical AD presentation was identified in female 5xFAD mice. Whole-cell patch-clamp of cells in the RSC revealed impaired electrophysiological properties of fast-spiking parvalbumin (PV) interneurons in female but not male 5xFAD mice. Non-fast spiking inhibitory interneurons were not impaired. Subsequent histology also revealed decreased PV interneuron density and decreased presynaptic PV boutons in female 5xFAD mice across the RSC. Chemogenetic inhibition of PV interneurons in the RSC was sufficient to induce cognitive impairment in young, naïve, C57BL/6J mice. Chemogenetic

stimulation of PV interneurons in the RSC of 5xFAD mice restored cognitive performance in female 5xFAD mice. These results suggest that the neuron types most vulnerable to the increased metabolic stress associated with early AD progression may be those with high energetic demands, such as PV interneurons. Preferential disruption of PV interneurons in the female brain during AD progression may explain the sex differences underlying cognitive decline.

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**Title:** Elucidating the effect of Bisphenol A in Alzheimer’s disease using label-free nonlinear optical microscopy

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**Abstract:** Alzheimer's disease (AD) is a neurodegenerative disorder caused by progressive brain cell degeneration, and influenced by a combination of genetic, environmental, and lifestyle factors. The aggregation of the Amyloid-Beta (Aβ) protein, leading to excitotoxicity and cell death, is considered a primary factor in the pathogenesis of AD. These aggregates serve as markers and aid in the diagnosis of AD. Recent studies suggest that environmental toxins such as endocrine-disrupting compounds (EDCs) are associated with a high risk of AD. Bisphenol A (BPA), commonly used in plastics, is an EDCs that can disrupt the normal functioning of the endocrine system by mimicking estrogen and disturbing hormonal balance, regulation, and homeostasis in the body. This hormonal disruption can have extensive effects on brain development and function. Animal studies have shown that early-life exposure to BPA can lead to alterations in neuronal connectivity and synaptic plasticity. Our lab has previously demonstrated that label-free nonlinear optical microscopy can provide unprecedented contrast in the AD brain, enabling precise identification of Aβ plaques. Through simultaneous label-free autofluorescence and multi-harmonic (SLAM) microscopy, we identified different morphological and molecular aspects of plaques (Fig.1A). In this study, we utilized SLAM microscopy to investigate the effect of BPA on AD progression in a murine AD model. Male and female mice were exposed to a high dosage of BPA in their drinking water and imaged at 22 weeks of age (mid-stage AD with substantial plaque formation). We examined four experimental groups: 1) no exposure, 2) pre-weaning exposure, 3) post-weaning exposure, and 4) pre- and post-weaning exposure. Analysis of plaque number and nonlinear signals revealed increased plaque numbers and larger plaque areas in the groups that received BPA water, suggesting BPA exposure exacerbates AD progression (Fig.1B-C).

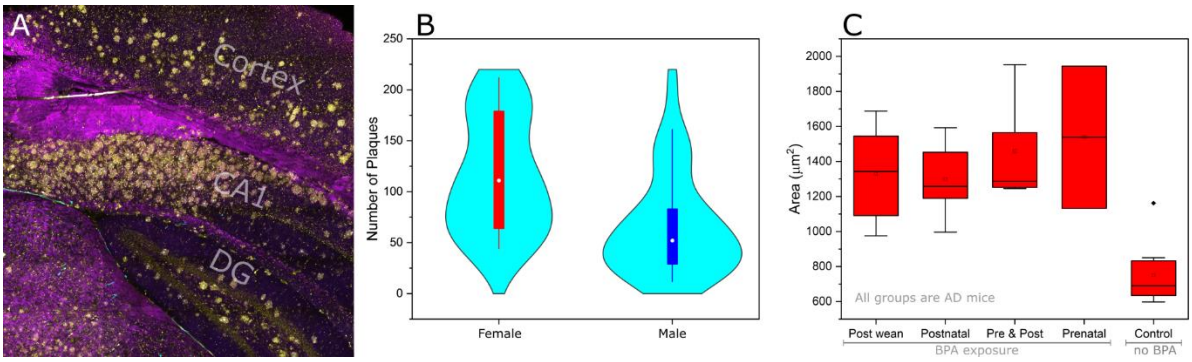


*Fig.1 (A) SLAM microscopy of an AD mouse received BPA. Number (B) and area (C) of Aβ plaques between different groups.*

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**Title:** Pathological tau differentially affects the composition and firing mode of neuronal subpopulations in a patient derived iPSC cortical neuronal model

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**Abstract:** Neural circuitry comprises different types of brain cells, which show distinct alterations during the progression of Alzheimer's disease and other tauopathies. Limited attention has been given to how pathological tau selectively influences the vulnerability and/or function of specific neuronal subpopulations. Using a human induced pluripotent stem cell (iPSC)-derived neuronal model, we comprehensively characterized changes in excitatory vs. inhibitory neurons with familial P301L tau mutation. We observed altered excitatory vs. inhibitory ratio in mutant neurons. Inhibitory marker GAD1 decreased by 42±8% ($p<0.0001$, t-test), while excitatory marker CaMK2a increased by 67±14% ($p=0.0002$, t-test) in mutant neurons, compared to the isogenic control. The fraction of inhibitory neurons with phosphorylated tau increased by 35±6% in mutant neurons ($p<0.0001$, t-test), but remained unchanged in excitatory neurons ($p=0.9956$, t-test). To evaluate the functional impact of mutant tau in these types of neurons, we conducted patch-clamp experiments. Mutant tau did not affect spontaneous firing in these neuronal subtypes. However, it significantly changed the firing modes of the evoked neuronal activity. When injected with a large depolarizing current, mutant excitatory neurons fired action potentials at a higher frequency than isogenic controls (isogenic vs. P301L: $p=0.002$, two-way ANOVA). The action potential waveform of mutant excitatory neurons had a larger afterhyperpolarization potential (isogenic: -16.9±1.6 mV, n=17; P301L: -21.3±1.4 mV, n=20, $p=0.043$, t-test) and shorter duration (isogenic: 1.05±0.06 ms, n=17; P301L:

0.89±0.05 ms, n=20, Mann-Whitney U test, p=0.014). In contrast, mutant inhibitory neurons fired action potentials at a lower frequency than isogenic controls (isogenic vs P301L: p=0.006, two-way ANOVA). Further analyses revealed that mutant inhibitory neurons fired spikes at a more depolarized threshold (isogenic: -32.4±1.7 mV, n=15; P301L: -24.2±1.9 mV, n=14, p=0.003, t-test), and finished with a larger afterhyperpolarization potential (isogenic: -15.9±1.9 mV, n=15; P301L: -27.5±1.5 mV, n=14, p<0.0001, t-test). In summary, these results indicate that tau-P301L affects neuronal composition as well as firing modes of excitatory and inhibitory neurons in this iPSC-derived tauopathy neuronal model. An altered excitatory/inhibitory balance is likely to be involved in neuronal circuitry dysfunction in Alzheimer's disease and other tauopathies.

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**Title:** Cell-type specific hippocampal changes are associated with memory deficits in novel Alzheimer's disease mice

**Authors: A. DEPRADA**[1,2], M. D. CAPILLA-LÓPEZ[1,2], M. MARCHENA[1], I. MARTÍNEZ-GALLEGO[3], A. RODRIGUEZ-MORENO[3], A. PARRA-DAMAS[1,2], *C. A. SAURA[1,2]; [1]Inst. de Neurociències, Univ. Autònoma de Barcelona, Bellaterra (Barcelona), Spain; [2]Ctr. de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain; [3]Physiology, Anat. and Cell Biol., Univ. Pablo de Olavide, Sevilla, Spain

**Abstract:** Excitatory/inhibitory neurotransmission imbalance due amyloid-β and tau pathologies has been postulated to underlie memory deficits in Alzheimer's disease (AD) but the cellular mechanisms by which these neuropathological hallmarks induce dysfunction of excitatory and inhibitory hippocampal neurons remain poorly understood. Here, we report differential deleterious effects of amyloid-β and tau on hippocampal-dependent memory and synaptic plasticity as well as cell type-specific pathology in novel APP/Tau transgenic mice expressing human familial AD-linked mutant amyloid precursor protein (APP) and microtubule-associated protein tau (MAPT) genes in glutamatergic neurons. Histopathological analyses reveal that Aβ and phosphorylated Tau are acumulated in excitatory neurons (CaMKIIα+) rather than inhibitory interneurons in the hippocampus of APP/Tau mice. Male and female Tau and APP/Tau mice show spatial learning and memory deficits, which are associated with reduced levels of synaptic proteins at excitatory synapses in the hippocampus. Importantly, synapse dysfunction coincides with the presence of tau pathology at synapses, as revealed by biochemical and expansion

microscopy analyses. Interestingly, APP/Tau mice show region-specific reduction and morphological changes of hippocampal inhibitory parvalbumin (Pvalb) interneurons, accompanied by age-dependent alterations in perineuronal nets (PNNs), the specialized extracellular matrix structures surrounding this neuronal population. Moreover, cell-type gene profiling using APP/Tau;Pvalb-Cre;RiboTag mice show transcriptional changes related to memory loss in Pvalb interneurons. In summary, APP/Tau mice show cell-type specific pathological alterations in memory neural circuits making this model a useful tool for studying the molecular mechanisms underlying selective cellular vulnerability in AD.

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**Title:** Activating TrkB and TrkC neurotrophin receptors prevents deficits in memory, synaptic plasticity, and synapse-associated gene expression in an amyloid mouse model of Alzheimer's Disease with late-stage pathology

**Authors: *A. LATIF-HERNANDEZ**[1], T. YANG[1], R. R. BUTLER, III[1], P. MORAN-LOSADA[1], P. S. MINHAS[1], H. WHITE[1], K. C. TRAN[1], H. LIU[1], D. A. SIMMONS[1], V. LANGNESS[1], S. M. MASSA[2], T. WYSS-CORAY[1], F. M. LONGO[1];
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**Abstract: Introduction:** Neurotrophin signaling via TrkB and TrkC receptors participates in long-term potentiation (LTP), a biological substrate of memory formation that is disrupted by amyloid-β (Aβ) in APP-mutant mice. Activating TrkB and/or TrkC could reduce Alzheimer's disease (AD)-related degenerative signaling, memory loss, and synaptic dysfunction. In this study, we ask whether there are alterations in activity-dependent protein and gene expression signatures in APP mutant mice, and if so, could elements of these alterations be restored by prior *in vivo* treatment with a small molecule TrkB/C partial agonist, PTX-BD10-2. **Methods:** PTX-BD10-2 (BD10-2), a small molecule partial agonist of TrkB and C or vehicle was administered daily for 3 months via oral gavage to London/Swedish-APP mutant (APP-L/S) and wild-type (WT) mice starting at age 13 months, an age exhibiting advanced pathology. One month after behavioral assays of memory, acute hippocampal slices were prepared at 24 hrs post-dosing. Theta Burst Stimulation (TBS) was applied to the slices to induce long-term potentiation (LTP). After recordings, the same hippocampal slices were used for RNA-sequencing to assess differential gene expression, and for western blotting and immunostaining to evaluate protein expression and activation. **Results:** Memory and LTP deficits were pronounced in vehicle-treated APP-L/S versus WT mice, and were attenuated after BD10-2 treatment. These effects

were associated with BD10-2's prevention of decreased activation of AKT, GluA1 and CaMKIIβ, and levels of PSD-95 and dendritic GluA1 observed in APP-L/S mice. In TBS-hippocampal slices from APP-L/S_VEH_TBS mice, genes in pathways known to be important for synapse function were significantly down-regulated while those in AD-related degenerative pathways were up-regulated compared to WT_VEH_TBS slices. Significantly, most of the altered gene expression patterns in these pathways were normalized to WT patterns in APP-L/S_BD10-2_TBS hippocampal slices. In addition, slices from APP-L/S_BD10-2_TBS mice showed enriched expression of memory-associated genes in Wnt, ERK and PI3/AKT pathways. **Conclusions:** Modulating TrkB and TrkC receptors with BD10-2 prevented Aβ-associated memory and LTP deficits and reduced abnormalities in structural and signaling components fundamental to synaptic function and synapse-relevant gene expression profiles. These findings suggest a therapeutic potential in targeting these Trks, and moreover, provide a cellular model to identify candidate signaling modules for understanding synaptic impairment and revealing novel therapeutic targets.

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**Title:** Specific physiologic and morphologic alterations in the hippocampal dentate gyrus mossy cells may constitute some of the earliest signs of hyperexcitability in the Tg2576 model of AD.

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**Abstract: Introduction.** Alzheimer's disease (AD) is a neurodegenerative illness characterized by progressive cognitive impairment and altered neural activity, associated with accumulation of amyloid beta (Aβ) and tau protein. Functional biomarkers like hyperexcitability have been described recently, which mainly characterize the early stages of AD, contributing to the further progression of the disease. However, the mechanisms involved are not clear. This project focuses on studying the physiological and morphological alterations of dentate gyrus (DG) mossy cells (MCs), which play a central role in the E/I balance on DG. In epilepsy, MCs contribute to hyperexcitability, but their role in AD has not been substantially explored.
**Methods.** MCs and GCs intrinsic and synaptic properties from wild type (WT) and Tg2576 mice at early ages (1-2 m.o.) were characterized by electrophysiological whole-cell patch-clamp recordings. Synaptic properties included the frequency and amplitude of spontaneous EPSPs and excitatory and inhibitory PSCs. MCs axonal distribution, soma size and pyknosis were evaluated using immunolabeling with calretinin and Nissl staining. Aβ accumulation was evaluated with the antibody McSA1, detecting intracellular and extracellular Aβ deposition.
**Results.** Compared with WT mice, MCs intrinsic properties from Tg2576 mice showed

depolarized RMP, reduced Tau, rheobase, AP peak amplitude, and time to peak ($p<0.05$ for all). The correlation between #APs generated by specific current injection showed Tg2576 MCs fired more APs ($z=-2.46$). Furthermore, Tg2576 MCs had augmented EPSP frequency ($p<0.05$), which correlates with reduced IPSC frequency and amplitude ($p<0.05$). Accordingly, increased expression of c-Fos was present in DG ($p<0.05$). No changes in cell area and pyknosis were found, but there was a significantly larger axon distribution in the DG IML ($p<0.05$). These changes suggest an increased E/I balance in MCs, which could cause increased GCs excitability and hippocampal hyperactivity. Aβ labeling showed robust intracellular aggregation in Tg2576 MCs, suggesting Aβ may underlie these alterations. Tg2576 GCs at 1 m.o. did not have any difference in their intrinsic properties. However, augmented IPSC and NMDA-mediated EPSC frequency ($p<0.05$) were found. These changes suggest that there is enhanced excitatory and inhibitory input to GCs in the 1-2 m.o. Tg2576 mice.
**Conclusion.** Aβ accumulation and physiological/morphological changes on MCs, occurring before GCs, suggest they may be the cause of increased excitability happening later in GCs, signifying MCs could be an important contributor to AD and be considered a new therapeutic target.

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**Title:** Compromised adult hippocampal neurogenesis contributes to memory impairments in APPKI preclinical mouse model of AD

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**Abstract:** Alzheimer's disease (AD), an age-related dementia characterized by the aberrant deposition of β-amyloid (Aβ) and neurofibrillary tangles leading to neuronal dysfunction, synaptic failure and ultimately progressive cognitive decline. Numerous studies have shown that adult hippocampal neurogenesis (AHN) is severely impacted in AD, both in rodents and humans and associated with learning and memory dysfunctions. Therefore, understanding the regulation of AHN could provide the framework for the rescue of memory impairments in AD. Transgenic AD mouse models widely used in the past decades share a common limitation of overexpression of APP and putative off-target effects, which could introduce artificial and non-clinically relevant phenotypes. Here, we analyzed the fate of AHN and its cognitive correlation in clinically relevant AD mouse model engineered to express normal physiological levels of APP

harboring humanized Swedish (K670N/M671L), Beyreuther/Iberian (I716F) and Arctic (E693G) mutations (App $^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$), termed APPKI mice. These mice showed a significant reduction in the pool of proliferating neural progenitor cells *in vitro* and *in vivo*, leading to reduced number of new neurons. Further, immunofluorescent staining and stereological analysis revealed that the density of newly mature neurons diminished in the neuronal ensemble activated during acquisition and retrieval phase of the memory formation, which may underlie for the poor performance of APPKI animals in hippocampus - dependent memory tasks. Additionally, the transcriptomic and epigenomic profiling of these newly born neurons in APPKI mice also showed altered molecular signatures responsible for memory impairments in these animals. Taken together, this study suggests that impairments in AHN is a contributing factor to learning and memory deficits in AD and implies that modulating adult neurogenesis is a potential therapeutic approach for AD-related cognitive decline.

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

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**Title:** Functional and molecular characterization of the hippocampal network failure during early amyloid pathology in the App$^{NL\text{-}G\text{-}F}$ mouse model of Alzheimer's disease

**Authors: *G. PIZZIRUSSO**[1], J. GOICOLEA[5], E. PREKA[2], C. AGUILAR RUIZ[6], P. RODRIGUEZ RODRIGUEZ[3], S. LATERZA[6], M. LATORRE LEAL[4], A. FRAGKOPOULOU[2], K. BLOMGREN[2], S. MAIOLI[7], P. NILSSON[6], A. FISAHN[6], L. ARROYO GARCÍA[1];
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**Abstract:** Alzheimer’s disease (AD) is a chronic multifactorial disorder characterized by progressive memory loss and cognitive impairment, preceded by a decades-long asymptomatic stage. The understanding of the mechanisms leading to cognitive decline and neurodegeneration in the late stages of the AD continuum remains minimal, which is reflected in the lack of successful and widely accepted disease-modifying treatment strategies to prevent the onset of dementia rather than slowing down its progression. Using a knock-in mouse model (App$^{NL\text{-}G\text{-}F}$), we have previously demonstrated that cognition-relevant gamma oscillations and GABAergic interneuron desynchronization in the hippocampus are the earliest detectable consequences of amyloidogenic progression, being apparent before widespread plaque load and behavioural changes. Here we aimed at characterizing this early network failure with a functional and molecular combinatorial approach. Using ex-vivo electrophysiology techniques, we discovered that the hippocampal network functionality, as judged by gamma oscillations, is progressively deteriorating in App$^{NL\text{-}G\text{-}F}$ mice until it reaches a breaking point at postnatal day (P)50 when it becomes significantly different from age-matched wild-type mice and younger mice from the App$^{NL\text{-}G\text{-}F}$ group. Additionally, patch-clamp recordings show that amyloidogenic progression

affects the intrinsic properties of a specific subgroup of GABAergic interneurons at this early stage. This provides for the first time a specific time window to investigate the functional and molecular changes happening in the early stage of amyloid pathology and potentially identify new targets that can prevent its progression. Indeed, we performed bulk RNA sequencing of the hippocampus with subsequent differential gene expression analysis and gene enrichment analysis in wild-type and $App^{NL-G-F}$ mice before (P30) and after (P60) the functional impairment. We found that multiple pathways involving inhibitory activity, synaptic communication, and microglial homeostatic and inflammatory responses change with the pathology progression in the $App^{NL-G-F}$ model. These transcriptional changes have been further investigated at the protein level by western blot and immunofluorescent staining. To our knowledge, this is the first functional and molecular study focused on hippocampal network dysfunction during the early stages of amyloidogenic progression. This understanding can provide new biomarkers and possible targets for the development of a new therapeutic approach to AD.

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**Title:** Decoding memory dysfunction in the neurons of entorhinal cortex and hippocampus of Alzheimer's disease mice

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**Abstract:** The entorhinal cortex and hippocampus (EC-HPC) are intimately involved in several memory functions, including spatial memory - a cognitive process affected early in Alzheimer’s disease. In this presentation we will show how amyloid beta and tau pathologies affect neuronal and network function. We will demonstrate how specific electrophysiological changes can be identified in the EC-HPC circuit using in vivo recordings in mice. Mouse models of Alzheimer's disease (App and Tau knockins) were implanted with electrodes in the hippocampus or the entorhinal cortex and recorded extracellularly to obtain single units and local field potentials. The data was analyzed to identify changes in properties of specific cell types such as grid cells, border cells, head direction and object cells in the EC and place cells in the hippocampus. Network activity was also analyzed to identify dysfunction in specific oscillation frequencies. We show that amyloid beta and tau pathologies have a detrimental impact on the neuronal and network function which possibly leads to impaired coding of neurons responsible for spatial and

non-spatial memory function. Specifically, we found that firing properties of hippocampal neurons (place cells), MEC neurons (grid cells) and LEC neurons (object, trace and odor-responsive cells) were affected. Analysis of high frequency oscillation revealed that network function was also affected. We also show that tau pathology in locus coeruleus- a region of the brainstem involved in arousal and sleep- has a detrimental impact on the downstream hippocampal neurons leading to reduced spatial information and unstable place fields. Finally, we show how spatial decoding using machine learning techniques could help identify subtle neuronal changes to help diagnose AD earlier than current methods and could potentially translate into a digital biomarker for clinical use.

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

**Support:** NIH grants 1RF1AG061706-01

**Title:** Targeted in vivo proximity labeling reveals synaptic dysfunction during early onset in $APP^{NL-G-F}/MAPT^{P301S}$ mouse model of Alzheimer's disease.

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**Abstract:** To investigate the molecular basis of synaptic dysfunction during early onset of Alzheimer's disease, we have applied TurboID driven proximity labeling workflow to identify altered protein-protein interactions in vivo. Using TurboID conjugated to key SNARE protein SNAP25, we demonstrated target selective biotinylation and recovery of both well described and previously unreported auxiliary proteins associated with neurotransmission. We have further used this chemico-genomic sensor to identify synaptic changes in protein interaction landscape during early disease onset in $APP^{NL-G-F}$/ $MAPT^{P301S}$ mouse model of Alzheimer's disease. Our results specifically suggest differential regulation in synaptotagmin family of calcium sensing proteins in the synapse. This leads to behavioral anomalies and synaptic transmission defects in mutants, validated by independent electrophysiology measurements. Moreover, our data Indicates Tau mediated dysfunction in neuronal protein homeostasis and lysosomal function under cellular stress.

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**Title:** Gabaergic interneuron-microglia crosstalk in a mouse model of neurodegenerative disease

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**Abstract:** Neurodegenerative diseases start developing years before symptoms appear, as the brain maintains cognitive function despite ongoing neurodegeneration. This makes it highly challenging to timely detect early functional impairments, leading to late interventions and prevalent failure in treatment approaches. Thus, it is imperative to find mechanisms that affect neuronal function during early pathological exposure. Recently we found that a subtype of GABAergic interneurons called “fast-spiking interneuron (FSI)” are functionally compromised in the early stages of the amyloidogenic progression in animal models of Alzheimer´s disease (AD). However, still today, there is no information on the mechanisms that drive the FSIs to functional impairment. Interestingly, new data has shown that microglia, the resident immune cells in the brain, interact with the somatic part of neurons to monitor and protect their function. Moreover, by targeting microglia inflammatory response we can recover to an extent FSI functionality and correlate with cognitive improvement in AD-like pathogenesis. Here, by using ex-vivo electrophysiological techniques, hippocampal bulk RNA sequencing, and immunohistochemistry, we investigate GABAergic interneuron-Microglia crosstalk in a knock-in mouse model of AD ($App^{NL-G-F}$). Our results show that microglia homeostatic-, and inflammatory-related pathways are decreased in pre-stages of high plaque deposition in the hippocampus. We found an impairment in the intrinsic properties of the FSI that impact their spike-timing function. Moreover, we found a decrease in the “surveillance” microglia to the FSIs in the CA3 area of the hippocampus. In conclusion, our study shows a loss of microglia-homeostatic surveillance to FSI in a critical stage of the pathology progression. This could serve as a target in the clinic to maintain or recover neuronal function during pathological events.

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

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**Title:** Developing a Tau Rhesus Monkey Model of Alzheimer's Disease

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**Abstract:** Our understanding of the pathological events in Alzheimer's Disease (AD) has advanced vastly in recent years, but successful translation from rodent models into efficient human therapies has been minimal. We recently described that injection of adeno-associated viruses expressing mutant tau (P301L/S320F) into the entorhinal cortex induces extensive tau pathology in the hippocampus of rhesus monkeys 3 months after injections. In the current study, MRI, Tau PET (18F-APN-1607), CSF, and plasma biomarkers were combined with quantitative microscopic analyses to longitudinally characterize tau propagation and neuroinflammation in the brain of treated rhesus monkeys at 3 and 6 months after viral delivery. Furthermore, a quantitative 3D analysis of misfolded tau and reactive glia was performed, revealing that a direct interplay between microglia and astrocytes helps coordinate misfolded tau spreading progression. Combining imaging and fluid biomarkers with high-resolution microscopy, we detailed the initial steps of misfolded tau spreading and neuroinflammation in a monkey model highly translatable to AD patients.

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**Nanosymposium**

**NANO15: Molecular and Cellular Mechanisms of Ischemia**

**Location:** WCC 146C

**Time:** Sunday, November 12, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO15.01

**Topic:** C.08. Ischemia

**Support:** EU ERDF KK.01.1.1.07.0071
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**Title:** Monitoring in vivo activity of Tlr2 promoter by bioluminescence imaging after ischemic brain lesion in the TLR2-deficient mice showed reduced neuroinflammation and its relation to the neurological recovery

**Authors:** S. SRAKOCIC, P. JOSIC, M. RADMILOVIC DOBRIVOJEVIC, S. SKOKIC, A. GLASNOVIC, ***S. GAJOVIC**;
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**Abstract:** Inflammatory response after ischemic brain injury is mediated by Toll-like receptors (TLR) located on the microglia. To monitor post-stroke inflammation longitudinally using *in vivo* bioluminescence we developed a novel mouse line with TLR2 deficiency that (although not having functional TLR2 protein) expresses luciferase under the *Tlr2* gene promoter. This allowed to characterize *in vivo* neuroinflammation following ischemia in TLR2-deficient mice. TLR2-deficient and WT control mice underwent 30 minutes tMCAO (transient middle cerebral artery occlusion) followed by reperfusion. The animals were imaged at multiple time points after tMCAO by magnetic resonance imaging using a 7T BioSpec 70/20 USR MRI system and by bioluminescence imaging with optical imager Perkin Elmer IVIS Spectrum to monitor the progression of ischemic damage and TLR2-mediated inflammation, respectively. Neurological deficits were also assessed. Flow cytometry and immunohistochemistry were used to determine microglial inflammatory markers, while cytokine levels in blood plasma were measured using multiplex ELISA assay LegendPLEX™. TLR2-deficient mice showed lower inflammation by in vivo imaging, which could be confirmed by cellular and tissue analysis, and which was reflected by the status of the inflammation markers in the blood. Statistical modeling by linear regression demonstrated the relation between ischemic damage and inflammation, as well as beneficial impact of inflammation reduction rate on the neurological recovery. In conclusion, TLR2 deficiency reduced the inflammatory processes following ischemic brain injury. Moreover, they had a positive impact on the neurological recovery rate after brain ischemia in the adult mice.

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**Presentation Number:** NANO15.02

**Topic:** C.08. Ischemia

**Support:** DBT/PR21413/MED/122/40

**Title:** Evaluating the neuroprotective role of astrocytes after hypoxic injury by evaluating glial cholesterol synthesis in vitro

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**Abstract:** Perinatal hypoxic brain injury leads to maturation arrest in developing oligodendrocytes (OL) in the fetal brain, resulting in loss of myelination, and long-term neurological deficits. In preterm infants, hypoxic injury to vulnerable pre-myelinating oligodendrocytes (OL) results in a failure of differentiation of these cells to mature myelinating OL. As OL maturation is influenced by neighboring astrocytic cells providing trophic and nutritional support, this study aimed to evaluate the changes in astrocytes after hypoxic injury, eventually identifying possible mechanisms influencing myelination. Human fetal neural stem cell-derived astrocytes were characterized by the increased expression of astrocyte-specific glial

fibrillary acidic protein (GFAP) and excitatory amino acid transporters (EAAT1 and EAAT2) using qPCR and flow cytometry. Differentiated astrocytes were exposed to severe hypoxia (0.2% oxygen) and normoxia (20% oxygen) for 48 hours. Hypoxia exposure was validated by the increased expression of hypoxia-responsive protein HIF-1α. Transcriptomic changes in hypoxic astrocytes were identified by RNA-sequencing and these findings were validated in primary FNSC-derived astrocytes by qPCR and western blotting. Hypoxic astrocytes showed increased expression of HIF-1α on western blot (n=3). RNA-sequencing (n=3 each for hypoxic and normoxic astrocytes) analysis showed the upregulation of pathways relating to cholesterol synthesis in hypoxic astrocytes. On further validation, expression of HMG-CoA reductase, squalene epoxidase, cholesterol transporter ABCA1 and apolipoprotein E, were increased in hypoxic astrocytes, as seen by qPCR and western blotting (n=5). Astrocytic cholesterol content and efflux were increased after exposure to hypoxia as measured using enzyme-based assays (n=4). Further, labelling astrocytic cell line (SVG) with fluorescent BODIPY-cholesterol showed transfer of astrocytic cholesterol to cocultured premyelinating OL cell line (Mo3.13). Differentiating pre-myelinating OL into mature OL (using 100 nM PMA, over 7 days) showed increased expression of HMG CoA reductase by western blot (n=2), thereby indicating that cholesterol is essential for OL maturation and myelination. Interestingly, hypoxic injury decreased the expression of HMG CoA reductase in premyelinating OL exposed to hypoxia (on western blot: n=2). Upregulation of astrocytic cholesterol synthesis and its efflux could potentially supplement oligodendroglial maturation and myelination, and could be an important neuroprotective mechanism in perinatal hypoxic brain injury.

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AHA 23TPA1069224

**Title:** Protein arginine methylation in serum/glucocorticoid regulated kinase 1-mediated ischemic brain injury

**Authors:** Y. ZHANG[1], P. ZOU[1], L. XU[1], C. Y.-C. WU[2], ***R. LEE**[1];
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**Abstract:** Cardiopulmonary arrest (CA) is a major cause of death in the U.S. with poor prognosis and survival rates. The current CA therapeutic challenges are physiologically complex because they involve hypoperfusion (decreased cerebral blood flow) and neuroinflammation. Our long-term goal is to identify these complex regulatory elements that ultimately control neuronal survival. We previously discovered novel serum/glucocorticoid-regulated kinase 1 (SGK1) is highly expressed in brain regions susceptible to ischemia (hippocampus and cortex). Inhibition of SGK1 via GSK (specific inhibitor) alleviated CA-induced hypoperfusion, neuroinflammation, neuronal cell death, and learning/memory deficits; this suggests SGK1 is detrimental during ischemia. This mechanism underlying SGK1-mediated brain injury is

unknown. Our preliminary data indicate protein arginine methyltransferase 1 (PRMT1, an enzyme that catalyzes methylation of arginine residues of histone/proteins) levels in the hippocampus were decreased with enhanced SGK1 levels after CA. Intriguingly, inhibition of SGK1 via GSK increased PRMT1 levels results in favorable neuronal survival and neurological outcomes. To explore the potential role of PRMT1 in SGK1-mediated brain injury following CA, we manipulated both SGK1 and PRMT1 levels following CA using pharmacological approaches (specific SGK1 and PRMT1 inhibitors). Intra-vital two-photon laser scanning microscopy, laser speckle contrast imaging, brain histology, and protein chip assays revealed that pre-treatment with specific SGK1 inhibitor, GSK 650394 (1.2 μg/kg, intracerebroventricular injection), increased PRMT1 levels against cortical hypoperfusion and neuroinflammation after CA. Interestingly, neuroprotection of GSK 650394 was abolished by specific PRMT1 inhibitors (TC-E5003 or C21) suggesting that SGK1 causes hypoperfusion and neuroinflammation via downregulation of PRMT1. Finally, rodents' neurological outcomes after CA were evaluated using Y maze. Rats pre-treated with GSK 650394 exhibited better neurological outcomes following CA as compared to untreated animals. Neuroprotection of GSK was eliminated in the present of PRMT1 inhibitor (TCE or C21). In conclusion, PRMT1 protein levels are attenuated via SGK1 following CA, which leads to hypoperfusion, neuroinflammation, neuronal cell death, and functional learning/memory deficits. Since the FDA has approved over 46 kinase-related drugs for the treatment of various diseases, targeting SGK1/PRMT1 as therapeutics will be promptly translated into human clinical trials for the patients suffering from CA.

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**Presentation Number:** NANO15.04

**Topic:** C.08. Ischemia

**Title:** Tenascin-c expression in microglia is induced by par-1 signaling in the post-ischemic brain

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**Abstract:** Stroke is one of the most common causes for mortality and morbidity worldwide. Following acute stroke onset, biochemical and cellular changes induce further brain injuries such as neuroinflammation, cell death, and blood-brain barrier disruption. As the resident immune cells of the central nervous system, microglia rapidly respond to brain insults, including stroke and traumatic brain injury. Evidences suggest that Protease Activated Receptor-1 (PAR-1) mediates neuronal injury in cerebral ischemia. We examined if PAR-1 signaling upregulates the expression of tenascin-C (TNC) in microglia. TNC, an extracellular matrix protein, is considered to be an important inducer in promoting neuroinflammatory cascades and the resultant pathology in stroke. We found that TNC is highly expressed in cultured primary microglia treated with PAR-1 peptide, which can be effectively blocked with PAR-1 antagonist. Upregulation of TNC exocytosis upon PAR-1 activation in microglia was also observed. To examine the expression of TNC in microglia in the ischemic brain, mice were subjected to 1.5 hours of transient middle cerebral artery occlusion (MCAO), followed by 6, 12, or 24 hours of reperfusion. Consistent with results from cultured microglia, a high level of expression of TNC was observed in activated microglia in the ischemic brain. These results suggest that PAR-1 signaling plays an

important role in neuronal injury in cerebral ischemia via inducing TNC expression and exocytosis in microglia.

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**Topic:** C.08. Ischemia

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**Title:** Microglia Utilize RIPK2 to Promote Stroke Injury

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**Abstract: Introduction**: Microglia are uniquely positioned to respond to brain injury resulting from ischemic stroke. Pattern recognition receptors (PRRs) allow microglia to respond rapidly to molecular patterns and elicit a prompt immune response. Dead/dying neurons release damage-associated molecular patterns (DAMPs), which are recognized by PRRs on immune cells, facilitating a rapid immune response to ischemia-induced cell death/cellular stress. Receptor-interacting protein kinase 2 (RIPK2) serves as a signaling serine/threonine kinase for members of the NOD family of intracellular PRRs. Our previous studies have identified a protective effect of *Ripk2*-global deficiency after stroke. We hypothesize that microglia play a role in the progression of stroke injury through utilization of RIPK2 and that specifically deleting *Ripk2* from microglia will result in beneficial outcomes for mice after stroke. **Methods**: We generated *Ripk2*$^{fl/fl}$;*Tmem119*$^{CreERT2/wt}$ (microglia-specific knockout, μKO) and *Ripk2*$^{fl/fl}$;*Tmem119*$^{wt/wt}$ (WT) mice (n=12/group, male mice) and subjected them to 45min-transient middle cerebral artery occlusion (tMCAO) following tamoxifen injection and 7d washout period. We then subjected these mice to a battery of behavioral tests, including open field, weight test, vertical grid test, and neurological deficit scoring at 24h and 48h-post stroke. We calculated the area of infarction at 48h using 2,3,5-triphenyltetrazolium chloride (TTC) staining and performed an edema calculation. Western blotting for MMP-9 (n=6/group) and ELISA for albumin (n=8/group) were performed using the ipsilateral cortex (CXI) tissue lysate derived from the area of infarction. Finally, we subjected CXI RNA from WT and μKO mice to a nanoString® neuroinflammation panel (n=6/group). Analyses and experiments were performed by blinded investigators. **Results**: μKO mice displayed smaller total infarct compared to WT at 48h post-stroke, concurrent with a reduced edema index. μKO mice had significantly lower levels of active MMP-9 and cortical albumin in the CXI compared to WT mice at 48h post-injury. μKO performed significantly better on behavioral tests during the acute phase of stroke injury compared to WT mice. We discovered a total of 31 differentially expressed genes in the CXI between the two genotypes, with decreases in genes associated with astrocytic function and inflammatory/cytokine signaling being the most greatly affected. **Conclusion**: This study identifies RIPK2 as a pathogenic protein utilized by microglia to promote post-stroke injury and that specifically deleting *Ripk2* from microglia improves outcomes for mice after ischemic stroke.

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**Title:** Microglial laminin repairs blood-brain barrier damage and modulates microglial biology in stroke brain

**Authors:** A. NIRWANE, ***Y. YAO**;
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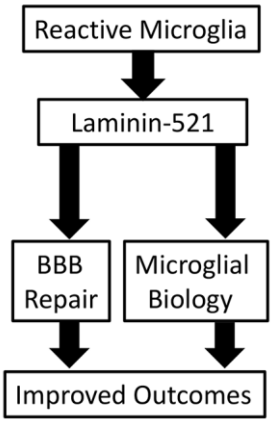
**Abstract:** Laminin, a major component of the vascular basement membrane, actively maintains the blood-brain barrier (BBB) integrity. Although vascular cells have been shown to synthesize and deposit laminin into the BM, it remains unclear whether microglia make laminin. Using an innovative cell-specific laminin reporter mouse line and in-situ hybridization, we reported that reactive rather than resting microglia produce laminin-521. To investigate the function of microglial laminin, we further generated microglial laminin conditional knockout mice. These mutants were grossly normal under homeostatic conditions, but developed exacerbated injury and all died by day 5 after ischemic stroke. Specifically, the mutants showed aggravated BBB damage, enlarged ischemic volume, and worse neurological outcome. Subsequent studies revealed that the enhanced BBB disruption is mainly mediated by the transcellular rather than paracellular transport. In addition, the mutants also showed abnormal microglial biology, including reduced microglial number, proliferation, and survival in the penumbra. The mutant microglia also exhibited diminished anti-inflammatory polarization, although pro-inflammatory polarization was unaffected. Furthermore, RNAseq analysis was performed to explore the underlying molecular mechanism. Compared to the controls, mutant microglia displayed a type-I interferon phenotype by up-regulating several key genes in the interferon signaling pathway. Interestingly, exogenous laminin-521 significantly attenuated BBB damage, reduced ischemic volume, reversed microglial changes, and improved stroke outcome in the mutants after stroke. Together, these results suggest that microglia-derived laminin plays a neuroprotective role in stroke by repairing BBB damage and regulating microglial biology (proliferation, survival, and polarization), and that microglial laminin may be targeted for the treatment of stroke.

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**Topic:** C.08. Ischemia

**Title:** Microglial&Macrophagic sik3 regulates phagocytosis through complemental system after ischemic stroke

**Authors: *C. WANG**, K. WANG, Y. GAO;
Fudan Univ., Shanghai, China

**Abstract: Background**In many central nervous system diseases, activated microglia/macrophages play a role of biphasic phagocytosis. Salt inducible kinase-3 (SIK3) is a kinase which highly expresses in microglia/macrophages after ischemic stroke then causes inflammatory responses. Knockdown SIK3 in microglia/macrophages converts microglia/macrophages anti-inflammatory heterogenization, then shifts excessive phagocytosis to normal phagocytosis and alleviate white matter injury. As widely known, complemental system regulates certain important biological functions, such as inflammation, phagocytosis and sterilization. Regulation of complement pathways' beginners and receptors may be a target for microglia/macrophages normal phagocytosis. **Aim**Since C1q and CR4 polarized microglia/macrophages to anti-inflammatory phenotype, we inferred maybe they were new targets which regulate normal phagocytosis in ischemic stroke. **Methods**In this research, we analyzed public single-cell RNA-seq datasets from the brain of ischemic stroke mice. We used microglia SIK3 conditional knockout (SIK3-cKO) mice and in vitro primary microglia culture to investigate the molecular mechanism of SIK3 on microglia phagocystois induced by transient focal cerebral ischemia. **Results**By scRNA-seq, we inferred that upregulating CR4 in microglia/macrophages would be a target for shifting excessive phagocytosis to normal phagocytosis. By immunoflurescence and in vitro experiments, we observed that upregulating C1q/CR3/CR4 by SIK3-cKO promoted microglia/macrophages phagocytosis. On the one hand, SIK3-C1q-CR3/CR4 enlarged contact area between microglia/macrophages and neuronal cell bodies after ischemic stroke. On the other hand, SIK3-C1q-CR3/CR4 enhanced the ability of microglia/macrophages normal phagocytosis of myelin debris. The progress of shifting excessive phagocytosis to normal phagocytosis preserved myelin integrity, then alleviated white matter injury in acute stage and long-term. **Conclusion**In this study, we investigated that SIK3

regulated normal phagocytosis and excessive phagocytosis through SIK3-C1q-CR3/CR4 pathway.

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**Title:** Oral contraceptive treatments increase cerebral sphingolipid and ceramide metabolites in female rats

**Authors: *S. SHARMA**[1], H. PRADHYUMNAN[1], S. H. PATEL[1], A. P. RAVAL[1,2]; [1]Peritz Scheinberg Cerebral Vascular Dis. Res. Labs. (CVDRL), Dept. of Neurol., Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; [2]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

**Abstract:** The Centers for Disease Control and Prevention estimates that 64.9% of women between 15 and 49 years of age use some form of contraception. In the United States, combined estrogen and progesterone oral contraceptive (OC) pills are the most commonly used form of contraception, and hence was the focus of the current study. Studies show that OC is associated with an increased risk of stroke, especially during the first year of use, potentially due deviation from hemostatic balance. Importantly, this study provides new insights that the pronounced effects of OC usage are seen in the beginning of treatment apart from long-term OC usage, which is an independent risk for myocardial infarction, venous thromboembolism, stroke, and more, most prevalently in smokers. OC exposure makes females more susceptible to stress hormone effects on episodic memory, fear conditioning, and cognitive emotion regulation. Our understanding regarding the contribution of short-term OC exposure on the severity and consequence of stroke injury is sparse, and hence is one of the goals of the current study. Adolescent and adult Sprague-Dawley female rats were randomly (n = 8/group) exposed to either placebo or OC for 16-21 days. At the end of the treatment, brain tissue was harvested to obtain an unbiased global metabolomic profile (performed by Metabolon Inc.) The metabolomic study was complemented with western blot analysis and enzyme activity measurements of key altered pathways. Metabolomics data using pathway enrichment analysis showed significant changes in sphingolipid metabolism. Sphingosine 1-phosphate (S1P) significantly ($p<0.05$) increased in the OC exposed brains as compared to placebo groups. The changes were more pronounced in adolescent rats as compared to adult. A growing body of literature implicates S1P as “a double-edged sword” in the pathogenesis of brain-related disorders [1]. Furthermore, S1P plays a key role in synaptic transmission, neuronal autophagy, and neuroinflammation [1]. Preliminary results indicate that there are four main proteins potentially affected by OC: Sphingosine Kinase 1 (SphK1), Sphingosine Kinase 2 (SphK2), S1P Receptor (S1PR1), and S1P Lyase (S1PL). Discerning the exact effects of OC on overall brain metabolism, and S1P metabolism, at different ages and for both long term and short-term periods of time will help us understand stroke risks in OC users. References: [1] Karunakaran, I. and van Echten-Deckert, G. Sphingosine 1-phosphate - A double edged sword in the brain. Biochim Biophys Acta Biomembr, 2017. 1859(9 Pt B): p. 1573-1582.

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**Title:** Spak inhibitor zt-1a improves cognitive function by preventing astrogliosis and oligodendrocyte cell deaths in a mouse model of vcid

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**Abstract: Background:** The key features of vascular contributions to cognitive impairment and dementia (VCID) are reactive astrogliosis, BBB breakdown and myelin loss. However, the underlying molecular and cellular mechanisms of VCID are not well understood and no treatment is available. Stimulation of Na-K-Cl cotransport 1 (NKCC1) and its downstream kinase SPAK (the STE20/SPS1-related proline/alanine-rich kinase) contribute to intracellular $Na^+$ overload, astrocytic hypertrophy and swelling. In this study, we investigated the effect of SPAK inhibitor ZT-1a on reactive astrogliosis, oligodendrocyte (OL) cell death, and cognitive function in a mouse model of VCID with bilateral carotid artery stenosis (BCAS). **Methods:** BCAS was induced in male mice by suture ligation of both carotid arteries (CA) guided by a needle or by using two metal micro coils twined around both CA. Sham or BCAS mice receiving vehicle or a selective SPAK inhibitor ZT-1a were monitored for changes in the regional cerebral blood flow (CBF) and neurological and cognitive functions by the Morris water maze (MWM) test. Expression of SPAK-NKCC1 cascade proteins, characterization of astrocyte polarization (A1/A2), and OL cell deaths were examined by western blotting and immunofluorescence staining. **Results:** BCAS mice displayed chronic CBF reduction and cognitive function deficits, along with significantly reduced myelin basic protein in the corpus callosum, and external capsule. Compared with the Sham control mice, the BCAS mice showed increased expression and activation of WNK-SPAK-NKCC1 signaling, an increase of $NKCC1^+GFAP^+$ astrocytes, $C3d^+$ cytotoxic A1 astrocytes, oligodendrocytes cell death, and decrease of $S100A10^+$ neurotrophic A2 astrocytes, in white matter tracts. Interestingly, early inhibition (2-4 wks) or delayed inhibition (4-8 wks) of SPAK kinase with ZT-1a prevented BCAS-induced increased NKCC1 expression, astrogliosis, OL cell deaths, and significantly improved CBF and cognitive functions. **Conclusion:** Our study demonstrates that the SPAK-NKCC1 cascade is activated in BCAS brains and contributed to the polarization of astrocytes to cytotoxic, OL cell deaths, and cognitive impairment. Pharmacological inhibition of the SPAK-NKCC1 cascade has therapeutic potential for VCID therapy. *This research was supported by NIH R01 166199 Grant (M.I.H.B.).*

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**Topic:** C.08. Ischemia

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**Title:** Role of STAT1 in ischemic preconditioning-induced microglial innate immune signaling

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**Abstract:** Ischemic preconditioning (IPC) is a robust protective phenomenon whereby brief ischemic exposure confers cyto- and axonal protection against a subsequent ischemic challenge. Innate immune pathways, notably Toll-Like Receptor 4 (TLR4) and type I interferon (IFN) signaling, specifically in microglia are critical in establishing this protection in both grey and white matter. Our previous in vivo studies demonstrated that type 1 IFN receptor (IFNAR1) in microglia is required for both IPC-induced axonal protection and interferon stimulated gene (ISG) expression. We also showed that exposure of cultured primary microglia to either IFN-beta or ischemia/reperfusion (I/R) results in phosphorylation of signal transducer and activator of transcription 1 (STAT1), a key signaling kinase downstream of IFNAR1. Here we report the impact of systemic STAT1 knock-out on IPC-induced microglial transcriptomic responses in vivo. We performed a transient (15 min) middle cerebral artery occlusion (MCAO) on wild-type (WT) and *Stat1*$^{-/-}$ mice and collected cortical tissues 72 hours later for single nucleus RNA-sequencing (snRNAseq) to identify microglial sub-cluster changes and gene expression changes (including ISGs). We describe global transcriptomic changes in microglial subpopulations in response to IPC and how STAT1-deficiency alters these responses. We also further characterize the impact of I/R, type 1 IFN signaling, and TLR4 stimulation on STAT1 signaling on microglia in vitro. We show that phosphorylation of STAT1 in microglia in response to I/R is dependent on both TLR4 and IFNAR1. We determine the time course of STAT1 phosphorylation and dephosphorylation in response to IFN-beta. We show that phosphorylation of STAT1 is also triggered by activation of TLR4 on a delayed time course compared to stimulation with either IFN-beta or I/R. The in vitro data suggest that TLR4 activation may concurrently lead to type I IFN signaling in microglia either via autocrine/paracrine mechanisms through the release of type I IFNs or via an alternative ligand-independent pathway directly activating IFNAR1. The findings also demonstrate novel dynamics of STAT1 signaling in microglia after exposure to a variety of stimuli and show that type I IFN signaling is robustly induced in microglia via multiple pathways. Overall, the results support a central role for STAT1 as a key mediator of microglial type 1 IFN signaling in both IPC and I/R.

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**Topic:** C.08. Ischemia

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**Title:** Genetic or pharmacological antagonism of system $x_c^-$ elicits ischemia-induced long-term potentiation

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**Abstract:** Stroke remains the most prevalent cause of adult disability in the world. Decreased blood flow (ischemia) to cerebral tissue can generate two distinct pathological regions: the ischemic *core* and the ischemic *penumbra*. The severe oxygen deprivation within the ischemic core precipitates excitotoxicity and rapid cell death, whereas hypoxic penumbral tissue can exhibit adaptations in neuronal excitability which may promote recovery. The cystine/glutamate antiporter, system $x_c^-$, with specific subunit xCT, supplies 60-80% of ambient extracellular glutamate in the brain**.** We previously employed hippocampal slice electrophysiology to reveal that tonic, system $x_c^-$ secreted glutamate drives the rapidity and synchrony of depolarizing events after total oxygen deprivation (anoxia) and thus exacerbates the ischemic core. Both genetic deletion and pharmacological antagonism of system $x_c^-$ provided ischemic neuroprotection by increasing latency to anoxic depolarization. The current study sought to extend these findings by scrutinizing system $x_c^-$ function during hypoxia as a means to explicate the antiporter's role in penumbral tissue. Hippocampal slices prepared from male WT and xCT KO ($xCT^{-/-}$) mice were challenged with an episode of hypoxia ($25\%O_2$ / $75\%N_2$), reoxygenated, and allowed to recover. Surprisingly, although the hypoxia-induced depression of synaptic transmission did not differ between genotypes, $xCT^{-/-}$ slices exhibited post-hypoxic long-term potentiation (LTP) upon reoxygenation. This initial finding was paradoxical as $xCT^{-/-}$ mice maintain decreased ambient glutamate concentrations: lower glutamate levels would tend to dampen synaptic transmission, not potentiate it. Remarkably, this LTP-effect was not driven by enhanced presynaptic glutamate release probability nor an adenosine-mediated pathway; moreover, theta-burst stimulation (TBS)-induced LTP was equivalent between genotypes. Post-hypoxic LTP in $xCT^{-/-}$ mice was, in fact, elicited by the activation of NMDARs and the influx of calcium, hence revealing a unique form of classical LTP. Importantly, the pharmacological inhibition of system $x_c^-$ in WT slices reproduced the post-hypoxic LTP observed in mutants. We conclude that system $x_c^-$ interference is an auspicious target for stroke therapeutics. Genetic or pharmacological antagonism of system $x_c^-$ provides neuroprotection for the ischemic core, where glutamate excitotoxicity is mitigated, as well as the ischemic penumbra, where post-hypoxic LTP is generated. Indeed, the current data uncover a possible underlying mechanism for improved post-stroke outcome in rodents when system $x_c^-$ is genetically deleted or inhibited.

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**Topic:** C.08. Ischemia

**Title:** Notch and p53-mediated regulation of cell death and glycolysis in ischemic stroke

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**Abstract:** Stroke remains a significant cause of mortality worldwide,leading to substantial morbidity. Notch, a well-characterized membrane receptorinvolved in cell differentiation and proliferation, has emerged as a criticalplayer in cell death during ischemic stroke. Inhibition of Notch signaling hasbeen shown to confer neuroprotection following stroke. Recent investigationshave shed light on the interplay between Notch and p53 under ischemicconditions. In this study, we aimed to investigate the mechanistic relationshipbetween Notch signaling and neuroprotection. Through pull-down analysis, we observed a significantincrease in the interaction between p53 and NICD1 (Notch1 Intracellular Domain)under oxygen-glucose deprivation (OGD) conditions. Furthermore, we found that the decrease inbinding between NICD1 and p53 in the OGD conditions resulted in a decrease inthe expression level of KFIS, a transcription factor. Subsequently, weconfirmed that the regulation of KFIS expression impacts cell death andglycolysis under OGD conditions. In addition, we confirmed this signaling in ananimal model of ischemic stroke.These findings underscore the importance of KFIS, regulatedby the interplay between Notch and p53, in the pathogenesis of ischemic stroke.Our study contributes to a better understanding of the molecular mechanismsinvolved in neuroprotection and suggests KFIS as a potential new therapeutictarget for stroke treatment. These insights may pave the way for thedevelopment of novel strategies to improve outcomes in patients suffering fromischemic stroke.

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**Nanosymposium**

**NANO16: The Functional Organization and Plasticity of the Ventral Stream of the Visual System**

**Location:** WCC 147A

**Time:** Sunday, November 12, 2023, 8:00 AM - 11:30 AM

**Presentation Number:** NANO16.01

**Topic:** D.06. Vision

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**Title:** The impact of strobe-rearing on the development of the macaque visual system

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**Abstract:** Experiencing temporal continuity of visual input or motion during early development plays an important role in the normal development of the visual system. Thus far, investigating

the impact of depriving animals of motion experience by rearing them in a stroboscopically illuminated housing room, has primarily focused on cats and other non-primates and targeted mainly the early visual cortex. The impact of strobe-rearing on the primate sensory system, particularly beyond the early visual areas, remains an open question. Here, we raised 2 infant macaques wearing helmets during the day fitted with optical shutters controlled by a small circuit that opens and closes at 1Hz (200ms open). Using such helmets allowed us to simulate stroboscopic vision, while maintaining a visually complex social environment, including interactions with other monkeys housed in the same room and humans. After 1.5 years of strobe-rearing, we conducted resting-state and task-based fMRI experiments under light sedation to investigate the brain-wide impact of strobe-rearing. We assessed visual motion responsivity by presenting 20s motion blocks where high-contrast random dot patterns changed motion direction every second and stationary blocks where random dots flashed on and off at 1Hz. Direction selectivity was assessed with an adaptation paradigm which included blocks of dot motion changing direction every second vs blocks where dot motion direction was constant for the full block duration. Resting state-fMRI indicated that area MT, a region critical for visual motion perception, showed largely typical correlations with striate and extrastriate visual, posterior parietal, and frontal cortices including LIP and FEF in strobe-reared monkeys. However, post hoc analysis suggested that while FEF showed significantly weaker correlations with the striate and extrastriate visual cortex compared to LIP in both strobe-reared monkeys, the opposite was observed in the control monkeys: FEF showed stronger correlations compared to LIP, though this difference was only significant in one control monkey. Task-based fMRI indicated that while strobe-reared monkeys showed motion responsivity across striate and extrastriate visual cortex including MT, no motion adaptation was found. Taken together, our results suggest that strobe-rearing does not affect the large-scale organization and functional connectivity of striate and extrastriate visual cortex. However, we find atypical parietal and frontal functional connectivity in addition to disrupted direction selectivity in MT, corroborating and extending previous findings in cats.

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**Presentation Number:** NANO16.02

**Topic:** D.06. Vision

**Title:** Cortical Representations Supporting Color-Shape Associations in Macaques

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**Abstract:** Humans use color and shape features to acquire different kinds of knowledge. Color might indicate object state while shape reveals object identity. Neurological cases together with functional imaging suggest that cortical representations of color and shape are somewhat separate, reflected as distinct color-biased regions (CBRs) and shape-biased regions in inferior temporal cortex. But the brain must connect color and shape because the meaning of color is shape dependent. To understand the neural basis of color-shape associations, quantitative data on stimuli likelihood, priors, and reward probabilities throughout life are needed, data largely unavailable in humans.
To address this, we deployed a learning paradigm in two juvenile macaques over four years,

where the monkeys learned about the colors and shapes of 14 colored objects using in-cage touchscreens. In each trial, a colored shape was presented, and liquid reward was provided for touching the matching color or shape. Subsequently, association trials were introduced: monkeys were cued with a shape or color and rewarded for touching the associated color or shape—this is analogous to tests of color-shape agnosia in humans. The monkeys achieved >90% accuracy on all trial types.
Three fMRI experiments were then conducted. A localizer identified CBRs and other category-selective regions of interest (ROIs). A block-design experiment measured responses to blobs of shape-associated color, achromatic color-associated shapes, and other uncolored shapes. Third, a version of the association task assessed task-related activity and was used as input for decoding models. A whole-brain convolutional decoder achieved shape-identity decoding with 66% accuracy and cross-decoding of associated colors with 52% accuracy (chance 20%). Linear Support Vector Classifiers trained on color-associated-shape responses in each ROI showed greater cross-decodability of color in more anterior temporal regions, including perirhinal cortex and the temporal pole. Among CBRs, univariate analyses showed that anterior CBRs responded best to color-associated shapes and color-blobs over unassociated shapes.
Overall, the results uncover a cortical network which engages category-selective regions, memory systems, and prefrontal cortex to represent learned color-shape-reward contingencies, and the approach provides a data-driven method to understand the circuit-level mechanisms that support the computational objectives of visual behavior.

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**Title:** Visual cortex transforms many features to many choices

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**Abstract:** The two pillars of flexible visual perception are ***generalized feature inference*** (we can just as easily rate the ripeness of mangoes as avocados) and ***generalized choice mapping*** (given the recipe, we can choose to slice or blend the mangoes). These can be thought of as flexibly mapping many (features) to one (inference) and one (feature) to many (choices) visual inputs to actions. Several challenges have limited progress understanding the neural mechanisms that support flexible visual-motor mappings. First, for practical reasons, most past research has tackled only one part of the problem at a time, studying variability in stimuli or actions, but not both. Second, the feedforward heuristics that have dominated the study of the visual system have meant that most studies look to association areas such as in parietal or premotor cortex for this sort of flexibility. Finally, the binary nature of most laboratory tasks limits the statistical power

to differentiate between opposing hypotheses.
We designed an experimental paradigm that solves these problems and allowed us to test the hypothesis that a single population of visual neurons could support both generalized inference and choice mapping. We recorded from populations of neurons in visual areas V1 and V4 while monkeys made veridical (continuous) judgments about an abstract property of 3D visual stimuli (medial-axis curvature). First, we tested the hypothesis that a single strategy for mapping visual neurons to behavior could account for generalized inference. We compared the ability of linear decoders of population activity to predict judgments about a single shape (shape-specific decoder) and many shapes that varied in task-irrelevant features (shape-agnostic decoder). We discovered that (a) while shape-specific decoders could better glean stimulus parameters, shape-agnostic decoders performed considerably better at predicting choice behavior, and (b) errors in perceptual decisions were attributable to systematic differences between the alignment of the shape's curvature representation and the shape-agnostic curvature decoder. Second, we tested the hypothesis that the same populations of visual neurons could mediate flexible choice mapping. When the animals had to flexibly plan eye movement responses after making a curvature judgment, the population responses rotated to encode the saccade direction. Together, these results suggest that visual cortex mediates many forms of cognitive flexibility that have traditionally been ascribed to downstream areas.

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**Presentation Number:** NANO16.04

**Topic:** D.06. Vision

**Title:** Supervised and unsupervised learning of neural representations for visual generalization

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**Abstract:** To produce appropriate behaviors in new situations, the brain must rely on neural representations that generalize widely and are invariant to a range of sensory transformations. Such invariant neural representations have been found for example in higher-order visual areas (HVAs) in primates. However, it is not known how the invariant neurons acquire their generalization properties, or how these neurons are used during behavior. Here we report that neurons with invariant visual coding emerge after learning in medial HVAs in mice. As a population, these neurons responded equally to learned and novel exemplars from visual categories such as “leaves” and “circles”. Mice also generalized behaviorally to the new visual stimuli, by licking in anticipation of reward. The emergent neural invariance did not depend on the mice learning our classification task, because similar properties were found in mice that experienced the visual stimuli in an unsupervised manner, without reinforcement. Unique to the supervised mice, we found a second neural population in frontal HVAs which encoded the anticipation of reward in a ramping manner. The activity of these neurons covaried with the decision of the animal, including on trials with the novel stimulus exemplars. Both the medial and frontal populations remained plastic after learning and continued to change while the mice learned a new, fine discrimination task. Throughout learning, the neural changes were consistent with a pattern of generalizing to new stimuli according to the rules of the respective task, even in the absence of an explicit task. We saw similar changes in dark-reared mice, in particular the

emergence of invariant neurons in HVAs. Thus, the unsupervised learning rules employed by the brain obey innate priors on generalization, a strategy which may allow animals to flexibly learn appropriate behavioral responses to a wide set of stimuli from relatively little direct reinforcement.

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**Title:** Neuronal tuning for configural vs. local features in the ventral stream

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**Abstract:** Visual cortex neurons are optimized to respond to natural images, realizing our ability to understand the world. In studies of the primate occipito-temporal visual cortex (commonly referred to as the "ventral stream"), there are two overarching theories about how neurons respond to natural images. One is that the ventral stream trends towards encoding complex features: neurons in V1, V2, and V4 respond to simple attributes like lines or curves, while neurons in inferotemporal cortex (IT) respond to complex combinations of simple features such as objects (*e.g., faces, places, bodies*). A second theory is that the visual system becomes increasingly tuned for niche features: as in V1-V4, most IT neurons remain tuned for relatively simple attributes, only rare and more cleverly diagnostic of objects. Do IT neurons preserve tuning for simple, local, yet statistically infrequent (niche) visual attributes? In this series of studies, we recorded from neuronal sites in V1, V4, IT, and prefrontal cortex (PFC) of multiple macaque monkeys, using an image-synthesis approach to identify each site's critical activating features. Image synthesis was achieved using different types of deep generative networks, and we specifically included networks that create highly photorealistic images in addition to networks that create more abstract, texture-like images. We found that neurons could drive image synthesis across both kinds of image spaces, resulting in images that were configurally distinct but shared local features. We further investigated how units in convolutional neural networks drive synthesis across both image spaces and found that texture-like images were more activating. Overall, these results are consistent with recent findings showing that neurons in anterior face patches respond to local features (Waidmann *et al.* and Leopold, 2022) and raise interesting implications for our understanding of visual perception, including the idea that much of our object recognition may rely on simpler features than previously assumed.

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**Presentation Number:** NANO16.06

**Topic:** D.06. Vision

**Support:** KAKENHI 19K12149
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**Title:** Comparison between deep neural network and neuronal responses in areas TE and TEO in pre- and post-visual categorical learning

**Authors: *N. MATSUMOTO**[1], Y. SUGASE-MIYAMOTO[1], K. HAYASHI[1], M. A. ELDRIDGE[2], B. J. RICHMOND[2];
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**Abstract:** Deep convolutional neural networks (DCNNs) categorize images with high accuracy. In the ventral visual pathway of macaque monkeys, areas TE and TEO are important for object recognition (Mishkin et al., 1983). A recent finding reveals that bilateral removals of both TE and TEO severely impairs the ability to categorize cat/dog images (Setogawa et al., 2021). To understand categorical representations in TE and TEO, we compared representations of visual categories in a DCNN with neuronal representations in TE and TEO before and after visual categorical learning. We recorded neuronal activity in TE and TEO of two Japanese monkeys ('R', 'X'), that performed a passive fixation task while 20 cat/20 dog images were presented. In this study, neuronal activities from two sessions ('pre', 'post') were analyzed. Between the pre and post sessions, the monkeys were given 8/3 sessions (R/X) of the task to train them to categorize the 20 cat/20 dog images. Activities of 93/97 (pre/post) neurons were simultaneously recorded from a Utah array embedded in TEO from Monkey X and activities of 298/272 neurons (pre/post) were recorded from three arrays in TE from Monkey R (148/137) and X (150/135). Population vectors were made from individual mean firing rates within a 100-ms time window while each image was presented. A representational dissimilarity matrix (RDM) was calculated using the vectors (Kriegeskorte et al., 2008). We used AlexNet, a DNN with five convolution layers and three fully-connected layers (Krizhevsky et al., 2012). The 20 cat/20 dog images were input to obtain feature vectors in each model layer. A RDM was also calculated using the feature vectors in each layer. Spearman correlation between a RDM of each model layer and that of TEO neurons reached a maximum in the fifth convolution layer in the pre and post sessions. Spearman correlation between a RDM of each model layer and that of TE neurons also reached a maximum in the fifth convolution layer in the pre session, but reached a maximum correlation in the first fully-connected layer in the post session. This result indicates visual categorical learning might change the categorical representation in TE neurons not in TEO neurons.

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**Title:** Neural activity in area TE, not TEO, supports monkeys' learning in a visual categorization task

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**Abstract: Title:** Neural activity in area TE, not TEO, supports monkeys' learning in a visual categorization task

**Authors:** Wenliang Wang, Bing Li, Mark AG Eldridge, Narihisa Matsumoto, Kazuko Hayashi, Kariely Martinez, Yasuko Sugase-Miyamoto, Barry J RichmondLab. of Neuropsychology, NIMH, NIH, Bethesda, MDHuman Informatics and Interaction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan.
**Abstract:** Primates, including old-world monkeys, can categorize images quickly based on similarity of visual features. We previously showed that two subregions of inferior temporal cortex, areas TEO and TE, contribute to visual categorization to differing extents. Monkeys with TE removals show a more sustained deficit than monkeys with TEO removals in the categorization of visual images. To learn more about how this difference in behavior arises, we recorded simultaneously from TE and TEO using implanted 64- or 96- channel Utah arrays while two old world monkeys were learning a visual categorization task. We found that the activity of neurons in TE, not TEO, becomes more strongly related toi by image category during during learning; this increasing modulation relatioinship is driven primarily by increasing stronger encoding of the visual category associated with a the larger reward. Using a one-layer neural network (no hidden layer) for population-level decoding, we found that decoding accuracy of TE neurons, not TEO neurons, increases monotonically during learning, approximately paralleling the monkeys' behavior. Furthermore, decoding accuracy of category in correct trials is consistently higher than error trials across days in TE, but not TEO. Consistent with the findings from lesions studies, our results suggest that area TE is the a key brain region supporting visual category learning.

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**Presentation Number:** NANO16.08

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**Title:** Neural activity changes in perirhinal cortex and area TEO during learning of a serial recognition task in Rhesus monkey

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**Abstract:** Visual recognition is an effortless process with near-unlimited capacity. Repetition suppression, the reduction of neural activity in response to repetitions of a previously novel stimulus, has been proposed as one of the potential neural mechanisms underlying visual

recognition. Repetition suppression has been observed in multiple brain areas, e.g., area TE, perirhinal cortex, entorhinal cortex, and hippocampus. Here we monitored activity in area TEO and in perirhinal cortex during a visual recognition task. We simultaneously recorded neuronal activity using Utah arrays implanted in each area, while the monkey was learning a serial recognition task. The monkey was trained to do a two-interval force choice task: release a touch bar during the second interval when a novel image is presented, and release during the first interval when it is repeated. Each image was shown exactly twice in a session. The number of intervening trials between the first and second presentation of a stimulus was increased as the monkeys' performance improved (to a threshold of 75% correct overall responses) throughout training. There were no intervening trials in the initial sessions, i.e., the stimulus was repeated immediately after it was first presented. The number of intervening trials was increased as performance improved, culminating in sessions in which repeat presentations could occur after as many as 128 trials after first presentation (the following interval sizes were interleaved in the final test sessions: 0,1,2,4,8,16,32,64,128). We found repetition suppression neurons in both perirhinal cortex and area TEO. The proportion was smaller in TEO. In both areas, the proportion of repetition suppression neurons was smaller later in training presumably when the monkey was more experienced with the task. Concomitantly, the average magnitude of the repetition suppression effect in individual neurons increased.

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**Title:** Distinct readouts for Image and category from neural representations in the inferior temporal cortex using deep neural networks

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**Abstract:** Neurons in the inferior temporal cortex (ITC), which is the final unimodal region of the ventral visual pathway, are known to respond to various visual features, from relatively simple graphic elements to complex stimuli such as faces. However, the neural mechanisms by which cognitive information can be extracted flexibly from ITC activity are not well understood. In this study, we investigated how information is read out from ITC during image perception, which involves reproducing the external world in detail, and during category cognition, which involves abstracting it. We utilized a deep neural network (DNN) approach. One DNN was trained for image reconstruction, while another DNN was trained for image categorization from brain activity. To achieve this, we employed electrocorticography (ECoG), which allowed us to record neural activity with high temporal and spatial resolution from a wide cortical area over an

extended period, providing sufficient data to train our deep neural network models for two macaque ITCs while they performed a visual fixation task. We curated a dataset comprising 12 categories of natural images, each containing 1000 images. Both category and reconstruction models demonstrated high performance in extracting information from ITC activities. The categorization model achieved a higher accuracy than the chance level (mon C: 62.7%, mon J: 40.3%, chance: 8.3%). To evaluate the quality of the reconstructed images, we used SSIM and LPIPS, as well as human psychological experiments. The indices showed significantly better evaluations compared to those obtained from noise images (Steel-Dwass test: $p < 0.001$). Conversely, the recordings from the prefrontal cortex resulted in significantly lower performance in both models. Having confirmed that both models successfully perform the reconstruction and categorization tasks, Input masking analyses were conducted to investigate the readout properties of DNN models from ITC representations. The channel contribution maps for the category and reconstruction model exhibited distinct characteristics. The category model showed more positive peaks with wider ranges and more channels with negative contributions. However, the channels that contributed the most were located similarly in both models. The results emphasize that accurate image reconstruction from higher-order visual areas using ECoG signals is feasible. Moreover, the input masking analysis highlights the importance of selectively extracting information from ITC representations for effective abstraction readout.

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**Topic:** D.06. Vision

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**Title:** Reliable high-throughput stimulation of inferior temporal cortex is possible using chronically implanted electrode arrays

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**Abstract:** We have made recent progress in characterizing the perceptual outcome of optogenetic stimulation of inferior temporal (IT) cortex. Optogenetic stimulation offers a significant advantage in its suitability for high throughput experiments and the ability to conduct a large number of trials. However, a major drawback of existing chronically implantable optogenetic tools is the inability to record the neural activity. This limitation obstructs the possibility of linking the perceptual outcome of brain stimulation with its underlying neural activity. Traditional electrical stimulation does not have this limitation, but given its more aggressive nature, the utility of chronically implanted electrodes for long-term brain stimulation has been questioned. Specifically, high-throughput electrical stimulation has been reported to lose effectiveness over time. To systematically assess this problem, we implanted a Utah-Array on the central IT region and trained a monkey to detect and report a 200ms electrical stimulation impulse while fixating on images. For stimulation, out of 64 electrodes on the array we utilized

45 electrodes that had feasible impedance for the stimulation currents up to 100 μA in two experiments. In the first experiment, we examined the behavioral detection thresholds of different electrode combinations and currents. Each stimulation trial involved a randomly selected combination of 1, 2, 4, 8, 16, or 32 electrodes, with currents ranging from 20 to 100 μAmp. The results revealed detection thresholds of 97, 72, 50, 31, 16, and 14 μA, respectively, for each number of electrodes. In the second experiment, we investigated the feasibility of conducting high-throughput experiments across multiple sessions, considering the potential impact of electrical stimulation on surrounding neurons and its potential to diminish effectiveness over time. The basic task for this experiment resembled the previous one, but with stimulation trials consisting of 1, 2, or 4 electrodes and currents ranging from 5 to 10 μA. We conducted 74 sessions throughout this experiment in which overall 95,956 stimulation trials were performed and each electrode was stimulated 4,967 times, on average. The findings demonstrated that not only did the performance remain significantly above chance level, but we also observed an increase in the detectability of electrical stimulation from 62% to 96% over the course of the experiment. In conclusion, our results indicate the potential of using chronically implanted electrode arrays for high-throughput cortical stimulation in the visual cortex, paving the road for research and development of effective prosthetic devices.

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**Topic:** D.06. Vision

**Support:** NIH R01EY032878
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**Title:** A role for the hippocampus in disambiguating familiarity from visual modulation.

**Authors: *S. BOHN**, C. M. HACKER, B. G. L. JANNUZI, T. MEYER, M. HAY, N. C. RUST; Psychology, Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Humans and other primates can reliably recognize whether they have seen an image before, even after seeing it briefly, only a single time. Within inferotemporal cortex (ITC), evidence points to ‘repetition suppression’ as the neural correlate of recognition memory, reflected as a reduction in the vigor of the ITC response to the repeated presentation of an image. However, a challenge to this proposal is the fact that in addition to memory, the vigor of the ITC response can be modulated by other visual properties of images. One example is “image memorability,” systematic variation in how well images will be remembered based on intrinsic properties of the images alone. In ITC, more memorable images produce more vigorous responses, and consequently, memory behavior cannot be decoded from ITC population response magnitude in the face of memorability variation. We hypothesized that in the context of a familiarity task, confounding visual magnitude modulation is eliminated such that when information arrives in the hippocampus (HC), memory alone is reflected in the magnitude of the population response. To test this hypothesis, one monkey performed a single exposure visual recognition memory task as we recorded neural signals from ITC and HC. Consistent with our hypothesis, we found that ITC population response magnitude was modulated by both memorability and memory whereas HC population response magnitude was only modulated by

memory. Moreover, memorability behavior could be decoded from population response magnitude in HC (but not ITC). These results imply the existence of computations in the medial temporal lobe that disambiguate memory from visual modulation, possibly in the hippocampus itself. To gain deeper insight into the nature of those computations, we examined how memory and memorability were reflected in individual units in ITC. We found that their strengths were only weakly correlated, and that this property facilitated the ability of downstream decoders to extract memory independently of memorability (because it led to partially non-overlapping representations within ITC). However, that fact also raises an intriguing question about how visual modulation is transformed into memory modulation, as it implies that this does not exclusively happen within individual ITC neurons (where more vigorous visual responses lead to more repetition suppression and thus stronger memory signals). Altogether, these findings shed insight into how memory is both encoded and decoded in ITC and the medial temporal lobe, including HC.

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**Title:** Probing the effects of object category learning on the macaque inferior temporal cortex

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**Abstract:** Like humans, adult non-human primates can learn to categorize visual objects. Much prior work shows that individual neurons in the inferior temporal (IT) cortex, which is critical for visual object discrimination, modestly increase their selectivity to objects from learned categories. While the field now has relatively accurate models of IT responses, we do not yet have a similar understanding of adult IT plasticity or its role in behavioral performance gains ("learning").
To begin to address this, we measured changes in IT due to object category learning and asked how those quantitatively relate to behavior. We performed multielectrode recordings in two groups of macaques (3 monkeys/group), while monkeys viewed naturalistic images (8 categories, 80 images/category, 100 ms). Prior to recording, one group (naive) was trained to fixate passively on images; the other group (trained) also learned to discriminate multiple object categories via operant conditioning. We randomly sampled 58 reliable, visually responsive sites from each monkey to construct two pools of IT activity (178 sites per pool/group).

Consistent with previous studies, a category-based representational similarity analysis revealed a small (13.81%) but significant difference in representation between the trained and naive IT population. Not surprisingly, this representational shift corresponded to a small, statistically significant increase (~ +10%) in IT-based linear decoding accuracy of learned categories. Notably, this inferred increase in the linearly separable category information in the trained IT was much smaller than improvements observed in the monkeys' behavior (~ +40% accuracy gain).
To probe the driving factors underlying these incommensurate changes across IT and behavior, we cast our learning paradigm as an extension of contemporary artificial neural networks (ANNs), the leading models of the ventral stream. We observed that IT layers in various task-optimized ANNs (different architectures, pre-training objectives, category learning schemes) showed monkey-IT-like increases in category information after training. Interestingly, akin to IT, where trained IT decodes were more consistent with image-level behavioral patterns than naive IT decodes, specific ANN-ITs were more aligned with monkey behavior after training.
In sum, our findings indicate that category learning produces modest changes in the IT cortex, enhancing category information readout. We developed a computational framework to simulate these transformations, enabling us to formulate testable hypotheses about other representational reconfigurations induced by category learning.

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**Title:** Distinct temporal scales of plasticity in macaque AM and AF face patches

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**Abstract:** The primate inferior temporal (IT) cortex processes various categories of complex visual objects such as faces, which are crucial for social life. Neurons in some portions of IT cortex are known to distinguish between old and new object images by diminishing responses to those that are already familiar. Through longitudinal recordings of individual neurons, we previously showed that this response attenuation arises gradually over a period of days to weeks when macaques were shown a few presentations of the same image each day. Other studies have demonstrated within-session adaptation, often termed repetition suppression, to multiple presentations of the same stimulus. Here we examined the relationships repetition suppression

and long-term familiarity-related plasticity in the same neurons. We recorded longitudinally recorded cells in the anterior medial (AM) and anterior fundus (AF) face patches, two anterior IT cortical areas thought to be important for facial identification. Single neuron responses were monitored across weeks using implanted microwire brush array electrodes. During this time, a wide range of face and body images were shown repeatedly within and across sessions. We found that AM neurons gradually decreased their responses to the repeated stimuli over weeks, with a relatively small degree of within-day repetition suppression. In contrast, AF neurons tended to show a rapid and clear repetition suppression within each session. Importantly, this repetition suppression recovered by the following day and did not appear related to gradual response changes, which were much weaker in AF. These contrasting patterns between AM and AF face patches indicate two distinct temporal scales of plasticity in the IT cortex and suggest that daily stimulus-specific adaptation observed in some IT neurons does not contribute directly to the more gradually established familiarity-based response attenuation.

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**Title:** Reduced object discrimination in autism is associated with structural properties of the Lateral Occipital Complex

**Authors: *T. KOLODNY**[1], X. FAN[2,1], K. WOODARD[1], A. TASEVAC[1], W. R. GANZ[1], H. REA[1], E. C. KURTZ-NELSON[3,1], S. J. WEBB[1], S. O. MURRAY[1];
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**Abstract:** There is evidence for atypical visual perception in autism spectrum disorder (ASD), especially in perception of complex stimuli such as faces. However, it is unclear whether face perception differences are related to the social content, or to the processing of complex visual stimuli more broadly. To investigate the latter possibility, we tested object discrimination in autistic and non-autistic young adults, using a combination of psychophysics and neuroimaging. We present preliminary results from 24 autistic and 20 non-autistic participants, who completed an object discrimination task as well as anatomical and functional MRI scans. The objects were novel complex shapes that required processing of fine spatial details to successfully determine whether two small objects presented near the fovea were the same or different. Discrimination performance (d') showed substantial individual differences, and was overall significantly lower in the autistic group compared to the non-autistic group ($t(42)=3.7$, $p<.001$).
To investigate possible neural substrates underlying individual differences in object processing, we examined associations between task performance and structural properties of object-selective cortical areas. A functional localizer, contrasting intact vs. scrambled real-life objects, was used to define object-selective regions within the occipital lateral and inferior temporal cortex in each individual participant. Cortical thickness within these regions was extracted from high-resolution T1-weighted images using a surface-based pipeline in Freesurfer. We found that among the

autistic participants, cortical thickness in object-selective areas in the left fusiform gyrus was strongly inversely correlated with object discrimination performance ($r=-.49$, $p<.05$). Exploratory whole-brain analyses confirmed this finding and revealed further areas within the lateral occipital complex (LOC) where cortical thickness is larger in autistics than in not-autistics, and is inversely correlated with task performance.
Overall, our results show reduced object discrimination ability in autism and indicate that individual differences within the autistic group are related to structural features of object-selective cortical areas. This association was not observed in non-autistic participants, suggesting that the neural mechanisms mediating object perception might differ between groups. Importantly, these results demonstrate that differences in visual perception in autism are not limited to the social domain.

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**Nanosymposium**

**NANO17: Psychostimulants, Drug-Seeking, and Underlying Mechanisms**

**Location:** WCC 140

**Time:** Sunday, November 12, 2023, 8:00 AM - 10:00 AM

**Presentation Number:** NANO17.01

**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** WSU Bridge Grants

**Title:** Ubiquitin-protein ligase parkin in methamphetamine use disorder

**Authors: T. ATASI**[1], K. G. FERREIRA[1], A. SHARMA[1], R. GARCIA MILIAN[2], T. LAM[2], *A. MOSZCZYNSKA[1];
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**Abstract:** In the United States, there are close to 1,000,000 people with methamphetamine (METH) use disorder (MUD). Despite numerous clinical trials conducted to date, there is no FDA-approved medication for MUD, needed especially for people who abuse METH heavily as they are at high risk for METH overdose. Our previous study demonstrated that overexpression of a ubiquitin-protein ligase parkin in the nucleus accumbens (NAc) decreased METH intake and relapse to METH seeking in rat model of heavy METH use. The goal of the current study was to elucidate molecular pathways underlying the anti-addictive properties of parkin in this model. Towards this goal, we generated and compared proteomes from wild type vs. parkin overexpressing NAc collected from saline-yoked rats, rats that self-administered METH and were withdrawn from the drug for 10 days, and from rats that relapsed to METH seeking. GSEA analysis of the proteomic data revealed that pathways most significantly upregulated by parkin in saline rats were stress response pathways, Notch1 and NFkappB, with leading-edge proteins belonging to the 19S proteasome. Major biological processes altered by parkin overexpression in the NAc of METH-withdrawn rats were processes involved in tonic motor seizures, GPCR

signaling, long term potentiation and inflammation. Leading-edge proteins in the second cluster were proteins mediating synaptic vesicle docking and exocytosis, and proteins mediating loading glutamate to the storage vesicles. In rats that relapsed to METH seeking, the most altered pathways were those mediating Notch response to chemical hypoxia. The leading-edge proteins within this cluster were proteins belonging the 19S proteasome and involved in Notch, NFT2L2, TNFR2, p53, and Hedgehog pathway. In adult brain, these pathways mediate DNA and brain tissue repair, maintenance of plasticity, inflammation, and antioxidant and anti-apoptotic processes. The pathway altered in all parkin overexpressing rats, as compared to WT rats, was the ELF2 pathway which integrates a diverse array of stress-related signals to regulate mRNA translation. Our results suggest that parkin decreases METH taking and seeking through regulation of synthesis and degradation of proteins belonging to the stress-response pathways.

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**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** NIH Intramural Program

**Title:** Compulsive methamphetamine is associated with differential hydroxymethylation of miRNAs in the rat nucleus accumbens

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**Abstract:** Methamphetamine (METH) use disorder (MUD) is a neuropsychiatric disorder characterized by compulsive and continued use despite adverse life consequences. Fifty percent of METH users develop MUD and these individuals often experience impairments in learning and memory functions. MUD is thought to be due to epigenetic and transcriptional changes that occur in various brain regions including the nucleus accumbens (NAc) after repeated exposure to the drug. A better understanding of these molecular alterations may help to develop therapeutic approaches to psychiatric patients with MUD. The present preclinical study was conducted to identify potential differences between rats that compulsively took METH from those that suppress their intake of the drug in the presence of foot-shocks used to mimic the 'adverse consequences' criteria of the psychiatric Diagnostic Statistical Manual (DSM). After rats increased METH self-administration (SA), they are then exposed to METH SA with contingent foot-shock punishment. In the presence of contingent foot-shocks, some rats suppressed their intake (shock-sensitive) whereas other rats kept on taking the drug (shock-resistant). Rats were euthanized 2 hours after the last METH SA plus foot-shock session. The NAc was immediately removed, frozen, and used in genome-wide hydroxymethylated DNA immunoprecipitation (hMeDIP) sequencing analysis. We found significant differentially hydroxymethylated peaks in the SRvCT, SSvCT, and SRvSS comparisons. Notably, DNA sequences near several microRNAs (MiRs) showed differentially hydroxymethylated peaks in these comparisons. These

include Mir17-1, Mir551b, and Mir708 that showed increased hydroxymethylation in the SRvCT comparison. In addition, sequences near Mir17-1, Mir29b1, Mir153, Mir181b1, and Mir3584 showed increased hydroxymethylation in the SSvCT comparison. In the SRvSS comparison, Mir124-1, Mir 145, Mir146a, Mir344b-1, Mir 3099, and Mir 3596a showed increased hydroxymethylated peaks. Our observations implicate altered DNA hydromethylation of MiRs in compulsive METH taking in the presence of adverse consequences. Non-coding small RNAs that participate in post-transcriptional modulation of synaptic plasticity might serve as targets to reduce compulsive METH taking this model of methamphetamine use disorder.
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**Title:** Nr4a2 dominant negative in medial habenula cholinergic neurons attenuates reinstatement of cocaine-self administration in mice

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**Abstract:** Substance use disorder is complicated by a lifelong risk of relapse. Relapse vulnerability is programed partly during drug use, when the action of drugs of abuse in the reward circuit enables the formation of abnormally strong context/reward memories. These memory processes underlie common relapse triggers such as exposure to drug-associated cues or environments and can be extremely long lasting. This persistence may be attributed to epigenetics (i.e. modulation of gene expression that occurs through altered chromatin structure without fundamental changes to the DNA sequence itself), which has been shown to establish stable changes in cell function, and changes in behavioral outcomes. Recent studies implicate the medial habenula (MHb) in cocaine-associated behaviors, yet the role of the MHb in regulating reinstatement of cocaine self-administration remains largely unknown. Recent work in the lab identified histone deacetylase 3 (HDAC3; a powerful epigenetic regulator of gene expression) target gene, *Nr4a2*, as an important regulator of cocaine-associated behavior. NR4A2 is a transcription factor that regulates dopamine signaling during development, and is densely expressed in the MHb. To study the effect of MHb *Nr4a2* on relapse to drug-seeking behavior, we expressed the dominant negative form of NR4A2 (NURR2C) in MHb cholinergic neurons of ChAT-Cre mice. Then, animals were trained to self-administer cocaine. To study reinstatement, we used an incubation of craving model wherein after self-administration, animals were given 30 days of homecage withdrawal. On the last day of testing, animals were extinguished for 5 hours,

and were then re-exposed to drug-associated cues to drive a cued reinstatement. We discovered that blunting the expression of NR4A2 in MHb cholinergic neurons dramatically reduced reinstatement of cocaine self-administration without affecting acquisition or extinction. Taken together, these findings demonstrate that the MHb is specifically involved in relapse, and that NR4A2 is a key regulator of this behavior. Because NR4A2 is a nuclear receptor, it is an excellent target for the development of small molecule therapeutics. Work to test the effect of these ligands on cocaine-associated behavior is ongoing. To better understand how NR4A2 affects the transcriptome during relapse, we performed single nucleus RNA sequencing of the MHb after reinstatement, and found that animals with reduced functional NR4A2 had generally reduced transcriptional activity. We also found key differences in gene ontology associated with addiction behavior, cholinergic signaling, glutamatergic signaling, and GABAergic signaling.

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**Title:** Astrocyte activity in the dorsal striatum regulates cue-induced reinstatement of cocaine seeking

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**Abstract:** The neuroscience community continues to face significant challenges in understanding cocaine use disorder (CUD). Recent literature indicates that astrocytes play an active role in drug seeking. Several of these studies, highlight that suppression of striatal astrocytic activity results in significant alterations in reinstatement of cocaine seeking, indicating their importance in regulating reinstatement. However, the effects of astrocytic suppression on neuronal signaling in CUD remain unclear. To investigate the roles of astrocytic suppression on behavioral patterns and neuronal activity we performed intracranial viral injections in the dorsal striatum in rats. Animals received injections of neuronal calcium reporter, GCaMP6f and "CalEx", which suppresses astrocyte activity by continually extruding cytosolic $Ca^{2+}$, or neuronal GCaMP6f along with a control astrocyte fluorescent tag, tdTomato. Animals then underwent jugular catheterization for cocaine self-administration training. Following recovery, animals underwent cocaine or saline self-administration, extinction, and cue-induced reinstatement. No significant alterations were observed between CalEx and tdTomato groups during saline self-administration, while the CalEx cocaine animals had increased self-administration relative to tdTomato cocaine animals. No alterations were observed between either group during extinction and no changes were found between the saline CalEx and saline tdTomato groups during cue induced reinstatement. However, the suppression of astrocytic activity led to increased cue-induced reinstatement in cocaine CalEx, relative to cocaine tdTomato animals. Subsequently, brain slices were collected from each animal for ex vivo calcium imaging. The suppression of astrocytic activity increased amplitude of neuronal $Ca^{2+}$

transients, an indirect measure of cell excitability, in the cocaine animals, but not the saline animals. The suppression of astrocytic activity also led to decreased duration of neuronal $Ca^{2+}$ events in the cocaine group, while once again no changes were observed within the saline groups. Furthermore, the addition of exogenous 10 μM cocaine HCl suppressed $Ca^{2+}$ transients in both CalEx and sham animals. These findings reveal that suppression of astrocytes in the dorsal striatum increases cue-induced reinstatement by magnifying neuronal excitability.

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**Title:** Binge sucrose-induced neuroadaptations: a focus on the orexin/hypocretin system

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**Abstract:** Binge eating disorder is the most common eating disorder. Animals, like humans, selectively binge on highly palatable food indicating that this behavior is driven by hedonic, rather than metabolic signals; the neuronal mechanisms involved in this maladaptive behavior are not well known. Given the links of the orexin/hypocretin system to both reward processing and food intake, this study examined its contribution to binge-like eating in female rats. In addition, behavioral and molecular adaptations induced by eating disorders share commonalities with those involved in addiction. Therefore, we also studied the impact of binge eating on cocaine demand. Separate groups were given intermittent (12h) or continuous (24h) access to 10% sucrose or 0.4% saccharin and food over 28 days. Only groups with intermittent access to either sucrose or saccharin displayed excessive intake within a discrete period of time (i.e., binge eating). Interestingly, all groups exhibited increased numbers of orexin A neurons compared to the group with limited access to food only. In parallel, different doses (10, 20 or 30mg/kg) of an orexin 1 receptor antagonist, SB334867, reduced binge-like intake in groups with intermittent access to sucrose or saccharin but not in rats with continuous access to sucrose. We then assessed whether binge-like intake alters economic demand for cocaine in females. Only intermittent access groups exhibited increased demand for cocaine. 10 and 30mg/kg doses of SB334867 decreased demand for cocaine in all groups compared to controls with food only. Interestingly, pre-exposure to cocaine increased saccharin bingeing and daily intake. Altogether, our findings indicate that sucrose and saccharin bingeing alter the orexin system similarly to drugs of abuse. Hence, our results broaden the understanding of neural alterations associated with binge eating pointing towards addictive-like properties of palatable foods.

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**Title:** Cortical correlates of the subthalamic pathological activity predicting compulsive like cocaine seeking in rats

**Authors:** J.-L. DE PERETTI, E. COMBRISSON, A. AWADA, *C. BAUNEZ, **M. DEGOULET**;
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**Abstract:** When it comes to of drugs addiction, all individuals are not equal. In fact, while most drug users can maintain a controlled pattern of intake, some individuals rapidly develop pathological behaviors, such as dysregulation of their intake and compulsive drug seeking/taking behaviors. Identifying these vulnerable individuals before they transition to more harmful patterns of use is a key challenge for addiction research. In rats, oscillatory activity within the subthalamic nucleus (STN) has been shown to predict cocaine compulsive-like seeking behavior. However, the necessity to implant electrodes within the STN to record this predictive biomarker somehow limits its translational potential. To circumvent this issue, we here performed concomitant recordings of STN local field potentials and electrocorticographic (ECoG) activities above prefrontal and motor territories. Recordings were performed during a cocaine escalation procedure, which allow rats to loss control over their intake. Cocaine compulsive-like seeking behavior was then assessed in the resistance to punishment test, in which cocaine seeking was randomly punished with a mild electrical shock on the paws, allowing the identification of compulsive individuals. Preliminary analyses confirmed that 'future' compulsive rats exhibit a progressive increase in STN low frequency oscillations, notably in the theta band (6-13Hz), during the escalation procedure. Likewise, we observed a parallel increase in prefrontal EcoG activity, also in the theta band, mirroring STN activity. No increase in STN LFP or prefrontal ECoG were observed in 'future' non-compulsive animals. These data strongly suggest that prefrontal cortical activity can be used as a readout for the deepest STN predictive biomarker of cocaine compulsive-like seeking behavior. Characterization of such a superficial biomarker in animals may facilitate its translational application in humans through non-invasive measurements, allowing the early detection of vulnerable individual, thereby improving their medical follow-up and developing new risk prevention strategies.

**Disclosures: J. de Peretti:** None. **E. Combrisson:** None. **A. Awada:** None. **C. Baunez:** None. **M. Degoulet:** None.

**Presentation Number:** NANO17.07

**Topic:** G.09. Drugs of Abuse and Addiction

**Title:** Reward-specific transcriptomes define cocaine- and sucrose-seeking ensembles in a sex-dependent manner

**Authors: *C. LITIF**[1,2], L. FLOM[3], K. SANDUM[3], S. HODGINS[3], A. BOBADILLA[3];
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**Abstract:** Cocaine use disorder (CUD) causes chronic cocaine seeking in millions of humans and is yet to have effective pharmacological treatments. The lack of efficacious therapeutics for

CUD is partly due to the lack of understanding of reward-specific mechanisms driving drug-seeking behavior apart from other biologically necessary reward-seeking behaviors, e.g., eating. Formation and maintenance of discrete neuronal networks, or ensembles, are known to underly drug-seeking and are partially shared with other biological rewards such as sucrose intake. We know ensembles in reward-associated regions, e.g., the nucleus accumbens core and prefrontal cortex, have genetic alterations coercing cocaine- and sucrose-seeking behavior. However, how discrete genetic factors regulate seeking behavior for each specific reward within an individual brain is poorly understood. We aimed to define and differentiate reward-specific genetic signatures underlying drug-seeking behavior using a polyreward-seeking model with alternating cocaine and sucrose self-administration sessions followed by extinction and cue-induced reinstatement. We utilized FosiCre/+/Ai14 transgenic mice to fluorescently sort and perform sex-specific RNA sequencing for genetic characterization of Fos-dependent reward-specific seeking ensembles. We found discrete and similar genetic components regulating sex- and region-dependent transcriptomes underlying cocaine- and sucrose-seeking ensembles. Our investigations expand our genetic understanding of CUD to attenuate cocaine seeking discretely without altering non-drug reward seeking. In parallel, our findings contribute to refining sex-considerate avenues for CUD therapeutics.

**Disclosures: C. Litif:** None. **L. Flom:** None. **K. Sandum:** None. **S. Hodgins:** None. **A. Bobadilla:** None.

**Presentation Number:** NANO17.08

**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** R01 DA037897

**Title:** Targeting inhibitory central amygdala GLP-1R-expressing circuits to ameliorate cocaine reinstatement and withdrawal

**Authors: *R. MERKEL**[1,2], C. CARTHY[1,2], H. D. SCHMIDT[1,2];
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[2]Dept. of Psychiatry, Univ. of Pennsylvania, Perelman Sch. of Med., Philadelphia, PA

**Abstract:** Activation of glucagon-like peptide-1 receptors (GLP-1Rs) attenuates cocaine-seeking behavior during abstinence in rats. However, the neural mechanisms mediating the effects of GLP-1R agonists on cocaine seeking have not been fully characterized. GLP-1Rs are expressed abundantly in the central nucleus of the amygdala (CeA), but the role of CeA GLP-1Rs in cocaine seeking is unclear. Given the role of the CeA in both drug- and stress-mediated behaviors, our hypothesis was that activation of GLP-1Rs in the CeA would attenuate the reinstatement of cocaine seeking. We showed that intra-CeA administration of the GLP-1R agonist exendin-4 (Ex-4) dose-dependently attenuated cocaine and cue-primed drug seeking without producing adverse malaise-like effects in both male and female rats. Neural tracing and fluorescent *in situ* hybridization approaches were used to determine the downstream targets of GLP-1R-expressing CeA neurons. We identified GLP-1Rs expressed on populations of CeA GABA neurons that project to the nucleus accumbens (NAc) and the bed nucleus of the stria terminalis (BNST). We then used viral-mediated chemogenetic techniques in transgenic rats to explore the cell type-specific role of CeA◊NAc GABA neurons in cocaine-seeking behavior.

Activation of CeA◊NAc GABA neurons was sufficient to attenuate cocaine reinstatement in rats. Next, *in vivo* calcium imaging was used to characterize the effects of cocaine and Ex-4 on Ca2+ dynamics in CeA GABAergic neurons. Our data show that Ex-4 pretreatment reversed cocaine-induced increases in activity of CeA GABA neurons during drug-seeking behavior. Finally, we explored the role of CeA GLP-1Rs in cocaine withdrawal-induced anxiety. It is possible that GLP-1R activation in the CeA attenuates the cocaine reinstatement by alleviating the anxiogenic effects of cocaine withdrawal. Our preliminary findings indicate that systemic Ex-4 pretreatment produces anxiolytic effects in cocaine-experienced rats. In contrast, activation of CeA GLP-1Rs promotes anxiety-like behaviors in drug-naïve rats. Currently, studies are being conducted to explore the role of CeA GABA neurons in the anxiolytic effects of Ex-4 in cocaine-experienced rats, as well as the functional contribution of GLP-1R-expressing CeA->BNST GABA neurons in cocaine seeking and anxiety-like behaviors during withdrawal. Together, these findings identify a novel GLP-1R-expressing circuit that could be targeted to reduce cocaine relapse and cocaine withdrawal-induced anxiety.

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**Nanosymposium**

**NANO18: Maintaining and Modulating Attention Across Species**

**Location:** WCC 201

**Time:** Sunday, November 12, 2023, 8:00 AM - 10:00 AM

**Presentation Number:** NANO18.01

**Topic:** H.01. Attention

**Support:** R01 DC017797

**Title:** Real-time signal detection theory reveals that reward history impacts performance on multiple time scales in a sustained attention-value task

**Authors: *M. MCGINLEY**[1], J. DE GEE[2];
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**Abstract:** The brain has a remarkable capacity to adaptively shift processing to support a diverse array of behavioral goals, contextual demands, and environmental changes. In sensory domains, the brain can enhance the processing of difficult-to-perceive stimuli when they are important (Kahneman, 1973; Miller & Cohen, 2001), such as when correct discrimination leads to a large reward. Matching attentional effort to potential gains is a key feature of adaptive behavior and must be continuously re-calibrated. To study how mice adapt processing to momentary attention value, we have developed an “sustained attention-value (SAV) task” for head-fixed mice (de Gee et al., 2022).
The SAV task is a quasi-continuous listening task, in which mice learn to lick for sugar-water reward to report detection of the unpredictable emergence of temporal coherence in an ongoing tone-cloud. To detect all weak-coherence signals, mice would need to sustain an infeasibly high level of attentional effort across the 90-minute sessions. Therefore, to probe adaptive effort

allocation, we switched the sugar-water reward size (droplet volume) between high and low values in blocks of 60 trials. Thus, mice should expend more attentional effort in blocks of high reward.
Here, we have developed a novel signal detection theory (SDT) framework for the SAV task, based on signal start time-matched sampling of a Kaplan-Meier survival function estimate of false alarm rate. We refer to this approach as real-time SDT (rt-SDT). rt-SDT is applicable to a broad range of quasi-continuous perceptual tasks and provides a simple, data-efficient, and statistically grounded estimates of sensitivity (d') and criterion (c, also called bias) for such tasks. Applying rt-SDT to data from the SAV task, we report signatures of rapid and adaptive shifts in performance in 88 mice. In the high vs low reward blocks, mice were both more liberal (loc) and had higher sensitive (d'). Furthermore, mice were also more liberal and sensitive after correctly licking (hit) on the previous trial. Ongoing analysis is determining the interaction of these two reward-history effects on performance.In sum, we find that application of rt-SDT reveals that mice adapt their allocation of cognitive resources to inferred changes in task utility on multiple time scales.
Kahneman, D. (1973). *Attention and effort*. Englewood Cliffs, NJ: Prentice-Hall.
Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual review of neurosci*, *24*(1), 167-202.
de Gee, J. W., Mridha, Z., Hudson, M., Shi, Y., Ramsaywak, H., Smith, S., ... & McGinley, M. J. (2022). Strategic self-control of arousal boosts sustained attention. *bioRxiv*, 2022.

**Disclosures: M. McGinley:** None. **J. de Gee:** None.

**Presentation Number:** NANO18.02

**Topic:** H.01. Attention

**Support:** NINDS R01 NS088661

**Title:** A disinhibitory basal forebrain to cortex projection supports sustained attention

**Authors: *S.-J. LI**[1,2], B. HANGYA[3], U. GUPTA[1], A. KEPECS[1];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary

**Abstract:** Sustained attention allows organisms to prioritize the processing of relevant information to guide behaviors in changing environments. The basal forebrain has been implicated in a wide range of cognitive processes, from regulating the sleep/wake cycle to controlling various forms of learning, memory and attention. However, the specific cell types and circuits supporting each function remain unknown. While cholinergic neurons have been extensively studied in the basal forebrain, their activity does not show features necessary for sustained attention. We hypothesized that non-cholinergic basal forebrain neurons, such as GABAergic projection neurons, may support sustained attention. Here, we examined the circuitry and function of parvalbumin-positive (acetylcholine-negative) cortex-projecting GABAergic neurons of the basal forebrain, which we term BF-PV neurons. First, we determined that BF-PV neurons project topographically to the neocortex and selectively target cortical PV+ inhibitory neurons and provide a disinhibitory control over cortical activity. To test their behavioral functions, we monitored their activity across behavioral tasks involving sustained

attention and value prediction. We recorded both the spikes of individual BF-PV neurons using optogenetically-assisted electrophysiology, and the corresponding cortical terminal activity in multiple cortical target regions (auditory and motor cortex) using fiber photometry. BF-PV neurons responded to salient, outcome-predictive cues, adapted to expected outcomes, and predicted reaction time and detection accuracy, on a trial-by-trial basis. We devised a computational model that explained these diverse responses as encoding value-guided sustained attention. Finally, we showed that optogenetic activation of BF-PV neurons accelerated responses and increased accuracy in the sustained attention task, while their inhibition dampened animals' performance, supporting this model and linking attention and value-guided decisions. These findings reveal that the basal forebrain utilizes long-range cortical disinhibitory projection neurons to modulate cortical activity and promotes sustained attention.

**Disclosures: S. Li:** None. **B. Hangya:** None. **U. Gupta:** None. **A. Kepecs:** None.

**Presentation Number:** NANO18.03

**Topic:** H.01. Attention

**Support:** CIHR

**Title:** Significant roles of supramammillary nucleus (SuM) glutamatergic neurons in alertness and memory deficits in an animal model of ADHD

**Authors: *X. QIN**[1], T. TIAN[2], X. YANG[2], Y. WANG[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Shenzhen Inst. of Advanced Technol., Shenzhen, China

**Abstract:** Attention-deficit/hyperactivity disorder (ADHD) affects a significant number of individuals, including children and adults, with considerable impact on executive function, memory, and alertness. Optimal stimulation theory suggests that the symptoms of ADHD arise from a deficiency in external stimulation. While interventions like multimedia learning and virtual reality have demonstrated some success in mitigating these symptoms, the underlying neural circuitry remains poorly understood.
In this study, we utilized looming stimulation and novel environment exposure tests to transiently enhance alertness in spontaneously hypertensive rats (SHR), an established model of ADHD in order to search for new neurosubstrates involved in ADHD. We observed that looming resulted in a temporary reduction in response time and improved recognition memory during a subsequent novel object recognition test in SHRs. Considering the involvement of the hypothalamic-pituitary-adrenal (HPA) axis in ADHD, we investigated the role of the hypothalamus in alertness modulation and discovered a direct association between the activity of glutamatergic neurons in the SuM and the animals' alerting level. By manipulating SuM neurons with optogenetics and chemogenetics, we achieved bidirectional controls over alertness, emphasizing the significance of these SuM neurons in altering responses and possibly in ADHD. Furthermore, through viral neural tracing, we identified the critical role of SuM neurons projecting to the dentate gyrus (DG) in the manifestation of recognition memory deficits observed in ADHD.
These findings offer valuable insights into the previously uncharacterized neural circuitry underlying alertness and cognitive impairments in an ADHD rat model. Additionally, the

identified glutamatergic neurons in the SuM as potential therapeutic targets for enhancing cognitive impairments associated with ADHD.

**Disclosures: X. Qin:** None. **T. Tian:** None. **X. Yang:** None. **Y. Wang:** None.

**Presentation Number:** NANO18.04

**Topic:** H.01. Attention

**Support:** NIH F32EY032776 (NBK)
NSF CAREER 2047298 (SPM)

**Title:** A dedicated midbrain inhibitory engine for the control of selective spatial attention

**Authors: *N. B. KOTHARI**[1], W.-K. YOU[3], A. BANERJEE[1], Q. ZHANG[2], S. P. MYSORE[1]; [1]Psychological and Brain Sci., [2]Johns Hopkins Univ., Baltimore, MD; [3]Natl. Def. Med. Ctr., Taipei City, Taiwan

**Abstract:** The selection and preferential processing of the highest priority ("most important") stimulus location amongst competing distractors is called selective spatial attention, and is critical for adaptive behavior. Importantly, to be evolutionarily advantageous, the selection of the attentional target needs to be accurate (with an appropriately positioned selection boundary between the highest priority target and lower priority distracters), and precise (with a categorical, selection boundary). Although decades of work have revealed the necessity of cortical (fronto-parietal) and midbrain (superior colliculus; SC) regions for selective spatial attention, a mechanistic understanding of attentional target selection has remained elusive. Specifically, neural circuit mechanisms for implementing accurate and precise selection boundaries between the attentional target and distracters are unknown. Additionally, because disruption of cortical and subcortical nodes of attention not only disrupts attention control, but also impairs perception of single targets, orienting behaviors, and motor plan selection, it is unclear whether dedicated circuits for attentional target selection might exist in the brain. Here, in freely behaving mice engaged in a human-inspired task of selective spatial attention, we demonstrate that a relatively unknown group of evolutionarily conserved inhibitory neurons in the vertebrate midbrain, called PLTi in mammals (parabigeminal lateral tegmental inhibitory neurons), controls distractor suppression and target selection for spatial attention. Several striking findings emerged upon bilateral silencing of PLTi neurons using cell-type specific chemogenetics. First, silencing PLTi neurons caused mice to become hyper-distractible, revealing a critical role for it in spatial attention control. Second, PLTi silencing severely disrupted both the accuracy and precision (categorical nature) of the selection boundary for target selection. Third, these behavioral deficits were closely matched by deficits in the accuracy and precision of the signaling of the most salient stimulus by neurons in the intermediate and deep layers of the SC (SCid) –well known to be critical for the control of selective attention – revealing a mechanistic route for PLTi action. Lastly, behavioral deficits following bilateral PLTi silencing occurred without affecting visual perception, motor plan selection, or task-related orienting behaviors. Taken together, our results discover PLTi as a dedicated midbrain seat for spatial attentional control, one that drives attentional target selection via its action on the SC.

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**Topic:** H.01. Attention

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Major Project of National Social Science Foundation (19ZDA363, 20&ZD153)
National Key Research and Development Program of China (2022ZD0211000)

**Title:** Developmental Shifts in Neurobehavioral Substrates of Attentional Networks: A Longitudinal fMRI Study

**Authors: *S. PENG**[1], R. DING[1], Y. ZHAO[1], L. HAO[2], S. QIN[1];
[1]Beijing Normal Univ., Beijing, China; [2]Southwest Univ., Chongqing, China

**Abstract: Developmental Shifts in Neurobehavioral Substrates of Attentional Networks: A Longitudinal fMRI Study**
**ABSTRACT**Human brain undergoes a prolonged maturation process to support nuanced cognitive functions, wherein developmental shifts may occur, giving rise to sensitive periods of development. However, hindered by small sample sizes, cross-sectional designs, and limited age range distribution, the impact of age maturation on the neurobehavioral substrates of attention networks, and dynamic shifts in these substrates during development, still remain elusive. Utilizing longitudinal data to capture developmental changes in attention can contribute to early clinical detection.
Based on the neural specialization perspective, we investigated changes in brain systems involved in three attentional components (i.e., alerting, orienting, and executive attention), separating age, performance and their interactions in an accelerated longitudinal design. A total of 846 scans were completed, spanning over ages 6 to 16, at an interval of one year for three years, with 400, 281, and 165 scans per year.
We observed behavioral trajectories of three attention processes converged toward adult levels. There is of a shifting point from 9 to 10 in age by performance interactions. The activation of the supplementary motor area and its connectivity with arousal and movement-related brain regions support the development of children with lower performance in alerting and orienting. Similarly, stronger activation of the right rostrolateral prefrontal cortex and temporoparietal junction, along with higher levels of ventral striatum-dorsomedial prefrontal cortex functional connectivity, support the development of children with poorer executive attention performance.
Overall, brain regions that facilitate the transition of higher cognitive functions and their co-activation patterns with other cortical/subcortical regions play a central role throughout development. And children with lower executive attention performance demonstrate an increased reliance on the functional connectivity of prefrontal-striatal pathway, indicating impairments related to goal-directed processes. Our findings provide insights into both the typical neurodevelopment of attention and potential early neurobiological targets for addressing

attention-related difficulties.
**Key words** attention, executive, development, children, fMRI

**Disclosures: S. Peng:** None. **R. Ding:** None. **Y. Zhao:** None. **L. Hao:** None. **S. Qin:** None.

**Presentation Number:** NANO18.06

**Topic:** H.01. Attention

**Support:** NIMH Intramural Research Program (ZIAMH002783)
NIMH Intramural Research Program (ZICMH002968)

**Title:** Contribution of In-Scanner Thoughts to Resting-State Functional Connectivity: How participants rest matters.

**Authors: *J. GONZALEZ-CASTILLO**[1], M. SPURNEY[1], K. LAM[2], F. PEREIRA[2], D. A. HANDWERKER[1], P. A. BANDETTINI[1];
[1]Section on Functional Imaging Methods, [2]Machine Learning Team, Natl. Inst. of Mental Hlth., Bethesda, MD

**Abstract:** Resting-state fMRI (rs-fMRI) scans are often used to identify aberrant patterns of functional connectivity (*FC*) in clinical populations and to reveal the neural correlates of specific phenotypes. To minimize interpretational uncertainty, researchers control for age, gender, co-morbidities, and motion. Yet, rarely considered is the role of systematic differences of in-scanner experience (i.e., what subjects are thinking during the scan). To evaluate this prospect, we used 471 publicly available rs-fMRI scans (MPI Mind-Brain-Body dataset) annotated with self-reports about the content and form of in-scanner thoughts, and perceived levels of wakefulness. Based on these self-reports, we subdivided our sample into groups with different in-scanner experience controlling for age, gender, and wakefulness. Group *G1* is characterized by reporting thoughts in the form of images, of positive valence and about other people. Group *G2* includes scans with thoughts focused primarily on the environment and of negative valence. For all scans, we estimated *FC* using the 400 *ROI Schaefer* Atlas augmented with 8 subcortical *ROIs*. Significant differences in *FC*across groups were estimated using Network Based Statistics. We found stronger *FC* between the *DMN* and somatosensory and attentional networks for the contrast *G1 > G2*. In addition, we observed significantly stronger *FC*between sensory regions and attentional regions for the contrast *G2 > G1*. These results show that internally vs. externally-oriented thought engagement modulates *FC* between attentional regions and the rest of the brain. Next, we asked if we could predict aspects of in-scanner experience using *FC*. Prediction targets included: wakefulness, individual descriptors of thought form (images, words, intrusive, vague) and content (surroundings, other people, oneself, future/past events, positive/negative valence). Using connectome-predictive modeling, we were able to significantly predict wakefulness, reported levels of visual imagery, and focus on surroundings and past events. Inspection of *FC* models contributing to each prediction agree with our current understanding of how these state-level aspects of cognition manifest in the brain. Together, these results highlight the key role of in-scanner experience in shaping rs-fMRI *FC* and motivate the practice of annotating rs-fMRI scans with first-person descriptions of in-scanner experience. Future work should elucidate if accounting for these state-level effects help characterize sources of inter- and intra-subject

variability that hinder our ability to interpret *FC* differences and develop rs-fMRI biomarkers of disease.

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**Presentation Number:** NANO18.07

**Topic:** H.01. Attention

**Support:** KAIST G-CORE Project

**Title:** Brain decoding of breathing meditation: an fNIRS pilot study

**Authors: *E. KIM**, S. KIM, S. HWANG, H.-M. BAE;
Korea Advanced Inst. of Sci. and Technol., Dajeon, Korea, Republic of

**Abstract:** For the last two decades, brain encoding research, connecting the target state with other well-known mental states, has been actively conducted to analyze meditation through neuroimaging and reveal the network of the meditation. However, for that same reason, the brain encoding method cannot classify the meditation state and other mental states. In order to distinguish the meditation from others, brain decoding is emerging as an alternative way. Therefore, we do brain decoding of meditation states and classify the meditation states for the beginner and expert using functional near-infrared spectroscopy(fNIRS).3 subjects have been recruited: 1 beginner (20s, F), 1 intermediate (50s, F), 1 expert practitioner (60s, M). The subjects were given a 15-channel prefrontal fNIRS device. They measured 3 times per week, each of them recording 18, 20, 49 sessions in total. Prior to each session, the subjects practiced breathing meditation(anapanasati) for 30 minutes. Throughout the session, the subjects were instructed to keep their eyes closed, and either enter a resting state or practice breathing meditation. Each resting or meditation task lasted 90 seconds, and were repeated 5 times alternatively.For HbO and HbR data of each task segment, parameters such as mean, slope were tested for 1. normality, 2. difference between resting-meditation conditions, and 3. correlation with repetition of sessions and blocks. Almost no parameter followed normal distribution for the exert's data ($p<.05$), while mean, slope, and skewness in many channels were normal for the beginner's data. Using the wilcoxon rank sum test, the HbO mean, HbR mean and HbR slope parameter for all channels show the differences between the resting and meditation tasks in the expert's data, and the result of the beginner's is exactly reversed.Furthermore, statistical distribution of each parameter and their impact in the classification model will be discussed. This study shows the feasibility of fNIRS as a tool for brain decoding of meditation, and within-subject variability of conventional fNIRS parameters presented in this study could help in calculating sample size requirement for brain-encoding studies.

**Disclosures: E. Kim:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; KAIST G-CORE Project. **S. Kim:** None. **S. Hwang:** None. **H. Bae:** None.

**Presentation Number:** NANO18.08

**Topic:** H.01. Attention

**Support:** IBS-R015-D1
NRF-2019M3E5D2A01060299
NRF-2019R1A2C1085566

**Title:** Dynamic modulation of neural representations in visual areas by time-varying attentional states

**Authors: *K. TARK**[1], W. SHIM[1,2,3];
[1]Ctr. for Neurosci. Imaging Research, Inst. for Basic Sci., Suwon, Korea, Republic of; [2]Biomed. Engin., [3]Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon, Korea, Republic of

**Abstract:** While sustaining attention at a certain level is crucial for successful cognitive task performance, attention intrinsically fluctuates between optimal and sub-optimal states over time. Previous research has identified two attentional states, “in the zone” and “out of the zone”, defined based on the behavioral variability, and has linked them to neural activity in high-order brain areas, such as the default mode network (DMN) and dorsal attention network regions. Here, we examined whether these time-varying attentional states modulate sensory representations in lower-level visual areas, and how this modulation relates to the interaction with higher brain regions involved in inherent attentional state changes. In the scanner, participants viewed two gradually appearing Gabor stimuli with different orientations and were instructed to detect changes in the spatial frequency of the attended stimulus, while ignoring the other one. Using an inverted encoding model, we reconstructed population-level, orientation-selective responses for each stimulus separately. We found robust orientation-selective responses to the attended Gabor in the primary and intermediate visual cortex, and such responses were stronger during zone-in compared to zone-out epochs, particularly in V3. Notably, in visual areas corresponding to the unattended stimulus, orientation-selective responses to both attended and unattended stimuli co-existed, indicating that the information of the attended stimulus was globally deployed. To examine the relationship between the modulation of feature-selective responses in V3 and the reconfiguration of attention-related functional networks involving higher-order brain regions, we computed the functional connectivity between V3 and the DMN regions. We found that during zone-in epochs, the representation of the attended stimulus in visual areas was more robust when V3 exhibited stronger connectivity with the medial prefrontal cortex, whereas this pattern was not observed during zone-out epochs. In sum, our results provide evidence that time-varying attentional states, even in the absence of external cues, can enhance or weaken sensory representations in visual areas. Furthermore, the feature-selective representation during optimal attentional states was associated with tight coupling between sensory areas and DMN sub-regions, suggesting a dynamic interplay between intrinsic attentional states, reflected by network activity in higher-order regions, and neural representation of sensory features in the visual cortex.

**Disclosures: K. Tark:** None. **W. Shim:** None.

**Nanosymposium**

**NANO19: Learning and Memory**

**Location:** WCC 150

**Time:** Sunday, November 12, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO19.01

**Topic:** H.10. Human Learning and Cognition

**Support:** NIH R01 MH074692

**Title:** Interactions between motor learning and novel episodic encoding

**Authors: *C. GASSER**, L. DAVACHI;
Psychology, Columbia Univ., New York, NY

**Abstract:** Behavior occupies a central role in human experience. Our behaviors are also highly repetitive, in that we regularly execute the same sequences of motor actions across time (e.g., commuting to work). However, layered upon these behaviors, we are bound to encounter a simultaneous stream of novel sensory information (e.g., hearing a new song on your commute). Despite the common co-occurrence of familiar behavior and novel experiences, very little research has investigated how our everyday actions affect the way we learn from and remember ongoing events. In this fMRI study (N = 30), we ask how the execution of a learned motor sequence facilitates episodic memory for novel, visual items that are encoded at the same time. Replicating our previously-published behavioral work, we first show that engaging in familiar motor sequences selectively benefits memory for the temporal order of novel items, relative to memory for items learned during the execution of a random, unstructured motor sequence. We then examine the neural underpinnings of this memory-enhancing effect. Specifically, we find that when participants encode novel items during the execution of a learned motor sequence, representations of that learned sequence become activated throughout a distributed cortical network. This network encompasses not only regions known to support motor learning, but also those associated more broadly with the representation of structured knowledge, such as medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC). Importantly, we find that the strength with which learned sequence representations are activated in mPFC and PCC is associated with better temporal order memory for novel items. Taken together, these results are consistent with the idea that familiar motor behavior functions as a kind of "scaffold" for incoming information. The activation of this knowledge representation in the brain, in turn, might specifically bolster the process by which novel information is weaved together across time into a cohesive episodic memory.

**Disclosures: C. Gasser:** None. **L. Davachi:** None.

**Presentation Number:** NANO19.02

**Topic:** H.10. Human Learning and Cognition

**Support:** 10400228

**Title:** Neural correlates of category representations following incidental category learning

**Authors: *T. HOUSER**, D. ZEITHAMOVA;
Univ. of Oregon, Eugene, OR

**Abstract:** Despite the ubiquity of categorization processes in everyday life, research has predominantly focused on one type of category learning task: training people to classify category exemplars with corrective feedback. While fruitful, this traditional approach does not capture the range of ways in which people acquire category knowledge in the real world. Can people acquire category knowledge incidentally by associating exemplars with other information that itself has category structure? Do people form cognitive and neural representations of incidentally-acquired category structure that are similar to those formed from classification training? To answer these questions, we ran a between-subjects study contrasting traditional classification learning and a novel specificity task, where participants were instructed to remember an exemplar's paired associate. We also tested for differences in how people used category structure to generalize to novel information. Generalization strategy was tested by fitting formal categorization models (i.e., prototype- and exemplar-based) to both behavioral responses and neural activity (acquired via fMRI) from brain regions of interest. Prototype models assume people extract a central tendency across multiple experiences while exemplar models assume people use individual instances to subsequently group information. We found that incidental category learning leads to very similar generalization performance. Consistent with prior literature, most participants used a prototype-based generalization strategy, though we also observed more exemplar-based generalizers after specificity training than classification training. This representational shift was even more pronounced in the brain where we observed more robust exemplar representations and less robust prototype representations than observed previously after traditional classification training. Overall, we found similarities and differences between incidental and more traditional category learning. This work informs a more nuanced understanding of the relationship between general and specific knowledge.

**Disclosures: T. Houser:** None. **D. Zeithamova:** None.

**Presentation Number:** NANO19.03

**Topic:** H.10. Human Learning and Cognition

**Title:** Overlap between events has different consequences for learning and memory when events overlap in location versus content information

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**Abstract:** Overlap with prior knowledge can facilitate new learning via integration across experiences, but also hinder learning and memory through interference. Research on facilitation and research on interference also implicate two distinct hippocampal codes to benefit memory. Facilitation research has shown that overlapping events can be integrated, accompanied by similar neural representations. In contrast, interference research has shown that overlapping events tend to get separated or represented as exceedingly dissimilar to resolve interference. We designed a task that allowed us to investigate these two disparate effects simultaneously. Participants learned a grid of object-location associations, then they learned a second grid that

overlapped with the first in objects, locations, both objects and locations, or did not overlap with the first (new objects, new locations). Behaviorally, we found that content overlap (re-use of the same objects) hindered learning of the second grid, while location overlap (re-use of the same locations) facilitated learning of the second grid, as compared to no-overlap baseline. We found no interaction, suggesting that these effects are independent and additive. To determine how overlap is represented in the brain, we scanned participants while they were engaged with the grid task and used pattern similarity analysis to measure hippocampal representations of each grid. We found that in anterior hippocampus, grids that overlapped in locations showed greater neural pattern similarity, in line with facilitation of overlapping events. Grids that overlapped in objects were not represented as any more or less similar than grids that contained different objects, suggesting pattern-separated representations. These results indicate that overlap between events may be represented differentially in the brain under conditions of facilitation versus interference. No reliable effects were found in the posterior hippocampus. Taken together, our results demonstrate the nuances of information overlap. Some types of overlap cause facilitatory effects on learning and memory while others cause detrimental effects. Additionally, neural representations of complex stimuli may differ depending on the behavioral relevance of different types of overlap.

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**Support:** NIH Grant MH129436

**Title:** Memory reactivation during sleep does not act holistically on object memory

**Authors: *E. M. SIEFERT**[1], S. UPPULURI[1], J. MU[2], M. C. TANDOC[1], J. W. ANTONY[3], A. C. SCHAPIRO[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]The Univ. of Texas at Austin, Austin, TX; [3]California Polytechnic State Univ., San Luis Obispo, CA

**Abstract:** Memory reactivation during sleep is thought to play an important role in memory consolidation. Most of the research on sleep-based memory consolidation, in both humans and animals, has examined memory for individual facts, items, or locations. However, our memories are not discrete items that exist in isolation but are rather constructed of multidimensional features that have structured relationships with one another. How does sleep-based reactivation act on these structured memories? We leverage the Targeted Memory Reactivation (TMR) method in an object category learning paradigm to examine how memory reactivation during sleep acts on different components of structured memories. Participants (N=36) came into the lab and learned three categories of novel objects. Each object had unique features as well as features shared with the other members of its category. As participants learned the visual features of an object, they also heard its spoken name, which was then used as a cue for reactivation during a subsequent nap (TMR). We developed a novel real-time EEG protocol to administer cues at the peaks of slow-wave oscillations while avoiding spindle refractory periods, mimicking the timing of endogenous reactivation. We administered cues for one category in interleaved order and one category in blocked order to test for potential impacts of reactivation order. The third category

was left uncued. We evaluated changes across the nap for unique and shared feature memory as a function of cueing condition. We found that cueing, under both interleaved and blocked orders, improved memory for unique features while hurting memory for shared features. These findings suggest that memory for different features within complex objects can be differentially impacted by reactivation, and in particular that cueing individual objects enhances memory for distinguishing features at the expense of shared features. The effects were especially strong for blocked reactivation on unique feature memory, consistent with evidence that blocking can drive differentiation. Together, the results indicate that reactivation does not act holistically on object memories, instead enhancing certain features at the expense of others.

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**Topic:** H.10. Human Learning and Cognition

**Support:** McDonnell Center for Systems Neuroscience
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**Title:** Cognitive and neural representations of cognitive maps underlying model-based planning

**Authors: *A. B. KARAGOZ**, W. KOOL, Z. M. REAGH;
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**Abstract:** When faced with a decision, we sometimes rely on habit, which serves us well in predictable situations. However, in more complex scenarios, we often need to plan towards a goal. Over the last decade, goal-directed planning has been studied using sequential decision-making tasks coupled with model-based reinforcement learning algorithms. However, in order to plan, one first needs useful a model, or a cognitive map, of the environment. There is surprising lack of knowledge on how these internal representations of task structure are constructed. This is partly because many prior studies ensured that participants had full knowledge of the structure of the task before having to make decisions. How do humans construct cognitive maps of their environment, and how is this instantiated in the brain? How do their cognitive maps relate to their ability to use model-based planning? To address these questions, we modified an established two-step decision-making task and conducted behavioral and neural representational similarity analyses over planning relevant versus irrelevant associations between choice options in the task. In a large behavioral sample, we found a strong association between representation of planning-relevant task representations and model-based control. In a separate sample of participants undergoing fMRI scanning, preliminary evidence suggests that the hippocampus and entorhinal cortex – regions previously implicated in constructing and representing cognitive maps – come to represent abstract planning-relevant relationships in the task. Our results suggest that cognitive maps represent multiple facets of task structure, which are in turn encoded by distinct neural regions.

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**Title:** Spaced learning over long timescales strengthens stimulus-specific representations in vmPFC

**Authors: *F. ZOU**, B. A. KUHL, S. DUBROW, J. HUTCHINSON;
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**Abstract:** One of the most widely known phenomena of human memory is that spacing learning events out over time can improve memory. While this phenomenon, known as the "spacing effect", has been studied across a wide range of paradigms and species over the past century, it remains unknown why spaced learning leads to such benefits. Recent evidence from rodents and humans has shown that spaced learning is associated with greater activity pattern similarity in the medial prefrontal cortex (mPFC), potentially suggesting that spaced learning enhances the reactivation of prior neural representations. However, these studies have focused only on short timescales, with repetitions occurring within a single experimental session (and day). In contrast, behavioral studies have consistently shown that the spacing effect operates at timescales spanning months and even years. This raises the important question of how neural representations are influenced by spaced learning over long timescales (days, weeks, months, and beyond). Here, we tested this question in a high-resolution (1.8 mm) human 7T fMRI study in which eight participants performed a continuous recognition memory task across 30-40 scan sessions distributed over 8-10 months. Across these sessions, thousands of natural scene images were pseudo-randomly presented up to three times, with spacing (i.e., the delay between the first two presentations) ranging from 4 seconds to 288 days. Consistent with prior behavioral evidence of spacing effects, greater spacing between the first two presentations was associated with better subsequent recognition. Strikingly, we found that this spaced learning increased stimulus-specific pattern similarity in vmPFC, but only when stimuli were successfully recognized at the second presentation. Moreover, vmPFC pattern similarity selectively predicted subsequent recognition when stimulus repetitions were separated by a day or more. Collectively, these findings suggest a potential role of vmPFC in mediating the benefit of spaced learning at long timescales.

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**Title:** Narratives shape the strength and structure of episodic memories

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**Abstract:** Episodic memory is a cornerstone of human experience, enabling us to transform a constant barrage of information into stored representations that can be later retrieved. Decades of highly controlled laboratory studies have identified core cognitive and neural mechanisms underlying episodic memory formation and retrieval. However, our everyday lives are inherently complex, dynamic, and multifaceted in ways that are not fully captured by such controlled experiments. Critically, in real-world experiences, we are guided by *meaning* and *structure* as we build and retrieve episodic memories. We use these features to assign significance to events, and to associate pieces of information in memory that were encoded at different times. Frequently, these features are used to construct narratives about our experiences. A growing literature suggests that many of the same regions of the brain involved in episodic memory are sensitive to narrative structure. Yet it remains unclear how narratives and their structure fundamentally shape the way episodic memories are encoded and organized. Across several experiments, we show that the structure of narratives and the use of a narrative format itself strongly shapes the strength of episodic memories, and the way those memories are organized.

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**Title:** A neural basis of distortions in episodic memories due to semantic knowledge

**Authors: *A. TOMPARY**[1], A. CLEMENTS[2], S. L. THOMPSON-SCHILL[3];
[1]Psychological and Brain Sci., Drexel Univ., Philadelphia, PA; [2]Comparative Human Develop., Univ. of Chicago, Chicago, IL; [3]Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Events are not remembered perfectly; rather, episodic memory retrieval involves the integration of specific details of the event with prior semantic knowledge. It is currently unknown how these sources of information are combined in the brain, whether the neural regions underlying the different components cooperate or compete during memory encoding and retrieval, and whether this might vary across individuals. We previously developed a location memory task wherein reliance on event-specific details (error) vs. semantic knowledge (bias) could be teased apart (Tompary & Thompson-Schill, 2021). In these experiments, participants could integrate across newly encoded image-location associations to learn that images' locations often clustered by their category membership. Results showed that images that were located far from their category clusters were consistently placed closer to their clusters at retrieval, suggesting a bias in memory due to prior knowledge of category membership. Here, we adapt this procedure for use with fMRI. With $n = 40$, we replicate the prior behavioral findings: participants consistently place images that are outside of their category clusters closer to the center of those clusters, and this effect is stronger for typical category members than atypical category members. This replication, despite several changes to the original paradigm to adapt the task for neuroimaging, demonstrates the robustness of the effect of distortion of episodic memories due to semantic knowledge. Preliminary fMRI analyses demonstrate that activity in hippocampus varies with the extent of error in behavioral responses, and activity in the left anterior temporal lobe varies with the extent of bias. Other analyses are currently underway to

test whether the balance in how whether these regions cooperate or compete at encoding determines participants' correlation between error and bias at retrieval.

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**Title:** The hippocampus and VMFPC represent abstract prototypes in category generalization

**Authors: *C. BOWMAN**[1], D. ZEITHAMOVA[2];
[1]UW-Milwaukee, Milwaukee, WI; [2]Univ. of Oregon, Eugene, OR

**Abstract:** While decades of research has established that the hippocampus supports detailed episodic memory, it is now known that it also plays a role in the ability to apply past experiences to new situations. In a series of fMRI studies, we have used category learning as a representative domain for understanding the types of representations that underlie hippocampal contributions to generalization. Whether concepts are represented by individual category members as posited by exemplar models or an abstracted central tendency as posited by prototype models has been debated for decades. Fitting these models to fMRI data acquired during category generalization tasks, we have shown that activation in the hippocampus and ventromedial prefrontal cortex (VMPFC) are consistent with predictions derived from the prototype model of categorization. These findings suggest that the hippocampus has a role in generalization above-and-beyond its known ability to represent individual instances, also forming abstract representations that link across separate experiences.

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**Title:** Repetition facilitates differentiation of neural representations in the hippocampus and MTL cortex

**Authors: *E. T. COWAN**, B. TANRIVERDI, V. P. MURTY, I. R. OLSON, J. M. CHEIN;
Psychology and Neurosci., Temple Univ., Philadelphia, PA

**Abstract:** While memory seems to benefit from repeated learning opportunities, it remains unclear how repetition affects the organization of memories in the brain. In an fMRI study, 25 participants viewed image-location pairs, each of which was repeated 4 times. To examine how the representational patterns change across repetitions, for each pair, we calculated pattern similarity between consecutive repetitions. In bilateral hippocampus, we found evidence of differentiation in the representational patterns evoked across repetitions, with a significant decrease in pattern similarity between the first two versus last two repetitions ($t(24)= 2.73$, $p=0.01$). This pattern of results was particularly evident in the anterior hippocampus ($t(24)= 2.38$, $p=0.03$). Beyond the hippocampus, there was also evidence for differentiation in medial temporal lobe cortical regions, with a significant decrease in pattern similarity in both parahippocampal cortex ($t(24)= 2.50$, $p=0.02$) and perirhinal cortex ($t(24)= 3.05$, $p=0.005$). Critically, this pattern was not significant in a control ROI in the motor cortex ($p=0.30$). Together, these results suggest that repeated exposures alter the underlying neural representation of the memoranda, potentially reflecting a learning-related process by which representations are refined in order to support memories for the long-term.

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**Title:** Episodic memories remain implemented in the episodic memory network when conscious access is lost

**Authors: *K. ZERVAS**[1], T. WILLEMS[1], F. RABE[1], A. FEDERSPIEL[2], K. HENKE[1]; [1]Dept. of Psychology, Univ. of Bern, Bern, Switzerland; [2]Support Ctr. for Advanced Neuroimaging, Inst. for Diagnos. and Interventional Neuroradiology - Univ. of Bern, Bern, Switzerland

**Abstract:** We previously showed that subliminal rapid associative encoding engages hippocampus and influences conscious decision-making (Pacozzi et al., 2022; Schneider et al., 2021). Here, we investigated whether consciously encoded supraliminal face-object pairs would still influence decision-making at a time, when participants can no longer consciously recollect face-object pairs. We hypothesized that decay of an episodic memory trace spans a continuum between consciously accessible over unconsciously accessible to completely forgotten. Hence, we expected that consciously inaccessible associative memories would still influence decision-making by engaging hippocampal-neocortical networks that had been recruited during encoding. Overnight memory consolidation was hypothesized to strengthen hippocampal-neocortical networks underlying unconscious memory retrieval.
20 healthy participants encoded 96 face-object pairs and retrieved the same pairs 30 mins and 24 hrs later during whole-brain functional magnetic resonance imaging at 7T field strength. For retrieval testing, the 96 faces were presented again for participants to retrieve either the associated object category (manmade versus natural) or the associated object (by dual-forced-

choice). Participants gave confidence judgments (sure, unsure, guess) after each retrieval attempt.
Retrieval accuracy for guessed responses failed to differ significantly from chance both at 30 mins and at 24 hrs following encoding. Nevertheless, a gPPI analysis for correct versus incorrect guess responses using a seed region in the right hippocampus revealed a larger functional connectivity with prefrontal areas (bilateral anterior cingulate and dorsomedial prefrontal cortex; 300 voxels, $T = 6.09$, $p_{FDR} < .001$), bilateral precuneus (40 voxels, $T = 7.32$, $p_{FDR} < .001$), and the left temporal pole (20 voxels, $T = 5.72$, $p_{FDR} < .001$) during the 30 mins retrieval. This larger hippocampal-neocortical connectivity correlated between-subjects with participants' guessing-accuracy during the 30 mins retrieval. This enhancement in functional connectivity for correct versus incorrect 30 mins guesses occurred already at encoding and correlated with the 30 mins guessing-accuracy. Overnight consolidation strengthened the functional connectivity between the right hippocampus and left temporal pole (14 voxels, $T = 5.06$, $p_{FDR} < .001$) for guess trials. Hence, consciously encoded episodic memories may remain implemented in the episodic memory network when conscious access is lost. These unconscious episodic memories appear to undergo overnight consolidation.

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**Title:** Gradual rather than sudden loss of hippocampal memory traces imaged with ultra-high field magnetic resonance imaging

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**Abstract:** Episodic memories are implemented in hippocampal-neocortical memory traces. These memory traces have a hippocampal trace component consisting in functional connectivity patterns between hippocampal neuronal ensembles. As memories slowly subside, their underlying memory traces become weaker, too. We hypothesize that before memories are forgotten and their traces dissolved, the memories might still be retrievable unconsciously and their traces still visible in the brain. Here, we aimed at tracking newly formed hippocampal memory traces in 20 healthy human participants over 2 days. Participants encoded 96 face-object pairs, retrieved the pairs 30 mins and again 24 hrs later during high-resolution medial temporal lobe fMRI at 7T field strength. For retrieval testing, the 96 faces were presented again for participants to retrieve either the associated object category ("manmade vs natural" decision) or the associated object (by dual-forced-choice). Participants gave confidence judgments (sure, unsure, guess) after each retrieval attempt. Retrieval accuracy of the face-associated object was above chance in the guess trials, but not the retrieval accuracy of the face-associated object category. A conjunction analysis of correct guess and correct sure responses vs baseline at the 24

hrs retrieval revealed activation within the right anterior hippocampus (152 voxels, peak at MNI 21.2, -14.8, -21.6; T = 3.71; $p_{uncorr} < 0.001$). The same conjunction for the 30 mins retrieval yielded a zero hippocampal result. Estimates of hippocampal activity during correct vs incorrect 30 mins-guess responses correlated between-subjects with 30 mins retrieval-accuracy in the right anterior hippocampus (431 voxels, peak at MNI 26.8, -7.6, -24.8; T = 6.32; $p_{uncorr} < 0.001$). This right anterior hippocampal cluster and two further clusters in the right and left posterior hippocampus measured during the 24 hrs-retrieval for correct vs incorrect guess responses correlated with the 24 hrs retrieval-guessing-accuracy. Following overnight consolidation, hippocampal retrieval activity was enhanced for correct guess responses but not for correct sure responses. This suggests a privileged consolidation of weak vs strong memory traces. Both in the guess and the sure trials, the face-associated object category was decodable from activity patterns in the medial temporal lobe independently of the correctness of the participants' response. Hence, when forgetting kicks in following episodic memory formation the underlying hippocampal memory traces initially persist, which speaks for a gradual rather than sudden loss of hippocampal memory traces.

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**Nanosymposium**

**NANO20: Network Analysis and Modeling**

**Location:** WCC 152B

**Time:** Sunday, November 12, 2023, 8:00 AM - 10:30 AM

**Presentation Number:** NANO20.01

**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** Learning brain states from resting state functional MRI data

**Authors: J. WANG**, H. LI, *Y. FAN;
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**Abstract:** Individualized functional networks provide rich measures for characterizing the brain functional organization and functional topography that can effectively quantify inter-individual differences and accurately predicts executive function, development, aging, and psychopathology at an individual subject level. However, the functional networks in existing studies are typically computed to capture an average effect of long time-series of fMRI scans or short temporal segments, not effective for delineating time-varying functional network information at the finest temporal scale, i.e., single time points. To characterize time-varying functional networks of single time points of resting state fMRI (rsfMRI) data, we develop a novel self-supervised deep learning method to build a model for learning individualized functional networks from single

time points of rsfMRI data. The model is trained to learn a set of functional networks from each time point of an individual's rsfMRI data by optimizing a data fitting loss function derived based on a nonnegative matrix factorization. The loss function is computed on all available time points of the same subject with attention to different time points so that distinctive time-varying activations of different functional networks of single time points can be highlighted. Validation experiments on rsfMRI data have demonstrated that the method can not only robustly characterize individualized, canonical functional networks, such as default mode network, with better accuracy than alternative methods based on all available time points of the same subjects, but also effectively capture instantaneously varying patterns of the functional networks at the finest temporal scale. Our method provides an effective means to characterize functional brain states of single time points of rsfMRI data based on individualized, canonical functional networks, facilitating "peeking" of brain activities of resting state fMRI data for individual subjects.

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**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** A novel graph diffusion framework for estimating neural communication with high temporal resolution

**Authors: *F. SCHWOCK**[1], J. BLOCH[1], K. KHATEEB[1], J. ZHOU[1], L. ATLAS[1], A. YAZDAN-SHAHMORAD[2];
[2]Bioengineering and Electrical Engin., [1]Univ. of Washington, Seattle, WA

**Abstract:** Understanding functional interactions between different brain regions or populations of neurons can provide fundamental insights into the functionality of both healthy and diseased brains. However, estimating these interactions is challenging as they cannot be observed directly; instead, they must be inferred from measured neural activity. Typically, this is done using techniques from statistical signal processing and multiple time series analysis, such as correlation, coherence, or Granger causality, which are collectively termed functional connectivity (FC). However, FC has two limitations: 1) Its calculation does not incorporate the underlying network structure through which signals can travel, and 2) its temporal resolution is generally well below that of transient network effects. Here we propose an alternative approach where communication in the brain can be estimated from recorded neural activity using a parameterized graph diffusion process that naturally produces a flow signal on the edges of a graph. This approach, when combined with state-of-the-art electrode arrays, enables estimation of neural communication with millisecond resolution rather than seconds or minutes such as for FC. We validate our framework on simulated neural activity obtained from a network of coupled Wilson-Cowan oscillators and demonstrate that our approach outperforms competing methods. Furthermore, we demonstrate the utility of our framework on two electrocorticography (ECoG)

datasets from macaque monkeys. First, using recordings from an optogenetic stimulation experiment, we analyze the neural communication dynamics evoked by paired stimulation. We found that the communication dynamics estimated by our framework align well with the experimental setup, a result unattained by alternative approaches. Second, we analyzed changes in neural communication following focal ischemic lesioning and electrical stimulation. We found that stimulation causes a local increase in neural communication near the stimulation location in both the ipsilesional and contralesional hemispheres that was not observed when analyzing various FC measures. In summary, our novel technique opens up opportunities for studying neural communication using diverse experimental setups at multiple spatiotemporal scales, thus offering unique insights into the interactions between different brain areas.

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**Presentation Number:** NANO20.03

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NSF 2207891
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NIH R01 EY017077

**Title:** Generative models of low-dimensional neural manifolds in brain activity

**Authors: *A. NANDA**[1], B. SINGH[1], Z. WANG[1], C. CONSTANTINIDIS[1], M. RUBINOV[1,2]; [1]Vanderbilt Univ., Nashville, TN; [2]Janelia research campus, Ashburn, VA

**Abstract:** Neural manifolds are low-dimensional embeddings of brain activity that may reveal interpretable structure related to ongoing behavior or task. These embeddings have been observed in neuronal firing of diverse animals, including macaque prefrontal cortex during a working memory task and anterior thalamus of freely moving mice. However, the significance of neural manifolds has not been rigorously tested against statistical models that preserve simpler and more interpretable structure in the data.
Here, we developed two independent methods that can generate synthetic brain activity with such preserved properties. We evaluated these models on spiking activity from the macaque prefrontal cortex during a working memory task where macaques remember stimuli over a delay period, and on spiking activity from anterior thalamus and head direction angle recording in freely moving mice. The first “pca model” constrains the first three temporal principal components for each dataset, while the second “tuning model” constrains neuronal tuning of each neuron to a working-memory task-trace (a linear projection of spiking activity across all units that is positive during delay periods and zero otherwise) or to the head direction angle, for monkey and mice brain activity, respectively. In addition, for both models, we constrained lag-1 autocorrelation and mean activity of each neuron. In each case, we used the UMAP algorithm on both empirical and model neuronal firing rates, to perform dimensionality reduction and extract the underlying manifolds.
Our results indicate that basic constraints account for manifold structure in a dataset-dependent manner (Figure 1). Specifically, our pca-model substantially recapitulates manifold in the working-memory task, but not in the freely moving task. Conversely, the tuning model is unable

to recapitulate this structure in both cases. These results demonstrate the importance of including basic-constraints in analyses of emergent phenomena, and our methods collectively pave the way towards doing such tests.

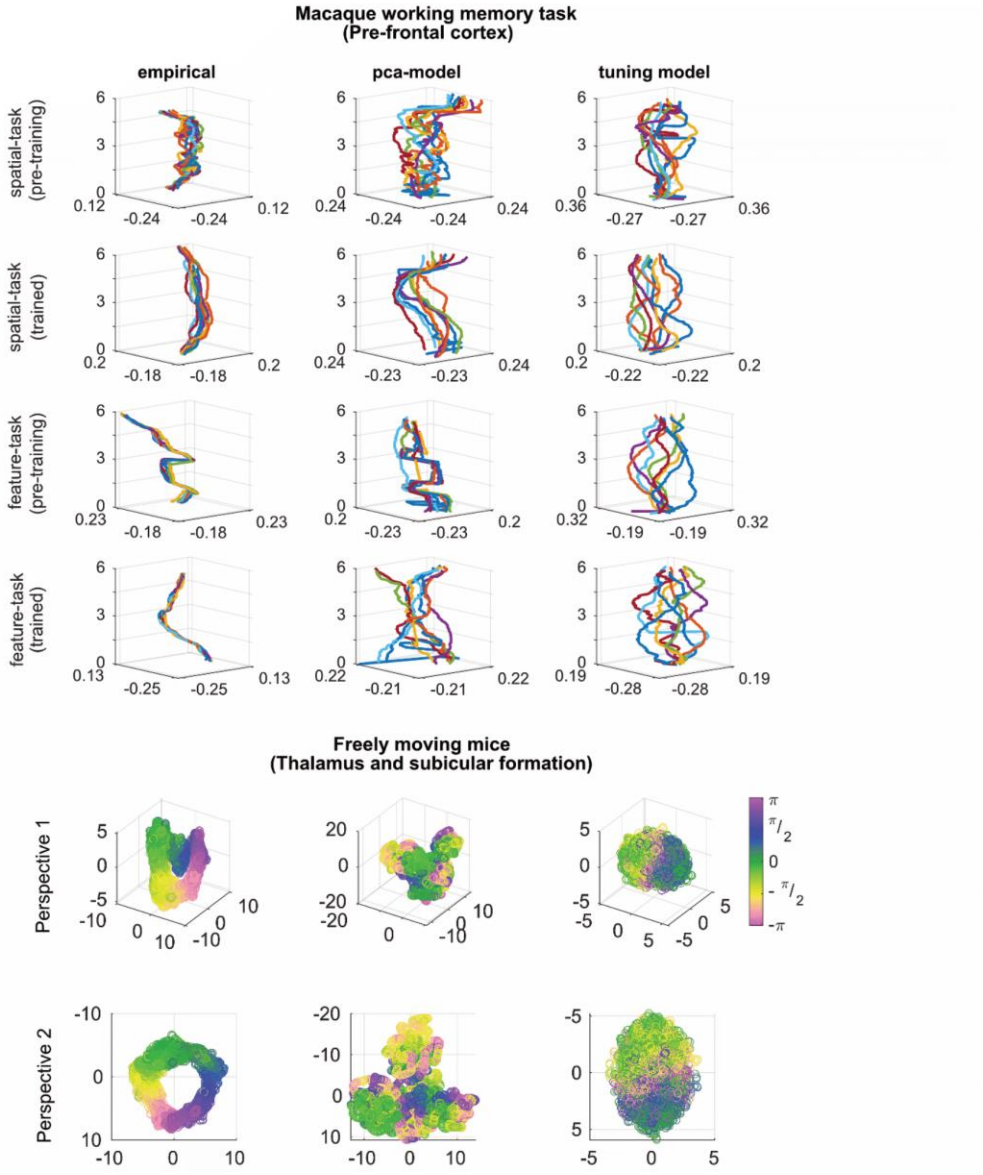


*Figure 1: Neural manifold (UMAP) in macaque PFC in working memory task and mouse anterior thalamus while freely moving. (top) The first column shows empirical manifold structure for spatial and feature tasks across the two conditions (Pre and post training). The second and third columns show manifolds for pca-model and tuning-model. (bottom) The first column UMAP embedding of empirical brain activity shows strong tuning to head-angle (colormap). The second and third columns show the manifolds for pca-model and tuning-model.*

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**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NIH Grant 5R01MH125429

**Title:** Predicting sensorimotor neuronal dynamics induced by optogenetic stimulation in non-human primates using statistical machine learning

**Authors: *R. MEHTA**, B. M. SMITH, F. SCHWOCK, N. STANIS, J. BLOCH, Z. HARCHAOUI, A. YAZDAN;
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**Abstract:** Optogenetics is a powerful tool that enables artifact-free recordings and millisecond-level control of neuronal activity within specific groups of neurons. It is ideal for relating brain function to behavior in animals with advanced behavioral capabilities such as non-human primates (NHPs). We have developed a large-scale optogenetic interface in NHPs enabling large-scale (>1cm2) stimulation and recording from the sensorimotor cortex. This interface creates an unprecedented opportunity for characterizing the sensorimotor cortex neural networks. In this study, we combine our large-scale optogenetic interface with graph artificial neural networks (GNNs) and sequence-to-sequence (Seq2Seq) modeling, two state-of-the-art machine learning (ML) and artificial intelligence (AI) statistical approaches, in order to predict the response of primary somatosensory (S1) and motor (M1) cortices following optogenetic stimulation. Two adult male rhesus monkeys were used for data collection. AAV-CamKIIa-C1V1-EYFP was expressed across primary somatosensory (S1) and motor (M1) cortices. Two semi-transparent 96 channel micro-electrocorticography (μECoG) arrays were implanted over the opsin-expressing areas to record light-evoked local field potentials (LFPs) at the network level. One location in either M1 or S1 was stimulated with one-second pulse trains. The pulse width (0.5, 1, 5, and 10 ms) and frequency (10, 20, 30, 35, 40, 50, 70, 90, 100, and 150 Hz) of these pulse trains were varied in an interleaved fashion. Each parameter was repeated 40-60 times. We first provide an exploratory analysis of changes in functional connectivity (as measured by the estimated coherence network of the LFP recordings) and changes in the raw 192-dimensional time series when the different stimulation parameters are applied. We then predict network-wide LFPs for each stimulation parameter from both stimulation and resting state recordings using GNNs and Seq2Seq modeling methods. We consider challenges such as missing data, batch effects, and faulty channels while suggesting ways to account for them when applying such methods. The long-term aim of this work is to provide accurate and robust prediction of stimulation induced neural activity in order to define underlying network dynamics with explainable ML and AI models. This will enable us to better understand how neuronal networks respond to stimulation, thereby enabling the generation of targeted neural states through stimulation. We can then leverage this understanding for guided rehabilitation from neurological diseases such as stroke and mental illness.

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**Presentation Number:** NANO20.05

**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** Switching Functional Network Models of Oscillatory Brain Dynamics

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**Abstract:** Rhythmic networks in brain activity may reflect several different mechanisms of coupling: for example, networks may be formed by a common source influencing rhythms in multiple areas, or rhythms may propagate through local networks either by driving each other or through a shared source of noise. In any case, we can assume that specific cognitive states and behavioral tasks may be mediated by a small number of network modes whose expression changes dynamically. In our previous work, we modeled the first mechanism using a "Common Oscillator Model," in which the activities across multiple electrodes are linked through a small number of latent oscillators, with each oscillator describing the associated activity of a single network mode (Hsin et al., 2022). In this work, we introduce two additional methods to capture propagating rhythms: the first "Correlated Noise" model where networks are defined by common structure in the noise driving the oscillations on each electrode. The second "Directed Influence" model allows the state of one oscillation to directly influence another. All these methods use the full data to estimate a discrete set of functional network modes and use local data over short time scales to estimate which network modes are being expressed at each moment. Each model is comprised of three components: (1) a set of latent switching states that represent transitions between the expression of each network mode; (2) a set of latent oscillators, each characterized by an estimated mean oscillation frequency and an instantaneous phase at each time point; and (3) an observation model that relates the observed activity at each electrode to a linear combination of the latent oscillators. We use switching Kalman filters and smoothers to estimate the instantaneous phase of each oscillator and the probability of each switching state at any given time. The estimated switching state characterizes which of the network modes is being expressed at each time point. We discuss the advantages of each model type for modeling different kinds of rhythmic functional connectivity and demonstrate differences between the methods in a variety of simulated scenarios. Statistical software implementing these methods will also be made available. Ultimately, we demonstrate that latent process models can be used to accurately estimate functional network structure across a wide range of coupling mechanisms and detect dynamic changes in network expression related to clinical or cognitive context.

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**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** Uncovering network motifs important for communication and control of network dynamics with maximum entropy models: application to the Drosophila connectome

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**Abstract:** Brain function producing sensations, cognition, and behaviors unfold overtime based on neuronal dynamics on structurally connected circuits. Modern connectomics is producing anatomical maps of connectivity between neurons at unprecedented resolution. These datasets offer novel opportunities to elucidate how patterns of connectivity shape the capacity of networks to transmit information and be controlled. Prior work has largely focused on global network properties such as wiring efficiency, modularity, and small-worldness. Yet, it has long been observed that neural connectomes exhibit particular patterns of local connectivity, or motifs, at rates higher than would be expected given the degree distribution. If and what role such motifs play in specific dynamical processes in brain circuits is unclear. In this work, we analyzed Drosophila Melanogaster nano-connectome. We extracted counts for motifs of size up to seven. We utilized maximum entropy models of random graphs to reveal if and how particular motifs facilitate network functions. These families of random graphs provide principled null models that constrain the frequency of various subgraphs but are otherwise unstructured. We applied scalable Monte Carlo methods to fit maximum entropy models that constrain motifs of size up to four, and develop a self-supervised boosting algorithm to iteratively increase the complexity of terms included in the models. We examined two related, but distinct, dynamical processes on the drosophila connectome: communicability and controllability. We quantified the information flow between different parts of the connectome using Estrada communicability. Likewise, to quantify the degree to which a network motifs enable control of network dynamics, we examined the spectrum of the controllability Grammian. We find that recapitulating communicability in the drosophila hemibrain involves constraining networks beyond degree distribution. Thus, high-order network motifs are important for communicability. We also uncover the minimum motif set needed to reproduce the controllability within the central fan-shaped body structure. Overall, our work provides a novel approach for linking connectomic datasets to dynamic functions, and reveals that high-order network motifs are important for these functions.

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**Title:** Link and node communities of the macaque interareal cortical network

**Authors: *J. S. MARTINEZ ARMAS**[1], P. MISERY[2], C. LAMY[2], K. KNOBLAUCH[2], Y. HOU[2], H. KENNEDY[2], Z. TOROCZKAI[1];

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**Abstract:** Retrograde tracers injected into a cortical area allow us to estimate the density of neurons projecting axons into that area from all the other cortical areas. Injections in multiple areas result in a weighted directed network whose nodes are the cortical areas, the edges are the neuronal connections, and the axonal densities represent the weights. Retrograde tract-tracing data from the macaque and marmoset monkeys and mice reveal that interareal networks are dense, directed, and their connection weights are heterogeneous, with values covering several orders of magnitude. Discovering communities in such complex networks is a challenging problem since such networks are not suited for current state-of-the-art community detection algorithms used in network science. For example, the standard algorithms are based on similarity measures that cannot handle weights varying across multiple scales. For these reasons, we extended the link community algorithm to be applicable to the study of anatomical neural networks, introducing novel methods to handle their directionality, density, and heterogeneous weights. Furthermore, our algorithm identifies link and node hierarchies, allowing us to analyze the cortical network's structure at multiple scales. Using benchmark networks with known community structures, we demonstrate that the node hierarchy encodes the information of the ground-truth partition with high accuracy. Using the extended link community algorithm, we infer the macaque anatomical neural network's areal (nodal) hierarchy with 47 injected areas from an atlas of 106. We validated its statistical significance using appropriate null models (omega index). These statistical tests show that the interareal physical distances partially explain the brain's community structure, information, which is lost if axonal projections are randomly swapped. Finally, using a recently introduced hierarchical entropy measure, we find that node and link hierarchies from the macaque data have a broad distribution, implying that the system comprises multiple branches and subbranches, an unusual property compared to other networked systems. We compare the hierarchy within the visual system to the one obtained by Felleman and Van Essen and others and show how the new hierarchy addresses some of the shortcomings of the previous hierarchies. Understanding the network connectivity patterns at a deeper level might provide valuable insights into understanding the information processing capabilities of the cortex.

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**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** Exploring Temporal and Structural Variability in Neural Ensembles Across Trials and Conditions

**Authors: *N. MUDRIK**[1], G. MISHNE[2], A. CHARLES[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Univ. of California San Diego, UCSD, La Jolla, CA

**Abstract:** The analysis of neural data in relation to behavior stands as a central pursuit in neuroscience. The intertwined impacts of observed and unobserved factors further complicate the analysis as they significantly contribute to trial variability within and between controlled conditions. Specifically, the challenge of identifying the underlying interpretable neural ensembles driving the observed activations is compounded by potential subtle variations, both in the temporal activity of the ensembles and in their structure/membership. However, existing computational methods for finding these hidden ensembles often assume that neurons can belong to one ensemble only (e.g., common clustering approaches) or rely on other non-biological plausible assumptions (e.g., fixed connectivity patterns across trials or equal contribution of all neurons in an ensemble). Here, we introduce a method for identifying unknown neural ensembles based on shared temporal activity across trials. Our method includes a multi-way graph-driven dictionary learning procedure where the graphs encode the inter- and intra-condition relationship. Our method thus enables the identification of interpretable underlying neural ensembles and characterizing their structural and temporal differences across trials. Our approach can extract ensembles with overlapping components, allows for variations in session counts and duration, can identify condition-specific vs condition-invariant ensembles, and offers both supervised and unsupervised data-driven approaches for controlling the level of ensemble similarity between conditions. Thus, we provide a foundation for studying temporal and structural variability across multiple trials and conditions, effectively accounting for the complexities of large-scale recordings. When applied to area 2 of the somatosensory cortex during a reaching-out task, we revealed dynamic and interpretable ensembles, capturing temporal and structural nuances across conditions. Additionally, we observed smaller intra-condition variability compared to cross-condition variability, highlighting the consistency of the identified ensembles within each condition. By leveraging the temporal traces of these identified ensembles, we achieved high accuracy in classifying the right task condition.

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**Title:** Resting-state alpha frequency band spatial pattern is predicted by the brain's anatomical connectivity and microarchitecture

**Authors: *P. VERMA**, B. SIPES, S. NAGARAJAN, A. RAJ;
Univ. of California San Francisco, San Francisco, CA

**Abstract:** Understanding how brain spatiotemporal patterns arise is a prevailing question in neuroscience. This work investigates how the alpha frequency band spatial patterns arise in the resting state as observed in magnetoencephalography (MEG). Specifically, we focus on the anterior-posterior spatial gradient observed in resting-state eyes-closed condition in MEG. Using whole-brain biophysical modeling, we show that the alpha band anterior-posterior spatial gradient is predictable from the brain's anatomical connectivity and microarchitecture. We show this finding in a young healthy (N=36) cohort. To evaluate whether the model can capture changes in alpha spatial patterning, similar to that observed with propofol, we modulated the inhibitory gains and time constants and found that the resulting anterior-posterior spatial gradient shifted with more positive anterior power and more negative posterior power. Interestingly, these spatial patterns are preserved even after removing connections from the thalamus, suggesting that the thalamocortical connections may not be necessary for these patterns to arise. Together, these results suggest a potential alternative explanation for the arousal and disappearance of the alpha frequency band spatial patterns.

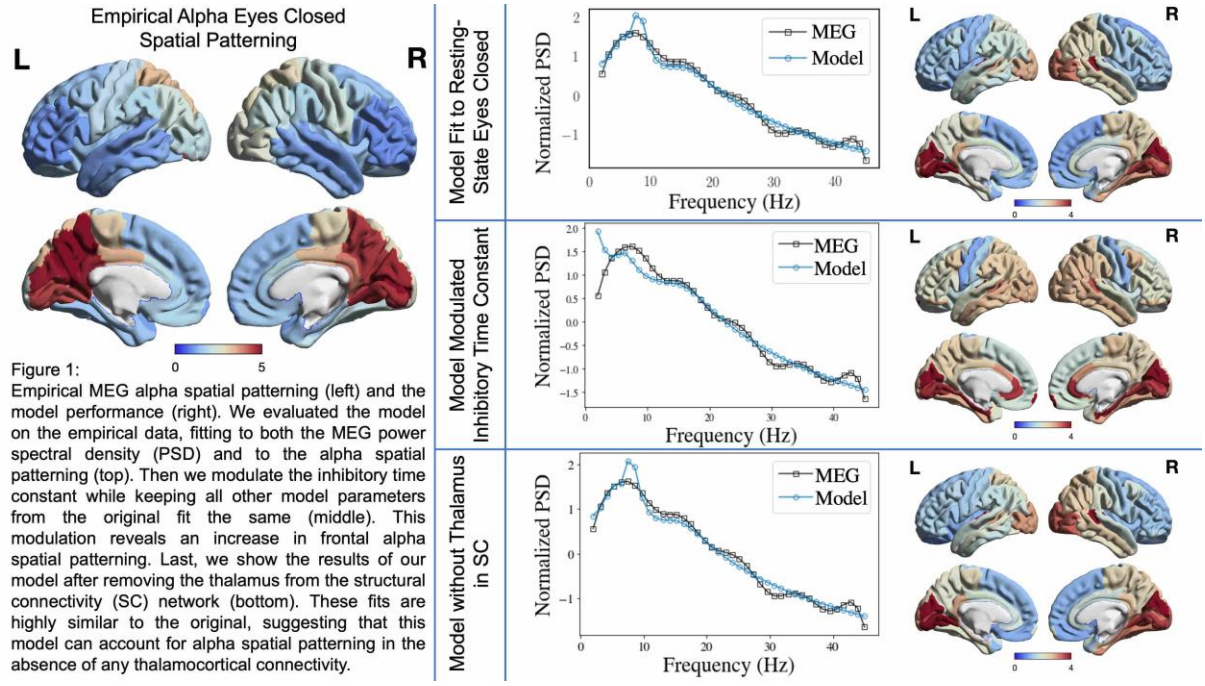


Figure 1:
Empirical MEG alpha spatial patterning (left) and the model performance (right). We evaluated the model on the empirical data, fitting to both the MEG power spectral density (PSD) and to the alpha spatial patterning (top). Then we modulate the inhibitory time constant while keeping all other model parameters from the original fit the same (middle). This modulation reveals an increase in frontal alpha spatial patterning. Last, we show the results of our model after removing the thalamus from the structural connectivity (SC) network (bottom). These fits are highly similar to the original, suggesting that this model can account for alpha spatial patterning in the absence of any thalamocortical connectivity.

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**Presentation Number:** NANO20.10

**Topic:** I.06. Computation, Modeling, and Simulation

**Title:** Enhancing neural encoding in naturalistic perception with a multi-level integration of deep neural networks and cortical networks

**Authors: *S. GU**[1], Y. LI[2];
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**Abstract:** Cognitive neuroscience aims to develop computational models that can accurately predict and explain neural responses to sensory inputs in the cortex. Recent studies attempt to leverage the representation power of deep neural networks (DNNs) to predict the brain response and suggest a correspondence between artificial and biological neural networks in their feature representations. However, typical voxel-wise encoding models tend to rely on specific networks designed for computer vision tasks, leading to suboptimal brain-wide correspondence during cognitive tasks. To address this challenge, this work proposes a novel approach that upgrades voxel-wise encoding models through multi-level integration of features from DNNs and information from brain networks. Our approach combines DNN feature-level ensemble learning and brain atlas-level model integration, resulting in significant improvements in predicting whole-brain neural activity during naturalistic video perception. Furthermore, this multi-level integration framework enables a deeper understanding of the brain's neural representation mechanism, accurately predicting the neural response to complex visual concepts.

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**Nanosymposium**

**NANO21: Neuronal Differentiation in Health and Disease**

**Location:** WCC 147A

**Time:** Sunday, November 12, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO21.01

**Topic:** A.01. Neurogenesis and Gliogenesis

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**Title:** A pan-neuronal alternative splicingevent triggers pan-neuronal gene transcription

**Authors: *E. LEYVA-DÍAZ**[1,2], M. CESAR[2], O. HOBERT[2];
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**Abstract:** Gene expression programs in differentiating neurons can be subdivided into at least two types, those that control expression of neuron type-specific proteins and those that control expression of proteins that are shared by all cells in a nervous system, such as proteins involved in the synaptic vesicle cycle or in neuropeptide biogenesis. Pan-neuronal gene expression is controlled by members of the CUT homeobox gene family, including the pan-neuronally expressed *ceh-44*. We address here how the expression of *ceh-44* is directed to the nervous system. Our studies show that *ceh-44* pan-neuronal expression is triggered by a pan-neuronal RNA splicing factor, UNC-75, the *C. elegans* homolog of vertebrate CELF proteins. UNC-75 spatially specifies the production of an alternative, CEH-44 homeobox gene-encoding transcript

from a ubiquitously expressed gene locus, which can also produce a conserved Golgi apparatus-localized Golgin protein, CONE-1 ("**C**ASP **o**f **ne**matodes"). During embryogenesis, before terminal tissue differentiation, the CONE-1/CEH-44 locus exclusively produces the Golgi-localized CONE-1 protein in all tissues, but upon the onset of postmitotic terminal differentiation of neurons, UNC-75 binds to the CONE-1/CEH-44 transcript to redirect the splicing machinery to now produce the alternative, CEH-44 CUT homeobox gene-encoding transcript, exclusively in the nervous system. CEH-44 subsequently controls the expression of pan-neuronal effectors genes, such as proteins of the synaptic vesicle cycle. Hence, UNC-75-mediated alternative splicing not only directs pan-neuronal gene expression, but also excludes a phylogenetically deeply conserved Golgin from the nervous system, paralleling surprising temporal and spatial specificities of other Golgins that we describe here as well. Remarkably, the unusual combination of Golgin and homeobox gene production from a single locus is conserved in vertebrates as well. Our findings provide novel insights into how all cells in a nervous system acquire pan-neuronal identity features.

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**Topic:** A.01. Neurogenesis and Gliogenesis

**Support:** NIH Grant 1F31NS124264
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**Title:** Foxp1 and Foxp2 regulate cerebellar hemisphere formation by controlling the diversification of Purkinje cells

**Authors: *N. KHOURI FARAH**[1], Q. GUO[2], J. Y. LI[3];
[1]UConn Hlth., Farmington, CT; [2]UConn Hlth., Farmingtonn, CT; [3]Uconn Hlth. Center/Department of Genet. and Genome Sci., Uconn Hlth. Center/Department of Genet. of Developmental Biol., Farmington, CT

**Abstract:** Purkinje cells (PCs) are a unique type of neurons found exclusively in the cerebellum and play a pivotal role in its operation. PCs also guide cerebellar development by expressing morphogens and managing the differentiation of other cerebellar cell types. Although all PC have similar morphology and distribute uniformly in a monolayer in the adult mammalian cerebellar cortex, transient molecular heterogeneity during embryonic stages has been previously reported. However, the understanding of PC heterogeneity is limited, and individual PC subtypes remain unidentified. Through single-cell RNA sequencing, we identified 11 molecularly distinct PC subtypes in the embryonic mouse cerebellum. Using CyCIF, a multiplexed immunofluorescence imaging method, and light-sheet fluorescent microscopy (LSFM), we assigned PC subtypes to their spatial locations and discerned their three-dimensional distribution in the cerebellar cortex. Different subtypes of PCs form distinct patterns along the anteroposterior and mediolateral axes of the developing cerebellum. Remarkably, PC subtypes express *Foxp1*,*Foxp2* and *Foxp4* transcription factors in various combinations and levels. *Foxp1* and *Foxp2* have been implicated in developmental speech and language disorders and Autism in humans. We generated cerebellum-specific *Foxp1*and *Foxp2* knockout mice and characterized their phenotypes. Strikingly, Foxp1/2 double knockout completely abolished the formation of the

cerebellar hemisphere, a distinct feature of the mammalian cerebellum involved in higher cognitive functions. Using single-cell multiomics and quantitative spatial transcriptomic analysis, we demonstrate that *Foxp1* and *Foxp2* knockouts disrupt a subset of lateral PC subtypes, thereby impeding the formation of the cerebellar hemisphere. Collectively, our findings illustrate that *Foxp1* and *Foxp2* collaboratively dictate the differentiation of PC subtypes to regulate the formation and expansion of the cerebellar hemispheres.

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**Title:** Loss of Ezh2 in MGE progenitors alters interneuron fate

**Authors:** C. RHODES[1], D. ASOKUMAR[1], M. SOHN[1], S. NASKAR[2], L. ELISHA[1], P. STEVENSON[2], D. R. LEE[1], Y. ZHANG[1], P. ROCHA[1], R. DALE[1], S. LEE[2], ***T. PETROS**[3]; [1]NICHD, Bethesda, MD; [2]NIMH, Bethesda, MD; [3]NICHD/Eunice Kennedy Shriver Nat'l Inst. of Child H, Bethesda, MD

**Abstract:** Enhancer of zeste homolog 2 (Ezh2) is responsible for trimethylation of histone 3 at lysine 27 (H3K27me3) resulting in gene repression. During neurogenesis, Ezh2 is critical for normal cell cycle dynamics and neuronal fate. Here, we explore the role of Ezh2 in forebrain GABAergic interneuron development by removing Ezh2 from the medial ganglionic eminence (MGE). We find that loss of Ezh2 increases somatostatin-expressing (SST+) and decreases parvalbumin-expressing (PV+) interneurons in multiple brain regions of the adult mouse. Intrinsic electrophysiological properties in SST+ and PV+ interneurons are normal, but PV+ interneurons display increased axonal complexity in *Ezh2* KO mice. We also observe fewer MGE-derived interneurons in the first postnatal week, indicating reduced interneuron production. Last, we performed single cell Multiome and CUT&Tag assays to characterize transcriptional and H3K27me3 differences in the MGE between WT and KO mice. Our results reveal a critical role for *Ezh2* in forebrain interneuron fate and maturation.

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**Title:** Neurodevelopmental abnormality associated with aberrant GPR110 signaling in human stem cells and mouse brain

**Authors: *Y. JOO**[1], E. AFLAKI[1], K. CHEN[2], G. PARRA-MERCADO[1], H.-Y. KIM[1]; [1]NIH, Natl. Inst. on Alcohol Abuse & Alcohol NIAAA, Rockville, MD; [2]NINDS, NIH, Bethesda, MD

**Abstract:** G-protein coupled receptor 110 (ADGRF1, GPR110) is an adhesion GPCR involved in the development of neurons and cognitive function. Synaptamide, an endogenous ligand for GPR110, binds to the *N*-terminal G-protein autoproteolysis-inducing (GAIN) domain of GPR110 and activates GPR110/cAMP signaling, promoting neurogenic differentiation of neural stem cells, neurite growth and synaptogenesis of developing neurons in mouse primary cells in culture. To examine the relevance of GPR110 in human neurodevelopment, we investigated the developmental phenotype and related molecular signaling pathways using human neural progenitor cells (hNPCs) along with GPR110 knockout (KO) mouse model. We found a high level of GPR110 expression in hNPCs and differentiated neurons while its expression was absent in astrocytes. Neuronal expression of GPR110 is predominantly in the membranes of soma and dendritic regions. GPR110 ligands dose-dependently increased cAMP production which was blocked by the pretreatment with *N*-terminal targeting GPR110 antibody. Introducing a point mutation F663S to GPR110, a mutant identified in a schizophrenic patient population, caused GPR110 to be trapped mainly inside the cells, leading to the loss of ligand-induced cAMP production capability. Ligand-induced GPR110 activation stimulated neurogenesis and neurite growth in hNPC-derived neurons. GPR110 KO *in vivo* or neurons derived from inactive F663S mutant hNPCs showed abnormal developmental phenotypes according to the RNA sequencing, imaging analysis and western blot analysis. In addition to the downregulated neurogenesis and neurite growth, the expression of glutamate receptors and synaptic molecules associated with postsynaptic density were significantly reduced in GPR110 KO mouse brain or neurons derived from hNPC with F663S mutation. In contrast, the F663S mutant neurons showed increased expression of genes implicated in neuropsychiatric disorders such as DLG2, FOXO1. Electrophysiological analysis using a microelectrode array assay also revealed decreased synaptic activity in the F663S mutant compared to the WT neurons. Our results indicate a significant role of GPR110 and its downstream signaling in neuronal differentiation and neuromaturation during development. The lack of GPR110 signaling leading to aberrant neuronal development may contribute to the psychiatric abnormality in adult stage.

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**Topic:** A.01. Neurogenesis and Gliogenesis

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**Title:** Non-classical ion channel function in human cortical histogenesis

**Authors: *N. K. HYLTON**[1,3], D. J. KANG[3], S. GOLINSKI[4], K. SORIANO[4], R. ANDERSEN[3], J. E. NEIL[3], M. TALUKDAR[3,2], S. K. AKULA[3], J. BAZAN[5], R. S. SMITH[4], C. A. WALSH[3]; [1]Harvard Med. Sch., Cambridge, MA; [2]Harvard Med. Sch., Boston, MA; [3]Boston Children's

Hosp., Boston, MA; [4]Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; [5]Vlaams Inst. voor Biotechnologie-UGent Ctr. for Inflammation Res., Ghent, Belgium

**Abstract:** Proper development of the human cortex is essential for brain function and depends on the synchronization of complex molecular and cellular processes. Malformations of cortical development may occur in the setting of genetic mutations that alter the activity of genes essential for this synchrony. Mutations in developmentally expressed ion channels have been increasingly recognized for their contribution to cortical malformations; however, the role of ion channels in cortical histogenesis—and their contribution to disease—remains poorly understood. Through whole exome sequencing of families with polymicrogyria (disordered cortical gyration), we identified three affected individuals with de novo missense variants in the gene PANX1, encoding the Pannexin 1 protein. PANX1 forms a heptameric ion channel of seven PANX1 subunits that releases small anions and ATP into the extracellular milieu, participating in purinergic signaling. The channel is further speculated to contribute to the propagation of calcium waves and form gap junctions, yet its definitive function in the fetal cortex is unknown. Exome analysis reveals each of the three variants identified are absent from the genome aggregation database (gnomAD) and are predicted deleterious based on in silico pathogenicity prediction tools. Each affected amino acid is highly conserved among mammalian orthologs, and these amino acids are in regions of the protein known to regulate channel gating. Bulk RNA-sequencing of the human cortex throughout gestation reveals preferential expression of PANX1 in early fetal cortical development, with decreased postnatal expression, correlating with the development of polymicrogyria. To study the effect of our PMG-associated variants on channel expression and activity, we designed plasmids containing wildtype or mutant PANX1 and GFP linked by the self-cleaving T2A peptide in the integration-coupled piggyBac transposition system. Expression of the mutant channel in HEK293T cells reveals disrupted complex glycosylation of PANX1, as well as increased ATP release compared to wildtype channels. Using AlphaFold, modeling of each PANX1 variant demonstrates alterations to channel topography and kinetics. Furthermore, we identified through structural homology additional uncharacterized proteins that share a high degree of structural similarity to pannexins and connexins, suggesting functional overlap and creating the human gap junction-ome. Through this study, we aim to further our understanding of how early electrical coupling and ion flux in the cortex shape key developmental processes.

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**Presentation Number:** NANO21.06

**Topic:** A.01. Neurogenesis and Gliogenesis

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**Title:** Decoding the development and potential of human neural stem and progenitor cells by multiomics

**Authors: *X. LI**;
Neurobiology, Care Sci. and Society, Karolinska Institutet, Stockholm, Sweden

**Abstract:** The spinal cord comprises the caudal region of the central nervous system and is responsible for conveying and processing motor and sensory information between the brain and the periphery. Any injury to the human spinal cord may result in irreservable functional loss due to the limited regenerative potential of the spinal cord. There is currently no cure for such conditions. Therefore, understanding how neurons and glia are generated from neural stem and progenitor cells (hNPCs) during development, and how hNPCs maintain or lose their stem cell potential during development and aging in the human spinal cord is an important yet understudied topic. Our recent work used single-cell and spatial omics has revealed the cell diversity and cell type specification of the developing human spinal cord during the first trimester. In this study, to further revealed the genetic and epigenetic regulation of human neural and stem progenitor cells for their self-renewal, differentiation and aged-related changes of stem cell potential, we used single-cell multiome-seq, spatial transcriptomics and epigeneomics to profile the spatiotemporal gene expression and regulations from developmental and adult human spinal cord at different ages. We revealed how different subtypes of human neural stem cells are genetically regulated during differentiation into different neurons and glia during development, and lost their stemness during aging. Furthermore, we observed chromatin accessibility of neural stem cells in the adult spinal cord stem cells, suggesting a therapeutic potential to recruit such cell population for stem cell therapy after injuries. Thus, we delineate spatiotemporal genetic regulation of human spinal cord across life span and leverage these data to gain insights into future therapeutic possibilities.

**Disclosures: X. Li:** None.

**Presentation Number:** NANO21.07

**Topic:** A.01. Neurogenesis and Gliogenesis

**Title:** Functional characterization of human and brain specific long noncoding RNAs associated with neurodegenerative diseases

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**Abstract: Background.** Long noncoding RNAs (lncRNAs) are important cellular regulators as they are involved in chromatin remodeling, transcription modulation, and post-transcriptional regulation through a variety of chromatin-based mechanisms and interplay with other RNA species. Studies have reported the functional implications of lncRNAs in the developing and mature brain, and lncRNA deregulations have been associated with many neurodegenerative diseases. Some lncRNAs are evolutionary conserved, suggesting a critical role for them across diverse species. **Objectives**. The goal was 1/ to identify lncRNAs that are human- and brain-specific, 2/ to investigate whether the expression of these lncRNAs was critical for neuronal differentiation, and 3/to determine whether deregulations of their expression could be associated with neurodegenerative diseases.**Methods.** The functional characterization of these lncRNAs

was performed using neurons derived from human induced pluripotent stem cells and brain tissue of patients with neurodegenerative diseases.**Results.** We performed a bioinformatic analysis on lncRNA expression in different tissues and organisms and identified 8 lncRNAs that are specifically expressed in humans and in a high abundance in brain tissue. Subsequent quantitative PCR analysis validated the expression of these lncRNAs in mature neurons and brain tissues. We focused this study on our lncRNA #1 candidate and demonstrated that its expression is dynamically regulated during neuronal differentiation. The knock-down of this lncRNA impaired the differentiation and maturation of primary neurons, and transcriptomics analysis confirmed that the absence of lncRNA #1 led to the dysregulation of key signature genes involved in neuronal differentiation. Finally, we showed that the expression of this lncRNA is deregulated in the brain tissue of patients with Alzheimer's , Huntington's disease, and Parkinson disease.**Conclusions**. We identified human- and brain-specific lncRNAs whose expression is critical for neuronal maturation and deregulations associated with neurodegenerative diseases. Our next step is to identify the molecular mechanisms by which they are important contributors to brain pathophysiology.

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**Title:** Single-cell genomics reveals region-specific developmental trajectories underlying neuronal diversity in the human hypothalamus

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**Abstract:** The development and diversity of neuronal subtypes in the human hypothalamus has been insufficiently characterized. We sequenced the transcriptomes of 126,840 cells from the prenatal and adult human hypothalamus, revealing a temporal trajectory from proliferative stem cell populations to mature neurons and glia. Developing hypothalamic neurons followed

branching trajectories ultimately leading to 370 transcriptionally distinct neuronal subtypes in eleven hypothalamic nuclei. Lineage analysis also revealed transcription factors (TFs) associated with the development or maintenance of distinct neural populations. The uniqueness of hypothalamic neuronal lineages was examined developmentally through comparisons to neurodevelopmental lineages in the cortex and ganglionic eminences, utilizing scRNA-seq in multiple brain regions from each donor, revealing both distinct and shared drivers of neuronal maturation across the human forebrain. Cross-species comparisons to published scRNA-seq from the mouse hypothalamus identified corresponding populations for most neuronal subtypes. Focusing on *POMC+* neurons, the major appetite-suppressing neurons in the arcuate nucleus, we found distinct groups of TFs active at early, middle, and late stages of the maturation of maturation. Sub-clustering of n=1656 *POMC+* human cells together with n=4,539 *POMC+* mouse cells revealed six evolutionarily conserved, transcriptionally distinct sub-populations. These results provide a comprehensive transcriptomic view of human hypothalamus development through gestation and adulthood at cellular resolution.

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**Title:** Somatic SNV revealed by duplex sequencing in fetal human brains

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**Abstract:** Recent work suggests that somatic mutations may be unexpectedly common in the human brain, though the extent and causes of such mutations are unknown. Somatic mosaicism in neurons has been found to have associations with neurodevelopmental and neuropsychiatric disorders, as well as normal aging, but there is currently not much knowledge of how many mutations human brains acquire during development. In order to determine the number of sSNV in individual neurons of human fetal brains, we used a single-cell whole genome amplification technique called META-CS, which amplifies the complementary strands of DNA and leverages these “duplexes” for accurate somatic SNV (sSNV) detection; thus we avoid false positive calls created by single-stranded DNA damage seen in other whole-genome amplification techniques.We applied this technique on single and bulk neurons from fetal and infant tissue. In

comparison, to postnatal tissues (teenager, middle aged, and elderly) sSNV burden is higher in fetal compared to infant neurons, then rises again with postnatal age. The variation in the SNV burden for the fetal brain samples is also much higher than any other age group. We analyzed the mutational spectra of human fetal sSNV, and showed that it predominates in a clock-like signature SBS5 as well as SBS1. However compared to postnatal neurons, the distribution of these signatures differ during prenatal life. In conclusion, our results indicate a surprising elevation of sSNV in fetal development of yet unclear etiology, and have the potential to shape our understanding of what a "normal" amount of sSNV are in fetal brains.

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**Title:** Non-coding variants alter GATA2 expression in rhombomere 4 motor neurons and cause dominant hereditary congenital facial paralysis

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**Abstract:** Our ability communicate verbally and non-verbally through facial expression is critically dependent on the nuanced control of facial muscles. Function of the facial motor system requires the coordination of facial branchiomotor neuron (FBMN) identity assignment, migration, and axon targeting. These processes are disrupted in hereditary congenital facial paresis type 1 (HCFP1), an autosomal dominant disorder of absent or limited facial movement that maps to chromosome 3q21-q22. Here we report that HCFP1 results from heterozygous duplications within a neuronal-specific *GATA2* regulatory region that includes two enhancers

and one silencer, and from non-coding single-nucleotide variants (SNVs) within the silencer. Some SNVs impair binding of NR2F1 to the silencer *in vitro* and *in vivo*, and attenuate *in vivo* enhancer reporter expression in FBMNs. *Gata2* and its effector *Gata3* are essential for inner ear efferent neuron (IEE) but not FBMN development in mice. A humanized HCFP1 mouse model extends *Gata2* expression, favors the formation of IEEs FBMNs, and is rescued by conditional loss of *Gata3*. These findings reveal a critical developmental switch required for facial motor function and highlight the importance of temporal gene regulation in development and of noncoding variation in rare Mendelian disease.

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**Nanosymposium**

**NANO22: Neuroinflammation and Microglia**

**Location:** WCC 144

**Time:** Sunday, November 12, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO22.01

**Topic:** B.09. Glial Mechanisms

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**Title:** Microglial P2Y6 calcium signaling promotes phagocytosis and shapes neuroimmune responses in epilepsy development

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**Abstract:** Microglial calcium signaling is engaged during early epilepsy development, but we do not know its underlying mechanism or purpose. After screening multiple receptors, we found that the activation of $P2Y_6$ receptors transduces calcium signaling in microglia during epileptogenesis through a Gq signaling pathway. UDP is the main molecular activator of $P2Y_6$ receptors, and its release is a conserved response to seizures and excitotoxicity. By knocking out the $P2Y_6$ receptor, we discovered that UDP-$P2Y_6$ signaling is necessary for microglial lysosome upregulation in multiple brain regions associated with epilepsy development. $P2Y_6$ activation also enhances pro-inflammatory cytokine production in hippocampus. Targeting a calcium extruder (CalEx) protein to microglia can also attenuate microglial calcium signaling, similar to the $P2Y_6$ KO mouse. In CalEx microglia, we similarly observe failures in lysosome upregulation during epilepsy development. In the hippocampus, only microglia with $P2Y_6$ expression can perform full neuronal engulfment, which substantially reduces CA3 neuron survival and impairs cognition. Our results demonstrate that calcium activity, driven by UDP-$P2Y_6$ signaling, is a signature of phagocytic and pro-inflammatory function in microglia during epileptogenesis.

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**Title:** Functional characterization of microglia derived Nlrp3 dependent extracellular vesicles in neuroinflammation

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**Abstract:** The activation of the NLRP3 inflammasome is widely implicated in the pathogenesis of neurodegenerative diseases including Parkinson's disease (PD) and Alzheimer's Disease (AD). The NLRP3 inflammasome is activated in microglia in response to danger signals which can include pathologic forms of amyloidogenic proteins, bacteria, or viruses. Activation of the inflammasome results in the release of proinflammatory cytokines as well as a distinct subpopulation of NLRP3 activation dependent extracellular vesicles (EVs). Inflammasome dependent EVs are poorly characterized but contain inflammasome related proteins and RNAs in addition to aberrant protein aggregates. A growing body of evidence indicates that inflammasome dependent EVs participate in cell-to-cell immune signaling. We used tandem-mass-tag (TMT) based, quantitative mass spectrometry to deeply characterize the proteome of EVs released from reactive primary microglia derived from wild-type and *Nlrp3*$^{-/-}$ mice and identified a subset of 338 proteins differentially packaged upon the loss of *Nlrp3*. We also identified a different subset of 265 proteins that are preferentially packaged upon *Nlrp3* activation in WT microglia. We further dissect the functional consequences of these differentially packaged EVs in cell to cell signaling and inflammasome dynamics. Finally, we investigate the utility of these unique NLRP3 activation dependent proteins as neuroinflammatory biomarkers. Our data include a deep, quantitative coverage of the NLRP3-dependent microglial EV proteome, provide evidence for the role of microglial EVs in immune signaling, and identify novel biomarkers for neuroinflammation.

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**Title:** Dysregulated CD200-CD200R signaling in early diabetes regulates microglia-mediated neuroretinopathy

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**Abstract:** Diabetic retinopathy (DR) is a leading cause of blindness that affects 1 in 3 diabetic adults. Diagnosis and treatment of DR primarily focuses on microvascular changes as they can be identified through clinical examination and targeted with current therapeutics. However, recent investigations have demonstrated that retinal inflammation and neuronal alterations precede vasculopathy and contribute to neurodegeneration and vision loss during early stages of DR. Microglia are the predominant immune cell type in the homeostatic retina and adopt dynamic roles in disease that impact pathogenesis. The role of inflammation in DR has been studied extensively but the unique contributions of microglia remain unclear. We have shown that chronic ablation of retinal microglia ameliorates visual dysfunction and neurodegeneration in a type I diabetes mouse model. Furthermore, we have found evidence of enhanced microglial contact and engulfment of retinal neurons and synapses, and transcriptomic alterations that drive inflammation and phagocytosis. In this study, we aimed to identify microglia-neuron signaling interactions affected by early DR to inform immunotherapeutic strategies for inhibiting microglia-mediated neuroretinopathy. Using RNA-Sequencing data from DR-associated retinal microglia, ligand-receptor databases, gene expression assays, and immunostaining we found that the immunomodulatory CD200-CD200R signaling axis shared between inner retinal neurons and microglia is dysregulated during early DR in a streptozotocin-induced (STZ) diabetes mouse model and correlates with neuroinflammation. Using the BV2 microglia cell line, we show that pharmacological targeting of CD200R with commercially available CD200 fusion protein (CD200Fc) can attenuate high glucose-induced inflammatory cytokine production and phagocytosis of pHrodo-tagged cell debris *in vitro.* Lastly, we show that intravitreal delivery of CD200Fc *in vivo* reduces inflammation in STZ mouse retinas. These studies provide a molecular framework for the pivotal role that microglia play in early DR pathogenesis. Furthermore, in demonstrating the immunotherapeutic value of CD200Fc *in vitro* and *in vivo*, we present a novel mechanistic target for potential neuroretinal preservation in patients.

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**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

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**Title:** Dual role of Microglial RGS10 in inflammation and metabolic homeostasis

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**Abstract:** Age-related changes in inflammation and metabolism in the brain have been implicated as risk factors for neurodegenerative diseases, including Parkinson's disease (PD), Lewy body dementia, and other related form of dementia. Microglia are immune cells that reside in the brain, and they play a critical role in the dynamic immune surveillance of the central nervous system (CNS) and maintenance of metabolic homeostasis. of Regulator of G-protein Signaling 10 (RGS10) is a homeostatic protein in microglia and its level is significantly decreased with chronic inflammation and aging. We hypothesize that age-associated decrease in the expression of RGS10 in microglia results in an exaggerated chronic inflammatory response and disrupts metabolic balance in the CNS. Our previous research showed that RGS10 knockout mice displayed impaired glucose tolerance and high-fat induced insulin resistance phenotypes, indicating a potential role for RGS10 in metabolic homeostasis. However, the specific association between microglial RGS10 and metabolic homeostasis, as well as its mechanistic connection with the inflammatory response, has not been clearly demonstrated. To address this gap, we conducted an investigation to explore the potential dual regulatory role of microglial RGS10. The focus was on determining whether microglial RGS10 is involved in regulating both inflammatory and metabolic responses. In this current study, we found that high glucose levels exacerbate the production of tumor necrosis factor (TNF) in BV2 cells lacking RGS10. This indicates that RGS10 has a regulatory role in suppressing TNF production in microglial cells under high-glucose conditions. Our data also indicate that microglia lacking RGS10 displays impaired synuclein uptake and clearance, and it is exacerbated under inflammatory and high glucose conditions. This finding supports the notion that RGS10 acts as a dual regulator of both inflammation and glucose homeostasis in microglia. This integrated approach would provide valuable insights into the potential therapeutic targeting of RGS10 which we are under the developing both gene and nanoparticle delivery of RGS10 for conditions involving dysregulated microglial function, inflammation, and metabolism.

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**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Clearance of senescent microglia improves remyelination in a mouse model of demyelination

**Authors: *P. S. GROSS**[1], V. DURAN LAFORET[2], Z. MANAVI[3], J. LEE[3], N. SHULTS[3], D. P. SCHAFER[4], J. K. HUANG[3];
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**Abstract:** Multiple Sclerosis (MS) is a demyelinating and degenerative disease that is the number one neurological disability among young adults. Myelin degeneration results in denuded axons that consequently conduct signals less efficiently and eventually degenerate. In early stages of the disease, oligodendrocytes, the myelinating cells of the central nervous system, and their progenitors (OPCs), can regenerate myelin, resulting in effective remyelination. However, at later stages of the disease and with increasing age, remyelination becomes inefficient, leading to degeneration of axons and worsening of symptoms. One of the key characteristics of aging is the accumulation of senescent cells, which are apoptosis-resistant cells locked in permanent cell cycle arrest that secrete an inflammatory milieu termed the senescence associated secretory phenotype (SASP). Here, we hypothesized that the age-associated accumulation of senescent cells limits the ability of OPCs to remyelinate. Using a murine reporter line with a fluorochrome indicator under the expression of the p16INK4a locus (p16-tdt), a common marker of senescence, we found increased markers of senescent cells present after toxin-induced demyelination and throughout the process of remyelination. As early as 5 days post lesion (dpl), we found large increases in p16-tdt positive cells and other senescence markers, with expression progressively decreasing throughout remyelination before plateauing, with low lingering levels present at later timepoints. These markers predominantly colocalized with microglia. Additionally, aged mice showed markedly increased markers of senescence in the lesion compared to their young counterparts at both early and late timepoints post lesion, suggesting the accumulation and subsequent failed clearance of senescent cells might contribute to their reduced remyelination. Interestingly, clearance of senescent cells in young and middle-aged mice resulted in increased remyelination in the lesion compared to vehicle-treated controls, and a reduction in inflammatory microglia. These results suggest that therapeutic targeting of cellular senescence, i.e. by senolytics or senomorphics, might promote remyelination in MS.

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

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**Title:** Acyl-coa:cholesterol acyltransferase 1 (ACAT1) inhibition modulates TREM2 and LRP1 to increase amyloid-β uptake in microglial cells

**Authors: *M. HOVDE**, A. MAASER-HECKER, D. KOVACS, R. E. TANZI;
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**Abstract:** Alzheimer’s disease (AD) is a neurodegenerative disease with hallmarks that include accumulation of extracellular amyloid plaques which contain mostly Aβ peptides, neurofibrillary tangles composed of hyperphosphorylated tau, and chronic neuroinflammation. Additionally late onset AD patients have been shown to have increased lipid droplet accumulation. Lipid droplets are made up of mostly neutral lipids such as triacylglycerols and cholesterol esters (CE). CE is the storage form of cholesterol and makes up less than 1% of total cholesterol content in the brain. However, in vulnerable brain regions of AD patients, CE levels are significantly increased. Acyl-CoA:cholesterol acyltransferase 1 (ACAT1) is a resident ER protein that converts free

cholesterol to CE that can no longer be sequestered in membrane and is, instead, stored in lipid droplets. Cholesterol homeostasis is vital for cellular health as high levels of cholesterol at the membrane can crystalize and cause damage to cell membranes, ultimately resulting in cell death. Previous work from our lab has shown ACAT inhibition decreases CE levels within cells and decreases amyloid precursor protein maturation leading to decreased amyloid-β (Aβ) generation in neurons. Additionally, ACAT inhibition *in vivo* has been shown to reduce levels of existing amyloid β plaques in AD mouse models. In AD, microglia cells play a major role in phagocytosis of Aβ plaque. Thus, we explored the role ACAT regulation in microglia Aβ uptake. Shibuya et al. (2014) reported ACAT inhibition in microglia increases Aβ uptake and phagocytosis, however the mechanism by which Aβ uptake is increased is unknown. To explore this, we targeted Triggering Receptor Expressed on Myeloid cells 2 (TREM2) and Low Density Lipoprotein Receptor Related Protein 1 (LRP1) both of which are involved in lipid signaling and bind multiple ligands including Aβ. We find ACAT inhibition increases Aβ uptake in BV2 microglial cells but when we knocked out TREM2 in BV2 cells, ACAT inhibition no longer led to increased Aβ uptake. When LRP1 was knocked out in BV2 cells, TREM2 protein levels were significantly decreased, however ACAT inhibition still led to increased Aβ uptake. Taken together we have determined that TREM2 plays a role in the mechanisms by which ACAT inhibition increases Aβ uptake, likely through downstream signaling events. In summary, we posit a complex relationship between ACAT regulation, TREM2, LRP1 signaling, and Aβ uptake in microglia.

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**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

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**Title:** Extracellular vesicles derived from clonally expanded immortalized mesenchymal stromal cells reduce neuroinflammation in a pre-clinical model of sepsis-associated encephalopathy

**Authors: *M. B. DA SILVA**[1], Y. MOULOUD[2], J. N. TSOPORIS[3], A. EKTESABI[4], C. M. VASWANI[5], S. YOUSEF[4], S. IZHAR[4], B. BERND GIEBEL[2], T. MARON-GUTIERREZ[1], C. C. DOS SANTOS[3,6,5];
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**Abstract:** Sepsis is an exacerbated host response against an infection. Sepsis-associated encephalopathies (SAE) are neurological complications which occur during or after sepsis

events. Currently, there is no treatment available for SAE-induced neurological damage. Therefore, it is necessary to investigate new therapeutic approaches. Mesenchymal stromal cells (MSCs) have a well-established immunomodulatory capacity. Our group demonstrated MSC administration reduces neuroinflammation and cognitive damage caused by SAE. Moreover, conditioned media from MSCs reduces astrogliosis *in vitro*, suggesting a paracrine mechanism of action. MSCs release extracellular vesicles (EVs), containing proteins, nucleic acids, and lipids that may confer therapeutic properties to recipient cells. The aim of the present study was to evaluate the effects of intravenous clonally-expanded immortalized MSC-derived EVs on sepsis-induced neuroinflammation. Our model consisted in male adult C57Bl0/6 mice that underwent cecal ligation and puncture (CLP) surgery for sepsis induction versus sham-surgeries. All mice received antibiotics, fluid resuscitation and pain medication. Six hours after surgery, mice were randomized to MSC-EVs versus placebo (platelet-derived EVs) or equal volume saline. Outcomes were measured 72 hours post-CLP. MSC-EV administration resulted in improved survival, reduced expression of inflammatory mediators in the circulation, lungs and hearts, and histological evidence of reduced astrogliosis and microglial activation in the cortex and hippocampus. At 72 hours, we did not detect changes in the local synthesis of pro-inflammatory mediators, assessed by qPCR. In cultured microglial cells (BV2 cells) stimulated with LPS, MSC-EVs resulted in decreased IL-6 and IL-1b gene expression; this effect could be blocked by Dynasore-an inhibitor of clathrin/dynamin-mediated endocytosis. Taken together our results suggest a single dose-therapy with MSC-EVs reduced peripheral inflammation, neuroinflammation, and improved survival from CLP-induced sepsis. In vitro, MSC-EV-mediated reduction in pro-inflammatory gene expression was in part dependent upon MSC-EV uptake, highlighting the potential of EVs as tissue-specific targeted therapies.

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**Title:** Multiscale and multiomic profiling of cellular signaling in pathologic neuroinflammation after West Nile virus encephalitis

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**Abstract:** In human brain infection (encephalitis) due to West Nile virus (WNV), patients often experience prolonged neurocognitive impairment that persists well after the virus has been eliminated. Recent evidence suggests an exaggerated inflammatory immune response in the brain (neuroinflammation) in these patients is a primary driver of neural injury. To model human

WNV encephalitis, we infected wild-type B6 mice via a footpad injection. In this model, we found that WNV RNA is first detectable in the brain at 6-8 days post infection (DPI), peaking at 10 days and declining steeply thereafter. Using immunohistochemistry, we found that astrocyte activation was coincident with viral RNA, while microglial activation occurred later and CD8 T cell recruitment occurred last. Microglial activation persisted longer than the other cell types. In the same animals, we performed noninvasive neuroimaging using positron emission tomography (PET) of translocator protein (TSPO) signal and found a strong correlation with Iba1 immunoreactivity, a marker of microglial activation.
However, immunohistochemical assays focusing on expression of single proteins provide limited insight into complex signaling pathways and direct cell to cell interactions. In order to investigate the inflammatory pathways associated with long term neural injury after infection, we used muliplex analyte detection with spatial context featuring the the CosMx$^{TM}$ Spatial Molecular Imager (Nanostring). We performed simultaneous proteomic and transcriptomic analysis on the same tissue section at a single-cell resolution. Greater than 68 proteins and 1000 RNAs are targeted on the same mouse brain section, covering 44 pathways. This platform enables robust neural and glial cell typing, and illuminates key aspects of neurodegeneration, neurodevelopment, cell state and signaling, including numerous ligands and receptors involved in neuron-glia communication. Our analysis identified pathways related to inflammation and cellular damage in neurons, astrocytes, microglia and T cells linked with WNV infection and infection sequelae.
By integrating immunohistochemistry, neuroimaging, and single cell high plex spatially resolved proteogenomics, we gain unprecedented holistic insight into the mechanisms driving pathologic neuroinflammation in post-viral encephalitis in an intact animal. Finally, we identify potential novel targets for treating neuroinflammation in WNV encephalitis in order to prevent long term neural injuries from infectious disease.

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**Presentation Number:** NANO22.09

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

**Support:** Restracomp Graduate Scholarship
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**Title:** Amyloidogenic cell culture models derived from human pluripotent stem cells implicate microglial phagocytosis and pro-inflammatory responses in AD pathogenesis

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**Abstract:** Alzheimer’s disease (AD) is a progressive neurodegenerative disease that is the most common cause of dementia. The aggregation of amyloid-β (Aβ) appears central to the disease, however treatments that target this phenomenon are largely ineffective at stopping disease progression. Genome-wide association studies of AD repeatedly highlight loci whose cerebral expression is limited to microglia. In this study, we aim to use human pluripotent stem cell (hPSC)-derived cell culture models of AD to characterize microglial reactivity to Aβ and to determine the functions of uncharacterized microglial AD risk loci. We have differentiated microglia from hPSCs and acutely exposed monotypic cultures to synthetic amyloid beta. We isolated total RNA after 24 hours and performed RNA-seq analysis to determine differentially expressed genes. We show that synthetic Aβ induces a pro-inflammatory response, demonstrated by the upregulated mRNA expression of cytokines IL1B and TNF, and monocytic/microglial-responsive chemokines CCL3, CCL8, and CCL13. We also embedded microglia into 3-dimensional co-cultures with other hPSC-derived neural cells to allow their maturation which we demonstrate through morphological and transcriptomic changes. We observed similar inflammatory responses to synthetic Aβ in these co-cultures evidenced by the upregulation of pro-inflammatory mRNA transcripts as measured through RT-qPCR analyses. To interrogate microglial responses to Aβ generated from within cell cultures, we built an inducible transgene that expresses familial mutations in amyloid precursor protein ($APP^{S/F/L}$) and presenilin-1 ($PSEN1^{M146L/L286V}$). We used ELISA and western blot analyses to demonstrate that cell cultures expressing this transgene produce high levels of Aβ only when induced. In parallel, we performed genome editing using CRISPR/Cas9 to generate hPSC lines with mutations in membrane-spanning 4-domains A6A (MS4A6A), a poorly characterized AD risk locus. In

MS4A6A$^{-/-}$ microglia, we observed changes in phagocytic responses to pHrodo-labeled particles. Together, our findings suggest that microglia mount inflammatory responses to Aβ, and we propose that MS4A6A affects AD risk by modulating the sensing or uptake of Aβ. In order to further interrogate the intersection of MS4A6A function and inflammatory responses to Aβ, we are generating 3-dimensional amyloidogenic neural cell cultures with wild-type or MS4A6A$^{-/-}$ microglia. These cultures will provide a platform to investigate the function of other risk loci in a genetically manipulable human model of AD.

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**Nanosymposium**

**NANO23: Imaging and Assessment of Stroke Damage**

**Location:** WCC 143

**Time:** Sunday, November 12, 2023, 1:00 PM - 3:45 PM

**Presentation Number:** NANO23.01

**Topic:** C.09.Stroke

**Support:** NIH Grant R00HD091375
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**Title:** Electroencephalography measurements of cross-frequency coupling in early stroke recovery

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**Abstract:** Neuroimaging-derived biomarkers may offer critical insight to inform post-stroke rehabilitation. Phase-amplitude coupling (PAC), a form of cross-frequency coupling, is a mechanism by which wavelets generated in the prefrontal cortex (PFC delta oscillations, 1-4 Hz) subserving executive control modulate activity in the primary motor cortex (M1 beta oscillations, 13-30 Hz). In this study, we acquired serial measurements of prefrontomotor delta-beta PAC (DB-PAC) in individuals early post-stroke to investigate coupling during inpatient rehabilitation. Participants with stroke admitted to an inpatient rehabilitation facility (IRF) completed two research visits near IRF admission and discharge. Visits involved motor behavioral assessments (Upper Extremity Fugl Meyer (UEFM) and Action Research Arm Test (ARAT)) and high-density electroencephalography (EEG) recordings at rest and during a 20% submaximal isometric grip task. Unimpaired controls completed a single EEG research visit. We calculated DB-PAC from leads overlying PFC and M1. Given greater cognitive demand during task vs. rest states, we hypothesized greater DB-PAC in the former. Consistent with the compensatory recruitment of cortical regions during movement post-stroke, we hypothesized greater DB-PAC during task performance in stroke vs. controls. Further, we expected greater coupling during

affected vs. less-affected extremity performance that related to motor behavior over hospitalization.
Of the 30 individuals with stroke enrolled (14 females, 66.8 ± 9 years of age, 10.4 ± 3 days post-stroke), 20 completed testing at IRF admission and 26 at discharge. A control group of 18 individuals (8 females, 75.3 ± 13 years of age) also participated. Across groups, DB-PAC was greater during the task condition as compared to rest at admission ($F(1,65.2)= 17.1$, $p< 0.0001$) and discharge ($F(1,71.3)= 19.6$, $p= 0.0001$). At admission, individuals with stroke demonstrated greater DB-PAC during task performance than controls ($F(1, 38.4)= 17.0124$, $p= 0.0002$) with higher coupling observed in the affected extremity ($t= 21.53$, $p< 0.0001$) that positively correlated with motor behavior at admission (UEFM: $\rho= 0.59$, $p= 0.026$; ARAT: $\rho= 0.55$, $p= 0.041$). Notably, coupling during task performance from the affected extremity decreased during hospitalization ($t= 15.13$, $p< 0.0001$).
These findings suggest an increased reliance on PFC control over M1 during early stroke rehabilitation, particularly evident during affected extremity task performance that decreases over hospitalization. Communication between PFC and M1 during early stroke recovery as captured by DB-PAC may be an informative motor biomarker.

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**Presentation Number:** NANO23.02

**Topic:** C.09.Stroke

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**Title:** Linking post-stroke neurophysiology to neuroanatomy: novel method to extend voxel-lesion mapping to multi-dimensional EEG data

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**Abstract:** EEG measures of neural activity contain rich spatio-temporal information across different channels and frequency bands. EEG recordings after stroke have shown group-level changes in both resting state and task-evoked activity. However, the neuroanatomical basis of these EEG changes remains poorly understood with relatively few studies attempting to link the neuroanatomy of lesions to the changes seen in EEG signals. Here we present a novel method to analyze the relationship between patterns of neuroanatomical injury and changes in neural activity across different cortical regions and frequency bands.
EEG-Voxel Lesion Mapping (EEG-VLM) builds on voxel-lesion symptom mapping (VLSM). In VLSM a single *t*-map is created by comparing values for a single "symptom" (i.e., one value per patient) at every voxel between those patients who do and don't have a lesion at that voxel. To incorporate the multi-dimensional nature of EEG signals (*n* channels x *m* frequencies), a separate *t*-map is first created for each pair (channel, frequency). Using adjacency maps (based on neighboring channels and frequencies), we designed a novel, tractable, clustering algorithm which finds maximal significant clusters (containing *x* voxels, *y* EEG channels and *z* frequencies). A cluster captures a relationship where lesions in any of the *x* voxels co-occur with significant changes at each EEG pair (channel, frequency). The significance of these clusters can then be assessed using permutation statistics.
We applied this method to a previously recorded dataset in which chronic stroke patients ($N$=30, 49.8 +/-12.1 years, 18 Male) with arm and hand motor impairments performed cued hand opening/attempted opening using their healthy or paretic hand. Initial findings show that during attempted paretic movement (in the first 500ms post-movement cue), EEG-VLM finds a significant cluster (permutation test, $p$=0.021) relating (a) neural activity in the beta range (22-32Hz) in lesioned hemisphere's motor cortex and (b) the presence of lesions in frontal white matter. The directionality of this relationship is that frontal white matter lesions predict reduced motor-related beta desynchronization. Given that frontal white matter lesions are implicated in impaired attention, alertness, and inhibition, our findings suggest cognitive factors may play a role in motor-related beta-desynchronization.
EEG-VLM is a novel and unbiased method for relating neurophysiologic changes after stroke with neuroanatomic lesions. We propose that this method will enable an improved mechanistic understanding of stroke-induced changes in EEG signals and improve detection of behaviorally relevant EEG-biomarkers.

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**Title:** Poor sleep efficiency 3 months post-stroke is associated with fibre density reduction in the cortico-ponto-cerebellar tract over 3 years.

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**Abstract:** Sleep disorders after stroke are highly prevalent at all post-stroke phases - acute, subacute and chronic. However, the pathogenesis of poststroke sleep dysfunction remains largely unclear. In our previous work, we demonstrated that during the subacute period (at 3 months), suboptimal sleep duration and poor sleep efficiency were associated with neurodegeneration affecting the reticular activating system, with the white matter fibre density reduction primarily occurring in the corticopontocerebellar tract. However, little is known about the longitudinal trajectories of white matter changes associated with sleep dysfunction. In the current study, we aimed to determine whether poor sleep at 3 months is associated with continued neurodegeneration in the cortico-reticular pathway over 3 years.Using the data from the Cognition And Neocortical Volume After Stroke (CANVAS) study, we categorised stroke participants as "good" sleepers (>80% sleep efficiency, N=47) or "poor" sleepers (<80% sleep efficiency, N=17) based on sleep actigraphy (SenseWear Armband) data at 3 months post-stroke. Using diffusion-weighted imaging data processed with fixel-based analysis in MRTRIX and tracts segmented with TractSeg, as in our previous work, we quantified fibre density changes between 3 months and 3 years post-stroke in 10 tracts of the cortico-pontine-cerebellar pathway, shown to have reduced fibre density at 3 months. For each of the tracts we compared fibre density between the two sleep groups, including age, sex, stroke severity and total intracranial volume as covariates. The poor sleep efficiency group showed continued fibre density decline in the right cortico-spinal ($F[1,58]=5.401$, $p=0.024$), fronto-pontine ($F[1,58]=4.019$, $p=0.05$) and parieto-occipital pontine tracts ($F[1,58]=5.331$, $p=0.025$), while no considerable change over 3 years was observed in the good sleeper group. A confirmatory correlational analysis in the whole stroke group showed an association between fibre density reduction and poor sleep efficiency in 7 of the 10 tracts, including bilateral pontine tracts and contralateral cerebellar peduncles.Our results suggest that the corticopontocerebellar tract functionally relevant for sleep is vulnerable to sustained degeneration in the chronic post-stroke phase (at least over 3 years) in stroke survivors experiencing poor sleep quality subacutely at 3 months. Treatments improving post-stroke sleep in the acute and subacute stages may be needed to prevent longer term neurodegeneration due to poor sleep.

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**Title:** Regional Brain Age Is Associated with Sensorimotor Outcomes in Stroke: An ENIGMA Stroke Recovery Analysis

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**Abstract:** Brain age, a global measure of neurobiological aging assessed from structural MRI, has recently been associated with post-stroke sensorimotor outcomes, explaining variance beyond that of focal metrics, such as lesion volume or load. However, it is unclear whether lesion location leads to more brain aging in some regions, or if greater aging in specific brain regions has a stronger impact on functional outcomes. We hypothesized that there should be more brain aging observed in regions closer to the lesion (e.g., ipsilesional cortex) compared to more distant, contralesional regions. We also expected that lesion damage in more globally-connected networks, such as the salience network, should have greater impacts on the rest of the brain. Finally, we expected that brain aging in sensorimotor networks should be most strongly related to sensorimotor outcomes. To examine these questions, we used cross-sectional, high-resolution brain structural MRIs and clinical measures from 432 chronic stroke patients from 27 cohorts in the ENIGMA Stroke Recovery consortium. Our deep learning model estimated regional brain age from cortical morphological metrics and was trained on 17,791 UK Biobank subjects without stroke, using 5-fold cross-validation. We extracted regional brain age estimates for 18 functionally-defined networks (ipsilesional and contralesional sensorimotor, frontoparietal, dorsal, ventral-language, default, salience, auditory, visual, and limbic networks). Mixed effects linear models were used to examine relationships between brain predicted age difference (brain-PAD), lesion size, and sensorimotor outcomes, FDR-corrected for multiple comparisons ($P<0.05$). Greater lesion volume was significantly associated with older brain age in 7 ipsilesional networks, and younger brain age in 1 contralesional network. Greater lesion load in ipsilesional networks was related to older brain age in other ipsilesional networks, with the most

wide-spread effects observed with lesion load on the ipsilesional salience network. Finally, older ipsilesional sensorimotor brain age, along with greater lesion load on ipsilesional sensorimotor, salience, and limbic networks, significantly improved prediction of sensorimotor outcomes ($R^2$ = 0.58, $p<0.001$). Overall, we demonstrate that lesion volume and location influence regional brain aging patterns with disproportionate effects in ipsilesional sensorimotor and salience networks. Damage to the salience network has the most extensive downstream impacts compared to other networks. Inclusion of regional MRI features may improve predictive models of post-stroke sensorimotor outcomes.

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**Topic:** C.09.Stroke

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**Title:** Cerebellar white matter and language recovery in post-stroke aphasia following transcranial direct current stimulation

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**Abstract:** This study aimed to investigate the neural substrates of language recovery in post-stroke aphasia (PSA) patients treated with transcranial direct current stimulation (tDCS). While tDCS has been found effective in enhancing language outcomes, the mechanisms underlying this improvement remain unclear. Here, we employed diffusion tensor imaging (DTI) to examine the correlation between language improvement and changes in infratentorial white matter regions, which are macrostructurally intact in PSA. This approach circumvents the methodological challenges posed by large supratentorial lesions in PSA, which complicate neuroimaging analysis.
DTI analysis was conducted on 33 PSA patients (16 tDCS; 17 sham), who received either real or sham tDCS (20 minutes per session over 5 days) as an adjunct to SLT, as part of a double-blind randomized controlled trial. Fractional anisotropy (FA) and mean diffusivity (MD) were calculated for selected white matter regions of interest, and changes in these metrics were correlated with changes in language outcomes following intervention.
After multiple comparisons correction, our results showed that improvement in spontaneous speech was significantly correlated with reduced MD in the left superior cerebellar peduncle

(SCP) following real tDCS, but not in the sham group. This finding suggests that the cerebellar output pathway could play a crucial role in language recovery, possibly by facilitating neuroplasticity and strengthening communication between the cerebellum and other speech-related brain regions.
These findings could inform the development of more targeted and effective treatment strategies for PSA, underscoring the value of exploring white matter changes as potential neural markers of recovery. This study provides a critical step toward understanding the complex neural mechanisms underlying tDCS-mediated language recovery in PSA.

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**Title:** Contributions of white matter hyperintensities to motor outcomes after stroke

**Authors: *J. FERRIS**[1], B. LO[2], A. BRODTMANN[3], C. M. BUETEFISCH[4], K. HAYWARD[5], A. CONFORTO[6], L. A. BOYD[7], S.-L. LIEW[2];
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**Abstract: Background:** Damage to the corticospinal tract (CST) is strongly associated with motor impairment after stroke[1,2]. Yet, measures of CST damage do not completely explain variance in motor outcomes, especially for individuals with severe motor impairment[3]. Recovery from stroke is mediated by surviving neural pathways that compensate for the stroke injury[4]. These compensatory pathways may be affected by concurrent age-related structural damage from white matter hyperintensities (WMHs)[5], which may account for additional variance in motor outcomes.
**Aim:** This study tested if WMH volumes modulate relationships between CST integrity and motor impairment after stroke. CST integrity was indexed by weighted lesion load (CST-LL), a weighted sum of overlapping voxels between the stroke lesion and an atlas-defined CST tract[1,2]. We hypothesized that whole-brain WMH volume would interact with CST-LL, such that individuals with more extensive CST-LL would show a stronger relationship between WMH volume and motor impairment.
**Methods:** We included data from the ENIGMA Stroke Recovery Working group. Our final sample included 161 individuals from 7 independent research sites meeting the following inclusion criteria: 1. availability of T1 and FLAIR/T2 scans; 2. >7 days post-stroke; 3. some degree of motor impairment on standardized motor assessment scales. Stroke lesions were manually drawn on T1-weighted scans and WMH volumes were extracted with FreeSurfer's SAMSEG[6]. We ran a linear mixed effect model with motor impairment as the outcome measure and a random effect of site, testing for an interaction between CST-LL and WMH volumes, with

age, sex, and days post-stroke as covariates.
**Results:** There were main effects of CST-LL ($b$ = 0.415, $p$ < 0.001) and WMH volume ($b$ = 0.203, $p$ = 0.011) on motor impairment, but no significant CST-LL x WMH volume interaction ($b$ = 0.034, $p$ = 0.556). A follow-up mediation analysis revealed that WMH volume partially mediates the effect of CST-LL on motor impairment (mediation effect of WMH: $b$ = 0.041; $p$ = 0.012; direct effect of CST-LL: $b$ = 0.417; $p$ < 0.001; total effect of CST-LL: $b$ = 0.459; $p$ < 0.001).
**Discussion:** WMH volumes relate to motor impairment after stroke, but contrary to our hypothesis this relationship does not vary based on the degree of CST lesion load. WMH volume partially mediates relationships between CST integrity and motor outcome, indicating that WMH-related damage may be clinically relevant for recovery processes after CST injury. Including WMHs in predictive models may help to characterize motor recovery outcomes after stroke; future studies should examine if this is useful in predictions for individuals with severe motor impairment.

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**Presentation Number:** NANO23.07

**Topic:** C.09.Stroke

**Support:** VA Rehabilitation Research & Development Service Grant I01 RX003511

**Title:** Enhancing motor recovery strategies by analyzing the functional connectivity changes in brain motor regional networks after subcortical stroke

**Authors: *X. FANG**[1,2,5], G. HADDADSHARGH[1,3], F. LIU[4,1,5], J. MAK[1,5,3,6], A. BOOS[1,2,5], G. F. WITTENBERG[1,5,3,6,2];
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**Abstract:** Functional magnetic resonance imaging (fMRI) is a useful tool to study changes in neural network interactions after subcortical stroke. Previous studies showed that central nervous system plasticity is associated with motor recovery after stroke, but has not lead to development of rehabilitation methods than effectively use this intrinsic plasticity to support motor recovery. Theoretically fMRI could be used to define how networks are modified by stroke lesions and plasticity, and theraby determine an optimal combination of non-invasive stimulation and motor practice to enhance motor recovery after stroke.
Here we quantify motor system regional connectivity with resting-state fMRI (rs-fMRI) and task-related fMRI (tr-fMRI), specifically focusing on functional connectivity among the bilateral dorsal premotor cortex (PMd), the ventral premotor cortex (PMv), and the primary motor cortex (M1). The fMRI was conducted with 3T Siemens Prisma scanners with a repetition time (TR) = 0.8 seconds on 21 subjects (18 healthy control, 3 stroke). The rs-fMRI data was acquired over two 9-minute runs while the subjects kept their eyes open, and the tr-fMRI data was recorded while the subjects performed 60 trials of 10-second planning and hand-movement tasks, which in

total take around 10 minutes.
The preprocessing and statistical analysis were completed with FSL and SPM12 to estimate the functional connectivity among our regions of interest (ROIs). For rs-fMRI data, we utilized independent component analysis (ICA) and dual regression to explore the seed-based correlation and generate spatial maps at $p < 0.001$ statistical significance level. For tr-fMRI data, we developed generalized linear models (GLM) to extract the stimulus-induced signals and separated those signals based on events to create activation maps at a corrected cluster significance threshold of $p = 0.05$. Then we created dynamic causal models (DCM) to study the effective connectivity among the ROIs. We found connectivity at varied strengths between unilateral premotor cortices and M1 in stroke patients, while in healthy controls, connectivity was established between bilateral premotor cortices and M1. These findings demonstrated the individual differences in network changes after subcortical stroke that suggest a need to develop customized rehabilitation methods, and the potential of using premotor area connectivity measures to design motor rehabilitation treatments that optimize motor recovery.

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**Presentation Number:** NANO23.08

**Topic:** C.09.Stroke

**Support:** VA Rehabilitation Research & Development Service Grant I01 RX003511

**Title:** Non-primary motor area involvement in reaching behavior after subcortical stroke

**Authors: *J. MAK**[1,2,6,7,3], A. F. BOOS[4,7,2], X. FANG[4,2,7], G. HADDADSHARGH[3,2,6], F. LIU[2,7,5], G. F. WITTENBERG[4,3,2,6,7];
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**Abstract:** Motor impairments following a stroke can be a source of decreased independence and quality of life for patients. Our overall goal is to improve motor function after stroke using a combination of neuromodulation and robot-guided practice. To do so, we must first understand the neural mechanisms underlying essential daily movements, particularly reaching. Literature shows that non-primary motor areas contribute to reach behavior, but how their connections to primary motor cortex (M1) change functionally after stroke remains unclear. In this study, we investigate premotor and parietal connectivity during reaching by disrupting these areas with a repetitive transcranial magnetic stimulation (rTMS) protocol in stroke and healthy participants. The results could provide the foundations for neuromodulatory strategies to maximize therapeutic outcomes. We recruited a preliminary cohort of 3 acute subcortical stroke patients and 13 healthy controls. Participants performed 36 trials of planar forward or backward reaches with their right or impaired hand while seated in a robotic exoskeleton. Visual feedback of the target and fingertip cursor disappeared after the go cue. We delivered triple-pulse rTMS 100ms before the reaction time at 80% resting motor threshold (RMT), 120% RMT, and no stimulation (control) over 7 locations: left and right dorsal premotor cortex (LPMd and RPMd), left and right

ventral premotor cortex (LPMv and RPMd), left and right posterior parietal cortex (LPPC and RPPC), and the post-central sulcus (PCS) (control region). Kruskal-Wallis tests were performed to evaluate the effects of stimulation location and intensity on kinematic metrics. In healthy controls, LPMd stimulation at 120% RMT increased the total path length ratio, endpoint error, and initial angle and decreased the percentage of total path length traveled at the time of maximum velocity. This means the LPMd to left M1 connection remains effective at the time of reaching initiation so that disruption by stimulation results in less efficient, less accurate, and less controlled reaches.In the stroke group, two patients showed no effects of stimulation on reaching, suggesting a loss of connectivity between these areas and M1. In the third, LPMv stimulation at 120% RMT appeared to decrease endpoint error while RPMv and RPPC stimulation at 80% increased the path length ratio at maximum velocity, indicating faster and more accurate reaches. These patient-specific results will be used towards the development of a treatment that synchronizes targeted, regional stimulation with practice.

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**Presentation Number:** NANO23.09

**Topic:** C.09.Stroke

**Support:** NICHD-R01HD096071

**Title:** Flexion synergy and paresis both contribute to reaching dysfunction in subacute stroke

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**Abstract:** Paresis and flexion synergy are two cardinal motor impairments that develop after stroke and impact reaching function in the chronic phase of recovery. However, less is known about the contributions of flexion synergy, or loss of independent joint control, in the early weeks after stroke and how it interacts with weakness of the arm over time. The purpose of this study was to explore the contributions of weakness and flexion synergy to reaching dysfunction over the first 100 days after stroke. Eleven individuals (mean FMA = 18, 56 ± 15 years old) with moderate to severe hemiparetic stroke were recruited within an inpatient rehabilitation facility an average of 15 ± 9 days post-stroke. Kinetics and kinematics were used to quantify strength, flexion synergy, and reaching function longitudinally up to 100 days post-stroke. Maximum voluntary torque production in the directions of shoulder abduction and elbow extension for the paretic arm were reported as normalized to the non-paretic side. Two force-based thresholds quantified flexion synergy expression, with the takeover threshold being more practical for early stroke than the emergence threshold. Reaching function was measured as maximum reaching distance at gravitational loading. Linear mixed-effects models explored the contributions of relative strength, synergy, and time to reaching function. Overall, participants produced more volitional torque with their paretic arm over time. Reaching function and the ability to move outside synergy patterns also improved in the weeks following stroke. Linear mixed-effects modeling demonstrated that time ($p = 0.027$), shoulder abduction strength ($p = 0.007$), and flexion synergy ($p = 0.004$) all had significant relationships with reaching function. The

estimates were especially large for shoulder abduction strength (5.01) and flexion synergy (2.56), suggesting strong positive slopes for each association. Additionally, the interaction term between these two fixed effects was significant and negative in directionality (-0.05, p = 0.012). This suggests that improvement in one impairment lessens the impact of the other impairment on reaching function, and vice versa. The final version of the model, which included participant as a random factor, explained 83% of the variance in reaching function. These results provide evidence that both paresis and flexion synergy expression negatively impact individuals' ability to reach against gravity during early stroke recovery. The findings emphasize the importance of restoring shoulder abduction strength and ameliorating the development of synergy through rehabilitation therapy that promotes recovery of reaching function.

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**Title:** A Novel Specific Aptamer Delivery to Cerebrovascular Endothelial Cells in Ischemic Stroke Brains

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**Abstract:** Background: Cerebrovascular endothelial cells (CECs) are key components of the blood-brain barrier (BBB) and are the first cells to respond to ischemic stroke. However, therapeutic strategies targeting CESs are underdeveloped. Vascular cell adhesion molecule-1 (VCAM-1; CD106) is a member of the immunoglobulin-like superfamily (IgSF) that shows increased expression in CECs after stroke. Herein, we test if an RNA aptamer targeting VCAM-1 can be specifically demonstrated to target CECs in stroke brains.Methods: Anti-VCAM-1 aptamer (conjugated with Cy5.5) and control aptamer (randomized oligonucleotides conjugated with Cy5.5) were chemically synthesized. A transient middle cerebral artery occlusion (tMCAO, 60 min occlusion) stroke model was induced in mice. Aptamers (0.5 nmol per mouse) were injected through their tail vein at 6 hours after stroke. Ex vivo whole brain images were acquired by IVIS imaging system. Then, the brain tissues were cryosectioned and stained against CD31 to visualize brain vasculature. Immunofluorescent images were acquired by Nikon confocal fluorescence microscope.Results: Higher fluorescence intensity was detected by IVIS images preferentially from the brain of stroke mice treated with anti-VCAM-1aptamer (n=4)- compared to the mice injected with control aptamer (n=4, $p<0.05$) or PBS (n=4, $p<0.01$). One mouse died in the PBS treated group but no mice died in aptamer treated group, demonstrating no toxicity of RNA. Quantified mean fluorescence intensity (MFI) showed the mice treated with anti-VCAM-1 aptamer had significantly higher MFI in ischemic hemisphere compared with stroke mice. Our confocal microscopy data from immunofluorescence staining against CD31 further confirmed that Cy5.5 signals were overlapped with CD31+ CECs. Conclusion: Our data suggest that CECs

affected by stroke can be selectively targeted with anti-VCAM-1 aptamer. The VACM-1 specific aptamer demonstrates an efficiency and safety of RNA-based aptamer as a useful delivery platform specifical into CECs after stroke. This method is expected to overcome the challenge from the lack of cell-specific targeting approaches needed to reduce off-target side-effects.

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**Title:** Optical Imaging Measures Predict Functional Recovery after Stroke

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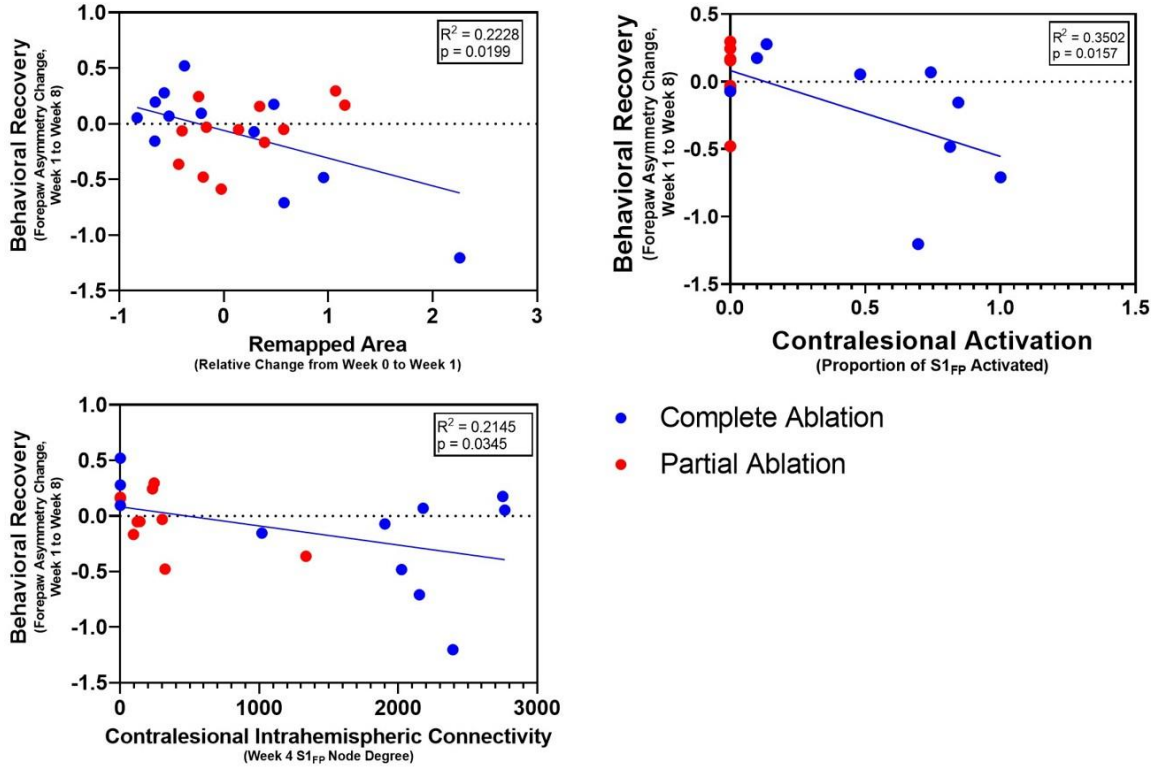
**Abstract:** Recovery from stroke, a major cause of chronic disability in adults, is often incomplete and unpredictable. Remapping of affected local neuronal circuits and repair of brain networks have been associated with enhanced behavioral recovery. However, it is unclear how stroke size affects remapping, network repair, and behavioral recovery. In this experiment, we set out to determine how the degree of injury within a somatotopic domain, which we define as the cortical area activated by stimulation of a given bodily region, affects remapping, network repair, and functional recovery, and to find optical imaging measures that predict recovery. We used *in vivo* wide-field optical imaging to examine longitudinal changes in local neuronal circuits and global neuronal network activity in mice expressing the genetically encoded calcium indicator, GCaMP, after photothrombotic stroke of variable size in the forepaw somatotopic domain. Local circuit activity was measured using evoked response recordings during anesthetized forepaw stimulation. Global network changes were measured using resting-state functional connectivity analysis in awake animals. Complete ablation of the forepaw somatotopic domain (infarcts 2 mm in diameter) caused spatially heterogeneous, bilateral, and multifocal remapping of forepaw somatosensory circuits compared to partial ablation of the forepaw somatotopic domain (infarcts 1 mm diameter), which caused spatially homogeneous, ipsilesional, and unifocal remapping. Larger areas of remapped somatotopic domains one week after stroke predicted poor behavioral recovery. Furthermore, the extent of contralesional activation in the unaffected forepaw somatotopic domain was also predictive of recovery, with greater contralesional activation predicting worsened recovery. Complete ablation of the forepaw somatotopic domain also disrupted global brain networks, causing hyperconnectivity within the contralesional hemisphere, with a high degree of contralesional hyperconnectivity predicting poor behavioral recovery, as well.




**Disclosures: R.M. Bowen:** None. **A.Q. Bauer:** None. **J. Lee:** None.

**Nanosymposium**

**NANO24: Neural Coding, Perception, and Plasticity**

**Location:** WCC 140

**Time:** Sunday, November 12, 2023, 1:00 PM - 3:45 PM

**Presentation Number:** NANO24.01

**Topic:** D.05. Auditory & Vestibular Systems

**Support:** NIH Grant DC003180

**Title:** Discrete patches of cortical pitch processing in the common marmosets

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**Abstract:** How the brain processes pitch on complex sounds has been one of auditory neuroscience's central questions due to the importance of pitch in music and speech perception. The cortical representation of pitch has been demonstrated by one pitch-sensitive region near the anterolateral border between A1 and R in the common marmoset (Bendor and Wang, 2005).

However, it's not clear if there exist other pitch-processing regions in the marmoset brain. Here, we performed optical imaging over the entire auditory cortex on the brain surface in awake marmosets. By contrasting responses to harmonic complex sounds with spectrally matched noises, we identified two discrete pitch-sensitive regions. One region is located anterolaterally to the A1 and R border and is consistent with the previously described "pitch-center" by single-unit recording. The second region is newly found at the location more anterior to the "pitch-center" and functionally overlaps with the RT field, referred to as "anterior pitch-region". When tested by synthetic tones comprised of low-numbered harmonics, these two pitch-sensitive regions only appear when the fundamental frequency (F0) is close to or higher than 400Hz, a phenomenon consistent with the estimated harmonic resolvability of the marmoset (Osamanski et al, 2013, Song et al, 2016). The response contrasts in these two pitch-sensitive regions were also robust when tested by more natural sounds such as a female's singing of a-cappella songs (F0 ~300-700Hz). Furthermore, the ratio between the singing contrast and the synthetic tone contrast is higher in the "anterior pitch-region" than in the anterolateral "pitch-center" in all tested subjects. Together, our results suggest that the cortical pitch processing in marmoset is organized into discrete regions with a functional hierarchy along the anterior direction for natural harmonic sounds.

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**Presentation Number:** NANO24.02

**Topic:** D.05. Auditory & Vestibular Systems

**Title:** The role of the harmonic sieve model in pitch perception of complex sound

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**Abstract:** Pitch perception plays a crucial role in auditory processing of real-world sounds that oftentimes do not have perfect harmonic structure. In the acoustic engineering field, the harmonic sieve model has been proposed to simulate human pitch perception of sounds with imperfect harmonic structures. However, evidence of the harmonic sieve model is lacking. Here, we systematically examined the harmonic sieve hypothesis. In a series of psychophysics tests, participants were asked to discriminate pitches of perfect harmonic tones and inharmonic tones with jittered harmonic frequency. Results showed that pitch perception was robust when harmonic frequency was jittered within 5%, whereas jitter above 5% significantly impaired pitch perception. These results suggest that 5% tolerance may represent a property of the 'harmonic sieve'. Furthermore, we recorded electroencephalography (EEG) from participants while they were listening to sounds with perfect harmonic structure (80% trials) and jittered harmonic frequency (5, 10, 20, and 30%, 5% trials each) through earphones. The amplitude of the event-related potential (ERP) at 100-220ms recorded from frontal-central electrodes decreased as jitter level increased. We found that 5% jitter did not result in significant change in the ERP amplitude, which together with the behavioral results, suggest that 5% jitter tolerance may represent the computational characteristic of the harmonic sieve model. Finally, we developed a computational model to simulate human pitch perception by implementing a specific Gaussian

sieve-filter with a biologically constrained cochlear model. Our results provide new evidence supporting the harmonic sieve hypothesis and its role in pitch perception.

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**Presentation Number:** NANO24.03

**Topic:** D.05. Auditory & Vestibular Systems

**Support:** NIH Brain Intiative U19 900937722

**Title:** Probing neural connectivity in auditory cortex for efficient functional processing

**Authors: *H. KANG**, T. A. BABOLA, P. O. KANOLD;
Johns Hopkins Univ., Baltimore, MD

**Abstract:** Sensory perception requires fast encoding of relevant input from a mixture of complex signals. In audition, for example, auditory cortex (AC) plays a key role for efficient processing of incoming acoustic signals. It has been suggested that sparse representation of co-activating neurons in AC for given inputs is highly related to efficient information coding strategy with reduced energy. Sparse coding is commonly represented by a small set of neurons in a local network showing active responses while other cells remain silent or show very low modulation, commonly measured by electrophysiology or calcium imaging. However, a causal effect of targeted neural activity manipulation on functionally connected neurons remains elusive. Here, we probe strong neural connectivity in AC based on its functional characteristics by using holographic optogenetic stimulation targeting only a small number of neurons along with in-vivo 2-photon imaging on awake mice. We hypothesize that strong neural connectivity exists among neurons sharing the same functional characteristics. Therefore, we expect that the activity manipulation on a small subset of targeted neurons can change activities of other neurons with the same functionality. To study this, we injected AAV9-hSyn-GCaMP8s-T2A-rsChRmine virus over AC of mice. We first imaged neural activities on a population of neurons to either 100-ms 16 kHz or 54 kHz pure tones using 2-photon imaging (baseline session). We then added the holographic stimulation on pre-selected 5 neurons that are responsive to a 16 kHz pure tone, along with pure tone presentations (experimental session). By doing so, we compare response changes from the baseline to the experimental sessions and detect activity changes to different frequencies due to the extra manipulation of the neural activity. Targeted neurons showed increased response amplitude during the experimental session compared to the baseline session due to the stimulation, regardless of the pure tone frequency. More importantly, for non-targeted neurons, we observed greater response amplitude decreases when 16 kHz was played along with stimulation, compared to 54 kHz presentation. Our results suggest that the network activity can be controlled by only a small subset of neurons, and the network change is highly related to the functional properties of neurons.

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**Topic:** D.05. Auditory & Vestibular Systems

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**Title:** Emergence of prominent brain state-dependence of sound responses in the inferior colliculus

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**Abstract:** Sound responses in auditory thalamus and cortex depend strongly on neuromodulatory brain state, as indexed by pupil size (McGinley et al., 2015). In particular, responses show an overall inverted-U shaped dependence on pupil size. However, at what stages, and with what pattern, arousal influences sensory processing remains largely unknown. Here, we performed pupil and sound response measurements in head-fixed awake mice, from the cochlea to the inferior colliculus (IC). Using optical coherence tomography (OCT) to measure the vibratory responses of basilar membrane for the first time in awake animals, we observed that responses to pure tones showed no evidence of modulation due to pupil-indexed brain state changes. Similarly, in preliminary results of subcutaneous auditory brainstem responses (ABR; N=2 mice, n=4 sessions), we did not see obvious state-dependence. We also tested whether inferior colliculus (IC) is influenced by pupil-indexed brain state. We used Neuropixels probes to record central IC (ICC) tone responses (N=7 mice, n=10 sessions, n=2131 neurons). Our results indicate strong state dependence in ICC. The population average showed an inverted-U shaped relationship. Moreover, using PCA followed by K-means clustering, we found subpopulations of units with distinct patterns of state-dependence. The largest group (49% of sound-responsive neurons) showed an inverted-U shaped relationship while the other major group (41%) showed monotonically reduced activity with increasing arousal. Interestingly, the inverted-U neurons had narrower bandwidth than the decreasing neurons ($p<0.05$), suggesting that low-arousal high-gain neurons play a role in ‘detection’ of sounds at low arousal, whereas the inverted-U neurons perform finer-grained stimulus analysis at mid-level arousal, which is optimal for sound processing (McGinley et al., 2015). To quantify the degree and pattern of state-dependence in ICC, we used a pupil-binned mean model to predict single-trial responses and compared to the grand average. Pupil-linked brain state explained a large fraction (49%) of response variance. Thus, prominent effects of brain state have emerged at the level of the IC. Future invasive recordings in sub-midbrain auditory structures will be needed to determine if the lack of state-dependence of the ABR results from the coarse and indirect nature of the measurements rather than a lack of state-dependence at the single-neuron level. McGinley, David, & McCormick (2015). Cortical membrane potential signature of optimal states for sensory signal detection. Neuron, 87(1).

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**Presentation Number:** NANO24.05

**Topic:** D.05. Auditory & Vestibular Systems

**Support:** THE ISRAEL SCIENCE FOUNDATION grant # 725/21

**Title:** The Impact of Emotional States on Sensory Processing and Perception

**Authors: *G. A. BISHARAT**, E. KAGANOVSKI, A. TEMNOGOROD, J. RESNIK;
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**Abstract:** Our emotional state is a potent driver of decision-making and multi-layered cognitive processes such as learning and memory. At a much more fundamental level, emotional states can interact with the way we perceive sensory inputs. For example, the doorbell will sound louder when we are stressed, and hills will seem steeper when we are despondent. So, to understand how emotional states shape behavior, we first need to understand how emotional states shape sensory processing and perception. *How is emotional state-information represented in the sensory systems? How do changes in emotional state shape the perception of specific stimuli? For how long?*
To tackle these questions, we developed a rodent auditory behavioral approach that enables direct evaluation of perception. We coupled these behavioral paradigms to manipulations of the animal's emotional state (inducing chronic stress) and, using two-photon calcium imaging, detailed probing of neural activity modulations at the network and cell-specific levels in the auditory cortex. Our findings indicate that chronic stress decreases sound-evoked activity in the auditory cortex. These alterations are not caused by acute stress and occur gradually over time. Moreover, we found changes in perception as a result of stress-induced changes to cortical activity. Our findings could have clinical implications by explaining how stressful states distort the neural representation of sensory stimuli and alter our perception of the world.

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**Topic:** D.05. Auditory & Vestibular Systems

**Support:** NIH Grant NS099288-07
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**Title:** Sensorimotor integration of vocal feedback in the songbird auditory cortex

**Authors: *F. DUARTE ORTIZ**, R. MOONEY;
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**Abstract:** Learning complex vocal behaviors requires the brain to generate a vocal motor program that matches an auditory target. One idea is that this process depends on neural circuits that compare vocal-related auditory feedback to an internal representation of the auditory target. One way that this comparison is thought to be made is through vocal motor signals that suppress auditory cortical responses to predictable features of vocal-related auditory feedback. Whether predictive suppression characterizes the auditory cortex in animals that produce learned vocalizations remains poorly understood. Here, we used pan-neuronal calcium imaging in freely behaving adult zebra finches to determine how vocal motor signals modulate auditory cortical activity when birds sing. We found that of those neurons in the primary and secondary auditory

cortex (i.e., Field L, CML, and NCM) that were modulated during singing, the majority were suppressed compared to activity evoked during non-singing epochs by playback of the bird's own song. Additionally, a small proportion of neurons in these regions were suppressed in a window up to 500ms prior to song onset. We then deafened the birds and tracked the same neurons to isolate their responses from auditory feedback. We found that after deafening, the majority of neurons were modulated in the same way during singing, despite the absence of auditory feedback. This persistent modulation suggests two possibilities: first, that vocal motor signals act independently of auditory feedback and operate to suppress vocal feedback, and second, that the auditory cortex is processing other signals such as proprioceptive feedback. Finally, we evaluated the modulation of neurons during the production of innate calls and learned song. When comparing the time-warped calcium signals of these types of vocalizations, we found that neurons that were suppressed during song were not so during calls. In summary, calcium imaging in the zebra finch auditory forebrain reveals evidence of a vocal motor signal that is learned and functions predictively to suppress singing-related auditory feedback. Additionally, these results show that the auditory cortex of the male finch is strongly modulated during singing even in the absence of hearing. This underscores the multisensory nature of activity in regions traditionally regarded as unimodal in nature.

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**Topic:** D.05. Auditory & Vestibular Systems

**Support:** NIH Grant DC008343

**Title:** Noncanonical auditory cortical plasticity for behaviorally synonymous social sounds

**Authors:** D. B. ANANDAKUMAR[1], C. BESOSA[2], ***R. C. LIU**[3];
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**Abstract:** As “survival machines” (Dawkins, 1976), animals evolved neural mechanisms for ethological signals like vocalizations to robustly trigger social behavioral responses that help organisms live and reproduce. However, these mechanisms need not be purely innate and fixed, since genetically “open programs” (Mayr, 1974) can be made more efficient through experience by learning that other novel cues can reliably predict the need for the same critical social behaviors. Thus, a baby’s cry and an alarm from a baby monitor can both elicit an urgent response from a new mother to her infant. Do the neural representations of such behaviorally synonymous signals remain independent during sensory processing to separately elicit responses via downstream motivational and motor areas, or does their convergent meaning emerge earlier within the sensory system itself? We answer this for acoustic cues linked to a natural reproductive behavior in mice: sound-guided retrieval of pups. Pup ultrasonic vocalizations (USVs) naturally elicit search and retrieval by mothers, but they can also learn in a T-maze (Dunlap et al, 2020) to be guided by a new synthetic Target (sinusoidal + linear FM-modulated tone) whose source location predicts pup delivery, similar to USVs. Auditory cortex (ACx) is necessary for trained mice to express the learned association. However, contrary to usual expectations that learning would increase cue-evoked excitation in ACx, noncanonical forms of ACx plasticity dominate, particularly in neurons responding to both cues. We recorded 992

neurons from 5 Trained and 5 Yoked (i.e. sound exposed in T-maze with pups but no retrieval) mothers awake and passively listening to the Target. In Core ACx of Trained mice, neurons that jointly responded to both cues and belonged to the Target's "lateral band" (i.e. best tone frequency outside the Target's spectrum) were tuned in their selective Target-evoked suppression rather than excitation, in contrast to Yoked mice. This result reinforces the hypothesis that behavioral meaning impacts sound encoding through selective ACx Core suppression (Galindo-Leon et al, 2009). Meanwhile in secondary ACx (A2), we saw an increased prevalence of OFF firing beyond chance occurrence specifically in neurons that exhibited Off firing to both Target and USV, and their OFF excitation was tuned around the Target. This broadens prior findings of A2 Off plasticity for USVs in mothers (Chong et al, 2020) by revealing individual A2 neurons as a de novo site of convergent excitation by behaviorally synonymous sounds that are acoustically distinct. Together, this work suggests that ACx plays an active role in constructing semantic categories from sounds.

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**Title:** Electrotonically isolated dendritic inhibition tunes temporal integration in a coincidence detector neuron

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**Abstract:** Octopus cells (OCs) of the mammalian cochlear nucleus are coincidence detectors that respond to rapid depolarizations characteristic of auditory onsets. Unusually fast membrane properties contribute to a short integration window (~1ms) for small EPSPs that arise from >1000 tightly packed synapses. When EPSPs from disparately tuned auditory nerve fibers (ANFs) sum precisely, the fast rate of depolarization elicits an action potential. As a result, OCs are excellent onset detectors, particularly for broadly tuned stimuli. OCs also respond to frequency modulations, providing evidence they detect coincident changes in frequency during ongoing stimuli. Our understanding of temporal integration in OCs has been restricted to excitation because, while there is anatomical evidence of inhibition, physiological evidence has remained elusive. We seek to understand how excitation and inhibition are integrated in OCs and if inhibition modulates timing. To define the anatomical excitation/inhibition (E/I) balance of OCs inputs we built a whole-OC input map from reconstructions of 40 *Thy1-YFP-H* labeled OCs in P28-35 mice. Cre-dependent expression of synaptophysin-tdT marked putative synapses in ANF subpopulations and glycinergic inputs. ANFs densely innervate OC somas (13 syn/10μm$^2$) and proximal dendrites (27 syn/10μm$^2$) and make fewer synapses distally (7 syn/10μm$^2$). Additionally, we found dense and evenly distributed glycinergic synapses on dendrites (4 syn/μm$^2$) yet surprisingly few on somas (<1 syn/10μm$^2$). This difference in somatic (20:1) and dendritic (3:1) E/I balance suggests separate functional roles for each compartment. To evaluate E/I balance in the two compartments, we targeted OCs for *in vitro* recordings and activated

inhibition with optical stimulation of ChR2+ glycinergic fibers (P35-50, 35°C). Despite the abundance of dendritic inhibition, IPSPs were undetectable in somatic recordings until dendritic isolation was abolished by blocking potassium and HCN channels. We hypothesized that inhibition is isolated by the electrotonic structure of OC dendrites and therefore can only shape EPSPs locally. With simultaneous electrical stimulation of excitatory ANFs during optical stimulation of ChR2+ glycinergic fibers, we found that coincident arrival of inhibition and excitation on OC dendrites modulates EPSP amplitudes and peak times recorded at the soma. We propose that inhibition can locally influence temporal summation as EPSPs travel through the dendritic arbor, allowing for targeted filtering of EPSPs. With extremely short integration windows, small changes in EPSP shape can impact spikes and detection of coincident activity.

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**Title:** Perinatal nicotine exposure disrupts nAChR functional expression and impairs glutamatergic synaptic transmission in the mouse auditory brainstem

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**Abstract:** Maternal smoking leads to chronic nicotine exposure in utero, increasing the risk of persistent auditory processing deficits in the developing brain. However, the impact of chronic nicotine exposure during the critical period on glutamatergic synaptic transmission and auditory processing in the brainstem remains poorly understood. We thoroughly investigated the functional expression of nicotinic acetylcholine receptors (nAChRs) at both post- and presynaptic terminals in the medial nucleus of the trapezoid body (MNTB) and assessed the impact of perinatal nicotine exposure (PNE) on nAChR mediated currents, glutamate release, and auditory brainstem responses in juvenile mice. Our findings show a switch in functional nAChR expression from primarily postsynaptic at MNTB neurons to primarily presynaptic expression at Calyx of Held terminals around hearing onset. Our findings revealed age-dependent changes in nAChR functional expression at postsynaptic MNTB neurons and presynaptic calyx terminals around hearing onset. Chronic nicotine exposure prior to hearing onset resulted in abnormally increased nAChR-mediated postsynaptic currents in MNTB neurons and compromised glutamatergic neurotransmission at the calyx-MNTB synapse. Notably, PNE significantly reduced readily releasable pool size and release probability, indicating that chronic activation of nAChRs during early postnatal development critically impacts presynaptic neurotransmitter release in the auditory brainstem. Furthermore, PNE mice exhibited increased auditory brainstem response (ABR) thresholds and reduced ABR peak amplitudes, suggesting impaired auditory processing without alterations in cochlear function. In summary, our findings reveal that PNE disrupts glutamatergic synaptic transmission at the calyx of Held-MNTB synapse and impairs auditory processing, providing novel insights into the underlying mechanisms of auditory deficits following chronic developmental nicotine exposure.

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**Title:** Functional implications of a patch/matrix-like compartmental organization in the mouse inferior colliculus

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**Abstract:** The inferior colliculus (IC) is an obligatory relay station and massive convergence center for auditory information. In addition to its role in sound processing, the IC receives inputs from diverse multisensory and neuromodulatory structures and is implicated in acoustico-motor behavior. The lateral cortex of the IC, a multisensory region, contains a network of neurochemical modules that subsect this structure into discrete processing regions. Somatosensory inputs to the IC target these modules, which stain heavily for markers of inhibition, plasticity, and metabolic processing, while auditory inputs target complementary extramodular zones. While these auditory inputs have been shown to mediate diverse functions, including predictive processing and flight behavior, the role of somatosensory inputs to the IC is unknown. Previous studies have shown that inputs from the somatosensory cortex target inhibitory colliculo-thalamic projection neurons in the neurochemical modules, leading to suppression of auditory responses in the auditory thalamus. These results suggest that modular regions of the IC may serve as somatosensory-driven gating regions for auditory information. To test this hypothesis, we trained mice to perform a go/no-go task in which they lick for a water reward after presentation of a noise target, while selectively activating somatosensory-inputs to the IC on a subset of behavioral trials. We also performed anterograde trans-synaptic labeling of somatosensory-recipient neurons in the IC. In addition to assessing the functional role of somatosensory inputs to the IC, we used two-photon imaging to determine the sound response properties of neurons in modular and extramodular regions of the IC. Movement behavior during imaging was recorded and analyzed using FaceMap and DeepLabCut. Preliminary data suggest that mice can learn the go/no-go task paradigm with high accuracy following ~3 weeks of training. Axon fibers from trans-synaptically labeled somatosensory-recipient IC neurons were found in known targets of the lateral cortex, including the medial geniculate body, laterodorsal tegmental nucleus, contralateral lateral cortex, and the superior colliculus. Two-photon imaging of IC responses to pure tones, FM sweeps, noise, and vocalizations was successfully performed. Sound, motion, and sound/motion responsive units were parsed using a generalized linear model. The results of the experiments will determine what effect somatosensory input to the IC has on sound processing and target detection and will show whether modular and extramodular regions of the IC have distinct sound and movement processing features.

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**Topic:** E.05. Brain-Machine Interface

**Title:** Platform for real-time closed-loop feedback of behavior and/or cortical GCaMP activity in mice using an all-optical strategy at the mesoscale

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**Abstract:** Mice can learn to control specific neuronal ensembles using sensory (eg. auditory) cues (Clancy et al. 2014, 2021) or even artificial optogenetic stimulation (Prsa et al. 2017). In an initial study, we found mice can also control GCaMP cortical activity at the mesoscale. The changes in fluorescence activity (ΔF/F) were mapped to ascending auditory cues as feedback that mice learned to control over days. Here, we are presenting an extension to this work by incorporating flexible real-time feedback on behavior (pose) tracking using DeepLabCut-live (Kane et al, 2020) as well as an all-optical strategy for imaging and manipulating cortical dynamics at the mesoscale using open-source Python code. Our multi-threaded program allows us to stream and save the images and pose, and employ conditions such as adaptive thresholding or movement-based logic for rewards. The system can employ a variety of platforms including compact footprint NVIDIA Jetson and Raspberry Pi. We measure behavioral parameters (speed, distance, and position) of mouse body parts (forelimbs, hindlimbs, and tail) and map them to a graded auditory cursor for feedback. Based on the behavioral parameter of interest, specific start and target conditions are specified (eg. start and target position of a forelimb) that mice need to learn to receive a reward. The task for mice is to learn a specific body movement, guided by auditory feedback. We intend to explore behavioral parameters that lead to faster learning of task-specific movements in these mice. In preliminary work consistent with published work from the lab (Forys et al. 2020) mice could learn task-specific movements to get an increasing number of rewards over days. In conclusion, we developed an open-source system for all-optical closed-loop feedback of the brain as well as behavior that can be added to experimental scenarios for activity training. We intend to further apply this technique to investigate functional outcomes and induce neuroplasticity in a mouse model of stroke.

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**Nanosymposium**

**NANO25: Neural Decoding and Neuroprosthetics**

**Location:** WCC 201

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**Title:** Error detection from intra-cortical activity is more accurate outside, rather than inside, the target

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**Abstract: Summary**: Brain machine interfaces (BMIs) provide direct pathways for controlling the external world and thus regaining motor capabilities. However, their effectiveness is hampered by decoding errors, which can cause frustration or potential harm to the user of a physical system. We have previously demonstrated the ability to detect and correct erroneous movements online while non-human primates (NHPs) operated intra-cortical BMIs (Wallace et al., SfN 2022). Here we report on further offline analysis, which indicates that error detection outside the target is more accurate than inside the target. **Methods:** One NHP was implanted with Utah microelectrode arrays in the motor cortex and trained to acquire virtual targets, presented on a screen, by controlling two virtual fingers with two finger groups. Online BMI control of the virtual fingers was performed using a Kalman filter trained on the 50 ms binned spike-band power (SBP) from 96 channels (Nason et al., Neuron 2021). Movement segments of N=4 bins with consistent finger movement (either flexion or extension), were classified as erroneous or correct (away or toward the target, respectively) based on the corresponding binned SBP activity. The effect of the location of the virtual fingers on error detection was evaluated by comparing the receiver operating curves (ROC) of three sets of classifiers, trained and validated on: (a) all the labeled segments, (b) labeled segments outside-the-target, and (c) labeled segments inside-the-target. For fair comparison, the same number of balanced training samples were used in each case. The resulting performance was evaluated using 5-fold cross-validation. **Results**: Classifiers trained and validated on segments outside the target had better ROCs (larger true-positive rates, TPRs, for same false-positive rates, FPRs) than classifiers trained and validated on all segments, or on segments inside the target. Limiting FPRs to below 5%, the resulting TPRs were always highest in ROCs computed from validation segments outside the target. Furthermore, TPRs obtained by classifiers that were trained and validated on segments outside the target were significantly larger than TPRs obtained by classifiers that were trained and validated on segments inside the target or on segments from both inside and outside the target (one sided t-test, p smaller than $10^{-14}$ ). **Conclusions**: Our analysis indicates that detecting erroneous movements outside the target is more accurate than inside the target. This suggests that erroneous movements outside the target evoke more distinct patterns of error processing, and thus that error detection is not limited to stop-detection.

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**Title:** Advances in speech and communication neuroprostheses using electrocorticography

**Authors: *D. MOSES**[1,2], S. METZGER[1,2,4], K. A. LITTLEJOHN[1,2,5], A. SILVA[1,2,4], J. LIU[1,2,4], R. WANG[1,2], M. SEATON[1], M. DOUGHERTY[1], A. TU-CHAN[3], P. WU[5], M. BERGER[6], I. ZHURAVLEVA[5], K. GANGULY[2,3], G. ANUMANCHIPALLI[1,2,5], E. CHANG[1,2,4];
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**Abstract:** Severe paralysis due to neurological injury or neurodegenerative disease can drastically restrict the capacity to interact and communicate with others, leading to a profound reduction in quality of life. Communication neuroprostheses have the potential to bypass damaged neural pathways and restore independence and interpersonal connectivity to affected persons. In particular, speech neuroprostheses may be able to decode intended or attempted speech from brain activity, enabling the user to communicate naturalistically and expressively at rates approaching spoken speech. Our research has focused on developing speech-neuroprosthetic technology driven by electorticographic (ECoG) recordings. In an ongoing clinical trial, we implant high-density ECoG arrays over the speech-motor cortex of participants with severe paralysis who are no longer able to speak intelligibly. Using deep learning and natural-language modeling, we show that it is possible to decode attempted speech from ECoG activity by leveraging speech-motor representations that have persisted for many years after paralysis. Decoded outputs include text, audible speech, and facial-avatar animations, demonstrating highly personalizable and flexible control of the speech neuroprosthesis. Stable signals and broad cortical coverage enable promising long-term decoding performance and decoding of non-speech motor attempts, respectively. Overall, ECoG-based speech neuroprostheses offer a promising pathway towards enabling long-term, embodying communication for persons who are unable to speak or type.

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**Topic:** E.05. Brain-Machine Interface

**Support:** NSF GRFP 2139841

**Title:** Learning on the manifold of human brain activity through real-time neurofeedback

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**Abstract:** Learning to perform a new behavior is constrained by the geometry, or intrinsic manifold, of neural population activity supporting that behavior. Recent work highlights the importance of manifolds that capture low-dimensional dynamics of neural population activity for brain-computer interface learning. Through invasive BCIs, humans and non-human primates can learn to operate neural prosthetics more efficiently with a device controlled via activity on the intrinsic neural manifold. Recently, we showed that manifold learning unveils behaviorally-relevant dynamics from fMRI, a safe, non-invasive imaging technique. Can manifolds be harnessed with neurofeedback training to enhance human learning? We present preliminary findings from a multi-session, real-time fMRI study in which participants learned to use their brain activity to control an avatar's movement through a virtual world. In session one, they were tasked with navigating the avatar to a goal location using a joystick while fMRI data were collected. From these data, we estimated a manifold of neural activity during the virtual navigation task from a network of regions including the retrosplenial cortex and hippocampus. We hypothesized these regions represent a space of brain states that can be manipulated with respect to the task (e.g., self-guided navigation, goal-directed behavior). Manifold learning was performed using T-PHATE (Busch et al., 2023), an algorithm designed for high-dimensional timeseries data such as fMRI. An autoencoder regularized with the T-PHATE manifold was constructed to extend the manifold to new datapoints. In all subsequent sessions, participants returned to the scanner and were trained using neurofeedback to perform the same task but now by controlling the direction of the avatar's movement with their brain activity. Using a closed-loop architecture, we acquired whole-brain fMRI volumes every two seconds and transmitted them to a compute cluster for processing and embedding onto the T-PHATE manifold. Then, embedded data were mapped to the direction of the avatar's next movement in the game. Neurofeedback training used a staircasing procedure to quantify how much control the participant's brain exerted over the avatar's movement. In an initial cohort, the accuracy of decoded movements improved with neurofeedback training, as reflected by an increase in the staircased control term. Neurofeedback training was more effective when the avatar's movement was based on a high-ranked manifold component relative to a low-ranked component. Preliminary results suggest that the fMRI activation manifolds serve as useful control spaces for neurofeedback training in humans.

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**Topic:** E.05. Brain-Machine Interface

**Title:** Comparison of time-frequency representations for deep learning-based epileptic EEG classification

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**Abstract:** Many approaches combined the traditional time-frequency transforms, such as short-time Fourier transform (STFT) and continuous wavelet transform (CWT), with deep neural networks have been developed for epileptic EEG classification. However, the optimal time-frequency method for this task is still unclear. The EEG data utilized in this study is obtained from the Bonn University repository, which contains five-class EEG data from healthy subjects with eyes open (Set A) or closed (Set B), interictal subjects with different onset regions (Set C and D), and ictal subjects (Set E) with a duration of 23.6 seconds. The present work first preprocessed the EEG data with a 5-point Butterworth filter within the 1-30 Hz frequency band and then segmented them into 1-second pieces. We established a deep learning architecture with time-frequency transform frontend module for five-class epileptic EEG identification. Firstly, multiple time-frequency transforms were applied to the EEG signal, including CWT with Meyer wavelet, Haar wavelet, Daubechies wavelet of order 4 (db4) and Morlet wavelet, STFT with Gaussian window, Rectangular window and Hamming window, and the Stockwell transform (S-transform) (Stockwell, 1996). The proposed deep learning network consists of two layers of 2-D Convolutional Neural Network (CNN) and one Bidirectional Long Short-Term Memory (BiLSTM) module, where each 1-s piece was treated as one time-step. The final output is determined by the majority voting of 23 outputs of the time steps. Meanwhile, 5-fold cross-validation was conducted, and the average five-class classification accuracy was reported. This study indicates that for CWT, the Meyer wavelet with 87.6% ± 3.1% surpasses Haar (84.0% ± 2.1%), db4 (86.6% ± 1.3%) and Morlet (87.2% ± 1.9%) wavelet. For STFT, the Kaiser window with 86.0%±2.0% overperforms Hamming (83.4% ± 2.5%), Gaussian (81.8% ± 1.1%) and Rectangular (88.4% ± 2.3%) window. And overall, S-transform achieves the best performance with (88.4% ± 1.5%). Our visualization results also demonstrate that the proposed model can effectively learn discriminative features from S-transform representation. This study suggests that S-transform outperforms all types of STFT and CWT in epileptic EEG classification tasks when combined with the CNN-BiLSTM model. The proposed method achieves a five-class classification accuracy of 88.4% on Bonn epileptic EEG dataset, which is competitive with other methods proposed by previous studies. This work can reveal more about the potential personalized treatment for epilepsy patients.

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**Title:** Learning-related dynamics of hierarchical motor control properties in the human cortico-basal ganglia network

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**Abstract:** The neural dynamics of fine motor control, particularly during coordinated sequential movements, are poorly understood due to low spatiotemporal resolution and difficulty separating learned encoding changes from altered dynamics resulting from altered behavior. Thus, we use chronic multisite neural implants to record high spatiotemporal resolution activity from the motor control network of four Parkinson's (PD) patients typing two sequences for multiple days on custom equipment that precisely captures movement - in the first neurophysiological investigation of prolonged motor sequence learning (MSL) in PD.
Leveraging biomarker search, supervised machine learning and unsupervised state space modeling, we assess the roles of motor cortex (MC) and basal ganglia (BG) in a learning-dependent, distributed representation of hierarchical motor control properties of sequential finger movements.
For low level properties - digit (finger) and element (serial position) - learning-related shifts in encoding occur, reflected by altered timing of significant amplitude differences and phase locking and by shifts in center frequency, for both low and high frequencies in both MC and BG. Supervised classifiers and unsupervised state space models trained on these features predict digit or element, with shifts across days.
For high level, sequence-specific motor planning prior to sequential movement onset, biomarker search selects features from oscillatory neural activity. Classification models, successfully predicting identity of the upcoming sequence, show motor planning signal content increases across days in BG, largely via phase locking of high frequency oscillations. Premotor cortex signal content is initially high, also driven by high frequency phase, but decreases across days.
In learners, sequence-general, low frequency phase resets develop during motor planning with multiple days of practice in both MC and BG. In non-learners, only MC shows consistent low frequency phase resets; BG phase remains disorganized. This suggests that BG low frequency phase locking during motor planning is critical for MSL in PD patients, perhaps by enabling cortico-subcortical tutoring.
We describe the involvement of MC and BG in the representation of low and high level motor control properties over the course of MSL, and we decode digit, element and sequence identity. Upper limb BCI decoders often use gamma amplitude as the sole feature but struggle to decode sequential finger movements. We show that, in addition to gamma amplitude, phase and other oscillatory frequencies are instrumental in motor decoding, in a manner that dynamically changes with learning.

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**Topic:** E.05. Brain-Machine Interface

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**Title:** Using stereo-electroencephalography to decode intended speech in an individual with aphasia

**Authors: *T. M. THOMAS**, O. WOOLNOUGH, N. TANDON;
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**Abstract:** Aphasia is a devastating speech disorder affecting more than one million people in the US alone. While speech therapy and alternative communication devices provide some improvement, many patients suffer from long-term impairments. Recent research on brain-computer interfaces (BCIs) aims to restore speech function by detecting the patient's intended speech based on the neural pattern of the language network. Speech-BCIs have been demonstrated to predict speech from non-verbal patients with no cortical damage, but have yet to be used with patients who have sustained damage to some portion of the cortical language network. We investigated speech decoding for a patient with non-fluent aphasia due to a traumatic brain injury, using recordings from language areas that remained intact. Using stereo-electroencephalographic (sEEG) electrodes, placed for the localization of epilepsy, we recorded broadband gamma activity (70-150Hz) from frontal and parietal cortex as the patient named pictures of eight common objects. In each trial, the patient was shown the picture for 2s, and then probed for a response after a 1s delay. The patient responded on 25% of trials with the correct response, 38% with phonemic errors, 6% with an incorrect response, and 31% with no response. We used non-linear classification to decode the patient's response from trials where a word was produced, with and without phonemic errors. The responses were classified with an accuracy of 43.1% (12.5% chance accuracy) during the articulation period, with the most accurate word classified at 70%. Prior to articulation, the response was classified with 16.2% accuracy during the delay period, and with 21.9% accuracy once the patient was probed for a response. We then extended the analysis to evaluate whether the model could also decode the patient's intended response from trials where no word was produced aloud. Since no response was given, the model was used to predict the name of the picture during the delay period with an accuracy of 21.4%, and 18.0% once the patient was probed for a response. These results demonstrate that the remaining intact language areas can provide sufficient information for a speech-BCI to decode intended words from neural population activity in an aphasic patient, despite errors that are common with non-fluent speech. Furthermore, due to high variability in aphasia-related cortical damage patterns, sEEG enables distributed electrode coverage to access as many widespread intact areas as possible with minimal risk. As such, sEEG-based speech-BCIs hold much potential as an assistive device for aphasic patients to overcome difficulties in expressing their thoughts aloud.

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**Title:** Finding the groove in neural space

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**Abstract:** From playing a musical instrument to sprinting across a field, rapid and precise rhythmic movements are an essential part of our behavioral repertoire. While a large body of research has investigated the neural basis of gross periodic movements, like walking or swimming, less is known about the cortical mechanisms underlying periodic movements of the hand. In the present study, we investigated the encoding of volitional rhythm in human sensorimotor cortex of participants with intracortical implants in sensorimotor cortices. We instructed the participants to tap a button with their hand to a specific frequency following a metronome. We then tested several hypotheses about the neural correlates of rhythm at both the single and population level. At the single neuron level, we expected to find one of the two strategies for frequency encoding. In the first strategy, individual neurons might exhibit periodic activity at the frequency of the rhythmic movement. Alternatively, individual neurons might not exhibit any periodic activity but rather respond preferentially to some frequencies and not others. We find that individual neurons do not exhibit periodic activity but rather respond preferentially to some rhythms. At the population level, we find that the neural trajectories are frequency specific, allowing for a decoding of the rhythmicity from these population level dynamics.

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**Title:** Enabling contextual communication and control for brain-computer interfaces using large language models

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**Abstract:** Locked-In Syndrome (LIS) is a neurological disorder in which cognition is intact but communication is impaired due to paralysis from a variety of causes, including brainstem stroke and amyotrophic lateral sclerosis. Recent advances in brain-computer interfaces (BCI) have shown great promise in directly decoding attempted speech output or motor gestures from severely paralyzed patients, but such methods rely on high-fidelity neural data obtainable only with neurosurgically implanted devices. Low-bit decoding approaches such as brain clicks are robust to different types of neural data, but suffer from limitations in speed and precision when used in communication and control applications. We have engineered a novel system which

leverages large-language models (LLM) to integrate an individual's cognitive state and user history to provide the user with highly tailored control and communication options. Despite the limited set of commands available to the user, the system enables the user to map their BCI output to prepopulated options, thereby dramatically enhancing communication throughput. We have taken rigorous measures to ensure the user privacy; a locally hosted system is employed to ensure all personal information and communications remains on-device, while the real-time sensing data for contextual information is acquired and used for processing, these data are not stored long-term. Our BCI communication and control system streamlines real world navigation for individuals with severe impairment. Operating in real time, the system presents the user with the most probable communication and control options based on their surrounding context and prior history. The user can select specific parameters using existing BCI technology, such as binary or multiclass brain clicks, facilitating interaction and feedback with the system. Long-term context retrieval is achieved by storing state information related to the user's interactions and actions, informing the selection of choices presented to the user. This research marks a substantial progression towards high-throughput communication, capitalizing on recent breakthroughs in artificial intelligence and computational hardware. The integration of AI-assisted communication enables users to employ simpler decoding strategies while retaining a swift and effective mode of communication and control. Further research will explore the potential for broadening the application and effectiveness of this system.

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**Nanosymposium**

**NANO26: Motivation—Social Communication and Behavior**

**Location:** WCC 147B

**Time:** Sunday, November 12, 2023, 1:00 PM - 4:30 PM

**Presentation Number:** NANO26.01

**Topic:** G.03. Motivation

**Support:** NIH Grant
Duke Biology Department
Duke Core Research Facilities

**Title:** The effect of social experience on gene regulation, neural activity and behavior of Drosophila melanogaster

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**Abstract:** Social behaviors of animals are modulated by signals from the environment. The molecular and neural circuit-based mechanisms underlying experience-dependent behavioral regulation remains poorly understood. Gene regulation plays important roles in controlling

animal behaviors. Emerging evidence from both mammals and insects indicates the intimate connection between behavioral modulation, neural transcription, and neuronal activities. The *Drosophila melanogaster* is an excellent model where links among stereotyped courtship behaviors, genes and circuits have been elucidated. Single-pair courtship assays showed that socially isolated male flies displayed more vigorous courtship behaviors compared to group housed males. The increase in courtship vigor in isolated males was accompanied by an increase in the response of P1 command neurons in the central brain. However, how social experience regulates master genes controlling courtship at the level of transcription in different courtship circuits remains unclear. The bulk RNA-seq of male fly brains showed that social isolation or disrupting pheromone receptor function in the olfactory system altered the transcription pattern of genes involved in immunity, metabolism, male courtship behaviors and so on, including a master regulatory transcription factor of courtship Dsx, a kind of neuropeptide DSK and one of its receptor CCKLR-17D3. These results suggest that social context modifies neuronal activities, alters different gene transcription in the courtship circuits, and ultimately regulates courtship behaviors.

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**Title:** Acute and chronic social isolation promote diverse behavior repertoires and differentially modify mPFC responses to social contact

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**Abstract:** Divergent social behaviors emerge from different durations of social isolation (Lee et al., 2021). However, the exact time course of isolation impacting social behavior and the neural systems and circuits maintaining social homeostasis remain elusive. Previously, we found that dorsal raphe nucleus dopamine neurons mediate a loneliness-like state and innervate the medial prefrontal cortex (mPFC) (Matthews et al., 2016).
To explore how the mPFC encodes social information and undergoes a state change following

isolation, we used cellular resolution calcium imaging, ultrasonic vocalization (USV) recordings, computer vision, and machine learning tools. To first determine how different durations (2hr, 6hr, 24hr, 7d, 14d, and 28d) of isolation impact social behavior, we performed a juvenile intruder task after isolating adult male mice. We performed pose estimation using SLEAP (Pereira et al., 2022) and a custom pipeline for behavioral feature extraction and unsupervised clustering for behavioral motif discovery. We found an inverse correlation between isolation duration and interaction time (n=108 mice, $R^2$=0.115, p=0.0023) and that 2 and 6 hours of isolation promotes social interaction with juvenile mice (n=108 mice, F(6,101)=4.715, p=0.0003; GH vs 2hr: p =0.0002; GH vs 6hr: p=0.0409). Despite not detecting differences in interaction time in chronically isolated mice compared to group-housed mice, unsupervised clustering of behavior features revealed changes in social behavior repertoire, promoting face sniffing and reducing chasing in 7 and 14 day isolated mice (n=54 mice, F(15,192)=2.806, p=0.0006). Next, we found that 2 hours of isolation increased call rate (n=108 mice, F(6,101)=2.831, p=0.0137; GH vs 2hr: p=0.0218) and changed USV acoustic features (n=90 mice, F(5,84)=4.444, p=0.0012; GH vs 2hr: p=0.0352). Finally, we performed calcium imaging using miniature endoscopes in the mPFC of mice engaged in social behavior after group-housing and isolation. We found that isolation (24hr and 7d) increased the responsiveness of mPFC neurons to social contact (n=18 mice, 3938 cells, GH vs 24hr: $\chi^2$ =9.090, p=0.0106; GH vs 7d: $\chi^2$=30.28, p<0.0001) by promoting excitatory responses (n=18 mice, F(2,15)=5.189, p=0.0194; GH vs 24hr: p=0.0548, GH vs 7d: p=0.0146). Additionally, we found an increase in mPFC population trajectory length following isolation compared to a group housed session prior, an effect reversible followed by re-housing (n=6 mice, F(2,6)=16.60, p=0.0036; GHD1 vs 24hr: p=0.0110; 24hr vs GHD2: **p=0.0055). Overall, our findings support a role for mPFC in promoting features of the response to novel social stimuli following social isolation.

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**Support:** Rockefeller University
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**Title:** Dissecting the relationship between speech and dance in humans: from brain pathways to clinical therapy

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**Abstract:** Evidence from different levels of analysis points to intriguing commonalities between vocal learning, a core feature of human speech, and beat synchronization, a core feature of human dance. Recent findings go beyond the fact that both behaviors rely on rhythmic motor control and on a tight auditory to motor integration. For example, parrots, who are complex vocal

learners, like humans, were shown to be able to entrain their body movements to a musical beat in a spontaneous and sporadic way (i.e., dance), something that led to the hypothesis that the ability to move in time with an auditory beat originated in the neural circuitry for complex vocal learning (Keehn et al. 2019; Patel et al. 2009). Further, developmental studies in human children showed that the development of the ability for a sustained beat perception and synchronization predicts the development for phonological production ability until late childhood (Nave, Snyder, and Hannon 2023).
In order to shed light on the relationship between speech and dance in humans, we sought to a) compare these behaviors' brain activity patterns (via a meta-analysis of ~50 studies); and b) test whether there were significant differences in the activity of the speech brain pathways of people with Parkinson's Disease before vs. during and after 8 months of a dance intervention (reanalysis of raw data provided by Barnstaple et al. 2022). Our large-scale meta-analysis (a) revealed that brain regions involved in the production of speech vocalizations (i.e., in the production of laryngeal movements) vs. in the production of rhythmic limb movements lie in adjacent or overlapping brain regions (e.g., in the Primary Motor Cortex). Our reanalysis (b) indicated that during their participation in dance classes, Parkinson's Disease patients showed significant activation changes in brain regions that have been shown to be involved in speech production, e.g., in the dorsal Laryngeal Motor Cortex.
Independently of the evolutionary hypothesis that links vocal learning and beat synchronization, our findings unravel a comparison between the neurobiology of two complex sensory motor behaviors (i.e., speech and dance) that serve human communication, as well as suggest the impact of dance training on modalities that have not been tested hitherto (i.e., in speech deficits).

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**Presentation Number:** NANO26.04

**Topic:** G.03. Motivation

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**Title:** Implantables to Correlate Social Interactions and Brain States in Free-living Animals

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**Abstract:** Wireless tracking devices have become indispensable tools in the analysis of free-living animal social networks. However, these devices, which rely on event-based data, do not provide insights into the bioelectric activity (e.g., body and brain) that occurs before, during, and after social interactions. To address this, we have transformed our wearable contact tracing technology into an implantable that can record neural or cardiac biopotentials, paving the way for richer animal behavior studies. We present this innovative form factor as one of the first to blend these technologies, developed using flexible circuit board substrates and biocompatible 3D printing. The result is a hormetically robust device applicable to a wide range of species. The device's functionality is complemented by a custom mobile application that allows users to configure device settings, establish recording schedules, and remotely upgrade the firmware as needed. We anticipate that this integrated platform will mark a significant step forward in the

field of neuroethology, offering the potential for an enhanced understanding of animal behavior or broadening the scope of laboratory experiments.

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**Topic:** G.03. Motivation

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**Title:** The role of the masculinization process in the gender bias of autism spectrum disorder: insights from a mouse model exposed to valproic acid

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**Abstract:** Autism spectrum disorder (ASD) is characterized by reduced sociability and the presence of repetitive behavior patterns. Remarkably, only one out of four affected children are girls. To uncover the mechanisms underlying this gender bias, we used a mouse model of autism that involves exposing mice to 600 mg/kg valproic acid (VPA) on gestational day 12.5. Notably, we previously observed that female mice exposed to VPA did not exhibit the same sociability deficits as VPA-exposed males in adulthood.One of the primary differences between male and female mammals is the masculinization process, wherein the male brain undergoes reorganization through hormone exposure during the perinatal period. It is worth mentioning that this developmental organization of the brain is essential for the proper display of sexual behaviors in adulthood. Since the masculinization process involves inflammatory signaling molecules and we previously observed immunological alterations in our VPA model, our hypothesis is that the masculinization process is necessary for VPA prenatal exposure to affect autism-related behaviors.To test this hypothesis, we exposed female mice in the VPA model to neonatal 17β-estradiol benzoate (E2) on postnatal days 2, 5 and 8 to mimic the hormone surge that males experience during the perinatal period. Then, we analyzed four experimental groups: SAL-OIL (n = 13), SAL-E2 (n = 17), VPA-OIL (n = 12) and VPA-E2 (n = 14). We observed that VPA-E2 females did not habituate to a social stimulus in either in a three-chamber social interaction test (three-way ANOVA followed by Fisher's LSD test, bin 1 vs 5; $p > 0.05$) or a social habituation test (three-way ANOVA followed by Fisher's LSD test, presentation 1 vs 4; $p > 0.05$). Furthermore, we evaluated cellular alterations in the brain and observed a distinct ellipsoidal cell aggregate of interneurons that were calbindin-positive in the sexually dimorphic nucleus of E2 females, similar to the pattern observed in males (two-way ANOVA, postnatal treatment: $p < 0.05$). Control females lacked such a structure. Furthermore, we found no differences between groups in the number of calbindin-positive cells in the piriform cortex (two-way ANOVA, $p > 0.05$) and seventh lobule of the cerebellum (two-way ANOVA, $p > 0.05$), which are other areas known to be affected in our model.Overall, our findings demonstrate that the postnatal period is a sensitive time to modulate adult ASD related behaviors, and that the masculinization process plays a critical role in determining the sexual bias observed in this mouse model of ASD.

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**Topic:** G.03. Motivation

**Title:** Acute social isolation acts on hypothalamic neurons to promote social behavior in female mice

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**Abstract:** Humans and other social animals are highly motivated to seek out social connections, and short-term social isolation is aversive and increases social motivation. How social isolation acts on the brain to promote social behavior remains incompletely understood. In previous work, we found that 3-day isolation exerts pronounced effects on social behavior in B6 mice, particularly in females, and that single-housed females that subsequently engage in same-sex interactions spend more time spent investigating and begin mounting female social partners and produce higher rates of social ultrasonic vocalizations (USVs) relative to group-housed females. To explore the neural circuit changes that underlie these effects on social behavior, we compared Fos expression in the brains of group-housed and single-housed females following same-sex social interactions. We found a population of neurons in the preoptic hypothalamus that exhibit increased Fos expression in single-housed females following same-sex social interactions ($POA_{iso}$ neurons), in a manner that correlates with time spent engaged in social investigation and with rates of mounting. To test the functional contributions of these $POA_{iso}$ neurons to changes in female social behavior, we employed an activity-dependent labeling strategy (TRAP2) to test whether silencing $POA_{iso}$ neurons in single-housed females reduces the effects of short-term isolation on social behavior. We found that both chemogenetic silencing and caspase-mediated ablation of $POA_{iso}$ neurons in single-housed reduced rates of mounting, although effects on social investigation and USV production were variable across manipulations. Conversely, optogenetic activation of $POA_{iso}$ neurons in group-housed females elicits social investigation, USV production, and mounting. In ongoing experiments, we are also investigating whether a similar population of POA neurons mediates the effects of short-term isolation on male social behavior during opposite-sex and same-sex social interactions. Our findings will inform our understanding of the neural circuit mechanisms through which social isolation alters social behavior, as well as whether these circuit mechanisms differ in females and males.

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**Title:** Defining the functional identity of the late-maturing paralaminar amygdala

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**Abstract:** The amygdala is a brain region involved in social and emotional processing. A key phase of amygdala development is adolescence, when the region grows in size and cell number. Dysfunction in this growth is associated with various neurodevelopmental disorders, including mood disorders and ASD, yet the cellular and circuit changes that occur during this period are unknown. The paralaminar nucleus of the amygdala (PL) contains a population of neurons that delay their maturation until adolescent ages. However, the identity, circuitry, and functional role PL neurons remains unknown. We recently identified and characterized the mouse PL, revealing close similarities to the human. Using this mouse model, we sought to uncover PL neuronal physiology, input connectivity, and responsiveness to sensory cues. By intrinsic electrophysiological properties and morphological profiles we observed multiple subtypes in the adult PL. Next, to uncover the sources of input to the PL we targeted the region with retrograde tracers; we revealed inputs from diverse brain regions within and outside the amygdala. We confirmed these findings using anterograde transsynaptic tracer injections to each input source. Finally, based on the connectivity of the PL to olfactory nuclei, we used fiber photometry to measure calcium dynamics in the adult PL we find that the PL is responsive to variety of salient olfactory sensory cues. Together, these experiments define the identity, connectivity and responsivity of mouse PL.

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**Title:** Chemogenetic inhibition of anterior cingulate cortex inhibits empathetic consoling behavior in prairie voles

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**Abstract:** Empathetic responses are disrupted in several psychiatric disorders, including autism spectrum disorder. Consolation, or comforting physical contact directed toward a distressed party, is an empathy-like response observed across species. Monogamous prairie voles reliably express consolation, in the form of allogrooming, towards their partner in distress. Consolation was found to induce neural activity in the anterior cingulate cortex (ACC) and blockade of ACC

oxytocin receptors (OXTR) ablated consoling. The ACC is also implicated in empathy in humans, suggesting conserved neurobiological mechanisms from rodents to humans. We aim to use prairie voles to investigate neural circuits involving the ACC in regulating consoling behavior. We examined general inputs to the ACC by administering a retrograde tracer (500 nL; AAVrg-CAG-GFP) unilaterally to the ACC in adult voles (N=6) and found projections from cortical structures including the claustrum and insular cortex. Projections from subcortical regions include the basolateral amygdala, hippocampus, and several thalamic nuclei. Next, as a first step towards cell-type specific and circuit manipulation of consolation, we used designer receptors exclusively activated by designer drugs (DREADDs) to inhibit general activity in the ACC. Animals received a control (AAV8-hSyn-mCherry; N=14) or inhibitory DREADD (AAV8-hSyn-hm4di-mCherry; N=9) virus bilaterally to the ACC (500 nL/side) and were later paired with an opposite-sex partner before undergoing a consolation test. In this test, the experimental vole was separated from its partner for 30 min and then reunited over two subsequent days. On the second day, the partner received mild foot shocks during separation. Experimental voles received a clozapine-N-oxide (CNO) injection prior to reunion on both days and allogrooming directed toward the partner was scored. Voles administered the control virus displayed an increase in allogrooming towards their partner after their partner was separated and shocked compared to separated only ($p < 0.01$). Voles administered the inhibitory DREADD failed to increase allogrooming in response to their partner's distress ($p > 0.05$), suggesting that disrupting general activity in the ACC prevents consolation. Finally, we used RNAscope to determine that OXTRs localize to layers 5 and 6 of the prairie vole ACC, and are expressed primarily on glutamatergic, but not GABAergic, cells. For future experiments, we have generated *Oxtr-P2A-Cre* voles using CRISPR to selectively trace and manipulate circuits containing ACC-OXTR cells to further our understanding of the ACC and oxytocin in empathy-like behavior.

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**Topic:** G.03. Motivation

**Title:** Sex differences in neural representations of social and nonsocial reward in the medial prefrontal cortex

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**Abstract:** The perception of social interactions as rewarding is necessary for appropriate social behavior. While there has been progress made towards understanding the neural circuits underlying social behavior, it remains unknown if social rewards are processed by the same or different neural populations as nonsocial rewards. One brain region that has been implicated in both social behavior and nonsocial reward processing is the medial prefrontal cortex (mPFC). We developed a novel two choice (social-sucrose) operant assay to directly compare the neural representations underlying social and nonsocial reward in the mPFC. We found that male and female mice seek a similar number of social and sucrose rewards under control conditions. We also performed cellular resolution calcium imaging of mPFC neurons in both male and female

mice (n = 459 neurons, 9 male; 570 neurons, 6 female) while they completed the two choice operant assay across various internal states (water restriction and social isolation). We identified non-overlapping populations of neurons that responded to social and nonsocial reward in a sex- and state-dependent manner. In particular, female mice had more distinct neural representations of social and nonsocial reward compared to male mice. Reward-seeking behavior and neural representations of reward changed with internal state. Following water restriction, both male and female mice showed increased sucrose reward-seeking behavior and had a greater proportion of sucrose reward responsive neurons. By tracking individual neurons across sessions, we found that this increase was driven by the recruitment of previously reward-unresponsive mPFC neurons. Following acute social isolation, the proportion of mPFC neurons that responded to social reward remained the same. However, the amplitude of the response to social reward varied in a sex-dependent manner, with the amplitude of social reward responsive neurons increasing in female mice and decreasing in male mice after social isolation. This sex-dependent change was also evident in reward-seeking behavior, with female mice seeking proportionally fewer social rewards and male mice seeking proportionally more social rewards relative to sucrose rewards after social isolation. Additionally, we optogenetically manipulated mPFC neurons during the reward period of the two choice operant assay and found that it disrupted reward-seeking behavior (n = 8 ChR2, 6 GFP). Thus, using a novel operant assay, we identified distinct and flexible neural representations of social and nonsocial reward in the mPFC that vary in a sex- and state-dependent manner and are essential for appropriate reward-seeking behavior.

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**Topic:** G.03. Motivation

**Title:** Modulation of serotoninergic transmission by galanin in social behavior

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**Abstract:** Galanin was initially identified as a ‘classic neuropeptide’ with actions primarily as a modulator of neurotransmission in the central and the peripheral nervous system. *In-situ* hybridization studies verified the co-existence of neuropeptide galanin with other neurotransmitters, opening new avenues for galanin research. Galanin and its three receptor subtypes are highly expressed in dorsal raphe nucleus (DRN), a region of the brain that contains a large population of serotonergic neurons. Serotonin (5-HT) is one of the neurotransmitters released in nucleus accumbens (NAc) from serotonergic terminals of DRN, where DRN-NAc projections mediate the release of 5-HT after presentation of a rewarding stimulus such as interaction with a conspecific (social reward). Furthermore, DRN is implicated in several behaviors such as those related to stress, anxiety, emotions and mood regulation, drug/reward seeking and social behaviors. The presence of galanin with the neurotransmitter 5-HT in DRN hints at the role of galanin in modulating the serotonergic system and hence in regulating DRN-mediated behaviors. However, the brain region contributing as a major source of galanin to DRN is still unknown.Previous reports suggest that GABAergic neurons in medial preoptic area (POA) of the hypothalamus express neuropeptide galanin, and POA is anatomically and functionally well-connected primarily to GABAergic neurons in DRN. Further, galanin effects

on local DRN circuitry and on 5-HT release downstream in NAc have not been studied. Thus, we propose that galanin neurons from POA inhibit GABAergic neurons in DRN, thereby, augmenting the activity of serotonergic neurons in DRN and promoting 5-HT release in the NAc. In the present study, using retrograde tracing, we dissect all major galanin inputs to DRN, and identify a major galaninergic input from POA to DRN. We plan to record the activity in this galanin input (POA-DRN) in real-time using fiber photometry to elucidate their role in social behaviors in both male and female mice. Next, we will optogenetically modulate (activate/inhibit) POA-DRN projections to investigate the impact of this specific modulation in regulating social behaviors and 5-HT release in NAc. Understanding sex-specific neurobiological mechanisms underlying social behaviors can lead to critical insights for molecular targeting of modulators involved in mediating optimal serotonergic tone in DRN, in turn directing various DRN-related behaviors.

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**Title:** A BNST-to-lateral septum vasopressin circuit modulates sex-specific social approach, communication, and anxiety-like behavior in mice

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**Abstract:** One of the largest sex differences in brain neurochemistry is the male-biased expression of the neuropeptide arginine vasopressin (AVP) within the vertebrate social brain. While AVP has long been implicated in social- and anxiety-like behavior, the exact circuitry and the anatomical substrate of AVP's control of social behavior is unclear. Here we show that optogenetic silencing of BNST AVP neurons in mice specifically reduced male investigation of other males in a three-chamber apparatus, whereas female investigation was unimpaired. Conversely, optogenetic stimulation of these cells in males increased investigation of both male and female conspecifics. Unexpectedly, BNST AVP cell stimulation in females also increased social investigation, albeit only toward males. Optogenetic stimulation of BNST AVP fibers in the lateral septum (LS), an area with the highest density of sexually-dimorphic AVP fibers, increased social investigation and anxiety-like behavior in males, but not in females. Blocking the vasopressin 1a receptor (V1aR) in the LS eliminated stimulation-mediated increases in these behaviors. Activation of a distinct BNST AVP → LS V1aR circuit modulates sex-specific social interest and anxiety-like behavior. This work suggests that sex differences in the neurochemical underpinnings of social behavior may contribute to sex differences in disorders of social behavior and communication.

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**Topic:** G.03. Motivation

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**Title:** Dopamine release within subregions of the nucleus accumbens (NAc) mediates the valence and salience of social stimuli

**Authors: *E. A. CROSS**[1], J. M. BORLAND[2], K. L. HUHMAN[3], H. ALBERS[4];
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**Abstract:** Social interactions are highly salient regardless of their valence (i.e., whether they are rewarding or aversive). The neural mechanisms underlying the processing of valence versus salience, however, are not well understood. The mesolimbic dopamine system (MDS), which includes the ventral tegmental area (VTA) and its dopamine (DA) projections to the nucleus accumbens (NAc), is the canonical "reward pathway" underlying motivated behavior. We propose that differential activity of specific subregions in the MDS encodes the salience and valence of social interactions. We tested the hypothesis that the NAc core encodes the salience of social stimuli while the NAc shell encodes the valence in male and female Syrian hamsters. We measured DA release in the NAc during social interactions using *in vivo* amperometry. We obtained DA measurements in the NAc core and shell in awake, behaving hamsters during socially rewarding interactions wherein the subject "won" (i.e., became dominant over a nonaggressive intruder) and during aversive interactions wherein the subject "lost" (i.e., was defeated by a larger, resident aggressor). In support of our hypothesis, there were no differences in number of DA transients in the NAc core during winning and losing, suggesting that agonistic encounters are associated with similar high salience and similar DA activity regardless of the outcome of the agonistic interaction. By contrast, tonic DA in the NAc shell was found to be higher in the rewarding social context, winning and lower in the aversive social context, losing. When the results were separated by sex, the same relationship between winners and losers was observed in the NAc shell, however, the effect was more pronounced in females. Baselines recorded before and after social sessions indicated that these DA changes were tied to the social experiences, and transients in the NAc core were time-locked to attacks. Electrodes outside the NAc (anatomical "miss" controls) did not show the same patterns, suggesting that the signals recorded in the core and shell were specific to DA release in those NAc subregions. These data suggest that DA release in NAc core and shell encode the valence and salience of social stimuli, respectively.

**Disclosures: E.A. Cross:** None. **J.M. Borland:** None. **K.L. Huhman:** None. **H. Albers:** None.

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**Topic:** G.03. Motivation

**Support:** R01MH101377
R01MH124927

**Title:** The Role of Dopamine in Modulating Aggression

**Authors: *B. DAI**[1], B. ZHENG[2], L. S. ZWEIFEL[3], D. LIN[4];
[1]Neurosci. Institute, New York Univ. Langone Med. Ctr., New York, NY; [2]Neurosci. Institute, New York Univ. Langone Med. Ctr., New York City, NY; [3]Pharmacol., Univ. of Washington, Seattle, WA; [4]New York Univ. Neurosci. & Physiol., New York, NY

**Abstract:** Excessive and maladaptive aggression is a common psychopathological symptom of various mental disorders. Despite the prevalence of aggression in mental disorders, our treatment options are limited and outdated. Only a few medicines were available, most developed decades ago. They are mainly dopamine receptor D2 (D2R) antagonists, often prescribed together with psychotherapy. However, the mechanism underlying D2R antagonists in suppressing aggression remains poorly understood. Moreover, these drugs cause sedation, leading to debates about the specificity of this treatment - whether it is specific to regulating aggression or aggression reduction is secondary to reduced movement. This project aims to elucidate the neural mechanims responsible for dopamine modulation of aggression in male mice. Through chemogenetic manipulation, we discovered that dopaminergic neurons in the ventral tegmental area (VTA) could bi-directionally modulate aggressive behaviors in an experience-dependent manner. Specifically, VTA dopamine cell activity strongly influences aggressive behaviors of rookie male aggressors (attack experience $\leq$ 2 days), but not expert aggressors (attack experience > 8 days). Furthermore, we utilized CRISPR-Cas9 to reduce or abolish tyrosine hydroxylase (TH) synthesis in VTA dopamine neurons of both rookie and expert mice. Consistent with our chemogenetic manipulation results, TH mutagenesis successfully eliminated aggression in rookie but not in expert aggressors, while other innate behaviors, such as sexual behavior and feeding, were unaffected. Lastly, we employed 6-OHDA lesion to screen various downstream targets of VTA dopamine neurons and identified the lateral septum as a crucial mediator of the VTA dopamine cell's effect. In summary, our findings shed new light onto the neural mechanisms underlying dopamine modulation on aggression.

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**Topic:** G.03. Motivation

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**Title:** Family matters: Biased brain and behavioral responses in the communally breeding spiny mice, Acomys cahirinus

**Authors: *B. A. FRICKER**[1], D. HO[1], A. W. SEIFERT[2], A. M. KELLY[1];
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**Abstract:** In complex social environments, individuals may interact with not only novel and familiar conspecifics but also kin and non-kin. The ability to distinguish between conspecific identities can have fitness consequences, yet how the brain processes conspecific type and how animals may alter behavior accordingly is not well known. We examined whether the communally breeding spiny mouse responds differently to conspecifics that vary in novelty and relatedness. In a group interaction task with familiar kin, novel kin, and novel non-kin, we found that males can distinguish novel kin from novel non-kin, and preferentially spend time with

novel kin over familiar kin and novel non-kin. To determine whether kinship and novelty status are differentially represented in the brain, we conducted immediate early gene tests, which revealed the dorsal, but not ventral, lateral septum differentially processes kinship, but not social novelty. Further, males did not exhibit differences in prosocial behavior toward novel and familiar conspecifics but exhibited more prosocial behavior with novel kin than novel non-kin. We then discuss the effects of CRISPR-cas9 mediated knock down of oxytocin receptors in the lateral septum on kin-biased behaviors. These results suggest that communally breeding species may have evolved specialized neural circuitry to facilitate a bias to be more affiliative with kin, regardless of whether they are novel or familiar, to enhance fitness and promote behaviors such as incest avoidance and nepotism.

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**Nanosymposium**

**NANO27: Addictive Drugs: Cell Signaling, Circuitry, and Neurophysiology**

**Location:** WCC 152B

**Time:** Sunday, November 12, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO27.01

**Topic:** G.09. Drugs of Abuse and Addiction

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Peter F. McManus Charitable Trust

**Title:** Diurnal variation in acetylcholine modulation of dopamine dynamics following chronic cocaine intake

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**Abstract:** Despite decades of research into its neurobiological mechanisms, cocaine use disorder (CUD) remains a major worldwide health problem. One variable that is often overlooked in CUD research is cocaine-induced disruption of diurnal (night/day) rhythms. The mesolimbic dopamine (DA) system is an important mediator of motivated and reward-associated behaviors that are maladapted in CUD. Acetylcholine (ACh) from striatal cholinergic interneurons (CINs) modulates mesolimbic DA release in the nucleus accumbens (NAc) core via nicotinic acetylcholine receptors (nAChRs) on DA terminals. Thus, ACh plays a critical role in these behaviors. Though the effect of chronic cocaine on DA signaling has been extensively studied at single time points, cocaine-induced diurnal disruptions of DA release as well as their mechanisms have not been investigated. Here, we used *ex vivo* fast scan cyclic voltammetry in a

rat model of cocaine self-administration under various access schedules [(**Short continuous access (ShA)**, **long continuous access (LgA)**, or **intermittent access (IntA)**] to test the hypothesis that diurnal rhythms of DA release and the CIN ACh influence on DA release will vary based on the pattern of cocaine availability. Consistent with the literature, we found that IntA resulted in cocaine intake that was comparable to ShA, but significantly less compared to LgA. Interestingly, IntA significantly increased DA release midway through the dark cycle while LgA significantly increased DA release midway through the light cycle compared to other groups. Furthermore, we found that the CIN ACh influence on DA release was dependent on stimulation frequency. IntA resulted in greater CIN ACh influence on DA at tonic-like frequencies while LgA resulted in greater CIN ACh influence on DA across both tonic- and phasic-like frequencies versus ShA and relative to controls across time points. Understanding the influence of diurnal rhythms and pattern of cocaine intake on NAc neurochemistry will provide a rationale for targeting these receptor systems as a mechanism for cocaine-induced disruptions.

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**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** R00DA045795
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**Title:** Zfp189 function in the nucleus accumbens regulates cocaine-induced transcription and behaviors in a cell type specific manner

**Authors: *G. M. SILVA**[1], J. PICONE[4], A. KAPLAN[6], N. L. TRUBY[2], R. K. KIM[5], R. L. NEVE[7], X. CUI[1], P. J. HAMILTON[3];
[2]Virginia Commonwealth Univ., [3]VCU, [1]Virginia Commonwealth Univ., Richmond, VA; [4]Virginia Commonwealth Univ. Neurosci. Grad. Program, [5]Virginia Commonwealth Univ., Virginia Commonwealth Univ. Neurosci. Grad. Program, Richmond, VA; [6]Virginia Tech. Univ., Blacksburg, VA; [7]Massachusetts Gen. Hosp., Boston, MA

**Abstract:** Previous work has demonstrated that *Zfp189* is a gene target through which the cAMP-response element binding (CREB) transcription factor (TF) regulates the reinforcing effects of cocaine within the nucleus accumbens (NAc). However, the exact NAc cell-type specific mechanisms through which *Zfp189* expression is able to regulate cocaine-induced neuroadaptations remains unclear. The *Zfp189* gene product is a Krüppel associated box (KRAB) zinc finger TF of unknown function. To directly interrogate the transcriptional function and gene targets of ZFP189, we reprogrammed the endogenous $ZFP189^{WT}$ by replacing the repressive KRAB domain with an enhanced transcriptional activation domain (VP64-p65-Rta ($ZFP189^{VPR}$) or by removing the functional moiety entirely ($ZFP189^{NFD}$). We demonstrate that these synthetic ZFP189 TFs exert divergent transcriptional regulation at a *luciferase* target gene, *in vitro*. Upon packaging these ZFP189 TF constructs in herpes viral vectors (HSVs) and surgically delivering to mouse NAc, we identify that the synthetic $ZFP189^{VPR}$ affects cocaine-, but not morphine- or saline-elicited behaviors. Further, in analyzing these tissues with bulk RNA sequencing (RNAseq) approaches, we see only mice with $ZFP189^{VPR}$ intra-NAc and treated with cocaine experience significant NAc transcriptional regulation. To understand the NAc cell-type specific

correlates of this drug-specific result, we performed single nuclei RNA sequencingon infected NAc tissues. We investigated the NAc cell type specific contribution of our ZFP189 variants to cocaine-induced locomotor behavior. We utilized transgenic mice that express Cre recombinase under the *Drd1*- or *Drd2*-promoter in combination with Cre-dependent expression vectors to express our synthetic ZFP189 TFs selectively in *Drd1*+ or *Drd2*+ NAc medium spiny neurons (MSNs). By delivering $ZFP189^{VPR}$ to *Drd1*+ MSNs, we observed an increase in cocaine-induced locomotor behavior. Interestingly, by delivering $ZFP189^{WT}$ to *Drd2*+ MSNs, we observed a similar cocaine-induced increase in locomotive behavior. Given the largely opposing roles of the two MSN subtypes in reward-related behaviors, and the observed opposite transcriptional control of our synthetic ZFP189 TFs, it is possible that we are dysregulating a ZFP189-governed opponent process between the MSN subtypes. Lastly, we investigated the consequences of altered ZFP189-mediated transcriptional function on dendritic spine density and morphology in *Drd1*+ or *Drd2*+ MSNs. Collectively, this work links the MSN-specific function of a drug-induced TF in governing lasting drug-related transcriptional neuroadaptations and behaviors.

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**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** Ministry of Science and Technology of China (STI2030-Major Projects 2021ZD0202900)
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**Title:** Inhibitory Microcircuit of the Orbitofrontal Cortex Regulated by Brevican of the Perineuronal Nets Modulates Conditioned Place Preference for Cocaine

**Authors: *W. ZHANG**[1], Y. FU[1], Z. HUANG[1], X. WEI[1], J. TIAN[1], Y. WANG[1], J. SHI[1], L. LU[2];
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**Abstract:** A hallmark of drug addiction is active or compulsive drug seeking, even with the knowledge of the negative or punishing consequences of the drug and such behavior, which suggests malfunction of the value-based decision-making of such individuals. The orbitofrontal cortex (OFC) is a brain region of the reward pathway important for value-based decision-making. Both clinical and animal studies have shown the impairments to the OFC after drug addiction. However, the underlying mechanism for prolonged OFC function alteration after drug addition still is not fully understood, and the possible neuronal circuit mechanism remains to be illustrated. To understand the underlying microcircuit change of the OFC in drug addiction, we studied male transgenic mice using a cocaine-conditioned place preference test. We combined it with electrophysiology, chemogenetic, optogenetic, and shRNA-interference techniques. We found that cocaine addiction chronically changed the GABAergic microcircuit, which reduced GABAergic input to the pyramidal neurons of the OFC. Such a change was mediated by the increased synaptic connections between inhibitory neurons (SST-PV), which decreased the strength of inhibitory circuit output. Brevican of the perineuronal nets, a PV+ neuron-specific extracellular matrix structure regulated the increased SST-PV synapses. Reversal of the

inhibitory synapse density increase with shRNA-interference restored microcircuit function and reduced the retention of CPP after cocaine withdrawal. These results showed inhibitory microcircuit function in the OFC of mice acquired cocaine-CPP, the dysfunction of this microcircuit impaired OFC function and contributed to the retention of CPP. Our study identified a novel circuit mechanism for the functional changes of the OFC in drug addiction and suggested a novel therapeutic target for drug addiction treatment.

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UM1 DA051410
U01 DA053625
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U01 DA056003

**Title:** A comparative single-cell multi-omic atlas of molecular adaptations associated with substance use and HIV infections in the prefrontal cortex of humans, non-human primates, and mice

**Authors: *S. A. AMENT**, B. HERB, J. P. RECEVEUR, O. R. WHITE, P. SCORCH CONSORTIUM;
Univ. of Maryland Sch. of Med., Baltimore, MD

**Abstract:** Substance use disorders (SUD) and addiction are associated with the dysregulation of neural circuits related to salience and habits, negative emotional states, and executive function, but the critical cell types within these brain regions are not fully described, and the best therapeutic targets within them are not known. The Single-Cell Opioid Responses in the Context of HIV (SCORCH) consortium was founded by the National Institute on Drug Abuse to conduct collaborative single-cell genomic studies of multiple brain regions implicated in SUD, utilizing both human post-mortem brain tissue and precisely controlled animal models of addiction. In addition, SCORCH will investigate the effects of SUD in the context of Human Immunodeficiency Virus (HIV) and associated animal models, as ~17% of people who inject drugs globally are persons with HIV, contributing to adverse outcomes in both conditions. Key

findings will be validated by independent approaches, and all data will be made publicly available as community resources. As an initial product of the SCORCH consortium, we present a single-cell multi-omic atlas of SUD and HIV-associated molecular adaptations in the prefrontal cortex (PFC), which controls executive functions that become progressively compromised in SUD, leading to impulsivity, perseveration, affective instability, and drug-overvaluation. Our current data version integrates single-cell multi-omic profiles of 367,219 cells from the dorsolateral PFC of 54 human donors and will soon grow to incorporate millions of cells from >150 humans, as well as from rhesus macaques and mice. Our analyses describe PFC cell type-specific gene expression and chromatin accessibility changes associated with SUD, HIV, and SUD+HIV. Comparisons between humans and animal models will enable us to identify gene networks associated with specific substances and stages of addiction, then evaluate the dynamics of these gene networks in human donors. These analyses provide insights into the mechanisms of SUD and HIV in the PFC and serve as a blueprint for collaborative studies of >15 brain regions. Overall, the SCORCH consortium seeks to address, at the single-cell level, critical gaps in our understanding of the molecular and cellular adaptations in the brains in individuals with SUD, HIV infection, and a confluence of these conditions.

**Disclosures: S.A. Ament:** None. **B. Herb:** None. **J.P. Receveur:** None. **O.R. White:** None. **P. SCORCH Consortium:** None.

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**Support:** NIH/NIDA Grant U01DA053600
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**Title:** Identifying cell-type-specific transcriptional changes in postmortem ventral midbrain with substance use disorder and long-term HIV infection: a cohort study at the Manhattan HIV Brain Bank

**Authors: *A. M. WILSON**[1], M. M. JACOBS[5], T. LAMBERT[2], A. VALADA[2], S. AKBARIAN[3], S. MORGELLO[4];
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**Abstract:** For people living with HIV (PLWHIV), substance use disorder (SUD) is a prevalent comorbidity and important neurological risk factor: in 2021, adults who injected drugs accounted for 10% of new HIV infections globally (18% outside sub-Saharan Africa) and were 35 times more likely to acquire HIV than others[1]. Even with antiretroviral therapy (ART), HIV persists in “reservoirs” including the brain and is associated long-term with neurocognitive disorders (experienced by ~50% of PLWHIV)[2]. Dopaminergic brain pathways carry a heavy HIV burden; SUD also interferes with dopaminergic function, complicating HIV progression. Insight into the cell-level changes associated with chronic SUD, HIV infection, or their co-occurrence is limited but would be valuable for shaping future interventions. Here, we explore cell-type-specific alterations in gene expression in the context of chronic, ART-treated HIV and SUD involving opioids, cocaine, or both, in the striatonigral dopaminergic pathway. We performed single-

nucleus RNA sequencing on postmortem ventral midbrain tissue from 90 donors (45 HIV+/SUD+; 17 H+/S-; 15 H-/S+; 13 H-/S-; ~200,000 nuclei total) and analyzed expression of 20,000 highly varying genes. We identified glial, neuronal, immune, and blood-brain-barrier cell types, including subtypes with inflammatory profiles, using an iterative marker-gene-based likelihood approach. For each cell type, we identified differentially expressed genes as a function of HIV and SUD presence, incorporating clinical information about HIV severity, other comorbidities, and demographic factors. We focus on expression changes in neurons (as central components of intercellular signaling) and microglia (as important mediators of inflammatory responses). We find that dopaminergic neuron function, including dopamine transport, is suppressed with undetectable or detectable HIV, and that SUD substantially worsens HIV-associated dysregulation in dopaminergic neurons, also upregulating genes associated with regrowth and synapse remodeling. Notably, we do not observe dysregulation of dopamine transport with SUD after controlling for HIV severity, suggesting that HIV may have a stronger influence there. In microglia, we observe differential expression suggesting elevated immune activation in patients with HIV and more aggressive, varied activation in HIV with SUD that includes anti-inflammatory responses. [1]UNAIDS Global AIDS Update, 2022. https://www.unaids.org/sites/default/files/media_asset/2022-global-aids-update_en.pdf. [2]Le L. and Spudich S. Semin Neurol 2016; 36(04): 373-381. DOI: 10.1055/s-0036-1585454.

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**Title:** Chronic drug use increases densities of perineuronal nets in the hippocampus of humans and non-human primates

**Authors: *J. VALERI**[1,2], S. O'DONOVAN[3], C. STIPLOSEK[2], K. GRANT[4], R. BOLLAVARAPU[2], D. M. PLATT[1,2], B. GISABELLA[1,2], C. A. STOCKMEIER[2], H. PANTAZOPOULOS[1,2];
[1]Grad. Program in Neurosci., Univ. Of Mississippi Med. Ctr., Jackson, MS; [2]Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Univ. of Toledo, Toledo, OH; [4]Div. of Neurosci., Oregon Natl. Primate Res. Ctr., Beaverton, OR

**Abstract:** **Background:** Emerging evidence points to a critical role of extracellular matrix (ECM) molecules in the regulation of reward memories. Chondroitin sulfate proteoglycans (CSPGs), a subset of ECM molecules, form perineuronal nets (PNN) around inhibitory neurons and restrict synaptic plasticity. Rodent models suggest that PNNs are degraded by endogenous proteases to allow for formation of reward memories and then reconsolidate around new synapses to strengthen these memories. Despite this compelling evidence, there is currently a

lack of information regarding PNNs in the brain of people with substance use disorders (SUD). Furthermore, SUD shares a high degree of comorbidity with major depressive disorder (MDD), complicating interpretation of human preclinical models. We tested the hypothesis that PNNs are increased in the hippocampus of subjects with SUD with and without MDD. **Methods**: PNNs and glial cells were labeled with Wisteria floribunda agglutinin histochemistry in a cohort of donors with SUD (n=20), SUD and comorbid MDD (n=24), MDD (n=20) and psychiatric controls (n=20), as well as hippocampal samples from adult male rhesus monkeys with (n=7) or without (n=5) chronic alcohol self-administration from the Monkey Alcohol Tissue Research Resource. PNNs and WFA labeled glial cells were quantified using stereology-based sampling. Stepwise linear regression analysis of covariance was used to test for effects of diagnosis group and confounding variables. QRT-PCR was conducted for genes involved in ECM regulation, synaptic markers, and neuronal populations associated with PNNs. **Results:** We observed increased densities of PNNs in CA1 stratum oriens ($p<0.007$) and WFA-labeled glial cells in CA4 ($p<0.003$) in subjects with SUD, coinciding with decreased mRNA expression of the ECM protease MMP9 ($p<0.05$), and increased mRNA expression of the excitatory synaptic marker VAMP2 ($p<0.04$). Increased density of PNNs was also detected in CA1 stratum oriens in rhesus monkeys with chronic alcohol self-administration ($p<0.04$) along with increased density of WFA-labeled glial cells in the dentate gyrus ($p<0.04$). In comparison, PNN density was decreased in CA1 stratum oriens of subjects with MDD ($p<0.05$). No changes were observed in subjects with comorbid SUD/MDD. **Conclusion:** Our findings represent the first evidence for PNN alterations in humans and nonhuman primates with chronic substance use. Increased PNNs and VAMP2 in SUD may stabilize contextual reward memories as suggested by preclinical studies. Targeting PNNs to weaken contextual reward memories may represent a promising therapeutic approach for SUD, however, comorbidity with MDD should be considered.

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**Presentation Number:** NANO27.07

**Topic:** G.09. Drugs of Abuse and Addiction

**Title:** The Tripnogram: AI assisted morphological, electroencephalographic, and behavioral signatures of diverse psychedelics

**Authors: *A. CONTRERAS**, E. AQUINO, R. M. HINES, D. J. HINES;
Univ. of Nevada, Las Vegas, Las Vegas, NV

**Abstract:** Serotonergic psychedelics are known to exert their hallucinogenic effects by activation of serotonin type 2 (5-$HT_2$) receptors, with serotonin 5-$HT_{2A}$ receptor (5-$HT_{2A}$R) activation considered central to their mechanism of action. The head-twitch response and behavioral arrest, as well as distinct waveform correlates in the cortical EEG of mice, are hallmarks that have been used to characterize the acute response to 5-$HT_{2A}$R activation, however, we currently lack a comprehensive and comparative characterization of psychedelic ligands. Additionally, while the rapid induction of novel synapses is a proposed major downstream mechanism for psychedelic therapies, the relative capacity for different psychedelics to trigger spine morphology changes remains uncharacterized. In the present study, we compared

psychedelics from distinct classes including psilocybin, LSD, mescaline, and 25I-NBOH, each at differing doses. While all psychedelics were capable of inducing plasticity of dendritic spines, they varied in the extent of spinogenesis and the morphology of resulting spines, particularly at high doses. We also found that all psychedelics induce common hallmarks in the cortical EEG that are distinguished by the temporal pattern and density of these waveforms. Finally, by examining a rich repertoire of behavioral indices we identified signatures in addition to the head-twitch response that allow further delineation of unique psychedelic features that scale with dose and map onto human subjective experience. We further used principal component analysis to interrogate the relationship between these signatures across all compounds. This work provides a platform for insight into the activity of diverse psychedelic substances, including novel chemical entities and intervention strategies being developed for potential therapeutic use.

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**Nanosymposium**

**NANO28: Neural Mechanisms of Decision-Making**

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**Topic:** H.03. Decision Making

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**Title:** Error-related signaling in nucleus accumbens D2 receptor-expressing neurons guides inhibition-based choice behavior

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**Abstract:** Learned associations between environmental cues and the outcomes they predict (cue-outcome associations) play a major role in behavioral control, guiding not only which responses we should perform, but also which we should inhibit, in order to achieve a specific goal. However, the neural mechanisms that underly the use of inhibition-based strategies for choice behavior are still unclear, and studies to date have primarily focused on investigating the reinforcement of behaviors that directly result in rewarding outcomes. The encoding of cue-outcome associations, as well as the performance of cue-guided choice behavior, is thought to involve dopamine D1 and D2 receptor-expressing medium spiny neurons (D1-/D2-MSNs) of the nucleus accumbens (NAc). Here, we developed a novel visual discrimination decision-making task in mice to assessed the role of NAc D1-/D2-MSNs in cue-guided inhibition of inappropriate behavioral responses. Cell-type specific optogenetic silencing and single cell-resolution in-vivo imaging revealed NAc D2-MSNs to contribute to inhibition of behavioral responses, with activation of NAc D2-MSNs following response errors playing an important role in optimizing future choice behavior. Our findings indicate that error signaling by NAc D2-MSNs contributes to the ability to use environmental cues to inhibit inappropriate behaviors. These findings are likely to have important implications for the treatment of neurodevelopmental and psychiatric conditions associated with impulse control and impaired decision-making, including ADHD, addiction, and schizophrenia.

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**Topic:** H.03. Decision Making

**Support:** ES/P000681/1 (Economic and Social Research Council UK)

**Title:** Frequency-specific EEG activity underlies both static and dynamic measures of metacognition

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**Abstract:** Metacognition allows us to introspect on our behaviour and decisions. It is crucial for self-evaluation and adaptive functioning. However, it can be suboptimal, and distortions in metacognitive judgements are associated with both clinical and subclinical psychopathology. To date, a clear understanding of the neurocomputational mechanisms underlying metacognition remains elusive. Previous studies have identified electroencephalography (EEG) signatures that predict model-free subjective performance, such as confidence ratings. However, it is currently unclear whether EEG activity relates to model-based measures of metacognition that offer additional insights into the latent processes underlying self-evaluation. Here, 40 human participants of both sexes performed a two-alternative forced-choice visual discrimination task with confidence while their EEG was recorded. 1$^{st}$-order and metacognitive performance were modelled with an extended signal detection theory framework as well as with a recently proposed dynamic evidence accumulation framework. Single-trial regression analyses revealed that stimulus-locked alpha/beta (8-30Hz) desynchronisation and low-frequency EEG activity predicted confidence ratings independently of accuracy and reaction times, extending previous

work. Confidence ratings were also linked to the aperiodic EEG component, which may represent a novel marker of subjective evaluation. Importantly, we found that the strength of the single-trial EEG-confidence relationship predicted participants' level of metacognitive efficiency, measured with two model-based measures (stationary: M-ratio, and dynamic: V-ratio). Specifically, those with stronger separation between confident and unconfident trials in the stimulus-locked alpha/beta EEG activity were better able to discriminate between correct and incorrect choices. This effect was independent of first-order performance, including type-1 sensitivity and drift rate. Instead, type-1 performance was linked to high-frequency (high beta/gamma) activity associated with reaction times. These findings provide new insights into the neural mechanisms of metacognition, suggesting it is underpinned by separable neural processes likely linked to excitability modulations in wide-spread neural networks. The signatures of these modulations, alpha/beta desynchronisation and altered aperiodic activity can thus be considered candidate markers of suboptimal metacognitive insight in general and clinical populations.

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**Title:** Dopamine regulates decision thresholds in human reinforcement learning.

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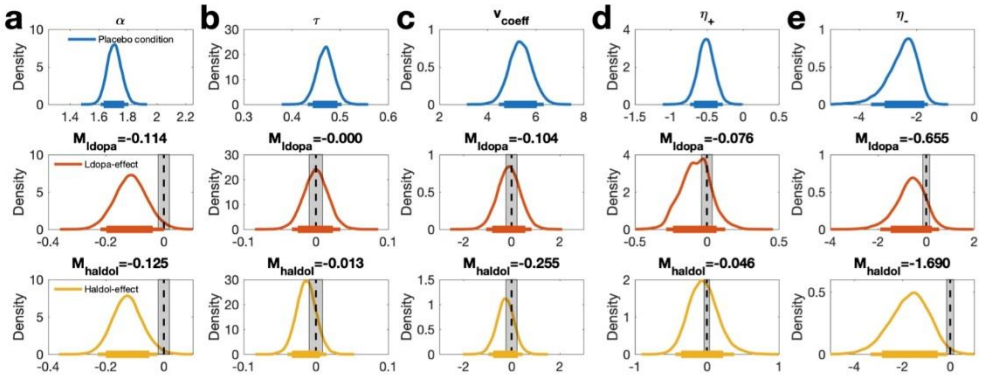
**Abstract:** Dopamine fundamentally contributes to reinforcement learning, but recent accounts suggest a contribution to specific action selection mechanisms. We examined dopaminergic mechanisms underlying human reinforcement learning and action selection via a combined pharmacological neuroimaging approach in male human volunteers (n=31, within-subjects; Placebo, 150mg L-dopa, 2mg Haloperidol). Previously reported beneficial effects of L-dopa vs. Haloperidol on reinforcement learning from gains and altered neural prediction error signals were not observed, which may be partly due to differences experimental design and/or drug dosages. Reinforcement learning drift diffusion models provided a good account of learning-related changes in accuracy and response times, and revealed consistent decision threshold reductions under both drugs, in line with the idea that lower dosages of D2 receptor antagonists increase striatal DA release via an autoreceptor-mediated feedback mechanism. Here we show

that dopamine regulates decision thresholds during reinforcement learning, a computational account that might bridge action selection and response vigour accounts of dopamine.

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**Title:** Boosting noradrenaline improves consistency of risky decision making by enhancing attention in healthy humans

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**Abstract:** Decisions should be consistent. According to economic decision theory, we should always select our most favoured option and, thereby avoid making mistakes and preference cycles (preferring A over B and B over C but also C over A). This principle of rational choice extends beyond economics and finds application in interdisciplinary fields such as political science, aging, mental health and biology. Yet our choices and those of other animals are often inconsistent. Here, we aim to investigate why this may be the case, and to establish a causal link between neurocognitive mechanisms and consistent choice. With the aim of increasing consistency of risky decisions, we used psychoactive drugs in a randomized placebo-controlled, double-blind, incentive-compatible design. One hundred and sixty (80 women) healthy volunteers, 40 per group, received either 4 mg reboxetine (REB), 20 mg methylphenidate (MPH), 2 mg nicotine (NIC) or placebo (PLB). We employed a previously validated task investigating risky decision-making (Kim et al., 2018), which also included options stochastically dominating other options. In accordance with the principles of rational decision-making, dominated options should never be chosen. We conducted Bayesian regression models where the dependent variable was explained with the drug (dummy) while accounting for age,

sex and cortisol level. We found that reboxetine significantly reduced the number of preference cycles and stochastic dominance violations, while no significant effects were observed with methylphenidate and nicotine ($\beta_{drug}$ - $\beta_{PLB}$ in preference cycles, REB = -59.92, 95% upper credible interval (CI) = -23.59, MPH = -20.26 and NIC = -20.53, both 95% upper CI > 0; $\beta_{drug}$ - $\beta_{PLB}$ in stochastic dominance violations, REB = -0.03, 95% upper CI = -0.01; MPH = -0.02 and NIC = -0.01, both 95% upper CI > 0). These findings are compatible with the notion that norepinephrine increases the consistency of risky decisions in humans. Next, we investigated drug effects on eye-based measures of attention. Only the reboxetine group fixated dominating options proportionally longer ($\beta_{REB}$ - $\beta_{PLB}$ = 0.06, 5% lower CI = 0.02; $\beta_{MPH}$ - $\beta_{PLB}$ = 0.02, 5% lower CI = -0.02; $\beta_{NIC}$ - $\beta_{PLB}$ = -0.01, 5% lower CI = -0.04 and more quickly ($\beta_{REB}$ - $\beta_{PLB}$ = -150.86, 95% upper CI = -66.54; $\beta_{MPH}$ - $\beta_{PLB}$ = -47.78, 95% upper CI = 31.93; $\beta_{NIC}$ - $\beta_{PLB}$ = -12.09, 95% upper CI = 66.03). Thus, pharmacological interventions can facilitate the optimal allocation of attention, resulting in heightened levels of consistency of decision-making. This selective attention to a subset of options has been recognized as an efficient normalization process for neural coding and representation (Glimcher and Tymula, 2023).

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**Title:** Dynamical shifts of mouse functional brain network during visual decision making

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**Abstract:** Learning to associate external stimuli with appropriate behavioral responses is a fundamental and crucial capability of the animal brain. In the processes of visual task learning and decision making, various regions of the mouse brain have been demonstrated to participate, with each region exhibiting distinctive dynamics in the representation of task-related information during the learning process. Nevertheless, the mechanism in which these regions interconnect dynamically as a mesoscale network remains an unresolved issue. In order to examine this particular mechanism, we divided 6 mice (C57BL6/J, male, 10 weeks) into two distinct cohorts: a training group and a control group. For each mouse within these cohorts, we implanted eight high-throughput ultra-flexible electrodes, forming a multi-electrode array in unilateral hemisphere with a total of 1024 channels to simultaneously recorded from 10 brain regions, including frontal regions (mPFC, OFC, ACC), motor cortices (M1, M2), visual cortices (V1,

V2M, V2L), and subcortical regions (striatum, MD thalamus), which have been implicated in visual decision learning, during a visual Go (hit)/No Go (correct rejection, CR) task. We found that mice gained proficiency in this task by learning to hold licking behavior at unrewarding No Go visual stimuli. Different visual regions and frontal regions were active at Go/No Go trials, and showed distinct functional connection dynamics in different trial conditions. For instance, the proportion of effective output of V1 to other brain regions increased after the appearance of the visual stimulus, but the increase in CR trials was relatively delayed compared to that in hit trials. Moreover, in hit trials, the increase of the proportion of effective output of V1 was transient, whereas in CR trials, the increase was maintained for a longer duration. As for the network depth, which has been widely used to assess network complexity, we revealed that the input center during the late stimulus and response phases of the hit trial were more prominent compared to those of the CR trial. Further, we found that the frequency of brain regions acting as input/output centers varied during different epochs within a trial, and gradually changed with the animal's proficiency during the task learning. These results suggest that the mesoscale functional brain network shifts fast and dynamically during visual task learning and decision making, recruits different brain regions at different stages of the task, integrates external stimuli information and priori experience, then determines the strategies and actions that need to be taken next.

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**Topic:** H.03. Decision Making

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**Title:** Characterizing the functional role of Interhemispheric Connections in value decisions using optical tools.

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**Abstract:** Animals foraging in the wild must rapidly detect and acquire valuable objects from their environment. A network of brain regions organized as two hemispheres are involved in processing value from the visual inputs arising from the contralateral side. The information processed in each hemisphere must be compared before a value-based motor decision. Our research showed that neurons in the oculomotor structure Superior Colliculus (SC) are sensitive not only to the value of objects present in the receptive field but also to the value of objects presented in the opposite visual field during a two-alternative forced choice task. The value information from the opposite visual field reaches these SC neurons, likely through the interhemispheric connections, such as the direct reciprocal connections between the hemispheres of SC. These inputs from the opposite hemisphere had an excitatory or inhibitory influence on the firing rates of SC neurons depending on the value of the object present in the opposite visual field. Here we propose to investigate the neural basis of this information transfer using

optogenetics to map the pattern of functional connections between the two hemispheres, which was previously untenable with simple anatomical or electrophysiological techniques. We injected anterograde (AAV2-CMV-ChR2) or retrograde(AAV2Retro-hSyn-ChR2) viral vectors in one of the SC hemispheres and stimulated the neurons in the opposite hemisphere. With anterograde viral vectors, we assessed the downstream effect of inter collicular connections and found that neurons were excited as well as inhibited when the axon terminals on the opposite side of the injection were stimulated. With retrograde viral vectors, we assessed the nature of neurons that send information to the opposite hemisphere and found that all three functional subtypes of SC neurons, i.e., visual, visuomotor, and motor neurons, project to the other side. To investigate the anatomical bases of these observations, we processed the tissue procured from the animal in which anterograde viral vectors were injected and found axonal fibers in the hemisphere opposite to the injection site. These fibers were localized in the intermediate and the deep layers of the opposite SC. We are now in the process of identifying potential excitatory and inhibitory synapses on these interhemispheric fibers that will corroborate our findings. The combination of optogenetics (anterograde/retrograde strategies) and anatomical tools will help in elucidating a detailed functional connectivity profile of the inter collicular pathway and its role in value-based decisions.

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**Title:** Neural mechanisms mediating biased beliefs about drug use

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**Abstract:** Despite efforts to educate people about the risks of opioid and other drug use, drug use remains common. There is a need to better understand how people think about these risks. Previous research suggests that people are generally optimistic and believe that good outcomes are more likely than bad outcomes. However, it is unclear whether people with addiction hold a specific form of optimism bias related to drug use. To address this, we used cognitive tasks and models to evaluate how people with opioid addiction and healthy individuals think about their chances of various negative outcomes due to drug use or other reasons. During fMRI, treatment-engaged individuals with opioid use disorder (n=29) and matched healthy controls (n=33) estimated the probability of drug- and nondrug-related negative events occurring to them (e.g., overdose, bone fracture). They then saw the actual likelihood of each outcome for someone in their demographic, before re-estimating its probability. Subjects were incentivized to be as accurate as possible in their estimates, which could earn them a bonus. We used a modified

learning model to assess how much likelihood estimates (indexing participants' beliefs) changed as a function of having received desirable vs. undesirable information (first estimate vs. true likelihood) and outcome type (drug vs. nondrug). A Bayesian belief updating model was also applied to capture how prior and new information is integrated into biased posterior beliefs. We found that most subjects held an optimism bias for nondrug outcomes, revising their estimates more after receiving desirable than undesirable information, and that only patients additionally held a drug-related optimism bias, suggesting this bias requires personal relevance. Biased belief updating was similarly captured by a model in which participants gave disproportionate weight to evidence that presented as "good news" regarding their personal risk. This bias was further mediated by opposing neural responses to receiving desirable compared to undesirable information in the inferior frontal gyrus and ventral striatum, with responses in the dorsomedial prefrontal cortex further reflecting the relative weighting and integration of this information into updated beliefs. These findings suggest the relevance of considering the cognitive neuroscience of optimism and belief change in opioid addiction and may inform efforts to educate people about the risks of drug use.

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**Topic:** H.03. Decision Making

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**Title:** Conceptual generalization in value-based decisions

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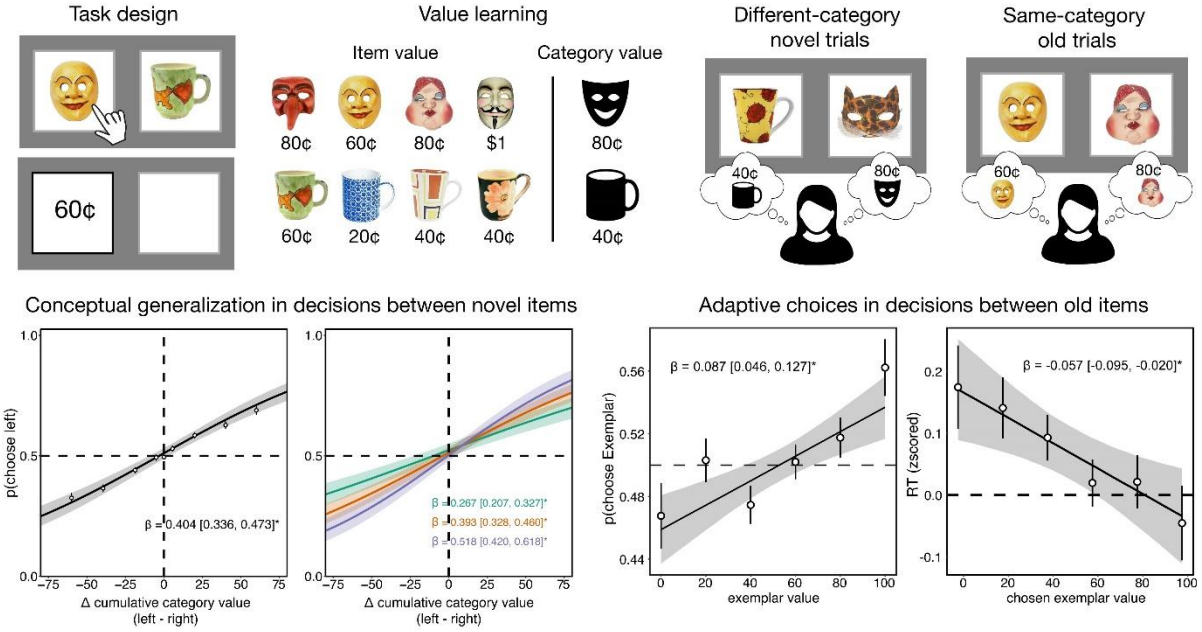
**Abstract:** Decisions are often guided by past experience. If a choice led to a good outcome, we are more likely to repeat it. However, many decisions require generalization of knowledge from a past instance to a new choice option. How does pre-existing knowledge interact with new learning to support such generalization? We hypothesized that conceptual knowledge about the world provides a scaffold for reward learning, thereby facilitating adaptive value-based decisions when presented with novel options. To test this, we designed a task that requires using previously formed category knowledge to generalize value across different instances of the same category. Participants made decisions between pairs of novel items for which value was unknown and received immediate feedback for their choice. Unbeknownst to them, the items belonged to a set of object categories (e.g. mugs, masks, etc.). Category value was learned over time and was operationalized as the cumulative average across items from the same category. Across two experiments (total n = 198), we found that when deciding between items for which value was unknown, participants generalized the category value to guide their choice and this conceptual generalization improved over time. To better understand how conceptual knowledge

is used, in Experiment 2 (n = 102) we contrasted category knowledge with the episodic representation of specific items, by asking participants to choose between two previously chosen items from the same category. The old items shared the same category value but had different item values. Even though participants were exposed to hundreds of items from dozens of categories, they successfully chose the more valuable item in same-category trials. Our findings point to a role of conceptual knowledge in generalization and inference and introduce a new perspective on the relationship between memory, decision-making and concept learning.

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**Topic:** H.03. Decision Making

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**Title:** A distinct neural code supports the prospection of future probabilities during instrumental information-seeking

**Authors: *N. M. SINGLETARY**[1], G. HORGA[2], J. GOTTLIEB[1];
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**Abstract:** To improve their decisions, decision-makers should seek instrumental information based on its value of information (VOI), which is modulated by expected information gain (EIG) and the possible decision outcomes. However, it is unknown how EIG is neurally computed. To investigate, we scanned humans (*N* = 23) using fMRI while they completed a modified Beads Task that measured demand for instrumental information. Participants were given an endowment ($30), and they had to infer whether a “hidden” gallery was a “portrait” gallery with more

pictures of faces than scenes or a “landscape” gallery with more scenes than faces. To improve their accuracy, they bid for one sample picture from the hidden gallery after being shown the penalty for an erroneous inference ($10 or $20), the prior probability of the gallery type (0.07-0.93), and the diagnosticity (predictive validity) of the sample (0.57-0.93). Together, the prior and diagnosticity modulated EIG.
Consistent with theory and predictions, bids increased with EIG and penalty. Then, we decomposed EIG into its components of prior and expected posterior certainty; bids rationally decreased with prior certainty of the hidden gallery and increased with expected posterior certainty of the sample. Lastly, we identified clusters of activation in the posterior parietal and extrastriate cortices in which we could decode expected posterior certainty with a neural code distinct from VOI. Therefore, these regions may compute the certainty provided by further sampling, an essential step to anticipate EIG. Our results suggest that neural computation of EIG is distinct from information value.

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**Topic:** H.03. Decision Making

**Support:** Canadian Institutes of Health Research

**Title:** Exploring the impact of Generalized Anxiety Disorder on the latent decision-making processes and neural correlates underlying approach-avoidance conflict decision-making

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**Abstract:** Approach-avoidance (AA) conflict arises when an organism is confronted with a stimulus imbued with both positive and negative valences, and its successful resolution is critical for survival. In humans, AA conflict processing is believed to be compromised in Generalized Anxiety Disorder (GAD), with avoidance tendencies being dominant in the face of motivational conflict. Relatively little, however, is known about the impact of GAD on the latent decision-making processes and neural correlates underlying AA conflict processing. To address this issue, we scanned 40 participants with GAD (33 female, mean age = 22.3 years) and 39 controls (26 female, mean age = 25.0 years) using functional magnetic resonance imaging (fMRI) during a AA conflict task in which participants approached or avoided indoor and outdoor scene stimuli according to a reward and punishment rule. GAD participants avoided motivationally conflicting stimuli more often compared to controls and Hierarchical Drift Diffusion Model (HDDM) parameters revealed that GAD was associated with quicker evidence accumulation for conflict avoidance. Across both groups, AA conflict processing was supported by a network of brain regions including the medial temporal lobe (MTL), striatum, and prefrontal cortex (PFC). Critically, GAD participants demonstrated decreased activity compared to controls in several areas including the perirhinal cortex and dorsolateral PFC. Computational modelling analyses combining neural data from key regions of interest with HDDM parameters revealed differential profiles of responding across the MTL, striatum, and PFC associated with group-based distinctions in evidence accumulation, response caution, and optimization of cognitive processes.

Our findings provide novel insight into the cognitive and neural changes that may underlie altered AA conflict processing in GAD.

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**Topic:** H.03. Decision Making

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**Title:** Choosing for others: neurocomputational mechanisms underlying risky choice

**Authors: *D. FARERI**[1], M. RUIZ[1], S. GALLARDO[1], C. LIMA[1], P. SOKOL-HESSNER[2], D. V. SMITH[3];
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**Abstract:** Extant literature points to the importance of social context in shaping our choices and how we process their consequences. Yet, our understanding of how the social world affects neurocomputational processes underlying risky choice is deeply lacking. We have previously shown that people become more loss averse and risk averse when evaluating risks that can affect others (close friends, strangers) relative to themselves (Fareri et al., 2022). Here, we begin to investigate the neural mechanisms supporting these computational changes (pre-registered N = 50 pairs of same-sex friends; https://osf.io/vk5rh). To date, 24 pairs of participants (17F) have completed a risky economic decision-making task. One person from each pair underwent fMRI while the other performed the same task in a behavioral testing room. Participants were endowed with $24 to use in the experiment. Across 3 rounds, (96 trials per round), participants chose to accept monetary gambles (e.g., 50% chance of receiving a monetary gain or loss) or reject them in favor of guaranteed outcomes (e.g., 100% chance of receiving $0). Critically, we varied the recipient of the monetary outcomes. Participants sometimes chose for: 1) themselves (baseline non-social condition); 2) a same-sex stranger; and 3) their close friend. Preliminary analyses focused on fMRI participants only, after excluding for motion (n=3) and poor behavioral variability (i.e., always gambled; n=1). Hierarchical Bayesian estimation of risk-related computations (risk-attitudes, loss aversion, choice consistency) revealed our sample to be risk neutral, gain-loss neutral and consistent in their behavior when choosing for themselves. Strikingly, participants became more loss averse [95% HDI: -0.2,-.023] and more consistent [95% HDI: -0.12,-.02] when choosing for friends relative to strangers. Neuroimaging analyses revealed increased ventral striatal (pre-registered ROI) activation to monetary gains relative to losses ($F(1,19) = 39.86$, $p<.001$), but no social modulation of this effect. Interestingly, the amygdala (exploratory ROI) was significantly modulated by social context ($F(2,38) = 3.56$, $p<.05$), showing greater deactivation when experiencing outcomes of choices for a friend relative to a stranger or computer. In sum, these results replicate and extend our prior work, showing that taking risks for others changes component processes supporting risk evaluation and modulates engagement of neural structures implicated in loss aversion in non-social contexts. Future analyses will probe parametric modulation of reward-related activation and functional connectivity by socially induced changes in loss aversion.

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**Presentation Number:** NANO28.12

**Topic:** H.03. Decision Making

**Support:** KAKEN 21H03492

**Title:** Neural Representation of Model-free and Inference-based Strategy in Mouse Brain

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**Abstract:** Humans and animals use various strategies for making choices. The inference-based strategy utilizes the hidden task structure for choice selection, whereas the model-free strategy relies on direct experiences of choices and outcomes. Since many studies have primarily focused on investigating the neural circuitry associated with a single strategy, it remains unclear whether distinct or unified neural circuits drive these two strategies. This study developed a tone frequency discrimination task for head-fixed mice. Mice selected either the left or right spout based on the low- or high-frequency tones to obtain a sucrose water reward. The task consisted of switch and repeat conditions, which dissociated the two strategies with different transition probabilities of tone stimuli. We found that mice optimally biased their choices depending on the transition probabilities in both conditions. The acquisition of choice biases was faster in the repeat than in the switch conditions, supporting the strategy differences in choices between the two conditions. We performed brain-wide electrophysiological recordings with Neuropixels 1.0, targeting the orbitofrontal cortex (OFC), posterior parietal cortex (PPC), hippocampus (HPC), striatum (STR), and auditory cortex (AC). We found that neurons in these regions increased the activity when their preferred tone or choice was expected in both conditions, suggesting the global encoding of model-free and inference-based strategies in the brain. Further experiments of optogenetic inhibition of neural activity in our task will provide evidence of whether cerebral cortical regions are necessary for inference-based and model-free strategies.

**Disclosures: S. Wang:** None. **K. Ishizu:** None. **A. Funamizu:** None.

**Presentation Number:** NANO28.13

**Topic:** H.03. Decision Making

**Title:** Effects of tyrosine on measures of physiological arousal and decision-making

**Authors: *A. BRANDS**, D. MATHAR, K. KNAUTH, K. LISNER, T. RÖDDER, J. PETERS;
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**Abstract:** Catecholamines are central to the flexible adaptation of physical and mental capacities in light of changing environmental demands. Most notably, Dopamine (DA) has been linked to learning and decision-making processes which map onto real-world outcomes and differentiate various states of mental health and disease. Balancing effortful, goal-directed vs. parsimonious, habitual modes of control has emerged as a robust transdiagnostic marker, recently shown to also

depend on DA functioning (Chakroun et al., 2020). Despite a growing demand for freely available supplements promising cognitive enhancing effects, their influence on these processes and physiological correlates remains unclear. In 2022 Mathar et al. found a single dose of the catecholamine-precursor tyrosine (2g) to reduce measures of sympathetic tone and reaction times (RT) across two decision-making tasks, while boosting goal-directed control. We aimed to replicate and extend these findings in a preregistered, double-blind, placebo-controlled study assessing a gender-balanced sample.OSF: https://tinyurl.com/5n7y3nae Methods: N=64 healthy participants (N=32 self-identified female) completed self-reports, baseline measures of physiological arousal, a delay-discounting and reinforcement learning (RL) task, once after ingestion of 2g tyrosine and placebo each. We complemented model-agnostic analyses of physiological and behavioural measures with extensive computational modelling of RL-task (restless bandit) data to identify mechanisms of exploration and exploitation and their dependence on supplementation.Results: Measures of autonomic functioning (HR, HRV, pupil dilatation, sEBR) did not differ between supplementation conditions. This was true across all participants regardless of gender. Ingestion of 2g of tyrosine (vs. placebo) however did significantly reduce RTs in the delay discounting task, while leaving task performance unaffected, thus replicating results from Mathar et al (2022). In the RL task performance indicated by most rewarding choices increased under tyrosine (vs. placebo), while reducing switching behaviour. Computational modelling mirrored these effects showing decreased random exploration (i.e. more exploitation) across both genders. Separate model components capturing directed exploration and perseveration suggest gender differences at baseline but were not notably affected by tyrosine. Conclusion: We largely replicated performance-enhancing effects of tyrosine on task performance, biasing subjects toward more rewarding and future-oriented choices. Autonomic functioning was not reliably influenced by supplementation

**Disclosures: A. Brands:** None. **D. Mathar:** None. **K. Knauth:** None. **K. Lisner:** None. **T. Rödder:** None. **J. Peters:** None.

**Presentation Number:** NANO28.14

**Topic:** H.03. Decision Making

**Support:** Simons Collaboration on the Global Brain Pilot Award

**Title:** Inter-regional neural dynamics underlying self-paced action decisions, from cortex to cerebellum

**Authors: *M. ELBAZ**[1], J. I. GLASER[2], J. A. MIRI[1];
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**Abstract:** Nearly sixty years after the initial observation of a slow monotonic drift in electroencephalographic recordings preceding voluntary self-paced actions (“the readiness potential”), its physiological underpinnings and relation to cognitive processes remain elusive. A classical interpretation holds that readiness potential onset reflects the decision to act, with the drift thereafter proceeding deterministically to movement onset. However, more recent analyses suggest that the readiness potential’s monotonicity may be an artifact of trial averaging. According to this alternative view, rather than a slow, deterministic process, a fast stochastic process underlies self-paced action decisions. In the absence of measurements resolving neural

dynamics during single trials, these competing accounts have persisted.
We have taken a comparative behavioral approach, developing a paradigm in which mice initiate an action in two decision-making contexts: in response to a cue (instructed) and in the absence of external cues (self-paced). This allows us to test whether there exist neuronal activity patterns specific to self-paced action decisions that are distinct from those driving movement itself. As mice behave in our paradigm, we use large-scale multi-electrode arrays (Neuropixels) to simultaneously record activity in medial prefrontal cortex, primary and secondary motor cortices, motor thalamus and deep cerebellar nuclei. Though movement kinematics are similar in both the instructed and self-paced contexts, the neuronal activity preceding movement onset is sufficient to classify above chance the decision-making context. In order to characterize the activity patterns enabling this classification, we identified subspaces that are either shared between contexts or specific to one. Above-chance classification is possible from both shared and unique subspaces. This implies that self-paced and instructed decisions differ from each other through: a) distinct temporal profiles within a shared neural activity subspace, and b) activity subspaces specific to either type of decisions. By projecting single trial activity onto the subspaces enabling above-chance classification, we are investigating whether and when stochastic processes are discernible in neural activity across regions, specifically prior to self-paced actions. This will help arbitrate between models underlying the neural basis of self-paced action decisions

**Disclosures: M. Elbaz:** None. **J.I. Glaser:** None. **J.A. Miri:** None.

**Nanosymposium**

**NANO29: Neural Circuits and Mechanisms of Language and Cognition**

**Location:** WCC 146C

**Time:** Sunday, November 12, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO29.01

**Topic:** H.11. Language

**Support:** American Association of University Women
NIH R01DC019653
NIH U01NS123130
NIH U01NS098968

**Title:** Neural dynamics of verbal communication: An intracranial recording and deep learning approach

**Authors: *J. CAI**[1,3], A. E. HADJINICOLAOU[2,3], A. C. PAULK[2,3], T. XIA[2], A. F. WANG[1], Z. M. WILLIAMS[1,3], S. S. CASH[2,3];
[1]Neurosurg., [2]Neurol., Massachusetts Gen. Hosp., Boston, MA; [3]Harvard Med. Sch., Boston, MA

**Abstract:** Human verbal communication is composed of the intricate interplay of speech planning, production, and comprehension, orchestrated by diverse neural dynamics across distributed brain regions. The precise representation of linguistic information during natural

conversations and the shared neural processes involved, however, remain poorly understood. In this study, we leveraged intracranial neural recordings from participants engaged in natural dialogue and compared them to pre-trained deep learning natural language processing models. Our analysis reveals a remarkable similarity not only between neural-to-artificial network activities but also in how linguistic information is encoded in the brain during both production and comprehension. We find that the patterns of neural activity that encoded linguistic information exhibited close alignment with speaker-listener transitions, diminishing after word utterance or during non-conversational periods. We also find that these patterns were observed across distinct mesoscopic areas and frequency bands, suggesting that they reflect the hierarchically structured information conveyed during dialogue. Collectively, our findings suggest that linguistic information during speaking and listening is represented similarly in the brain, shedding light on the distributed neural dynamics underpinning human communication.

**Disclosures: J. Cai:** None. **A.E. Hadjinicolaou:** None. **A.C. Paulk:** None. **T. Xia:** None. **A.F. Wang:** None. **Z.M. Williams:** None. **S.S. Cash:** None.

**Presentation Number:** NANO29.02

**Topic:** H.11. Language

**Support:** NIH NIDCD F30 DC017083
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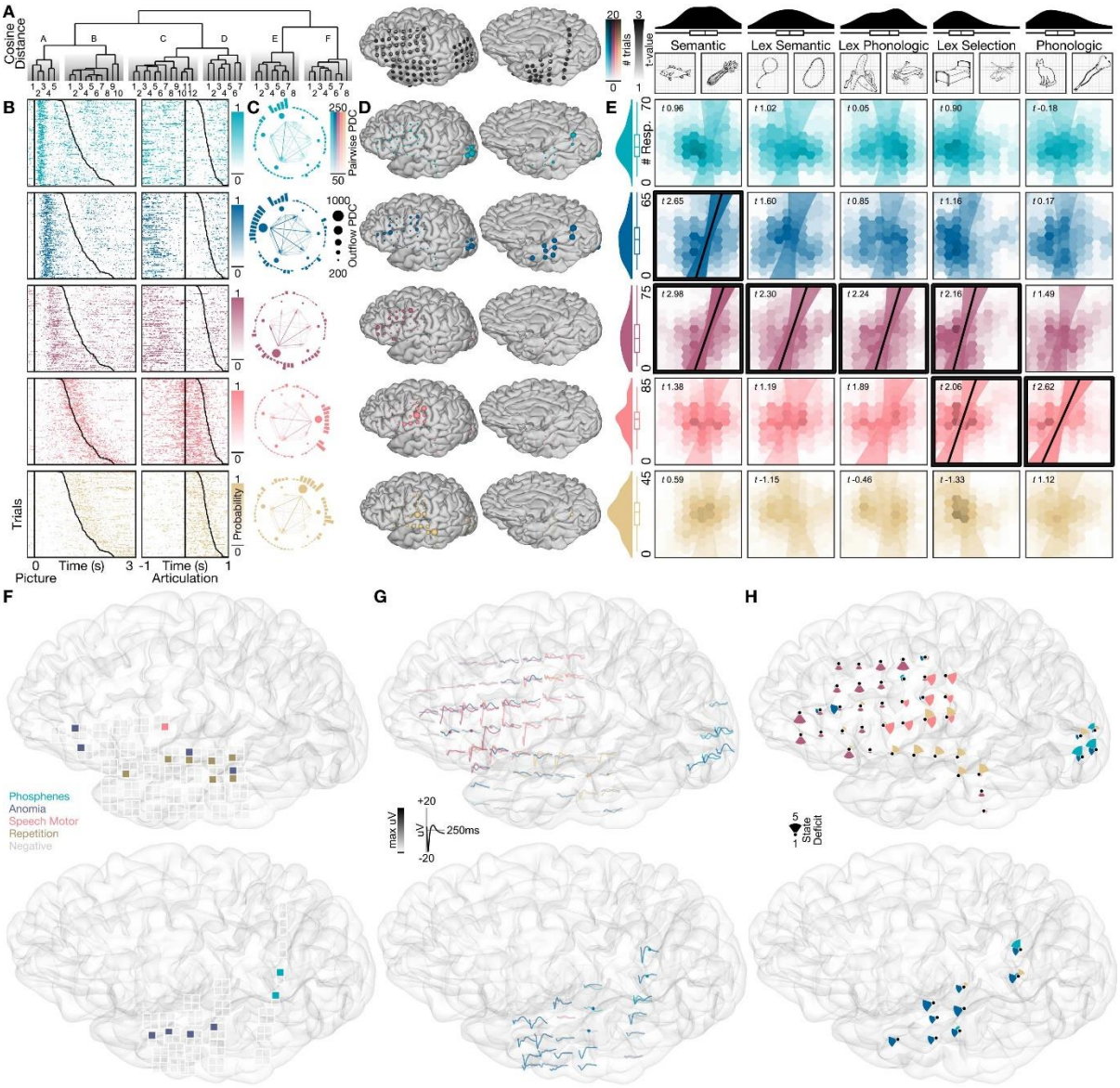
NIH NIDCD R21 DC016715
NIH NIDCD U01 NS098981

**Title:** Computational lesions of distributed state dynamics simulate focal aphasias

**Authors: *K. FORSETH**[1], N. TANDON[2];
[1]Univ. of California San Diego, San Diego, CA; [2]Neurolog. Surgery, Univ. of Texas Med. Sch. at Houston, Houston, TX

**Abstract:** Introduction: The cortical basis of speech production has been probed by lesion-symptom mapping, as well as by analyses of intact language systems through functional imaging, structural mapping, and electrophysiology. We sought a unified perspective that integrates rapid, transient, and distributed dynamics for language with isolated patterns of functional disruption in aphasia. Methods: Intracranial electrodes (n=25810, 134 patients) were implanted for the evaluation of epilepsy. Patients performed picture naming of common objects and underwent systematic stimulation mapping. We used cross-validated autoregressive hidden Markov models - combining the interpretability of multivariate autoregressive analysis with the nonlinear switching Markov characteristic - to distinguish cognitive states defined by causal interactional motifs of distributed cortical substrates. We then derived an *in silico* lesion model from complete patient-specific state dynamics and compared this to *in vivo* causal perturbation by direct cortical stimulation. Results: We created a detailed spatiotemporal atlas of word production spanning the entire language-dominant cortex. From this map, we resolved network dynamics comprising sequences of state space transitions for each trial. This identified five essential states for speech production, each defined by a unique pattern of directed interactions within the language network. We then derived a computational lesion model for state dynamics and compared its predictions with causal perturbation by direct cortical stimulation. Conclusion:

The distributed network dynamics in this comprehensive interactional map of speech production advance our understanding of how both local and disconnection injuries yield complex neurological deficits, facilitating the development of novel therapeutic approaches for aphasia.




**Disclosures: K. Forseth:** None. **N. Tandon:** None.

**Presentation Number:** NANO29.03

**Topic:** H.11. Language

**Support:** NIH Grant NINDS 1R01NS109367
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NIH Grant NIDCD R01DC018805
NIH Grant NIDCD F32DC019533

**Title:** The neural dynamics of sentence production: ECoG reveals sentence-specific networks and temporal patterns

**Authors: *A. MORGAN**[1], W. K. DOYLE[2], O. DEVINSKY[2], A. FLINKER[3];
[1]NYU Sch. of Med., New York, NY; [3]Neurol., [2]NYU Langone, New York, NY

**Abstract:** While significant progress has been made on single word production, a similarly nuanced understanding of sentence production remains elusive. Here, we collect electrocorticographic (ECoG) recordings from nine awake neurosurgical patients during a controlled sentence production experiment with 3 blocks: picture naming, sentence production, and list production - a control task.
Employing unsupervised machine learning (non-negative matrix factorization), we clustered electrodes into six functional networks. Four of these patterned with previously-characterized word production processes: stimulus processing, motor planning, articulation, and auditory feedback. However, two previously undescribed networks were active for sentences but not lists or words. Subsequent analysis of these networks, primarily distributed across middle and inferior frontal gyri, revealed sensitivity to two sentence-specific processes: event semantics (i.e., whether the event involved a physical action) and syntax (whether the upcoming sentence was active/passive).
Next, we assessed whether sentence production can be adequately characterized as a sequence of single word productions. If so, then different stages of word representation (e.g., conceptual, phonological, etc.) should come online in the same order when words are said in isolation and in sentences. We trained multiple classifiers (specific to patients and representational stages) to predict word identity using data from the picture naming task (10-fold cross validation; accuracy ~30%, p < .01 relative to permutation distribution), and used the classifiers to predict what word patients were saying throughout sentence production.
The majority of classifiers (89%) successfully generalized from single word production to sentence production data, accurately predicting what words the patient said during sentences, with different stages of word representation largely following the same temporal order. Intriguingly, however, we also observed a systematic exception: Objects — the last word of the sentence — were often predicted far earlier relative to their articulation in sentences than in single word production. In fact, 14% of classifiers predicted objects even before the onset of the first word in the sentence, pointing toward fundamentally different processes in sentence vs. single word production.
Our findings emphasize the importance of expanding beyond single word production studies. Moreover, these findings hold potentially important implications for clinical practice, which still relies heavily on single word production paradigms.

**Disclosures: A. Morgan:** None. **W.K. Doyle:** None. **O. Devinsky:** None. **A. Flinker:** None.

**Presentation Number:** NANO29.04

**Topic:** H.11. Language

**Title:** Exploring Speech Decoding Using Stereo-Electroencephalography: Mapping Neural Representations of Articulation

**Authors: *A. SINGH**[1], T. THOMAS[2], N. TANDON[3];
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**Abstract:** Brain-computer interfaces (BCIs) offer a promising avenue for decoding speech from neural activity, aiming to assist individuals with speech impairments. We present a novel approach utilizing stereo-electroencephalography (sEEG) to map and decode neural representations of speech, specifically focusing on tongue twisters as a controlled speech task. Our study includes 25 patients with sEEG electrodes implanted for seizure localization, engaging in a tongue twister production task involving silent reading, aloud reading, and memory-based production. Linear classification models trained on broadband gamma activity (BGA: 70-150 Hz) from sEEG recordings were used to decode phonemes, with separate analysis of covert trials using an encoder-decoder model.Decoding was conducted during articulation and pre-articulatory periods to explore latent neural information. The best-performing patient achieved a 25.2% macro F1 decoding score during articulation and 22.6% during the pre-articulatory phase (chance at 7.7%). Across all patients, the average F1 macro score was 13.3% +/- 4.4% during articulation and 11.6% +/- 5% during pre-articulatory periods, surpassing results from similar studies utilizing ECoG and MEA-based decoding approaches. Notably, sEEG offers improved safety with electrodes.We observed robust activation in posterior middle temporal gyrus, dorsal frontal cortex, inferior frontal gyrus, and superior parietal cortex, highlighting their role in predictive encoding and monitoring of articulation. The superior temporal gyrus and sensorimotor cortex exhibited distinct separation in the latent articulatory space, particularly related to manner and place of articulation. Furthermore, our findings unveiled information-rich sites in frontal and parietal regions, including deep sulcal sites inaccessible to subdural grids, validated by spatial decoding patterns.These results contribute to the development of precise and high-fidelity assistive communication devices for individuals with neurodegenerative speech disorders, refining existing models of the speech network derived from extracranial neuroimaging techniques. By leveraging sEEG and analyzing tongue twisters, we offer novel insights into decoding speech representations, advancing the field of brain-computer interfaces for speech rehabilitation and augmentative communication.

**Disclosures: A. Singh:** None. **T. Thomas:** None. **N. Tandon:** None.

**Presentation Number:** NANO29.05

**Topic:** H.11. Language

**Title:** An inclusive multivariate approach to neural localization of language components

**Authors: *W. GRAVES**[1], H. LEVINSON[1], R. STAPLES[1], O. BOUKRINA[2], D. ROTHLEIN[3], J. PURCELL[4];
[1]Rutgers Univ. -- Newark, Newark, NJ; [2]Kessler Fndn., West Orange, NJ; [3]VA Boston Healthcare Syst., Boston, MA; [4]Univ. of Maryland, College Park, MD

**Abstract:** Mapping language areas in the brain is relevant to any condition involving disrupted language (aphasia). For basic research, investigators want to understand the brain basis of language in terms of its parts. Existing protocols for localizing language are typically univariate. Univariate methods treat each small unit of brain volume (voxel) as independent. One prominent

example in functional magnetic resonance imaging (fMRI) contrasts brain areas responding to sentences with length-matched sets of pseudowords (pronounceable nonwords). By subtraction logic, this should highlight areas responding to coherent syntax (grammar) and semantics (word meanings), while subtracting away areas responding to phonology (speech sounds), orthography (visual word form), and similar perceptual stimulation. Defining language areas in this way misses smaller units of syllables, phonemes, and graphemes, along with brain areas that map between those language units and corresponding semantic and syntactic representations. Here we define multivariate regions of interest (mROI) based on areas showing high reproducibility across runs within each participant. Univariate regions of interest (uROI) are taken from previous results by other groups of the sentences > pseudowords contrast. We then compare univariate and multivariate results. Using Representational Similarity Analysis of an fMRI dataset (N = 20) in which neurotypical participants make familiarity judgments on visual words, patterns defined in terms of semantic distance (differences in concreteness between words) showed significantly greater correspondence with neural patterns in mROI than uROI (all $p < 0.001$). Patterns defined in terms of phonemic edit distance (number of phonemes that would have to be changed for each pair of stimuli to make them identical) also showed significantly greater correspondence with mROI than uROI. The mROI also showed greater left lateralization than the uROI. Conversely, in a different, previously published dataset (N = 22) where participants made meaningfulness judgments on noun-noun phrases, the contrast of meaningful phrases > length-matched pseudowords showed significantly greater activation in the uROI than mROI, along with greater left-lateralization. In all cases, areas of spatial overlap between mROI and uROI showed the greatest neural association and left lateralization. This suggests than ROIs defined in terms of multivariate reproducibility can be used to localize components of language such as semantics and phonology, and that the multivariate approach can be used productively along with the univariate approach for inclusively mapping language cortex.

**Disclosures: W. Graves:** None. **H. Levinson:** None. **R. Staples:** None. **O. Boukrina:** None. **D. Rothlein:** None. **J. Purcell:** None.

**Presentation Number:** NANO29.06

**Topic:** H.11. Language

**Support:** National Institute of Neurological Disorders and Stroke NS098981

**Title:** Human intracranial signatures of syntax without conceptual semantics

**Authors: *E. MURPHY**[1], O. WOOLNOUGH[2], C. MORSE[3], N. TANDON[4];
[1]Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [2]UT Hlth., UTHealth Houston, Houston, TX; [3]Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [4]Univ. of Texas Med. Sch. at Houston, McGovern Med. Sch. at UT Hlth., Houston, TX

**Abstract:** How the human brain constructs syntactic structures remains perhaps the most challenging topic in the neuroscience of language. We present an intracranial investigation of the spatiotemporal dynamics of basic syntactic structure processing in a cohort of seven epilepsy patients (1461 electrodes). Data were acquired from subdural grid electrodes or stereotactically placed depth electrodes (sEEG). Coverage was frontotemporal and parietal. Two patients also had sEEG probes in anterior and centromedian thalamus. One patient was subject to an awake

intraoperative surgery and conducted the task in the OR. We utilized a novel phrase reading paradigm involving Function/Content word pairs. We mixed combinations of these words, followed by a third phrase-completing word to remove wrap-up effects, analyzing effects only at the structure-marking second word. Patients were instructed to judge whether the phrase was acceptable (2AFC). Stimuli were presented in RSVP (500ms per word). 50% of trials included a grammatical or semantic violation, either at the second or third word. Behavioral performance was high (>95%) and only correct trials were analyzed. We contrasted grammatical Function-Function trials ('to the car') with ungrammatical ('they the car') and asemantic ('ti the car') trials. After isolating structure-sensitive electrodes (>10% amplitude increase from baseline for active channels, then a two-sided paired t-test at an alpha-level of 0.01), we applied a more stringent test by isolating electrodes that were also sensitive to the contrast between Content-Function ('swim the ocean') vs. unacceptable Function-Function conditions. We isolated sites in left posterior superior temporal sulcus that exhibited increased high gamma activity initiating between the 100-400ms window after the onset of the second word for grammatical structures, with effects lasting around 200ms. We found later effects in left anterior inferior frontal sulcus that began around 700ms after the onset of the second word that lasted approximately 150ms. Greater activity for unacceptable trials was found in lateral parietal cortex. We also discovered thalamocortical low-frequency phase-locking during the composition of functional grammatical structure, specifically to frontal sites. These results indicate the rapid sensitivity of posterior temporal cortex to minimal syntactic structure building in the absence of conceptual semantics, with subsequent transitions to inferior frontal regions potentially indexing a separable role for thalamocortical control. This research thus provides neurobiologically plausible signatures of natural language syntax.

**Disclosures: E. Murphy:** None. **O. Woolnough:** None. **C. Morse:** None. **N. Tandon:** None.

**Presentation Number:** NANO29.07

**Topic:** H.04. Executive Functions

**Support:** NIH Grant MH124004
NIH Shared Instrumentation Grant S10OD020039
Paul and Daisy Soros Foundation

**Title:** Verbal vs Non-Verbal Processing Leads to Generalized Laterality Effects That Span Multiple Networks

**Authors: *W. SUN**[1,2], L. M. DINICOLA[1], M. C. ELDAIEF[2,3], R. L. BUCKNER[1,2,3];
[1]Harvard Univ., Cambridge, MA; [2]Harvard Med. Sch., Boston, MA; [3]Massachusetts Gen. Hosp., Charlestown, MA

**Abstract:** Human systems neuroscience approaches have yielded important insights into the organizational and functional properties of association networks. While all association networks involve bilateral regions, some show strong laterality (cortical regions in one hemisphere are larger than the other). For example, a network involving canonical language regions is left-lateralized and preferentially recruited during tasks involving processing of sentences (e.g., Fedorenko et al. 2010). While the network is lateralized, the smaller right hemisphere regions of the language network also increase activity during sentence processing (Braga et al. 2020).

Neuropsychological investigations have long revealed laterality effects between verbal and non-verbal materials, raising the question of whether such materials are processed differentially by the lateralized networks. Here we explored the selectivity of association networks to verbal vs non-verbal processing using precision MRI approaches. Across two studies (n = 11, n = 15), individuals were repeatedly scanned during fixation (to define networks) and while performing an N-Back working memory task with both verbal (rhyming words, letters) and non-verbal (unfamiliar faces, scenes) materials. Multiple networks were defined within individuals including the left-lateralized Language network, a left-lateralized Frontoparietal Control Network (FPN), and a right-lateralized FPN. Analysis of the Study 1 task data yielded an unexpected result: activity modulated in a lateralized manner across networks based on stimulus domain. That is, the left hemisphere of each network demonstrated selectivity for verbal materials (rhymes), while the right hemisphere of each network demonstrated selectivity for non-verbal materials (faces). The lateralization effect spanned both the left- and right-lateralized networks, functionally dissociating the left and right regions. Prospective replication of these results is underway for Study 2 data. Our findings suggest a laterality effect that transcends network boundaries, which has implications for understanding the functional-anatomic organization of cognitive processes and potential targets for neuromodulation. In addition to specialization of anatomically distinct networks, there may also be mechanisms that broadly modulate hemispheric processing.

**Disclosures: W. Sun:** None. **L.M. DiNicola:** None. **M.C. Eldaief:** None. **R.L. Buckner:** None.

**Nanosymposium**

**NANO30: Probe Design and Engineering**

**Location:** WCC 152A

**Time:** Sunday, November 12, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO30.01

**Topic:** I.04. Physiological Methods

**Support:** NIH Grant 1RF1NS113303-01

**Title:** Steeltrode: Microfabricated stainless steel neural interface for high density recording in non-human primates and rodents.

**Authors: Z. AHMED**[1], I. KIMUKIN[1], *V. JAIN[1], K. GURNSEY[2], T. TEICHERT[2], M. CHAMANZAR[1];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Univ. of Pittsburgh, Pittsburgh, PA

**Abstract:** Robust microfabricated neural interfaces for rodents and non-human primates (NHP) are crucial for neuroscience research. While there have been significant advancements in the manufacturing of high density silicon-based neural probes for rodents, the inherent brittleness of silicon limits its use in neural probes with long shanks necessary for probing deep regions of the non-human primate brain. Low fracture toughness of silicon makes probes with long implantable shanks susceptible to breakage during and after insertion. To address this, we have developed a

novel neural probe platform using flexible polymers and stainless steel, called "steeltrode." Stainless steel offers mechanical robustness and biocompatibility, making it suitable for ultra-high aspect ratio NHP neural devices. With our novel microfabrication process, we have successfully produced monolithically integrated stainless steel probes featuring long shanks, reaching up to 12 centimeters in length, with a small cross-section measuring 250 um x 150 um that accommodate 16-102 densely distributed electrodes, with inter-electrode pitch as small as 25 um. We have rigorously validated the functionality of steeltrodes by recording high fidelity spontaneous and evoked responses in the macaque auditory cortex. We successfully recorded frequency following responses (FFR) and extracted tonal response fields from frequency-tuned neurons in the primary auditory cortex using various auditory stimulation paradigms. Utilizing the same microfabrication process, we developed steeltrode for rodents with shanks measuring 1-2 cm in length. Leveraging the robustness of stainless steel shank, we have demonstrated that these probes can penetrate intact rat dura without damage to the functional components of the probe. Upon puncturing rat dura with steeltrode, we have successfully recorded spontaneous single and multi-unit activity from rat hippocampus. Histological analysis using NeuN immunostaining following probe implantation showed a significant reduction in cellular damage in the superficial cortical layers when the steeltrode pierced the dura, compared to probe insertion after surgical dura removal. This presentation will focus on the design, characterization and in-vivo validation of steeltrodes in macaque and rodent models.

**Disclosures: Z. Ahmed:** None. **I. Kimukin:** None. **V. Jain:** None. **K. Gurnsey:** None. **T. Teichert:** None. **M. Chamanzar:** None.

**Presentation Number:** NANO30.02

**Topic:** I.04. Physiological Methods

**Title:** Measuring Auditory ERPs in the Fetal Brain Using Non-invasive Transabdominal Fetal Electroencephalography

**Authors: *J. CORTES**, E. BERI, C. OUCHIDA, J. URRUTIA-GANDOLFO, E. LEE;
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**Abstract:** There are no easily accessible non-invasive techniques for studying the electrical activity of the fetal brain. This lack is hindering research into fetal brain development and keeps us from monitoring fetal brain activity during pregnancy and childbirth. Measuring fetal electroencephalography noninvasively with abdominal electrodes has been unfeasible due to the high levels of artifactual contamination resulting from maternal and fetal movement, muscle activity, and maternal and fetal electrocardiogram signal, among others. Our work overcomes this challenge by harnessing advances in artificial intelligence to identify and filter out non-fetal EEG signals from electrophysiological signals non-invasively collected on the maternal abdomen, allowing fetal neurologic activity to be accurately and non-invasively measured. We used our approach to measure auditory evoked-related potentials (ERP) in the fetal brain in a sample of 10 pregnant women in their last trimester. The results showed classical time-locked ERP responses to auditory stimuli similar to those measured in age-matched premature neonates using direct scalp electrodes. Altogether, our preliminary work establishes the ability of our approach to reconstruct fetal neurologic activity from abdominal signals. This system has the potential to give unprecedented access to human fetal brain development in utero.

**Disclosures: J. Cortes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending for fetal EEG system. **E. Beri:** None. **C. Ouchida:** None. **J. Urrutia-Gandolfo:** None. **E. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent pending for fetal EEG system.

**Presentation Number:** NANO30.03

**Topic:** I.04. Physiological Methods

**Support:** NIH Grant 5R21EY033084-02

**Title:** An Optomechanical Neural Probe Using Wavelength Division Multiplexing

**Authors: *A. COCHRAN**, H. GUPTA, M. CHAMANZAR, G. PIAZZA;
Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA

**Abstract:** To unravel the neural basis of brain function, high resolution, simultaneous recording of neural activity from thousands of neurons across different regions of the brain is desirable. Traditional passive neural probes require one wire per recording electrode, therefore reaching scaling limits as increasing the number of wires renders the probe prohibitively large. Shrinking the size of wires is limited by the resolution of lithography processes and the gaps that need to be maintained to avoid crosstalk between channels. Time-division multiplexing (TDM) has been used in active neural probes to increase the number of channels without increasing the number of wires. Scaling up the number of channels so that they enable simultaneous acquisition of data requires fast sampling rates, thus compromising the signal-to-noise ratio (SNR) and the power consumption efficiency. Massively scaling the number of recording channels to meet the needs of the scientific community requires a disruptive technology capable of truly simultaneous multiplexing of neural recording with high density within dimensional, bandwidth, and power consumption limits.
Inspired by advances in MicroElectroMechanical Systems (MEMS) and integrated silicon photonics, we have designed and fabricated novel opto-mechanical modulators that can achieve dense multiplexing on neural probes without the use of active circuitry. This approach provides a paradigm shift from the previous tradeoffs between the number of channels, bandwidth, SNR, and power consumption by using wavelength division multiplexing (WDM). By encoding neural signals to the optical domain on the probe, we also make recordings less sensitive to external noise sources. Our device consists of a nanoscale electrostatic comb-drive actuator that converts neural electrophysiological signals into a mechanical displacement, which is upconverted in the optical domain by perturbing a photonic resonator. The sensor units are fabricated using a 220 nm Silicon-on-Insulator (SOI) platform and have been designed to maximize sensitivity with a bandwidth of up to 40 kHz and a footprint smaller than 200 x 200um. The modulators are characterized using emulated local field potentials (LFPs) to simulate neural recording. From simulations, we expect to resolve emulated LFPs with an SNR greater than 20dB. The results from this study will demonstrate the feasibility of massively scaling up the neural recording density using an opto-mechanical platform compatible with dense WDM. This new platform for neural recording based on optically multiplexed sensor arrays can be scaled up with hundreds to thousands of sensors.

**Disclosures: A. Cochran:** None. **H. Gupta:** None. **M. Chamanzar:** None. **G. Piazza:** None.

**Presentation Number:** NANO30.04

**Topic:** I.04. Physiological Methods

**Support:** Wu Tsai Neurosciences Institute Seed Grant

**Title:** Perpendicular Anisotropy Magnetic Tunnel Junction Sensors with Vertical Flux Concentrators for Neural Magnetic Recording

**Authors: *Z. ALI**[1], S. WANG[2], H. NAGANUMA[3], A. POON[1];
[1]Electrical Engin., [2]Materials Sci. and Engin., Stanford Univ., Stanford, CA; [3]Tohoku Univ., Sendai, Japan

**Abstract:** Brain implants that use microelectrode arrays to record neural activity suffer from longevity and signal limitations that impede their ability to provide clinical and experimental neuroscience insights. However, magnetic sensors are hypothesized to address longevity issues because they do not require a direct conductive path with tissue. In addition, neural magnetic recording is expected to provide insights into neural activity unattainable with conventional voltage recordings. To replace microelectrodes for *in vitro* experiments, magnetic sensors need to be 1) sensitive enough to detect picotesla-scale neural magnetic fields and 2) responsive to out-of-plane magnetic fields (perpendicular anisotropy). We investigated how the fabrication of novel vertical flux concentrators, integrated on-chip with magnetic tunnel junction (MTJ) sensors, enables us to move closer to realizing both of these aims. Using COMSOL simulations, we first demonstrated that the height of a typical MTJ stack (~30-50 nm) is small enough that high-permeability, T-bar flux concentrators situated above and below the stack enhance the magnetic flux density through the sensors by over an order of magnitude. We then showed that this amplification diminishes only slightly when the bottom flux concentrator is changed to a flat plane (more conducive to film deposition). Using COMSOL, we optimized the design of the flux concentrators within the design constraints set by the fabrication and film deposition equipment with the goal of measuring the magnetic field of a rat sciatic nerve spike. We then fabricated our sensors by depositing a 100 nm-thick layer of CoFeB as our bottom flux concentrator, followed by our MTJ material stack, followed by a 1-3 um thick x 1-10 um wide layer of iron for the narrow, bottom portion of our top flux concentrator's T-bar, followed by a second, 1-3 um thick x 10-100 um wide layer of iron for the top portion of the top flux concentrator's T-bar. We then demonstrated the enhanced sensitivity obtained by using this flux concentrator design compared to a normal perpendicular anisotropy MTJ, and applied it to measuring a rat sciatic nerve extracellular action potential. These results suggest that with continued work and design, magnetic sensors for *in vitro* neural sensing can eventually become sensitive enough to detect single-cell action potentials, a breakthrough that would facilitate greater understanding of neural network architecture, current flow, and spike propagation.

**Disclosures: Z. Ali:** None. **S. Wang:** None. **H. Naganuma:** None. **A. Poon:** None.

**Presentation Number:** NANO30.05

**Topic:** I.04. Physiological Methods

**Support:** NSF CAREER AWARD 2048012

**Title:** Optically multiplexed neural recording using graphene-integrated electro-optic sensor for massive scaling of electrophysiology recording

**Authors: *Z. AHMED**, V. HASSANZADE, K. SARNA, V. JAIN, H. GUPTA, M. CHAMANZAR;
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**Abstract:** Our ability to record from large ensembles of neurons with high spatio-temporal resolution can profoundly improve our understanding of the neural basis of brain function and help with developing next generation neural prostheses and devise novel treatments for neurological disorders. While significant progress has been made in simultaneously recording from large populations of neurons, the high neuronal density in the brain calls for a substantial scaling of neural recording density to capture the complexity of neural circuits. In traditional passive neural probes, using a one-wire-per-channel scheme, higher channel densities are achieved through ultra-high-resolution lithography. However, this approach is reaching its scalability limits due to increased channel crosstalk and signal attenuation. The introduction of CMOS-based active probes has helped boost the channel count of active neural probes to ~1000 channels on a single shank. However, there are power consumption constraints of the active circuits to limit tissue heating and trade-offs associated with noise and bandwidth in time domain multiplexing. In such architectures, often only a small subset of these ~1000 channels on each probe shank can be simultaneously addressed to capture neural signals. To overcome these scalability limits of concurrent recording from thousands of channels, an innovative neural signal transduction and multiplexing scheme is required. We present a high-density optically multiplexed neural interface based on a novel electro-optical sensing paradigm. This highly sensitive transducer is based on a monolayer graphene integrated microresonator. We have experimentally demonstrated highly sensitive detection of signals as low as 125 μV using a 50-μm diameter electro-optic sensor. By employing wavelength division multiplexing, an array of these wavelength-selective transducers can transmit optically encoded neural signals via a single bus waveguide. Our simulations indicate that approximately 300 electro-optic neural transducers with 8-μm diameter can be coupled to a single 500-nm wide silicon bus waveguide. With just 7 of such waveguides, we can have ~2100 simultaneously addressable channels on an ultra-compact shank measuring 70 μm x 20 μm x 6.5 mm. This innovative neural recording architecture ensures high scalability with significantly reduced power consumption per channel compared to conventional active CMOS probes. In our presentation, we will discuss the design, benchtop and ex-vivo characterization of the electro-optic transducers, as well as the system-level design of the optically multiplexed recording scheme.

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**Presentation Number:** NANO30.06

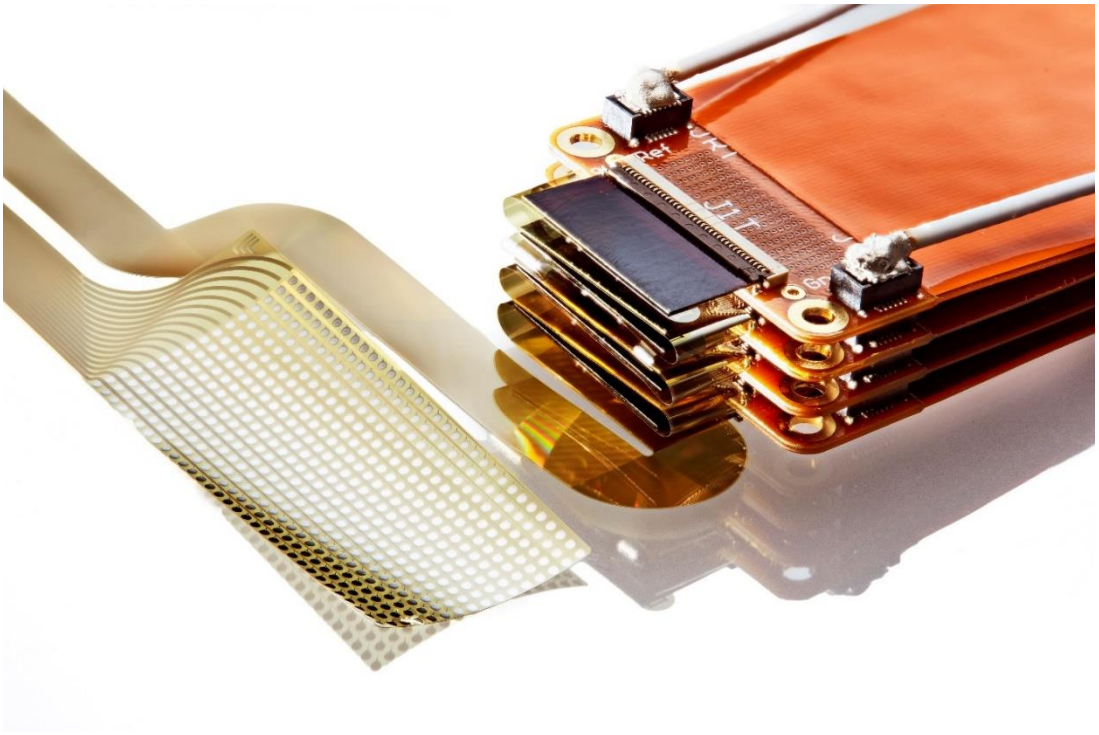
**Topic:** I.04. Physiological Methods

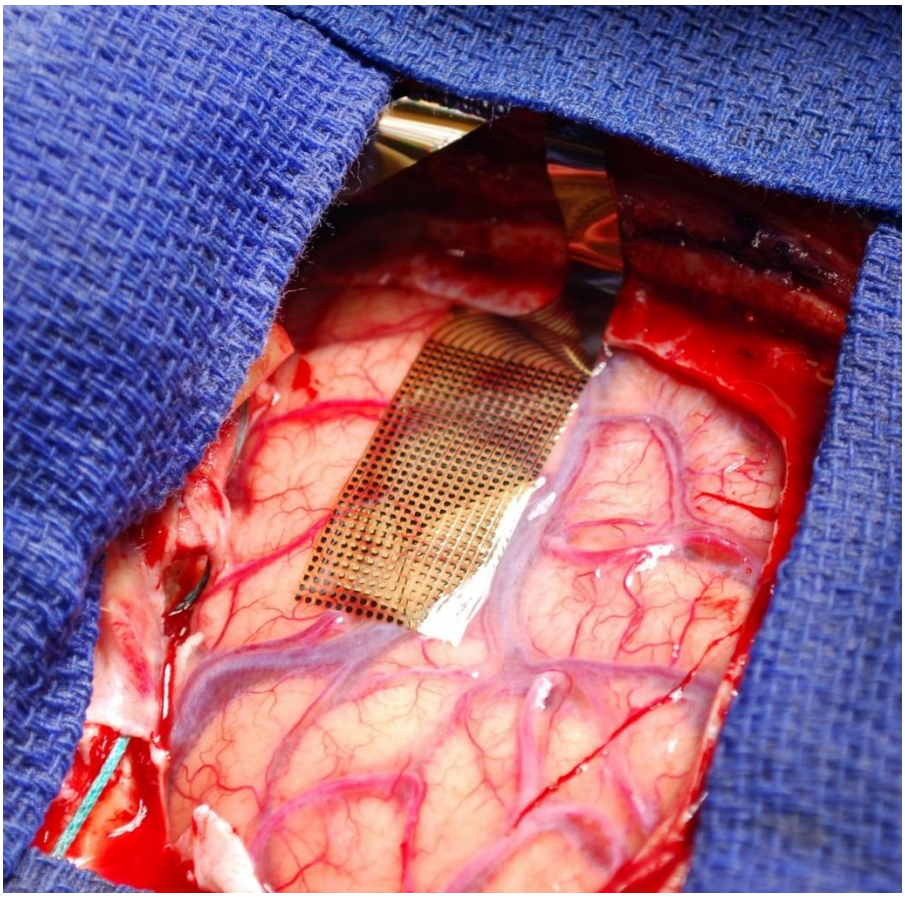
**Support:** NIH Grant U01NS117765
NIH IAA ADC15001001

**Title:** First-in-human validation of modular 512-channel thin-film polyimide microelectrocorticography (μECOG) arrays for intraoperative recording of speech activity

**Authors: *T. L. MASSEY**[1], R. MOUGHAN[1], K. SELLERS[2], J. R. GLEICK[1], J. ZHOU[1], J. GONZALES[1], M. K. LEONARD[3], E. F. CHANG[3], R.-U. M. HAQUE[1];
[1]Lawrence Livermore Natl. Lab., Livermore, CA; [2]UCSF, San Francisco, CA; [3]Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA

**Abstract:** Flexible polyimide neural interfaces conform to the surface of the brain and move freely with pulsatile micromotion, leading to high quality recordings that can be chronically stable. We present developments from 512-channel microelectrocorticography (μECoG) arrays microfabricated in LLNL's ISO 13485-compliant biomedical device foundry for intraoperative human recording. In this work, we show first-in-human 0-200 Hz activity recorded from the speech auditory cortex during speaking tasks in one of several languages, including English, Spanish, Mandarin, and Tagalog. We show that unique high gamma information content characteristic of speech is captured on each 0.5 mm electrode spaced at 1 mm pitch, highlighting the value of ever-higher channel counts and densities for speech recording and decoding applications. These arrays are modular and are designed to be implanted bilaterally for 1024 channels of simultaneous recording. Further, we demonstrate that our electrode yield is >99% as fabricated, and the interface from the probe to headstage is compact, sterilizable and reusable, robust to electromagnetic interference, and designed to be quick and simple to connect in the operating room. This is a significant step forward in the development of high density and high channel count neural interface tools for the clinical research community. [Prepared by LLNL under Contract DE-AC52-07NA27344.]





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**Presentation Number:** NANO30.07

**Topic:** I.04. Physiological Methods

**Support:** Grant agreement ID: 101070908 HOTIZON-EIC-2021-PATHFINDERCHALLENGES-01-02

**Title:** Crossbrain: toward self-standing micro-scale neuroelectronic implantable devices

**Authors: *J. F. RIBEIRO**[1], A. PERNA[1], T. GIANNATTASIO[2], A. CONTI[2], N. TOSCHI[2], G. ANGOTZI[1], L. BERDONDINI[1];
[1]Italian Inst. of Technol., Genova, Italy; [2]3Department of Biomedicine and Prevention, Univ. of Rome "Tor Vergata", Rome, Italy

**Abstract:** Aberrant electrical brain activity is a hallmark of numerous pathological conditions, necessitating the development of tailored, adaptive, and spatiotemporally localized functional interventions. In CROSSBRAIN we seek to create self-contained microscale devices (microbots) with a volume of 100x100x100 μm³ by harnessing cutting-edge nanomaterials, SiNAPS-derived circuits for neural activity sensing, nanoelectronic circuits for neuromodulation, learning, and

control, as well as miniaturized wireless power and communication technologies. The motivation to move toward such novel generation of neurotechnology relies on the hypothesis that such untethered microscale devices may achieve superior signal quality, stability and low foreign body reaction (FBR) than conventional implantable neural probes. Here we will report on two key neurotechnology challenges of this project. The first is the development of highly integrated front-end CMOS circuits for LFPs and APs recordings and neuromodulation that can fit the requirements of these microscale wireless microbots. Based on our experience on front-end circuits for bi-directional SiNAPS CMOS neural probes, we will discuss strategies to develop low-noise bidirectional circuits that can fit the limited area and low power imposed by microbots. The second challenge concerns the development of strategies for the implantation of such devices and the evaluation of FBR induced by micro-scale devices with respect to implantable neural probes. To investigate this second challenge we have realized micro-scale dummy Si devices of 100x100x100 $\mu m^3$ and evaluated strategies for insertion in agar-gel models, as well as protocols to assess FBR of micro-scale devices in the mouse brain.

**Disclosures: J.F. Ribeiro:** None. **A. Perna:** None. **T. Giannattasio:** None. **A. Conti:** None. **N. Toschi:** None. **G. Angotzi:** None. **L. Berdondini:** None.

**Presentation Number:** NANO30.08

**Topic:** I.04. Physiological Methods

**Title:** Tissue-susceptibility matched electrodes for simultaneous magnetic resonance imaging

**Authors: *A. VON RAVEN**, A. OELTERMANN, J. ENGELMANN, R. POHMANN, K. SCHEFFLER;
Max Planck Inst. for biological cybernetics, Tuebingen, Germany

**Abstract:** Functional magnetic resonance imaging (fMRI) is commonly used to study the operational organization of the brain, although the relationship between the measured fMRI signal and underlying neural activity remains unclear. In this study, we propose a novel approach utilizing an intracortical electrophysiology setup, enabling simultaneous recording of neural signals during fMRI measurements at a high field strength of 14.1 Tesla. While existing silicon-based NeuroNexus and Pt/Ir electrodes are effective up to 7 Tesla, our aim is to develop new electrodes with improved MRI characteristics for higher field strengths. By combining materials with complementary properties, we designed electrodes using the excellent conductivity and susceptibility properties of copper, in conjunction with polyurethane insulation and additional stabilizing fibers. We conducted susceptibility tests comparing established and prototype electrodes using gradient echo imaging, demonstrating reduced imaging artifacts with the prototype electrode (von Raven et al. SfN 2022). Biocompatibility tests performed on U-87 MG glioblastoma cells revealed that the prototype electrode did not cause significant cell death, unlike pure copper wires (figure 1a-d). The imaging artefact caused by the prototype electrode, consisting of a copper wire of 25µmØ and stabilization fibers using a gradient echo sequence was less than 200µmØ (figure 2). Noise level recorded by the electrophysiology setup during simultaneous echo planar imaging was less than 300µV at the input (figure 3). The use of insulated copper wire attached to stabilization fibers offers a promising alternative to conventional electrodes, particularly at higher field strengths. Animal experiments utilizing these electrodes could provide valuable functional information, and advanced post-processing

techniques may further reduce gradient-induced noise. This approach of combining different materials in electrode design to minimize susceptibility artifacts and enhance signal quality holds potential for future clinical applications.

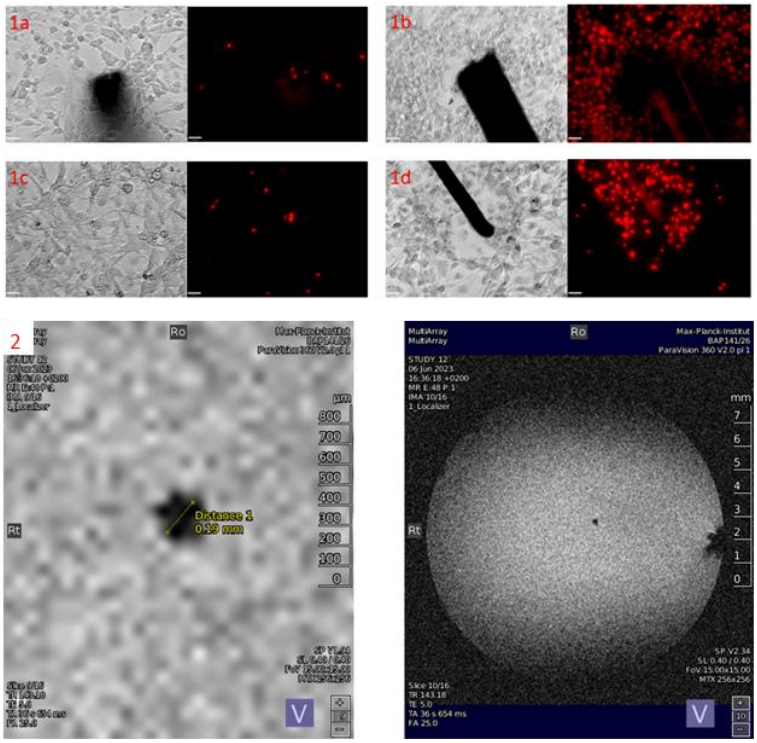


*Figure 1: Microscopic images of in U-87 MG glioblastoma cells incubated for 44h in the presence and absence of probes; phase contrast images (left) fluorescence images (right). Red fluorescence indicates nuclei of dead cells a) prototype Cu tip electrode; b) 100µmØ Cu wire not insulated; c) control without probe; d) 25µmØ Cu wire not insulated.*

*Figure 2: Field distortion artifact by the prototype electrode in cross section during simultaneous recording in saline; Gradient echo imaging; TR: 143ms; TE: 5ms; FA: 25 degree*

*Figure 3: Noise induced by the gradients during echo-planar imaging in saline. Up: peak to peak noise of 300µV at the input over the timespan of 100s. Down: Noise during two echo-planar imaging slides, repeating pattern. Gain: 24000, broadband 6Hz – 3kHz, sampling rate: 25000 S/s.*

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**Presentation Number:** NANO30.09

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH R01NS112176,
NIH R42NS125895
NIH R01NS129549

**Title:** A Development of a Device for Simultaneous Fast Scan Cyclic Voltammetry, Multi-Cyclic Square Wave Voltammetry, Electrophysiology, and Stimulation

**Authors:** *Y. OH[1], **H. SHIN**[2], J. ROJAS-CABERERA[1], K. SCHEITLER[1], G. CAMERON[1], Y. JASON[1], W. DENNIS[1], D. EAKER[1], J. BOESCHE[1], I. MANDYBUR[1], B. SHARAF[1], D. JANG[3], C. BLAHA[1], K. BENNET[1], K. LEE[1];
[2]Neurologic Surgery, [1]Mayo Clin., Rochester, MN; [3]Hanyang Univ., Seoul, Korea, Republic of

**Abstract:** Background: There has been significant progress in understanding the role of neurotransmitters and their release in the context of normal and pathologic brain function. However, little is known about real-time in vivo neurochemical changes as a function of dynamic brain processes such as disease progression and response to pharmacologic, cognitive, behavioral, and neuromodulation therapies. This is due at least in part to a lack of research tools capable of measuring these dynamic changes in brain activity in vivo. Method: Here, we present a research platform, WINCS MAVEN developed by Mayo Clinic Neural Engineering Laboratories and Division of Engineering, which can measure with four independent sensor channels and four independent stimulation channels and characterize real-time in vivo changes in neurochemical and electrophysiological activity across multiple anatomical targets to study normal and pathologic brain function Results: Main acquisition, Field Programmable Gate Arrays and stimulation circuits are being developed for multi-modal recordings and electrical stimulation. The battery-powered MAVEN device communicates with the base station using optical cable for MCSWV and via Bluetooth for all other functionalities. We demonstrate several key features of the MAVEN system in different functionalities. These features include measurement and characterization of neurochemical signals, real-time synchronization with therapeutic interventions such as electrical stimulation enabling users to act on these changes to provide real-time feedback to control neurochemical levels and aid in optimizing therapeutic efficacy. Conclusion: The MAVEN system described here will improve understanding of the dynamics of brain physiology in the context of neurologic disease and therapeutic interventions, which may lead to the development of precision medicine and personalized therapies for optimal therapeutic efficacy.

**Disclosures: Y. Oh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NaviNetics NeuroModulation, Inc.. **H. Shin:** None. **J. Rojas-Caberera:** None. **K. Scheitler:** None. **G. Cameron:** None. **Y. Jason:** None. **W. Dennis:** None. **D. Eaker:** None. **J. Boesche:** None. **I. Mandybur:** None. **B. Sharaf:** None. **D. Jang:** None. **C. Blaha:** None. **K. Bennet:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

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**Presentation Number:** NANO30.10

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH Grant 1R41NS113702-01
NSF Grant 1936173
Pittcon SACP Starter Grant
ACS PRF
DC CFAR Pilot Award

**Title:** Multiplexing Neurochemical and Neuropeptide Detection with Carbon Fiber Multielectrode Arrays and Fast-Scan Cyclic Voltammetry

**Authors: *A. G. ZESTOS**[1], N. ALYAMNI[3], C. ABDULLAEVA[2], H. VUONG[2];
[1]Chem. and Ctr. for Neurosci. and Behavior, [2]Chem., American Univ., Washington, DC; [3]Chem. and Biomed. Engin., American Univ. and Catholic Univ., Washington, DC

**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 characterized a novel carbon fiber multielectrode array, a 4-channel electrode, and found it comparable to the single channel CFME in sensitivity and selectivity. The multielectrode array, along with a multichannel potentiostat, had the additional capability of multiplexing neurotransmitter measurements in vitro by multi-waveform application. We utilized the multielectrode array and four-channel potentiostat to measure potassium chloride (KCl) stimulated release of dopamine and serotonin in the caudate putamen in coronal mouse brain slices ex vivo. We have extended this work to measure larger molecule neuropeptides such as 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 Neuropeptide Y and oxytocin.

**Disclosures: A.G. Zestos:** None. **N. Alyamni:** None. **C. Abdullaeva:** None. **H. Vuong:** None.

**Nanosymposium**

**NANO31: Development of Sensory Systems**

**Location:** WCC 152B

**Time:** Monday, November 13, 2023, 8:00 AM - 9:45 AM

**Presentation Number:** NANO31.01

**Topic:** A.08. Development of Neural Systems

**Support:** NIH F32 NS117723
NIH F32 NS127854
NIH S10 OD016167
NIH IDDRC P50 HD103555

**Title:** A single cell transcriptomic map of the developing Atoh1-lineage uncovers neural fate decisions and neuronal diversity in the hindbrain.

**Authors: *J. BUTTS**[1], S.-R. WU[1], M. A. DURHAM[1], R. DHINDSA[1], J.-P. REVELLI[2], H. Y. ZOGHBI[3];
[2]Dept. of Mol. and Human Genet., [1]Baylor Col. of Med., Houston, TX; [3]Molec Human Genetics/ Neurosci, Baylor Col. Med. Howard Hughes Med. Inst., Houston, TX

**Abstract:** Proneural transcription factors set up the molecular cascade to orchestrate neuronal diversity. One such transcription factor, *Atoh1*, gives rise to cerebellar excitatory neurons and over 30 distinct nuclei in the brainstem critical for hearing, breathing, and balance. Although neurons that arise from the *Atoh1*-lineage have been qualitatively described, the transcriptional programs that drive their fate decisions and the full extent of their diversity remain unknown. Here, we analyzed single-cell RNA-sequencing (scRNA-seq) and ATOH1 DNA binding in *Atoh1*-lineage neurons of the developing mouse hindbrain to elucidate transcriptional programs of neuronal fate decisions.To profile *Atoh1*-lineage neurons, we used two florescent genetic mouse models to detect *Atoh1*-expressing cells in real-time and to trace cells derived from the *Atoh1*-lineage. Single cell RNA-seq was performed in *Atoh1*-lineage cells from genetically modified E9.5 to E16.5 embryos to reveal the transcriptomic cascade driving developmental neural fate decisions. We also profiled DNA binding of ATOH1 using CUT&RUN at E12.5 and E14.5 to understand the role of ATOH1 binding in regulating transcriptomic changes.We analyzed 183,027 cells across 7 embryonic timepoints to build a transcriptomic map of *Atoh1*-lineage development by annotating the progenitors, migrating neurons, and mature *Atoh1*-derived nuclei. Excitingly, we discovered new markers for brainstem nuclei including *Tcf24* that is expressed in the intermediate lateral lemniscus and *Spx* that is expressed in the pedunculopontine tegmental nucleus. Further, we found the *Atoh1* progenitor pool changes throughout development as evidenced by progenitors expressing *Nes* at early developmental stages and switch to expressing *Pax6* at later developmental stages. Next, ATOH1 binding was significantly enriched in the differentially expressed genes of the migrating cells but not the progenitors. *In vivo,* we found without functioning ATOH1, the *Atoh1* progenitors were born but were unable to migrate, confirming the computational finding that ATOH1 is functionally critical during migration. Lastly, we identified a sizable population of proliferating unipolar

brush cell progenitor in the mouse *Atoh1*-lineage that was described in humans as the origin of one medulloblastoma subtype, but initially thought to be small in the mouse.Collectively, our data reveal unprecedented insight into the developing mouse hindbrain, provides markers for functional assessment of less studied neuronal populations, and provides evidence that the mouse is a relevant model to study medulloblastoma.

**Disclosures: J. Butts:** None. **S. Wu:** None. **M.A. Durham:** None. **R. Dhindsa:** F. Consulting Fees (e.g., advisory boards); AstraZeneca. **J. Revelli:** None. **H.Y. Zoghbi:** F. Consulting Fees (e.g., advisory boards); Regeneron, Column group. Other; Co-founder of Cajal Neuroscience.

**Presentation Number:** NANO31.02

**Topic:** A.08. Development of Neural Systems

**Support:** Leverhulme Trust RPG-2022-061
BBSRC BH163322
Newcastle University Faculty of Medical Sciences

**Title:** Novel transient cell clusters provide a possible link between early neural activity and angiogenesis in the neonatal mouse retina

**Authors: *E. SERNAGOR**[1], M. SAVAGE[1], C. BERTRAM[1], J. DE MONTIGNY[1], C. THORNE[2], Y. TAN[2];
[1]Biosci. INstitute, [2]Biosci. Inst., Newcastle Univ., Newcastle upon Tyne, United Kingdom

**Abstract:** By continuously producing electrical signals, neurons are amongst the most energy-demanding cells in the organism. Resting ionic levels are restored via metabolic pumps that receive the necessary energy from oxygen supplied by blood vessels. Intense spontaneous neural activity is omnipresent in the developing central nervous system. It occurs during short, well-defined periods that coincide precisely with the timing of angiogenesis. However, surprisingly little is known about the role of neural activity *per se* in guiding angiogenesis. We investigate these questions in the neonatal mouse retina, where blood vessels initially grow in a plane, while waves of spontaneous activity sweep across the retinal ganglion cell layer (GCL), just underneath the growing vasculature. We discovered transient clusters of auto-fluorescent cells in the GCL, forming an annulus around the optic disc, gradually expanding to the periphery. Remarkably, they appear locked to the frontline of the growing vasculature, reaching the periphery by P7-8. Blood vessels density is higher in cluster areas than in-between clusters at matching eccentricities. Large-scale pan-retinal multielectrode array recordings of the waves reveal that their initiation points follow a developmental center-to-periphery pattern similar to the clusters and blood vessels. Moreover, calcium imaging demonstrates that more spontaneous network events are initiated in regions supplied by vasculature than further out. The cluster cells seem to be eliminated by microglial activity, with the latter exhibiting more pronounced phagocytic features in their vicinity. Blocking Pannexin1 (PANX1) hemichannels activity with probenecid significantly decreases wave frequency (but not areas) and reverts active microglia to resting state in proximity to the cluster cells. Prolonged exposure to probenecid results in cluster cells disappearance. We suggest that these transient cells are specialized, hyperactive neurons residing in the GCL. They generate spontaneous activity hotspots, thereby triggering retinal waves through the release of ATP via PANX1 hemichannels. These activity hotspots attract new

blood vessels to enhance local oxygen supply. Signaling through PANX1 attracts microglia that establish contact with these cells, eventually eliminating them once blood vessels have reached their vicinity. The auto fluorescence that characterizes the cell clusters may develop because of metabolic stress developing once the process of microglial phagocytosis is initiated.

**Disclosures: E. Sernagor:** None. **M. Savage:** None. **C. Bertram:** None. **J. de Montigny:** None. **C. Thorne:** None. **Y. Tan:** None.

**Presentation Number:** NANO31.03

**Topic:** A.08. Development of Neural Systems

**Support:** NIH 5R01DC017489
NIH F99NS129179
NIH T32NS086750-06A1

**Title:** The organization and assembly of the gaze stabilization circuit in the larval zebrafish

**Authors: *D. GOLDBLATT**[1], S. HUANG[1], K. HAMLING[2], P. LEARY[3], M. LI[1], H. PANCHAL[1], D. SCHOPPIK[4];
[1]New York Univ., New York, NY; [2]New York Univ. Med. Ctr., New York, NY; [3]New York Univ. Neurosci. & Physiol., NEW YORK, NY; [4]Otolaryngology and Neurosci. & Physiol., NYU Sch. of Med., New York, NY

**Abstract:** Behavioral dysfunction in neurodevelopmental diseases often arises from aberrant neural circuit assembly. However, the developmental logic that dictates circuit organization, function, and ultimately behavior remains unresolved due to the complexity of most circuits. Gaze stabilization behavior in the larval zebrafish is an ideal model to elucidate mechanisms for neural circuit assembly. Vertical gaze stabilization uses simple architecture: all vertebrates use three cellular populations (peripheral sensory neurons, central vestibular projection neurons, and extraocular motor neurons) to transform nose-up or nose-down head movements into compensatory eye rotations. The mechanisms that organize subtypes of gaze-stabilizing neurons into circuit architecture remains elusive. By measuring calcium activity in developmentally “birthdated” neurons, we discovered that the gaze stabilization circuit is topographically organized, and that this topography emerges in a distinct temporal progression. With a genetic loss-of-function tool, we then demonstrated that motor partner populations are dispensable for this organization. Our data suggests that, like invertebrates, temporal mechanisms may assemble vertebrate sensorimotor circuit architecture. Recently, we used RNA sequencing to define the nature of these temporal forces. Several candidates for fate determination and wiring specificity have emerged from our screen, including the class 3 Semaphorins, the Cadm family, and the transcription factor evx2. We are currently performing CRISPR knockout screens to validate a role for candidates in circuit assembly. Our work speaks to general developmental principles for vestibular reflex circuit organization and function.

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**Presentation Number:** NANO31.04

**Topic:** A.08. Development of Neural Systems

**Support:** SNSF Synergia grant (CRSII3_141801)
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Louis-Jeantet Foundation award
Körber Foundation award
SNSF grant (31003A_182523)
NCCR 'Molecular Systems Engineering' grant

**Title:** Pyramidal neurons form active embryonic circuits perturbed by autism-associated mutations at the inception of neocortex in vivo

**Authors: *A. BHARIOKE**[1], M. MUNZ[1], G. KOSCHE[1], V. MORENO-JUAN[1], A. BRIGNALL[1], T. RODRIGUES[1], A. GRAFF MEYER[2], S. PICELLI[1], C. COWAN[1], B. ROSKA[1];
[1]Inst. of Mol. and Clin. Ophthalmology Basel, Basel, Switzerland; [2]Friedrich Miescher Inst., Basel, Switzerland

**Abstract:** Pyramidal-to-pyramidal neuron connections comprise a majority of the connections within cortical circuits, yet their initial assembly during embryonic development remains poorly understood. Here, we demonstrate that embryonic neurons labeled in Rbp4-Cre mice, that are transcriptomically closest to layer 5 pyramidal neurons, were both active at the inception of neocortex, and showed two phases of circuit assembly. The first phase, at E14.5, consisted of transient two-layered circuits spanning the neocortex. 2p calcium imaging in living embryos showed that neurons within these circuits had increased activity and high pairwise correlations. It is only following this phase of circuit assembly that embryonic layer 5 pyramidal neurons migrate to form layer 5. Migration is accompanied by decreased activity but, once assembled, neurons within layer 5 showed increased activity again. Using in vivo patch clamp recordings and pharmacology, as well as electron microscopy, we found that, in both phases, Rbp4-Cre neurons displayed voltage-gated sodium conductances, physical synapses, responded to glutamate agonists, and showed synaptic potentials. Further, embryonic Rbp4-Cre neurons already divide into three transcriptomic types, with each closest to one of the three adult layer 5 pyramidal neuron types (near-projecting (NP), pyramidal tract (PT), and intratelencephalic (IT)). Interestingly, the early transient circuits involved only embryonic NP-type neurons, with embryonic IT- and PT-types incorporated only later. Perturbing the activity selectively in postmitotic Rbp4-Cre neurons results in fine-scale changes to the location of neurons within layer 5. Additionally, given that embryonic Rbp4-Cre neurons preferentially express autism-related genes, we selectively perturbed two such genes in these neurons and found increased activity during the migratory phase, as well as a patchy disorganization of cortex, resembling that found in autistic patients. Hence, pyramidal neurons form active, transient pyramidal-to-pyramidal neuron circuits during embryonic development, at the inception of neocortex, and the activity in these circuits is reflected in cortical organization, with potential insights into the etiology of autism.

**Disclosures: A. Bharioke:** None. **M. Munz:** None. **G. Kosche:** None. **V. Moreno-Juan:** None. **A. Brignall:** None. **T. Rodrigues:** None. **A. Graff Meyer:** None. **S. Picelli:** None. **C. Cowan:** None. **B. Roska:** None.

**Presentation Number:** NANO31.05

**Topic:** A.08. Development of Neural Systems

**Support:** NIH Grant R15HD101974
NIH Grant P20GM144230
NIH Grant T32GM81741

**Title:** Genomic screen homeobox 1 is required for neural circuit assembly and function in the zebrafish visual system

**Authors: *S. A. BERGERON**, A. SCHMIDT, H. PLACER, A. CLUTTER, I. MUHAMMAD; Biol., West Virginia Univ., Morgantown, WV

**Abstract:** Early visual ability depends on the proper assembly of neural circuits during development. When visual system neurodevelopment is impaired due to genetic and environmental factors, it can result in lifelong adverse or adaptive changes in an organism's brain and behavior. *genomic screen homeobox 1* (*gsx1*) encodes a transcription factor that is expressed in developing visual system areas in zebrafish and mouse. However, despite its documented expression there, its functional role in the visual system was underexplored until recently. Our lab and others determined that *gsx1* is required for differentiation of glutamatergic neurons across the CNS. Using zebrafish *gsx1* mutants that we generated using TALENS and their wild type siblings for comparison, we found a reduction in vesicular glutamate transporter (*vglut2a*/*slc17a6*) expression in select CNS regions including the visual processing centers of the brain, the pretectum (Pr) and optic tectum (TeO). Retinal ganglion cell (RGC) axons connect the eyes to the brain in zebrafish by forming functionally distinct arborization fields (AFs) in these affected regions. AFs1-9 form in the Pr, and AF10 forms in the primary and predominantly studied visual processing center, the TeO which is analogous to the mammalian superior colliculus. We also observed a loss and reduction in size of select AFs in zebrafish *gsx1* mutants including Pr AF7, AF8, and TeO AF10. Loss of Pr AF7 has been linked to a reduction in prey capture ability which is also consistent with our observations during visual behavior testing of *gsx1* mutants. Two-photon mediated laser ablation of *vglut2a*-expressing neurons early in development also results in failure to form AF7 over time and visual behavior changes. Overall, *gsx1* primarily affects visual neural circuits downstream of the eye and forebrain as mutants maintain proper retinal morphology and display normal optic nerve and optic chiasm formation despite bands of *gsx1* expression in the developing forebrain where RGC axons navigate to their final trajectories. Our preliminary data also suggests that upon *gsx1* overexpression, *vglut2a* expression is expanded ectopically, and RGC axon tracks are disrupted in the TeO. This work has led us to identify for the first time in any vertebrate a novel and essential role for *gsx1* in visual system neural circuit assembly and function. As a result, many possible and testable RGC and other axon termination mechanisms can now be examined across all retinorecipient and other CNS areas that express *gsx1* and/or *vglut2a* which demarcates excitatory glutamatergic neurons and further implicates them as essential for axonogenesis and axon regeneration.

**Disclosures: S.A. Bergeron:** None. **A. Schmidt:** None. **H. Placer:** None. **A. Clutter:** None. **I. Muhammad:** None.

**Presentation Number:** NANO31.06

**Topic:** A.08. Development of Neural Systems

**Support:** K Lisa Yang ICoN Center, MIT
Simons Foundation
HHMI

**Title:** Self-organized emergence of modularity, hierarchy, and topography from competitive synaptic growth in a developmental model of the visual pathway

**Authors: *M. KHONA**[1], S. CHANDRA[4], T. A. KONKLE[5], I. R. FIETE[2,3];
[2]McGovern Institute, Brain and Cognitive Sci., [3]Brain and Cognitive Sci., [1]MIT, Cambridge, MA; [4]McGovern Institute, Brain and Cognitive Sci., Massachussetts Inst. of Technol., Cambridge, MA; [5]Psychology Dept, Harvard Univ., Cambridge, MA

**Abstract:** A hallmark of the primate visual system is its organization into multiple distinct areas that connect hierarchically. These areas exhibit specific spatial structure, with primary visual cortex at the center and subsequent regions in the hierarchy encircling the earlier one, and detailed topological structure, with retinotopic organization per area but striking mirror reversals across area boundaries. The developmental rules that drive the formation of these architectural, spatial, and topographic features of organization are unknown.Here we demonstrate that a simple synaptic growth rule driven by spontaneous activity, with heterosynaptic competition, can lead to emergence of all three levels of organization. We identify a theoretical principle, greedy local wiring length minimization, that captures the essential mechanism, and use it to propose mathematically similar but biologically distinct growth rules that yield a similar endpoint but take distinguishable developmental trajectories. The models explain additional properties of sensory hierarchies including convolution-like connectivity between layers. The few parameters governing structure emergence constitute simple knobs for rich control, enabling a shift from projection neuron-like connectivity patterns to interneuron-like ones and a shift from concentric to linear spatial organization as seen in visual to auditory cortex. In all, the presented rules can parsimoniously explain the organization of sensory cortical hierarchies and provide predictions for anatomical and functional motifs over normal and perturbed development.

**Disclosures: M. Khona:** None. **S. Chandra:** None. **T.A. Konkle:** None. **I.R. Fiete:** None.

**Presentation Number:** NANO31.07

**Topic:** A.08. Development of Neural Systems

**Support:** NIH GRANT RO1 EY033772
NIH GRANT 1F32EY034358-01

**Title:** A simple twist of fate: The serine threonine kinase LKB1 drives dopaminergic neurotransmitter switching in the visual system.

**Authors: *R. MACKIN**[1], M. SAMUEL[1], J. LIANG[2];
[1]Neurosci., [2]Baylor Col. of Med., Baylor Col. of Med., Houston, TX

**Abstract:** Neural circuits rely on transmission between neurons of specific types that can be defined by the neurotransmitters they produce. In turn, declines in specific subtypes can lead to

brain diseases, and neurons that produce dopamine are especially vulnerable. To reverse these changes, it is possible to confer the functions of degenerating cells to unaffected cells via engaging a unique type of neural plasticity called neurotransmitter switching. However, the mechanism that govern these molecular switches are unknown. Here, we show that the serine-threonine kinase LKB1 is a restrictive cue for dopamine neuron identity in the visual system. Removal of LKB1 from all retinal neurons or from interneuron subsets converts cholinergic neurons into dopaminergic neurons. Furthermore, removal of LKB1 from cholinergic neurons specifically is sufficient to induce this conversion, which increases dopamine production. Thus, interneurons require LKB1 to regulate post-mitotic neural identify, which may improve therapeutic options in dopamine-related brain diseases.

**Disclosures: R. Mackin:** None. **M. Samuel:** None. **J. Liang:** None.

**Nanosymposium**

**NANO32: Catecholamines and Purines**

**Location:** WCC 152A

**Time:** Monday, November 13, 2023, 8:00 AM - 10:15 AM

**Presentation Number:** NANO32.01

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

**Support:** This work was funded by a TIFR intramural grant to V.A.V.

**Title:** Norepinephrine regulates mitochondrial biogenesis and function in the hippocampus

**Authors:** D. KAPRI[1], P. TIWARI[1], A. B..J[1], A. SINGLA[1], A. BALAKRISHNAN[1], M. SHARMA[1], S. FANIBUNDA[1,2], U. KOLTHUR SEETHARAM[1], A. VAIDYA[2], ***V. A. VAIDYA**[3];
[1]Tata Inst. of Fundamental Res., Mumbai, India; [2]Kasturba Hlth. Society-MRC, Mumbai, India; [3]Tata Inst. Fundamental Rese, Mumbai, India

**Abstract:** Neuronal mitochondria are central to not only maintaining cellular bioenergetics, calcium dynamics, and modulation of ROS signalling but are also critical for specialized functions including synaptic plasticity and neurotransmission. While mitochondria are postulated to have a fundamental role in the functioning of neurons, it's only recently that factors that influence mitochondria in neurons and the central nervous system are being investigated. Here, we identify a critical role of the neurotransmitter Norepinephrine in modulating mitochondria in rodent hippocampal neurons. Norepinephrine increases mitochondrial biogenesis, enhances the expression of regulators of mitochondrial biogenesis, regulates ATP synthesis, and influences mitochondrial function. Increasing Norepinephrine content at the synapse via treatment with selective norepinephrine reuptake inhibitors also evoked robust increases in mitochondrial biogenesis, ATP content, higher mitochondrial respiratory capacity, and oxidative phosphorylation (OXPHOS) efficiency. These effects of Norepinephrine appear to be mediated by the β adrenergic receptor subtype, β2, and involve a critical role of the master modulator of mitochondrial biogenesis PGC1a. NE also reduces cellular reactive oxygen species and exerts

potent neuroprotective action in neurons challenged with excitotoxic stress, an effect that requires PGC1a. These findings identify a novel and exciting role for Norepinephrine in impacting mitochondrial turnover and biogenesis in the hippocampus.

**Disclosures: D. Kapri:** None. **P. Tiwari:** None. **A. B..j:** None. **A. Singla:** None. **A. Balakrishnan:** None. **M. Sharma:** None. **S. Fanibunda:** F. Consulting Fees (e.g., advisory boards); S.E.F serves as a consultant to Beckley Psytech which is not relevant to the current work.. **U. Kolthur Seetharam:** None. **A. Vaidya:** None. **V.A. Vaidya:** None.

**Presentation Number:** NANO32.02

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

**Support:** CURA
BYU Mentored Research Award to JTY

**Title:** Estrous cycle-dependent modulation of psychostimulant effects on striatal neurotransmitter release

**Authors: *L. FORD**[1], J. WOOLLEY[1], R. POWERS[3], P. MEDELLIN[1], H. WADSWORTH[1], J. YORGASON[2];
[2]Dept. of Cell Biol. and Physiol., [1]Brigham Young Univ., Provo, UT; [3]Noorda Col. of Osteo. Med., Provo, UT

**Abstract:** Women prescribed psychostimulants have self-reported changes in drug efficacy that coincide with menstrual cycling. Furthermore, cocaine and amphetamine effects on dopamine (DA) transmission are more potent in female rodents. However, it is unknown if psychostimulant effects in general are influenced by the estrous cycle, or if effects are specific to cocaine and amphetamine. The present study examines dopamine release kinetics across various stages of the estrous cycle in the nucleus accumbens, a key region for dopamine-mediated learning. The effects of cocaine, methamphetamine, and methylphenidate on dopamine release and clearance are examined using voltammetry. While estradiol and progesterone have been identified as major contributing factors to past findings, naturally-cycling mice were chosen over hormone-replaced mice for this study in order to holistically investigate estrous cycle effects on addiction circuitry. Dopamine terminals have recently been identified as co-releasing adenosine triphosphate (ATP). Dopamine and ATP release and clearance can be collected simultaneously. The effects of psychostimulants on ATP transmission are generally unknown, and may be altered considering the known interactions of vesicle packing between the transmitters. Slices in estrus had a greater decrease in dopamine clearance in response to methamphetamine than male, proestrus, and diestrus slices. Unexpectedly, in response to methylphenidate estrus slices were less affected compared to the other groups. In cocaine experiments, no sex- or phase-specific differences in clearance rate were observed. In response to multiple pulse stimulations, the rate of dopamine reuptake in males decreased with low frequency 5Hz stimulation, while slices in estrus seemed to require a minimum threshold of 20Hz to see a change in response. Methamphetamine and methylphenidate had no effect on single-pulse DA release. Cocaine increased release in male, estrus, and diestrus slices, but not in proestrus slices. The present findings support current literature showing the attenuating effects of female sex hormones on psychostimulant-mediated dopamine release. Furthermore, they suggest that dopamine release kinetics are sexually

dimorphic throughout the estrous cycle, even in the absence of exogenous drugs. Though investigation into the role of estradiol and progesterone in this pathway is already well underway, there are likely additional factors contributing to a complex model deserving of further exploration. I understand that efforts to ensure scientific rigor—including blinding, statistics, sample sizes, and replication—should be explicitly stated.

**Disclosures: L. Ford:** None. **J. Woolley:** None. **R. Powers:** None. **P. Medellin:** None. **H. Wadsworth:** None. **J. Yorgason:** None.

**Presentation Number:** NANO32.03

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

**Support:** NIH grant AA020919
NIH grant DA035958
Brigham Young University Young Investigator award

**Title:** Interleukin-10 enhances activity of ventral tegmental area dopamine neurons resulting in increased dopamine release.

**Authors: *J. P. RONSTRÖM**[1], S. B. WILLIAMS[1], A. PAYNE[1], D. OBRAY[1], C. HAFEN[1], M. BURRIS[1], S. WEBER[2], S. C. STEFFENSEN[1], J. YORGASON[1];
[1]Neurosci., [2]Microbiology and Mol. Biol., Brigham Young Univ., Provo, UT

**Abstract:** Dopamine transmission from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) plays a vital role in various motivational and learning related processes that may be influenced by immune activity. The neuroimmune system comprises leukocytes (e.g. microglia) and astrocytes, which function to detect and eliminate foreign threats while communicating through cytokine signaling to regulate other immune cells and neurons. The specific effects of anti-inflammatory immune cytokines on VTA circuitry are not well understood. Therefore, electrophysiology, neurochemical, immunohistochemistry, and behavioral experiments were performed to investigate the effects of the anti-inflammatory cytokine interleukin-10 (IL-10) on mesolimbic circuitry and related behavior. IL-10 enhanced VTA dopamine firing by reducing postsynaptic GABA currents onto dopamine neurons. The presence of IL-10 receptors was identified on both dopamine and non-dopamine cells in the VTA via IL-10Rα immunohistochemistry experiments. Furthermore, the effects of IL-10 on dopamine neurons occurred partially through post-synaptic phosphoinositide 3-kinase. Paired-pulse ratio voltammetry studies in brain slices revealed increased dopamine release at later intervals, suggestive of facilitated release at terminals independent of somatic activity. Furthermore, *in vivo* microdialysis experiments revealed elevated NAc dopamine levels in response to intracranial IL-10 administration. Furthermore, place conditioning induced aversive behavioral responses were enhanced by intracranial IL-10 administration. These findings shed light on the regulatory role of IL-10 on VTA dopamine circuitry and highlight the intricate interplay between the neuroimmune system, dopamine transmission and related behavior. By understanding the specific mechanisms through which cytokines influence dopamine activity, we can further unravel the complex relationship between the immune system and the nervous system. Efforts to ensure scientific rigor including blinding, statistics, sample sizes, and replication were employed throughout this study.

**Disclosures: J.P. Ronström:** None. **S.B. Williams:** None. **A. Payne:** None. **D. Obray:** None. **C. Hafen:** None. **M. Burris:** None. **S. Weber:** None. **S.C. Steffensen:** None. **J. Yorgason:** None.

**Presentation Number:** NANO32.04

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

**Support:** BYU Mentored Research Awards to JTY
R00DA045103 to CAS

**Title:** Dopamine and ATP accumbal co-release and neuroimmune interactions

**Authors: *J. T. YORGASON**[1], S. LINDERMAN[2], L. FORD[3], E. WHITE[4], E. TAYLOR[2], M. BURRIS[2], L. HAWLEY[2], C. SICILIANO[6], H. A. WADSWORTH[5];
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**Abstract:** Mesolimbic dopamine neurons are highly tuned regulators of reinforcement learning and implicated in motivational and emotional disorders. Many dopamine neurons co-release other transmitters, including glutamate and GABA. The present work explores ATP and dopamine co-release within the nucleus accumbens (NAc) and interactions with local microglia. We find that spontaneous and evoked ATP and dopamine co-release are mediated via similar vesicular release mechanisms and are co-regulated by autoinhibitory processes. Lipopolysaccharide (LPS), a well-known immunoactivator, transitions NAc microglia to de-ramified “activated” states. In contrast, ATP acts as a chemoattractant to microglia. Interestingly, LPS increases ATP and dopamine co-release, suggesting that dopamine may have some counter-chemoattraction effects. Microglia express dopamine receptors in immunohistochemistry assays. Iontophoretic drug application was used in multiphoton microscopy assays to study dopamine and ATP co-application effects on labeled microglia. Methamphetamine produced increases in dopamine release that were impaired in LPS treated brain slices. Furthermore, methamphetamine acutely reduced microglia ramification, but appeared to increase ramification across multiple exposures. Together, these data highlight the complex signaling that occurs between NAc dopamine terminals and local microglia. The present work was performed using experimenter single and double blinding when possible, statistical analysis, power analysis and appropriate sample sizes and replication for hypothesis testing.

**Disclosures: J.T. Yorgason:** None. **S. Linderman:** None. **L. Ford:** None. **E. White:** None. **E. Taylor:** None. **M. Burris:** None. **L. Hawley:** None. **C. Siciliano:** None. **H.A. Wadsworth:** None.

**Presentation Number:** NANO32.05

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

**Support:** BYU Mentored Research Award to JTY

**Title:** An exploration of microglial and neuronal cross-talk in the nucleus accumbens and methamphetamine effects

**Authors: *H. A. WADSWORTH**[1], L. H. FORD[1], N. SHEETS[2], L. R. HAWLEY[1], E. B. TAYLOR[1], E. R. WHITE[1], M. D. BURRIS[2], J. M. HANSEN[2], J. T. YORGASON[2];
[1]Neurosci. Ctr., [2]CELL, Brigham Young Univ., Provo, UT

**Abstract:** Microglia are monocyte derived immune cells and exhibit complex signaling behavior that include phagocytic activity to threats and prolonged neuronal activity. ATP (adenosine triphosphate) is a known chemoattractant for microglia, but how chemoattraction is modulated by other transmitters is not well understood. ATP is co-packaged and released with dopamine, thus the present work examines microglia morphology and motility in the context of these two transmitters. Microelectroiontophoresis and multiphoton microscopy were used in brain slices from transgenic mice to examine effects of dopamine and ATP signaling on microglia. Lipopolysaccharide (LPS) transitioned the microglia from ramified to amoeboid morphology over a period of 4 hours. LPS increased both dopamine and ATP release, as measured by fast scan cyclic voltammetry on a similar time course. Surprisingly, dopamine itself did not act as a chemoattractant to microglia, despite increasing after LPS treatment. Methamphetamine application decreased microglia ramification and impaired dopamine release and reuptake. Application of glucose oxidase increased reactive oxygen species (ROS) production and decreased dopamine release but had little-to-no effect on ATP release nor microglia morphology, suggesting that methamphetamine effects on ramification were not directly due to ROS production. Mice injected with methamphetamine had increased microglial ramification compared to saline injected mice. Methamphetamine injected animals also had attenuated glucose oxidase effects on dopamine release compared to saline injected controls. By understanding how neuronal outputs affect microglia activity in the context of psychostimulant use we can better parse out how the mechanisms of addiction are connected to immune system function. To ensure scientific rigor, analyzers were blinded for microscopy and electrochemistry analysis, and statistics, sample sizes and replication was specifically designed to verify results.

**Disclosures: H.A. Wadsworth:** None. **L.H. Ford:** None. **N. Sheets:** None. **L.R. Hawley:** None. **E.B. Taylor:** None. **E.R. White:** None. **M.D. Burris:** None. **J.M. Hansen:** None. **J.T. Yorgason:** None.

**Presentation Number:** NANO32.06

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

**Support:** NIH Grant R01NS121426

**Title:** Subsecond monitoring of neuroimmune communication

**Authors: *A. E. ROSS**;
Univ. of Cincinnati, Cincinnati, OH

**Abstract:** Understanding the dynamics and mechanisms of neuron-immune communication is vital for advancing our knowledge of neuroinflammation and neurodegenerative disease. The brain produces a robust and immediate response to attacks in order to mitigate the damage response. Ischemic stroke is of particular importance due to its prevalence in society yet the

molecular underpinnings of the brain's immediate response to ischemia-induced damage is not well understand. My lab has focused the last several years developing tools to measure immediate neuroprotective signaling at the site of localized ischemia in the brain. We have developed a robust microfluidic platform to enable localized and sustained delivery of ischemia to subregions of the brain ex vivo with simultaneous coupling to fast-scan cyclic voltammetry for subsecond monitoring of fluctuations in neurochemicals at the site of injury. With these tools, we have discovered endogenous subsecond release of guanosine that significantly increases as a function of ischemic severity for the first time. The majority of work to date in purine neurobiology has focused on adenosine; however little is known regarding the role of extracellular guanosine in the brain. We provide evidence suggesting that rapid release of guanosine in the brain functions as a neuroprotector to mitigate inflammation during ischemia. This talk will highlight some of our latest work in this area.

**Disclosures: A.E. Ross:** None.

**Presentation Number:** NANO32.07

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

**Support:** NIH grant NS112390
NIH Diversity Supplement 3R01NS112390-02S1
Rutgers Brain Health Institute Pilot grant

**Title:** Synaptojanin1 regulates dopamine transporter trafficking via the $PIP_2$ pathway

**Authors:** J. SAENZ[1], M. AGGARWAL[1], A. SHAIKH[2], G. CAMACHO HERNANDEZ[3], F. HERBORG[4], A. H. NEWMAN[5], ***P.-Y. PAN**[6];
[1]Rutgers Robert Wood Johnson Med. Sch., Piscataway Township, NJ; [2]Rutgers Univ., Piscataway Township, NJ; [3]NIH/NIDA, Bethesda, MD; [4]Blegdamsvej 3B build. 07.05, Univ. of Copenhagen, Copenhagen N, Denmark; [5]NIDA-IRP, NIH, NIDA IRP, Baltimore, MD; [6]Rutgers Univ. Robert Wood Johnson Med. Sch., Piscataway, NJ

**Abstract:** Parkinson's disease (PD) is a prominent neurodegenerative disease in the aging population characterized by the deterioration of dopaminergic neurons in the substation nigra. Mutations of *SYNJ1* (synaptojanin1/Synj1), an essential lipid phosphatase at the synapse, are linked to families with Parkinsonism. Recent studies from our lab and others have shown that Synj1 deficiency in mice led to dopamine neuron-specific synaptic defects such as impaired synaptic vesicle endocytosis and abnormal dopamine transporter (DAT) clusters. To determine that Synj1 regulates DAT trafficking and to identify the molecular underpinnings of this regulation we used multiple imaging strategies. Our data showed that in the striatum of aged *Synj1*+/- male mice DAT immunofluorescence was increased. Similarly, DAT was increased in the soma and axons of cultured *Synj1*+/- dopamine neurons. However, using a combination of antibody staining, fluorescent DAT ligand, JHC1-64, and expressing our recently engineered DAT reporter, DAT-pHluorin, we show that the steady state surface DAT (sDAT) was reduced in the axons of *Synj1*+/- neurons. The reduction of sDAT is further accompanied by an altered dopamine-induced DAT trafficking revealed by DAT-pHluorin live imaging. We found that protein kinase C beta (PKCβ), a dopamine neuron-specific isoform of PKC implicated in DAT internalization, was not increased in *Synj1*+/- axons. However, expressing human SYNJ1

harboring a PD mutation in the 5'-phosphatase but not the SAC1 domain resulted in a significantly decrease in sDAT expression in N2a cells. Consistently, pharmacological reagents that transiently increase neuronal $PI(4,5)P_2$, the enzymatic substrate of Synj1's 5'-phosphatase, led to a gradual loss of DAT-pHluorin fluorescence, suggesting DAT internalization. Thus, our study using a combination of novel imaging methods suggests Synj1 regulated sDAT maintenance via membrane $PI(4,5)P_2$ metabolism.

**Disclosures: J. Saenz:** None. **M. Aggarwal:** None. **A. Shaikh:** None. **G. Camacho Hernandez:** None. **F. Herborg:** None. **A.H. Newman:** None. **P. Pan:** None.

**Presentation Number:** NANO32.08

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

**Support:** Intramural Research

**Title:** Mdma modulates social interactions through taar1-mediated internalization of serotonin and glutamate transporters

**Authors: *S. UNDERHILL**[1], C.-Y. SUN[2], S. G. AMARA[3];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD; [3]NIMH, NIMH, Bethesda, MD

**Abstract:** MDMA (3,4-methylenedioxymethamphetamine, ecstasy) has recently been reevaluated as a therapeutic tool for the treatment of post-traumatic stress disorder, however the mechanism of action of this drug has not yet been fully elucidated. In previous studies, we have shown that a related compound, amphetamine (AMPH), enters neurons through the dopamine and norepinephrine transporters (DAT and NET) and activates TAAR1, which in turn increases the activity of the small GTPase RhoA and triggers internalization of DAT, NET and a neuronal glutamate transporter, EAAT3. Trafficking of these transporters from the plasma membrane results in increases in extracellular dopamine, norepinephrine as well as glutamate. Here we show that MDMA enters serotonin neurons through SERT and activates similar TAAR1-mediated signaling cascades to regulate transporter trafficking and MDMA-associated behaviors.We measured SERT activity in primary Raphe cultures and found that pretreatment of these cultures for thirty minutes with MDMA resulted in a decrease of SERT activity. This was blocked by an inhibitor of RhoA activity. Using total internal reflection fluorescence (TIRF) microscopy in HEK293 cells we examined the effects of MDMA on SERT and EAAT3 and found that both carriers were internalized by the psychostimulant in wildtype cells but not in a HEK293 cell line with a CRISPR-Cas9 TAAR1 gene deletion. We also assessed transporter trafficking in acute brain slices from adult mice. Slices containing the Raphe nucleus were treated with either vehicle or MDMA and subsequently treated with a cell-impermeable biotin reagent that labels all surface proteins. By western blot analysis of biotinylated proteins from these tissue lysates, we were able to detect significant SERT and EAAT3 internalization in response to MDMA treatment. There was no MDMA-induced trafficking observed in Raphe slices from TAAR1 knockout mice.Previous studies have demonstrated a vital role for EAAT3 in dopamine-linked behaviors; in contrast to wildtype littermates, EAAT3 knockout mice do not hyperlocomote in response to AMPH. MDMA's actions on serotonergic systems has been clearly linked to increases in social interaction (SI). Here, we examined the impact of MDMA-

induced EAAT3 trafficking on social behavior. Using a three-chamber social approach test to assess SI we found that male wildtype mice exhibited increased SI whereas EAAT3 knockout mice did not. These data indicate that MDMA can signal through TAAR1 in serotonergic neurons to alter trafficking of serotonin and glutamate transporters and regulate MDMA-induced behaviors.

**Disclosures: S. Underhill:** None. **C. Sun:** None. **S.G. Amara:** None.

**Nanosymposium**

**NANO33: Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models**

**Location:** WCC 146C

**Time:** Monday, November 13, 2023, 8:00 AM - 10:45 AM

**Presentation Number:** NANO33.01

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

**Support:** 1R01ES032440-01A1
1F31AG076332-01A1

**Title:** Alzheimer's disease-associated gut microbes shape microglia activation and disease outcomes in mouse models

**Authors: *L. BLACKMER-RAYNOLDS**, M. SAMPSON, A. HAMILTON, A. YANG, A. KOZLOV, L. LIPSON, J. CHANG, S. SLOAN, T. SAMPSON;
Emory Univ., Atlanta, GA

**Abstract:** Microglia have the capacity to play dual roles in Alzheimer’s disease (AD), providing neurotrophic support and/or furthering neurotoxicity. Despite growing evidence of the importance of microglia responses for AD outcomes, extrinsic factors that modulate microglia activation remain poorly understood. The gut microbiome is one such factor, with the ability to modify both microglia state and AD pathologies. However, the link between specific AD-associated gut bacteria and microglia activation remains unknown. Therefore, the present study mono-colonized wildtype germ-free mice with type strains of bacterial species of interest (*Escherichia coli, Bacteroides thetaiotaomicron, Clostridium celatum,* and *Lactobacillus johnsonii)* for 2 weeks before assessing microglia state. A bacterial-dependent shift in local intestinal inflammatory cytokines was observed, but these changes did not directly correlate with changes in the serum or brain. Further, RNA-seq analysis demonstrated sex and bacteria specific effects of mono-colonization on microglia transcriptional state, with *E. coli* mono-colonized mice displaying a more active, phagocytic, and disease-associated gene expression profile. Thus, to determine whether *E. coli* can modify AD associated outcomes, conventionally raised 5xFAD mice were enriched with E. *coli* into their native microbiomes for one month. *E. coli* exposed 5xFAD mice displayed accelerated cognitive decline compared to vehicle controls. Amyloid levels and inflammatory outcomes were also evaluated to determine whether *E. coli* exposure exacerbates AD pathology. Together, these results suggest that carriage of non-pathogenic *E.*

*coli* within the gut microbiome has the ability to modify microglia state, cognition, and pathology, highlighting the potential importance of this microbe for AD.

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

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**Title:** Microglia-specific deletion of ESCRT-I molecule Tsg101 suppresses microglial activation and restores the cognitive function and neuropathology in animal models of tauopathy

**Authors:** Z. RUAN[1,2], S. HERRON[2], Z. ZHANG[3], S. VENKATESAN KALAVAI[4,2], W. JOHNSON[5], S. IKEZU[6,2], ***T. IKEZU**[7,2];
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**Abstract: Background:** Extracellular vesicles (EVs) carry pathogenic molecules and play a role in the disease spread, including aggregated tau proteins. The Endosomal Sorting Complexes Required for Transport (ESCRT) machinery is responsible for the biogenesis of small EVs (exosomes), thus targeting critical ESCRT molecules can disrupt EV synthesis. We hypothesize that microglia-specific targeting of ESCRT-I molecule Tsg101 suppresses microglia-derived EV-mediated propagation of tau pathology, leading to amelioration of the disease phenotype of the tauopathy mouse model. **Methods:** PS19 tau transgenic mouse line was crossed with $Cx3cr1^{CreERT2/+}$:$Tsg101^{fl/fl}$ lines for tamoxifen-inducible Cx3cr1-specific deletion of *Tsg101* (Tsg101 cKO) in PS19 mice to generate WT, Tsg101 cKO, PS19, and PS19:Tsg101cKO groups. The animals were treated with tamoxifen or corn oil at 2 months of age and subjected to comprehensive behavioral, neuropathological, biochemical, and molecular biological assessments at 6-7 months of age. The microglia isolated from $Cx3cr1^{CreERT2/+}$:$Tsg101^{fl/fl}$ pups were subjected to *in vitro* synaptosome uptake analysis with or without 4-Hydroxytamoxifen treatment. **Results:** PS19 mice develop cognitive impairment as determined by Y-maze, forced alternation, novel object recognition and fear conditioning, which are reversed in PS19:Tsg101cKO mice. This is correlated with reduced $Alz50^{+}$ tau accumulation, neurodegenerative microglial activation, neuroinflammation and complement pathway activation as determined by bulk RNA sequencing, ELISA and neuropathology. Primary cultured

$Cx3cr1^{CreERT2/+}$:$Tsg101^{fl/fl}$ microglia with 4-Hydroxytamoxifen treatment show reduced phagocytosis of *E. coli*particles and synaptosome in C1q dependent manner. Tsg101 cKO microglia show reduced expression of C3aR1 and CD68, and secretion of total and $Tau^{+}$ EVs *in vivo*. **Summary:** Microglia-specific targeting of Tsg101 show beneficial effect for ameliorating the disease progression of tauopathy mouse model via suppression of EV secretion, microglial activation, tau accumulation and complement-dependent synaptic pruning. Microglial Tsg101 is a potential therapeutic target of Alzheimer's disease and related tauopathy.

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**Presentation Number:** NANO33.03

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

**Support:** NIH/NIA grant AG056114

**Title:** Plcg2-P522R remodels microglial transcriptomic states and ameliorates amyloid-related neuropathology in AD mice

**Authors:** X. GU[1,5], ***C. LEE**[2,1,5], P. LANGFELDER[1,5], A. DE LA ROCHA[1,5], Z. PAMONANG[1,5], L. RAMANATHAN[1,5], W. GE[3], A. BHADURI[3], X. YANG[1,2,4,5]; [1]Ctr. for Neurobehavioral Genetics, Semel Inst. of Neurosci. and Human Behavior, [3]Dept. of Biol. Chem., [4]Brain Res. Inst., [2]UCLA, Los Angeles, CA; [5]Dept. of Psychiatry, David Geffen Sch. of Med. at UCLA, Los Angeles, CA

**Abstract:** A rare variant in the phospholipase C-γ2 (PLCG2) coding region (rs72824905: p.Pro522Arg; referred to as PLCG2-P522R hereafter) is significantly associated with a reduced risk of Alzheimer's disease (AD). PLCG2 hydrolyzes phospholipid PIP2 to IP3 and DAG, leading to the release of intracellular calcium and subsequent signaling. In the brain, PLCG2 is selectively expressed in microglia and is essential for innate immune signaling. To investigate how the Plcg2-P522R variant may modify AD pathogenesis, we generated a novel Plcg2-P522R knockin mouse model (Plcg2-$P522R^{KI/KI}$) using CRISPR/Cas9 genome editing, and bred it onto 5xFAD, a transgenic amyloidosis mouse model of AD. The 5xFAD/Plcg2-$P522R^{KI/KI}$ mice exhibited a significant reduction in amyloid plaque and dystrophic neurite pathology compared to 5xFAD. Single-nuclei RNA-sequencing revealed that Plcg2-P522R reduced the population of damage-associated microglia (DAM) and remodeled transcriptomic states of both DAM and homeostatic microglia. Functionally, we observed increased Aβ phagocytosis by Plcg2-$P522R^{KI/KI}$ microglia in vitro and upregulated phagocytic marker proteins in plaque-associated microglia in vivo. Overall, our study demonstrates that the AD-protective Plcg2-P522R variant remodels the molecular and functional states of microglia to ameliorate amyloid plaque burden and plaque-associated neuropathologies, providing novel insights into this potential target for AD therapy.

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**Presentation Number:** NANO33.04

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

**Title:** Ubiquitin proteasome system and other PTMs in neurodegenerative disorders

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**Abstract:** In the cellular milieu, several proteins are present in the misfiled or in non functional form. In order to combat this burden, cells have an orchestrated machinery to provide proper shape of proteins from non-functional to functional form for the smooth Cellular functioning. However, beyond a threshold, these proteins are burden for the cells and cause accumulation in the form of aggregates, inclusion bodies. This is one of the major hallmarks in the neurodegenerative disorders. The Aβ burden, tauopathy and α-synuclein accumulation is playing a pivotal role in Alzheimer's disease (AD) and Parkinson's disease (PD) proteinopathy. The ubiquitin proteasome system (UPS) has a decisive mechanism in clearing the toxic metabolites and by products from the cells.Herein, we have unravelled the intricate mechanism of different amino acid residues that participate in UPS mediated clearance together with several post translational modifications through which toxic metabolites are released from the cells. Further we have tested the regime of post translational modifications, for instance, ubiquitination, acetylation and sumo-modifications in the neurodegenerative disorders along with drugs in neuroprotection.

**Disclosures: P. Kumar:** None.

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**Title:** Identification of a protective microglial state mediated by miR-155 and interferon-γ signaling in a mouse model of Alzheimer's disease

**Authors: *Z. YIN**;
Neurol., Brigham and Women's Hosp., Boston, MA

**Abstract: Background:** Microglia, the resident brain immune cells, play a critical role in brain homeostasis and disease progression. In neurodegenerative conditions, microglia acquire the neurodegenerative phenotype (MGnD), whose function is poorly understood. MicroRNA-155 (miR-155), enriched in immune cells, critically regulates MGnD. However, its role in Alzheimer's disease (AD) pathogenesis remains unclear. **Methods:** We used RNAseq and immunohistochemistry (n = 6-8 mice per sex per group) to investigate the gene expression profile and AD pathology. We further utilized single-cell RNAseq (n = 5 mice per group) to identify microglial clusters. Whole-tissue proteomics (n = 4 mice per group) was applied to detect the protein changes in brain milieu. Moreover, we used spontaneous alternation and forced alternation tests to evaluate the cognition (n = 33-36 mice per group). **Result:** We report that microglial deletion of miR-155 induces a pre-MGnD activation state via interferon-γ (IFNγ) signaling and blocking IFNγ signaling attenuates MGnD induction and microglial phagocytosis. Single-cell RNAseq analysis of microglia from AD mouse model identifies *Stat1* and *Clec2d* as pre-MGnD markers. This phenotypic transition enhances amyloid plaque compaction, reduces dystrophic neurites, attenuates plaque-associated synaptic degradation, and improves cognition. **Conclusion:** Our study demonstrates a novel miR-155-mediated regulatory mechanism of MGnD and the beneficial role of IFNγ-responsive pre-MGnD in restricting neurodegenerative pathology and preserving cognitive function in an AD mouse model, highlighting miR-155 and IFNγ as potential therapeutic targets for AD.

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NIA R21 1R21AG071884; PI: McAllister

**Title:** Major histocompatibility complex I mediates synaptic and behavioral deficits in Alzheimer's disease model

**Authors: *G. L. SELL**[1], D. R. NORTHCROFT[2], C. N. MCCORMACK[2], V. FLORES[2], A. MCALLISTER[3];
[1]Univ. of California-Davis Ctr. for Neurosci., Davis, CA; [2]Univ. of California-Davis, Davis, CA; [3]UC Davis, UC Davis, Davis, CA

**Abstract:** Alzheimer's disease (AD) is the most common neurodegenerative disease affecting the human population, with cases expected to triple between 2010 and 2050 (NIA). Despite this health crisis, there are few treatments and no cure for AD. Evidence from humans and animal models indicates that AD is associated with alterations in both synaptic and immune signaling, but the crosstalk between these two pathways in AD is not well understood. Recent work from our lab indicates that the classical major histocompatibility complex I (MHCI) molecules represent attractive candidates for linking immune signaling to synaptic deficits in AD. MHCI molecules act in the immune system to mediate the adaptive immune response, they are also expressed on neurons in the brain where they negatively regulate glutamatergic synapse number

and function (Needleman et al., 2010; Glynn et al., 2011), and they have recently been associated with APOE4 in AD (Zalocusky et al., 2021). Using biochemical and immunocytochemical approaches, we found that MHCI is elevated in the brains of advanced human AD cases and APP/PS1 mice, as well as on the surface of cultured neurons following $A\beta_{42}$ treatment. This elevation in sMHCI in cultured neurons is necessary for Aβ-induced synapse loss *in vitro* since Aβ failed to decrease synapses on neurons from mice lacking all classical MHCI on the surface (β2m-KO mice) or classical MHCI completely (KbDb-KO). To determine whether MHCI is necessary for Aβ-induced memory deficits *in vivo*, we generated mice from the APP/PS1 genetic line crossed to transgenic mice lacking surface MHCI expression (β2m-KO). Removal of sMHCI *in vivo* rescued deficits in novel object preference and context-dependent freezing in aged APP/PS1 animals. Finally, in order to start to determine how MHCI mediates these changes, we hypothesized that MHCI may cause synapse loss through decreasing levels of AD-related synaptogenic molecules, with a focus on neuroligin-1 (NL1) (Bie et al., 2012; Dinamarca et al., 2011; Dufort-Gervais et al., 2020). Indeed, MHCI bidirectionally regulates NL1 levels *in vitro* and *in vivo* and MHCI binds directly to NL1 in heterologous cells, dissociated cultures, and *in vivo*. Ongoing experiments are aimed at determining if this novel MHCI-NL1 signaling pathway is necessary for Aβ-induced synapse loss and memory deficits. Together, our results indicate that MHCI contributes to both synaptic and behavioral deficits in AD models potentially through a novel interaction with NL1, making MHCI a tantalizing target for future therapeutic studies as an intersection between inflammation and synaptic signaling in neurodegeneration.

**Disclosures: G.L. Sell:** None. **D.R. Northcroft:** None. **C.N. McCormack:** None. **V. Flores:** None. **A. McAllister:** None.

**Presentation Number:** NANO33.07

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

**Title:** Pf-04691502, a pi3k/mtor dual inhibitor, improves learning deficits in app/ps1 mice

**Authors: *M. LANZA**, G. CASILI, E. ESPOSITO, S. CUZZOCREA;
Univ. of Messina, Messina, Italy

**Abstract: PF-04691502, a PI3K/mTOR dual inhibitor, improves learning deficits in APP/PS1 mice.**
**Background.** Aging is the greatest risk factor for several neurodegenerative disorders, including Alzheimer’s disease (AD). Overwhelming evidence indicates that reducing mTOR signaling improves health span and lifespan in a multitude of organisms. PI3K is a key regulator of mTOR activity; the PI3K/mTOR signaling pathway regulates several key biological mechanisms related to cell development, cell survival, protein synthesis, autophagy, metabolism, and learning and memory. To this end, up-regulation of the PI3K/mTOR signaling contributes to AD neuropathology and causes neurodegeneration and learning and memory deficits. In this study, we sought to determine the molecular correlates of memory deficits in APP/PS1 mice, a widely used animal model AD. **Methods.** 18-month-old APP/PS1 and WT mice were dosed orally with 1 mg/Kg PF-04691502, an ATP-competitive PI3K/mTOR dual inhibitor, for 12 weeks. At the end of the treatment, we assessed changes in spatial learning and memory using the Morris water maze. We then processed their brains for neuropathological and biochemical assessment of amyloid-β (Aβ). **Results.** We found that PF-04691502 improved learning and memory in

APP/PS1 mice. Currently, we are processing the tissue to assess potential changes in brain Aβ deposits and soluble and insoluble Aβ levels. We will also assess the effects of reducing PIK3/mTOR signaling on inflammation. **Conclusions**. These results provide preclinical data indicating that PF-04691502 may be a valid therapeutic approach for AD and other neurodegenerative disorders associated with aging and mTOR hyperactivity.

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

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Alzheimer's association AARF-21-846786

**Title:** Apoe4 puts breaks on neutrophil-microglia crosstalk to accelerate alzheimer’s disease

**Authors: *N. ROSENZWEIG**, K. KLEEMAN, M. GRUCCI, M. ARONCHIK, O. BUTOVSKY;
Brigham and Women's Hosp., BOSTON, MA

**Abstract:** APOE4 is the strongest genetic risk factor for late-onset Alzheimer’s disease (AD). The role of human APOE variants in AD has been studied extensively in the regulation of brain cells but not in peripheral immunity. APOE is also expressed in neutrophils and controls their activation. Moreover, neutrophils have been shown to play a negative role in AD mice via the induction of neutrophil degranulation and extracellular traps. Here we aimed to dissect the impact of APOE4 on neutrophil phenotypes and functions in the transition from unimpaired cognition to mild cognitive impairment (MCI) and AD. RNAseq and pathway analysis of sorted neutrophils isolated from MCI, AD and aged-matched healthy control (HC) subjects, identified APOE4 sex-dependent induction of neutrophil degranulation in HC females associated with upregulation of immunosuppressive cytokines, including IL10, IL17 and TGFb. Using Cytek Aurora we extended these findings and identified a novel immunosuppressive neutrophil subset that is expended in HC female APOE4 carriers and is enriched during the transition to MCI/AD. APOE4 neutrophils accumulated in the brains of AD mice and in AD patients, associated with microglia at sites of plaque pathology. Furthermore, we show impaired induction of disease associated microglia (DAM/MGnD) signature in AD brains of APOE4 carriers. Utilizing Ly6g-CRE mice crossed to *APOE*-KI(E3 and E4)$^{fl/fl}$:APP/PS1 mice, we identified a cell intrinsic role of APOE3 in controlling neutrophil aging and degranulation, which is exacerbated in APOE4-KI neutrophils and reduced in APOE4-KO neutrophils. Here we show that genetic deletion of *APOE4* in neutrophils suppressed their activation and reduced their recruitment to the brains of APP/PS1 mice. RNAseq analysis showed increased proportion of MGnD in APP/PS1: Ly6g-CRE:APOE4$^{fl/fl}$ mice, associated with reduced plaque pathology. Taken together, these findings identify a cell-intrinsic role of APOE4 in the induction of immunosuppressive neutrophils recruited to the brain, which may provide new molecular targets to modulate and restore functional microglia in AD.

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U24AG07245801

**Title:** Pathological Tau and Cellular Senescence in Alzheimers disease

**Authors: *R. KAYED**[1], S. GAIKWAD[2];
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**Abstract:** Aging, pathological tau oligomers (TauO), and chronic inflammation in the brain play a central role in tauopathies, including Alzheimer's disease (AD). However, the underlying mechanism of TauO-induced aging-related neuroinflammation remains unclear. Here, we show that TauO-associated astrocytes display a senescence-like phenotype in brains AD patients. TauO exposure triggers astrocyte senescence through high mobility group box 1 (HMGB1) release and inflammatory senescence-associated secretory phenotype (SASP), which mediates paracrine senescence in adjacent cells. HMGB1 release inhibition using ethyl pyruvate (EP) and glycyrrhizic acid (GA) prevents TauO-induced senescence through inhibition of p38-mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB)-the essential signaling pathways for SASP development. Despite the developed tauopathy in hTau mice, EP+GA treatment significantly decreases TauO and senescent cell loads in the brain, reduces neuroinflammation, and thus ameliorates cognitive functions. Collectively, TauO-induced HMGB1 release promotes cellular senescence and neuropathology, which could represent an important common pathomechanism in tauopathies including AD.

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

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**Title:** Autophagy enables microglia to engage amyloid plaques and prevents microglial senescence

**Authors: *I. CHOI**, M. WANG, B. ZHANG, Z. YUE;
Icahn Sch. of Med. At Mount Sinai, New York, NY

**Abstract:** Autophagy enables microglia to engage amyloid plaques and prevents microglial senescence
Insup Choi[1], Minghui Wang[2], Bin Zhang[2], Zhenyu Yue[1]
[1] Department of Neurology and Neuroscience, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY10029[2] Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, 1425 Madison Avenue, New York, NY10029, USA
Dysfunctional autophagy has been implicated in the pathogenesis of Alzheimer’s disease (AD).

Previous evidence suggested disruptions of multiple stages of the autophagy-lysosomal pathway in affected neurons. However, whether and how deregulated autophagy in microglia, a cell type with an important link to AD, contributes to AD progression remains elusive. Here we report that autophagy is activated in microglia, particularly of disease-associated microglia surrounding amyloid plaques in AD mouse models. Inhibition of microglial autophagy causes disengagement of microglia from amyloid plaques, suppression of disease-associated microglia, and aggravation of neuropathology in AD mice. Mechanistically, autophagy deficiency promotes senescence-associated microglia as evidenced by reduced proliferation, increased *Cdkn1a*/p21$^{Cip1}$, dystrophic morphologies and senescence-associated secretory phenotype. Pharmacological treatment removes autophagy-deficient senescent microglia and alleviates neuropathology in AD mice. Our study demonstrates the protective role of microglial autophagy in regulating the homeostasis of amyloid plaques and preventing senescence; removal of senescent microglia is a promising therapeutic strategy.

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**Presentation Number:** NANO33.11

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

**Support:** UL1TR002373

**Title:** A Braak I/II stage mouse model for Alzheimer's disease

**Authors: *Z. HUANG**[1], H. KWON[2], D. SANTHOSH[2];
[1]UW-Madison, Madison, WI; [2]Univ. of Wisconsin-Madison, Madison, WI

**Abstract:** Alzheimer’s disease (AD) has three pathological hallmarks, amyloidosis, tauopathy, and neuroinflammation, that re-enforce each other during AD development through a three-way interaction and are generally agreed to be the most likely cause of AD. Because of their three-way re-enforcing relations, it is predicted a severe deterioration in just one may, alone, be sufficient to precipitate the deterioration of all three and cause AD. Indeed, in humans, mutations in familial AD genes that increase Aβ oligomer formation cause early-onset AD with near 100% certainty. However, in numerous mouse models where these mutations are overexpressed, the engineered amyloidosis has consistently failed to induce robust tauopathy.
Studies show that Aβ aggregation is regulated by a self-amplifying mechanism in which the initial formation of oligomers and fibrils promotes further aggregation by catalyzing secondary nucleation reactions that precipitously increase oligomerization. Aβ oligomerization in the brain not only consumes Aβ monomers (as substrates), but may also create fields of Aβ aggregates that trap and restrict monomer movement and in effect deplete Aβ monomers. Based on these and other observations, we hypothesize that amyloidosis in AD may not only produce Aβ oligomers that are neurotoxic and pro-inflammatory but may also deplete the effective pool of monomers that may be neuroprotective and anti-inflammatory, and this dual effect may be more potent than Aβ overexpression alone in inducing lasting brain neuroinflammation and be responsible for AD development. In support, several studies have shown that Aβ monomers are neuroprotective and synaptogenic. In pilot studies, we found Aβ monomers are also strongly anti-inflammatory. To further test this hypothesis, we also generated a microglia-specific ric8a/b mutant to mimic the proposed dual effect of amyloidosis, by blocking both GPCR signaling pathways that are known

to be key to brain immune homeostasis and a novel Aβ monomer-activated pathway that we discover that inhibits microglial inflammatory activation. We found this result in heightened brain neuroinflammation as well as strong **neuronal tau phosphorylation** at S202/T205/T231 (AT8 and AT180 staining) in the aging mouse brain. The tau hyperphosphorylation is observed specifically in and associated with **atrophy of the entorhinal/piriform cortex**. Entorhinal tauopathy and atrophy are defining features of Braak I/II stage AD pathology that determine the course of AD development in humans. The ric8a/b mutant thus provides a unique opportunity to determine mechanisms of tauopathy initiation and spread in AD.

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**Nanosymposium**

**NANO34: Microglial Roles in Alzheimer's Disease**

**Location:** WCC 150

**Time:** Monday, November 13, 2023, 8:00 AM - 10:45 AM

**Presentation Number:** NANO34.01

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

**Support:** RO1 NIH/NINDS # R21NS127211

**Title:** Defining a cell-autonomous role for DNAJC5 in microglia in Alzheimer’s disease

**Authors: *M. J. ROSENE**[1], L. BRASE[2], O. HARARI[2], B. A. BENITEZ[1];
[1]Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Washington Univ. in St. Louis, St. Louis, MO

**Abstract: Background:** *DNAJC5* encodes co-chaperone cysteine-string protein alpha (CSPα), a synaptic and endolysosomal protein whose mutations cause adult-onset neuronal ceroid lipofuscinosis (ANCL). Characterized by endolysosomal dysfunction, microgliosis, synaptic loss, and protein aggregation, ANCL shares pathological features with Alzheimer’s disease (AD). The endolysosomal role of CSPα in non-neuronal cell types has been understudied. Here, we aimed to address this gap and hypothesized that CSPα plays a yet undefined microglial role in AD. **Methods:** Aβ levels and plaques in human tissue were assessed through ELISA and immunohistochemistry, respectively. Bulk-RNAseq data from AD and ANCL patients with controls (GSE5281; GSE15222) were analyzed. To assess cell-specific expression, single-nuclei RNAseq (snRNA-seq) was mined from the parietal cortex of AD patients with APP and PSEN1 mutations (n=16) along with sporadic AD (n=31), non-AD (n=9), presymptomatic (n=3), and control patients (n=8). Key findings were replicated in an independent cohort (GSE138852). The Connectivity Map (CMap) was used to predict the preapproved drugs or existing medications that can reverse the transcriptomic profile from *DNAJC5* mutants in myeloid cells. ANCL RNAseq key findings were cross-analyzed using trascriptome from iPSC-derived microglia (GSE178317). **Results:** ANCL patients’ brain tissue exhibits nearly nonexistent soluble or insoluble Aβ levels and reduced synaptic protein levels compared to control individuals. However, there is intraneuronal APP/Aβ accumulation. *DNAJC5* transcript levels were reduced

in two independent RNAseq cohorts of AD patients compared to controls. *DNAJC5* transcript levels are higher in microglia compared with neurons in snRNA-seq from parietal cortex. ANCL patients exhibit 35 differentially expressed genes associated with proliferative state and 32 genes associated with the interferon/chemokine response iPSC-derived microglia. CMap indicated that HSP inhibitors (BIIB-021, geldanamycin), an opioid receptor antagonist (BNTX), and a proteasome inhibitor (bortezomib) could reverse the upregulated genes in ANCL patients. ANCL-patient transcriptomics displayed reduced *TSPAN14*, which facilitates ADAM10 maturation, and elevated *APH1B* and *GSAP*, enzymes that promote γ-secretase activity. **Conclusions:** In this study, we present transcriptomic and biochemical evidence suggesting that *DNAJC5* modulates APP metabolism and amyloidogenesis. Furthermore, we also show snRNAseq data that suggests a novel cell-autonomous role for *DNAJC5* in microglia and neuroinflammation.

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

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**Title:** Mitochondrial control of microglial function in Alzheimer's disease

**Authors:** K. LAI, J. WONG, L. H. FAIRLEY, W. CHONG, ***A. M. BARRON**;
Nanyang Technological Univ., Singapore, Singapore

**Abstract:** Mitochondria are emerging as the command centers of innate immune responses, controlling inflammatory responses via metabolic programming. Recent evidence suggests that in the Alzheimer’s brain, microglia shift from mitochondrial respiration to inefficient energy production via glycolysis. With their metabolism in overdrive, microglia fail to carry out phagocytosis, an important protective function in Alzheimer’s disease. We demonstrate that the translocator protein (TSPO) and a member of its mitochondrial complex, hexokinase-2 (HK), play critical roles in microglial respiratory-glycolytic metabolism and phagocytosis. Microglia lacking TSPO resembled those observed in aging and Alzheimer’s disease, exhibiting severe impairment of respiratory metabolism and phagocytosis. TSPO expression was associated with reduced phosphorylation of the voltage dependent anion channel (VDAC). Since VDAC is the major channel for mitochondrial supply of respiratory substrates and member of the TSPO-complex, we speculate TSPO may regulate microglial respiration via phosphorylation of VDAC. TSPO deletion was also associated with increased mitochondrial recruitment of the key glycolytic enzyme, HK. To determine the functional significance of mitochondrial HK recruitment, we developed an optogenetic tool for light-activated, reversible control of HK localization. Mitochondrial HK recruitment coordinated the inflammatory switch to glycolysis in mouse microglia, while displacement of mitochondrial HK inhibited glycolysis and increased phagocytosis. We find that targeting mitochondrial HK binding may offer an immunotherapeutic

approach to inhibit glycolysis and promote microglial phagocytosis with potential application in Alzheimer's disease.

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NMSS fellowship FG-2108-38372

**Title:** Determine the fate of Clec7a+ MGnD-microglia in neurodegenerative diseases

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**Abstract: Rational:** Microglia, the primary immune cells of the brain, play a pivotal role in the maintenance of brain homeostasis but lose key functions during the course of neurodegenerative diseases. How microglial function is maintained in the healthy brain and becomes prone to dysregulation in neurodegenerative diseases such as Alzheimer's disease (AD) and Multiple sclerosis (MS) remains unclear. In recent years more studies are consolidating two major identified microglia phenotypes, homeostatic microglia (M0) present in the healthy adult brain and microglia associated with neurodegenerative disease (MGnD), also known as disease-associated microglia (DAM). Understanding whether the MGnD phenotype is beneficial or detrimental in neurodegenerative disease progression is currently the breakpoint question for neuroinflammation-targeted therapeutic approaches. **Objective:** Define the contribution of MGnD microglia to the development and progression of neurodegenerative diseases by employing our novel mouse model, which can specifically target the MGnD-microglia subset. **Methods:** To specifically target MGnD-microglia, our group recently generated new transgenic Clec7a-$Cre^{ERT2}$ mice. These animals were crossed with $ROSA26^{TdTomato}$ and $DTR^{Flox}$ mice to address their efficacy and MGnD microglia fate map. To evaluate the MGnD response in a disease context, these mice were crossed with APP/PS1 and 5xFAD mice or immunized with MOG to induce an encephalomyelitis autoimmune experimental (EAE). **Results:** We validated that MGnD ($Clec7a^{+}$/ $TdTomato^{+}$) microglia are present at the site of axonal damage in the spinal cord white matter, and their numbers directly correlated with the disease clinical score in EAE mice. Moreover, $ROSA26^{DTR}$ mice showed it is possible specifically to ablate MGnD-microglia during EAE development. In APP/PS1 and 5xFAD mice (Alzheimer's diseases mouse models), we observed $Clec7a^{+}$ microglia exclusively in cells associated with amyloid beta (Ab) plaques. These microglia showed increased expression levels of MHC-II, GMCSF and TNFa, whereas LAP was suppressed. Interestingly, we found a decreased expression of MGnD Clec7a-$TdTomato^{+}$ microglia in males of 4 months of age compared to females, associated with a decreased load of Ab-plaques and dystrophic neurons. Fate-map analysis showed that Clec7a-TdTomato+ microglia turnover is around three months after activation. **Conclusion:** Clec7a-CreERT2 mice is a new mouse model to study an MGnD microglia subset in neurodegeneration.

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

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**Title:** Elucidating the neuroprotective mechanisms of the APOE3 Christchurch mutation in Alzheimer's Disease

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**Abstract:** Alzheimer's Disease (AD) is the most prevalent form of late-onset dementia, known for the presence of amyloid plaques and neurofibrillary tangles of hyperphosphorylated tau. Mutations in their presenilin-1 (*PSEN1*) gene causes familial early-onset AD with an average age of onset of 40s. One patient with this mutation interestingly did not develop any memory deficits until her 70s and had a significantly reduced tau load in comparison to other patients at her age; she was also homozygous for a R136S mutation in her APOE3 gene, known as the Christchurch mutation (*APOE3CC*). To study how *APOE3CC* could lead to protect effects against tau pathology, we established a mouse model of human *APOE3CC*. Using CRISPR-Cas9, we replaced mouse *ApoE* alleles with human *APOE3* or *APOE3CC* coding sequence: APOE3-R136R (*ApoE3*$^{WT/WT}$) or APOE3-R136S (*ApoE3*$^{CC/CC}$), which were then crossed with a transgenic mouse model overexpressing human tau carrying the P301S mutation to induce tau pathology.To determine if *APOE3CC* protects against tauopathy, we first performed a Morris Water Maze test, which measures spatial learning and memory. In *ApoE3*$^{WT/WT}$ mice, we showed that expression of P301S tau impaired spatial learning with a higher cumulative search error. In contrast, the tau-induced deficits were abolished in *ApoE3*$^{CC/CC}$ mice. *ApoE3*$^{CC/CC}$ also abolished tau-induced synaptic loss in the CA1 hippocampus compared to *ApoE3*$^{WT/WT}$*/P301S mice*. Lastly, we leveraged single nuclei RNA sequencing (snRNAseq) from mouse hippocampi to identify cell types that could be responsible for *APOE3CC*'s neuronal resilience. Our snRNAseq data showed that *ApoE3*$^{CC/CC}$*/P301S* hippocampal microglia significantly downregulate type I interferon signaling, suggesting that the protective effects of *APOE3CC* could be attributed to suppression of toxic inflammatory response in microglia.Overall, we expect that our model will allow us to probe the underlying mechanisms from the *APOE3CC* patient. A better understanding of how the Christchurch mutation yields resilience against neurodegeneration could be a promising target for AD therapy.

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

**Support:** NIH grant R03 AG070710

**Title:** Microglial TYROBP/DAP12 modulates the effects of gut microbiota manipulation on learning behavior, amyloid pathology, and gene expression in the APP/PS1 mouse model of Alzheimer's disease.

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**Abstract:** Gut microbiome has emerged as a modulator of brain functions, microglial activity, and Alzheimer's disease (AD) pathogenesis. Human and rodent studies indicate that gut microbiota composition and diversity are altered during aging and AD. In mouse models of AD-type pathology, gut microbiota transfer from aged mice to young ones has been shown to increase gliosis, accelerate AD pathogenesis and learning behavior deficit in young mice. However, the underlying mechanisms by which the gut microbiota influences microglia activity and the progression of neurodegenerative diseases remain poorly understood. TYROBP is a transmembrane polypeptide that acts as an adaptor for several receptors (including SIRP1β, CD33, and TREM2) on microglia and myeloid cells. We have previously shown that TYROBP plays a key driver role in microglia activation, the induction of disease-associated microglia genes (DAM), and AD pathogenesis. Recent reports have also suggested activation of the TREM1/2;TYROBP pathway in monocytes and macrophages in response to oral or gut dysbiosis.
We sought to investigate the role of TYROBP in modulating the effects of gut microbiota on learning behavior, gliosis, amyloid-ß deposition, and brain transcriptome in the *APP/PS1* mouse model of AD.
We manipulated the gut microbiota of *APP/PS1* mice WT (*APP/PS1*) or KO for *Tyrobp* (*APP/PS1;Tyrobp*$^{-/-}$) using fecal microbiota transplantation (FMT). FMT was achieved by weekly transfer of soiled bedding from aged *APP/PS1* mice (12-month-old) to young *APP/PS1* mice WT or KO for *Tyrobp* (starting at 3 months of age). We performed a panel of learning behavior (Novel Object Recognition), biochemical, and transcriptomic assays on 8-month-old *APP/PS1* and *APP/PS1;Tyrobp*$^{-/-}$ with or without FMT.
Absence of TYROBP in the *APP/PS1* mice prevented the learning behavior deficits and gliosis induced by FMT. FMT had limited impact on amyloid-ß deposition in *APP/PS1* and *APP/PS1;Tyrobp*$^{-/-}$ mice. Transcriptomic analyses identified unique transcriptomic signatures in the hippocampi of 8-month-old *APP/PS1* and *APP/PS1;Tyrobp*$^{-/-}$ mice after FMT. Notably, Ingenuity Pathway Analysis suggested that FMT increased phagosome formation, FAK signaling, and decreased neurodegeneration in the *APP/PS1* mice lacking TYROBP whereas these pathways were reversed in the *APP/PS1* WT for TYROBP.
Our results in the *APP/PS1* mouse model of Alzheimer's pathology suggest a role of TYROBP in modulating the effects of the gut microbiota on learning behavior, gliosis, and AD pathogenesis.

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

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**Title:** Xenon gas treatment suppresses neuroinflammation and ameliorate Alzheimer's disease

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**Abstract: Rational**: Microglia play an essential role in supporting normal brain functions, but in disease may contribute to neurodegeneration. We identified that neurodegenerative microglia (MGnD), also referred to as disease associated microglia (DAM), are regulated by the reciprocal suppression of TGFb and induction of APOE signaling in different neurodegenerative models including Alzheimer's disease (AD). Genome-wide association meta-analysis studies (GWAS) and an interactome investigation identified microglia as the only cell type in the central nervous system (CNS) whose gene expression pattern correlated with late onset of AD, however no treatment focused on microglia modulation is available. Xenon (**Xe**) gas is currently used in human patients as an anesthetic and as a neuroprotectant in treatment of brain injuries, however its effects on microglia are still unknown. **Objectives:** evaluate if Xe-gas treatment has a protective immunomodulatory role to restore homeostatic microglia phenotype and ameliorates AD pathology. **Methods:**Through a special chamber that can control the supply of Xe-gas in a closed-circuit system, we evaluated the Xe effect in microglia biology in models of acute and chronic neurodegeneration including humanized AD mice. The Xe concentration were determined by kinetics and dose-response experiments. The chronic neurodegeneration experiments were performed in female mice ($n$ = 8-9) from 3 independent experiments. To evaluate the Xe effects in human microglia in vivo, 5xFAD:MITRG mice injected with IPS-derived microglia (iMGL). Microglia were evaluated by IHC, flow cytometry, Smartseq2 and scRNA-seq analysis. **Results:** We found that Xenon-treatment can directly modulate the microglia phenotype, by increasing their phagocytic response and decreasing their proinflammatory signature. Weekly-based Xenon inhalation modulated mouse and human microglia activation towards a homeostatic phenotype in an acute neurodegeneration model and in AD mice. It led to amelioration of AD-like pathology, such as Ab-plaques and dystrophic neurons in APP/PS1 and humanized 5xFAD:MITRG mice. Mechanistically, we found that Xe treatment polarizes microglia toward an intermediate state, via induction of microglial responses to IFNg signaling, which maintain the phagocytic response with a suppression in pro-inflammatory cytokines. **Conclusion:** Xe-gas treatment directly induces microglia protective functions and reduces amyloid beta load to treat AD. **Disclosure**: this work was performed in collaboration with General Biophysics.

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

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**Title:** Apoe4 impairs the microglial response in alzheimer's disease by inducing tgfb-mediated checkpoints

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**Abstract: Background:** APOE ε4 is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). The contribution of microglial APOE4 to AD pathogenesis is unknown, although APOE has the most enriched gene expression in neurodegenerative microglia (MGnD). **Methods:** Generation of CX3CR1-CRE$^{ERT2}$ mice crossed to *APOE*-KI(E3 and E4)$^{fl/fl}$:APP/PS1 and *APOE*-KI(E3 and E4)$^{fl/fl}$:P301S mice. Microglia-astrocyte crosstalk was determined using scRNAseq and NichenetR database and validated in the brain of AD donors carrying the APOE ε3/3 and ε3/4 alleles. **Results:** Here, we show, in mice and in humans, a negative role of microglial APOE4 in the induction of MGnD response to neurodegeneration. Deletion of microglial *APOE4* restores MGnD phenotype, associated with neuroprotection in P301S tau transgenic mice and decreases pathology in APP/PS1 mice. MGnD-astrocyte crosstalk associated with β-amyloid (Aβ) plaque encapsulation and clearance are mediated via Lgals3 signaling following microglial *APOE4* deletion. In the brain of AD donors carrying the APOE ε4 allele, we found a sex-dependent reciprocal induction of AD risk factors associated with suppression of MGnD genes in females, including *LGALS3*, as compared to APOE ε3/3 carriers. Mechanistically, APOE4-mediated induction of ITGB8-TGFβ signaling impairs MGnD response via upregulation of microglial homeostatic checkpoints, including *INPP5D* in mice. Microglial deletion of *Inpp5d* restores MGnD-astrocyte crosstalk and facilitates plaque clearance in APP/PS1 mice. **Conclusions:** We identify the microglia APOE4-ITGB8-TGFβ pathway as a negative regulator of microglial response to AD pathology, and restoring MGnD phenotype via blocking ITGB8-TGFβ signaling provides a promising therapeutic intervention for AD.

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**Title:** Apoe4 genotype and aging affect spontaneous and responsive microglia motility in ex-vivo brain slices

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**Abstract:** Aging and *APOE4* genotype are the strongest risk factors for Alzheimer's Disease (AD), but how they affect microglial function prior to AD pathology remains unclear. To examine microglia physiology, we developed a preclinical mouse model that expresses human APOE3 or APOE4, with GFP under the CX3CR1 promoter. Using *ex-vivo* confocal microscopy in 6-month-old mice, we imaged the extension and retraction of microglial processes for 20 minutes. We showed that spontaneous motility of APOE4 microglia was significantly lower (27%) than APOE3 microglia in the entorhinal cortex but not in the hippocampus. To study the effect of APOE genotype in microglia response to damage, we imaged processes motility in response to ATP for 30 minutes and measured the velocity. APOE4 microglia extended processes toward 3mM ATP significantly slower (0.9 μm/min, $p<0.005$,) than APOE3 microglia (1.1 μm/min) in the entorhinal cortex. Similar results were observed in the hippocampus. We also assessed homeostatic microglia function in 12- and 21-month-old mice and found that the altered response to ATP in APOE4 microglia was reproducible in 12- and 21-months old mice, and aging further exacerbated this effect in APOE4 microglia. To further examine if the effect of *APOE4* on microglial response to damage was present in response to amyloid β (Aβ), we infused Hi-Lyte Fluor 555-labeled Aβ in acute brain slices of 6-months old mice and imaged microglia movement for 2 hours. APOE4 microglia showed a significantly slower process movement toward the Aβ, and less Aβ coverage two hours after the appearance of the Aβ in the brain. Microglial chemotactic receptors P2Y13 and P2Y12 were measured through qPCR and immunohistochemistry in 6-month-old mice. P2Y13 RNA levels in APOE4 brains were 20% lower ($p<0.05$) than in APOE3 brains. Total P2Y12 protein levels were the same across genotype; however, APOE4 microglia had significantly more P2Y12 localized to the cell soma compared to APOE3 microglia. Together, our findings demonstrated that APOE4 microglia exhibit reduced brain surveillance and altered response to ATP and Aβ. In addition, aging exacerbates alteration in homeostatic microglial function. This alteration in spontaneous and

responsive microglia motility may be due to genotypic differences in P2Y12 and P2Y13. Based on these data, we will further use this powerful *ex-vivo* model to investigate how APOE protein interacts with Aβ to facilitate phagocytosis. These impairments in microglial surveillance and response to damage due to *APOE4* and aging before gross Aβ pathology can help explain how APOE4 brains are more susceptible to AD pathogenesis.

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**Title:** Arachidonic acid remodeling and peroxidation shape microglia response to Amyloid pathology

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**Abstract:** The dysregulation of arachidonic acid (AA) metabolism is intricately linked to the pathology of Alzheimer's disease (AD); however, the underlying causal mechanism remains poorly understood. In this study, we investigate the impact of altered AA remodeling in microglia on Aβ pathology. In the microglia from *App*$^{NL-GF}$mice, we observed a significant increase in the levels of free AA and lysophospholipids (LPLs). By examinating enzymes involved in AA remodeling within phospholipids, we found increased activity of cytosolic Phospholipase A2 (cPLA2) and decreased expression of Lysophosphatidylcholine Acyltransferase 3 (LPCAT3), and this combination of alterations is likely responsible for the rise of free AA and LPLs levels. To elucidate the consequences of this dysregulated AA remodeling, we selectively manipulated the levels of AA-containing phospholipids in microglia through chronic deletion of Lpcat3. Lipidomics analysis reveals that loss of LPCAT3 leads to reduced levels of AA-containing phospholipids and ether lipids, resulting in decreased levels of free AA and lysophospholipids in microglia from adult *App*$^{NL-GF}$ mice. Notably, the loss of Lpcat3 in microglia effectively reduces oxidative stress and inflammatory responses while enhancing the phagocytosis of Aβ plaques and promoting the compaction of Aβ deposits. Intriguingly, the loss of Lpcat3 does not impact microglia's acquisition of the transcriptional signature associated with disease-associated microglia (DAM), but it increases the expression of genes involved in cholesterol and fatty acid de novo synthesis. Consistent with our *in vivo* findings, we demonstrate in primary microglia culture that exogenous AA or its main peroxidation derivative, 4-hydroxynonenal (4-HNE), exacerbates inflammatory responses and suppresses phagocytosis. Collectively, our study establishes a causal relationship between altered AA remodeling and microglial dysfunction in AD, providing insights into potential therapeutic strategies targeting AA levels through pharmacological and dietary interventions in AD patients.

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**Title:** G Protein-Coupled Receptor-Mediated Microglial Function: A Novel Gateway to Limit Alzheimer's Disease Progression

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**Abstract:** Alzheimer's disease (AD) is a devastating neurodegenerative disease with virtually no therapeutic interventions to reverse its pathology. Recent studies emphasize the role of glial cells, particularly microglia, in maintaining brain homeostasis and impacting AD progression. The adhesion G protein-coupled receptor GPR56 (also known as ADGRG1) is one of the critical genes defining "true" microglia: *Gpr56* is only expressed in yolk sac-derived microglia but not in fetal liver- and bone marrow-derived microglia-like cells, even after long-term adaptation in the central nervous system *in vivo* (Bennett et al., Neuron 2018). A recent study from Mathys *et al.* highlighted GPR56 as one of the top five genes upregulated in microglia in individuals with early-stage AD compared to those with no pathology or late-stage AD. Importantly, their data were generated from participants in a community-based cohort study, the Religious Order Study (ROS)/Rush Memory and Aging Project (MAP), collectively known as ROSMAP. Of those autopsied, the mean age was 89 years. This observation suggests that individuals with upregulated microglial GPR56 may have survived to an advanced age with mild AD pathology. In the present study, we test the hypothesis that microglial GPR56 prevents AD progression by employing both mouse models and human AD brain samples. Utilizing a new AD mouse model, in which microglial GPR56 is specifically deleted in 5xFAD mice (termed as AD-cKO and AD-control), we observed an exacerbated AD pathology in the absence of microglial GPR56. This was characterized by impaired microglial response, increased plaque burden, more severe neuronal pathology, and cognitive deficits. Single-nucleus RNA sequencing (snRNAseq) revealed a downregulation of genes linked to microglia homeostasis, phagocytosis, and lysosomal functions in AD-cKO microglia. In addition, our studies in human AD patients revealed the highest GPR56 expression in the microglia of individuals with mild cognitive impairments (MCI), compared to control and severe AD individuals. Pearson correlation coefficient analysis using several published human sequencing databases corroborated our results in mouse models. In conclusion, our study results support that microglial GPR56 plays an indispensable role in curtailing AD progression, presenting a potential new therapeutic target in combating AD.

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**Title:** Development of a novel targeted single-cell profiling method to assess microglial states in the context of amyloid pathology

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**Abstract:** Historically, the microglial state has been described as either “activated” (M1) or “homeostatic” (M2) microglia. With the advent of single-cell RNA sequencing technology, unique subtypes of microglia such as disease-associated microglia (DAM), Alzheimer's Disease 1 and 2 (AD1, AD2) microglia, and lipid-droplet accumulating microglia, have been identified and profiled. Researchers now question whether such microglial subtypes are unique and differentiated forms of microglia, or whether microglia exist in a dynamic state capable of shifting between transcriptional phenotypes in response to their environment.
Microglia are poorly represented in single-nucleus studies of the brain compared to neurons and astrocytes. Furthermore, whole-transcriptome data is sparse, meaning that many genes go undetected from cell to cell. To overcome these critical technical hurdles, we optimized a single-cell dissociation protocol that depletes neurons and enriches for microglia without any additional cell sorting steps. We also designed a novel, targeted single-cell RNA-sequencing panel to profile 598 genes associated with different microglial subtypes/states at dramatically increased depth and sensitivity.
Using this unique targeted scRNA-seq approach, we profiled microglia from three different models of amyloidosis (5XFAD, APP-NL-G-F, APPPS1) with approximately equal levels of amyloid burden. We then compared microglial state signature scores between amyloid and control mice, and assessed overlap between the models. This well-powered study (18 animals, 163,370 cells after passing quality control steps) shows changes in the DAM population between the 5XFAD model and other models, as well as complex shifts in microglial states across all three comparisons.

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**Nanosymposium**

**NANO35: Chronic Pain**

**Location:** WCC 140

**Time:** Monday, November 13, 2023, 8:00 AM - 10:45 AM

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**Topic:** D.02. Somatosensation – Pain

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**Title:** Enhancing fractalkine signaling normalizes the expression of CCI-induced differentially expressed genes and attenuates neuropathic pain in mice

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**Abstract:** Objective: Neuropathic pain (NP) is one of the most prevalent and incapacitating forms of chronic pain. A better understanding of its pathogenesis at cellular and molecular levels is crucial to the development of new therapeutics. A major focus has been on the roles of Fractalkine (CX3CL1) signaling between neurons and immune cells in the pathogenesis of NP. In this study, we over-expressed CX3CL1 in mice and investigated the impact of over-expressing CX3CL1 on neuropathic pain and on nerve injury-induced differentially expressed genes (DEGs). Methods: Chronic constriction injury (CCI) was performed on wild-type (WT) and CX3CL1 overexpressing (CX3CL1-Tg/CX3CR1$^{GFP/+}$) mice. Mechanical hyperalgesia was evaluated using the von-Frey Filament test. CCI-induced DEGs in the injured sciatic nerve in WT and CX3CL1-Tg mice in RNA-Sequencing were analyzed at post-CCI day 28. Results: Compared to WT mice, mice with full-length CX3CL1 overexpression showed significantly reduced pain behavior after CCI, as reflected by dramatically elevated paw withdrawal thresholds in the von Frey test in both male and female animals. We identified 5,882 CCI-induced DEGs (FDR<0.05 and a threshold of $|\log_2 FC| > 1$) in WT mice (CCI vs. Control). Of these genes, 3,224 (52.5%) were upregulated and 2,658 (47.5 %) were downregulated. The upregulated genes showed significant enrichment in multiple immune-related biological processes including cytokine/chemokine-mediated signaling and immune cell activation in Gene ontology (GO) enrichment analysis. CX3CL1 over-expression significantly normalized CCI-induced DEGs and signaling of relevant pathways. We identified 3,181 DEGs (FDR<0.05 and a threshold of $|\log_2 FC| > 1$) by comparing data from WT and CX3CL1-Tg mice; 1,442 (43.7%) were upregulated and 1,706 (56.3%) were downregulated after CCI. CX3CL1 over-expression almost fully reversed the upregulated genes in immune-related biological processes and partially reversed the downregulated genes related to synaptic transmission, synaptic organization, axonal outgrowth, and neuronal function in GO analysis. CX3CL1 over-expression also fully reversed the key upstream cytokine transcription levels that are upregulated by CCI in WT mice in IPA analysis. Most CCI-induced DEGs (2,854) overlap between WT and CX3CL1-Tg mice. CX3CL1 over-expression significantly reversed the key processes seen in WT-CCI mice. Conclusion: We concluded that enhancing CX3CL1 signaling significantly mitigated neuropathic pain and dramatically normalized nerve injury-induced DEGs that regulate a wide range of neuroimmune functions.

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**Topic:** D.02. Somatosensation – Pain

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**Title:** Neurokinin 1 (NK1) receptor antagonist delayed the development of osteoarthritic pain via attenuating neurogenic inflammation in rat models.

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**Abstract:** Osteoarthritis (OA) is one of the most common degenerative joint diseases. Chronic OA pain limits the daily activities of patients, which increases societal burden. Substance P (SP) and neurokinin 1 (NK1) receptor are related to inflammation leading to knee joint damage following OA. SP-NK1 signal are involved in neurogenic inflammation via a neuroimmune interactions, leading to hyperexcitability of dorsal horn neurons which is a causative mechanism of chronic pain. In the present study, we investigated the role of SP-NK1 signal on the induction of OA pain related to central sensitization via neurogenic inflammation by using NK1 receptor antagonist.GR 82334, one of NK1 receptor antagonists, or saline were intra-articularly injected 30 minutes before and 1 day after OA to monosodium iodoacetate (MIA, 4 mg/50 μl)-induced OA rats. Inflammation and pain-related behavioral tests were performed before and after NK1 antagonist administration in OA rats. The expression of pro-inflammatory cytokines in knee joints and L4-5 dorsal root ganglions (DRGs) was measured through western blot. Immunofluorescence was preformed to observe the M1 macrophage activation in knee joints. The expression of SP and calcitonin gene related-peptide (CGRP) in L3-5 segment of spinal cord was quantified through immunohistochemistry. Intra-articularly injected GR 82334 before and 1 day after MIA-induced OA significantly decreased knee joint diameter ratio and knee bending score and delayed the decrease of PWT compared to control. The expression of pro-inflammatory cytokines in the knee joint and DRGs was reduced and the M1 macrophage activation in the knee joint was relatively reduced in the GR 82334-administered group at 14 days after MIA-induced OA. The expression of SP and CGRP in spinal cord was significantly decreased by GR 82334 injection at day 14.The present data showed that NK1 receptor antagonist administration at both 30 minutes before and 1 day after of OA delayed the induction of OA pain and alleviated the neurogenic inflammation via reducing the acute inflammation and M1 macrophage activation. These results suggest that SP-NK1 signal might participate in the pathological process from acute inflammation to development of chronic pain related to central sensitization following OA, proposing that NK1 receptor would have a potential as therapeutic target for fundamental treatment of OA.

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**Title:** Acute modulation of Nav1.7 in inflammatory pain by Tumor Necrosis Factor-alpha

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**Abstract:** Inflammatory pain is mediated through the action of pro-inflammatory biomolecules on peripheral nociceptors. TNF-a is a prominent cytokine that is upregulated in a variety of inflammatory pain states and sensitizes nociceptors through its action on a variety of neuronal ion channels. In particular, chronic elevation of TNF-a in DRG neurons has been shown to drive increased transcription and translation of a peripheral voltage-gated sodium channel isoform, Nav1.7, that is well established as an obligate mediator of pain. However, TNF-a is released acutely from macrophages and inflammatory pain develops at timescales faster than can be accounted for by *de novo* channel biogenesis. In this study, we explore the previously undescribed acute effects of TNF-a on Nav1.7. We demonstrate that Nav1.7 activity is upregulated in response to short periods of TNF-a exposure. Further, we show that this increase in current rapidly generates sensory neuron hyperexcitability, and that these effects are dependent on the p38 MAPK cascade. Next, we systematically probed the intracellular regions of the Nav1.7 channel and identified the specific amino acid responsible for this phosphorylation-dependent regulation. Finally, using a variety of high-resolution confocal microscopy techniques and Nav1.7 channels affixed with a HaloTag enzyme, we investigated the acute of effects of TNF-a on the lifecycle of Nav1.7 channels, probing surface expression, vesicular trafficking, and insertion of channels into the neuronal membrane. Critically, we investigated these properties in both the cell bodies and the distal axonal terminals of dorsal root ganglion neurons.

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**Topic:** D.02. Somatosensation – Pain

**Support:** NIH R01NS112266
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**Title:** Preoperative acute sleep deprivation prolongs immune response and postoperative pain hypersensitivity

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**Abstract:** Background: Clinical and pre-clinical data have commonly reported perioperative sleep disturbances and loss of sleep in studies subjects. Furthermore, insufficient sleep increases long-lasting postoperative pain symptoms. However, the mechanisms by which sleep loss can affect long-term postoperative pain are still poorly understood. Because sleep and sleep loss are strongly linked to inflammatory processes, we hypothesized that insufficient sleep before a surgical procedure could hinder tissue healing by prolonging local inflammation, thereby extending the duration of pain symptoms. Methods: Adult female and male C57/BL6j mice were sleep-deprived for 9 hours using a gentle, minimally stressful protocol, and immediately subjected to plantar incision of the left hindpaw, together with non-sleep-deprived animals. We performed histological analysis of the operated hind paw skin at different time-points after incision using H&E staining to assess tissue damage and immune cells infiltration, in both well-rested and sleep-deprived animals. Mechanical allodynia and heat hyperalgesia were also assessed. All procedures were performed blinded to experimental conditions and approved by JHACUC. Results: Although preoperative sleep deprivation did not alter the intensity of postoperative mechanical allodynia, it markedly prolonged its duration compared to well-rested mice. The same trend was also observed for thermal hyperalgesia. Sleep deprivation did not change the number of immune cells in the skin of uninjured mice, it significantly altered the degree and intensity of the immune response after skin incision. The number of immune cells in the dermis of sleep-deprived mice significantly increased 14, 21, and 42 days after skin injury compared to well-rested animals. At that latest time points, sleep-deprived animals also displayed an infiltration of immune cells into the hindpaw adjacent muscle layers and a persistent edema, suggesting a spreading of the immune response. Conclusion: Our current data suggests preoperative sleep loss aggravates postoperative immune responses resulting in persistent postoperative incisional pain in mice.

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**Topic:** D.02. Somatosensation – Pain

**Support:** AA029925

**Title:** Alcohol consumption modulates the development of chronic pain in COVID-19 patients

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**Abstract:** Case reports have shown that a significant population of COVID-19 patients developed chronic pain. However, the mechanisms underlying the onset and progression of pain during COVID-19 are under studied. Using network meta-analysis, we previously demonstrated alcohol augmentation of COVID-19 pathologies. Previous reports suggest alcohol consumption exaggerates COVID-19 symptoms including severe cytokine storm and may even result in mortality. We and others have also reported that acute alcohol consumption produces analgesic effects while chronic alcohol consumption results in chronic pain and hyperalgesia. This study aimed to identify the influence of alcohol consumption and COVID-19 on pain including neuropathic pain and inflammatory pain. Using various GSE datasets, we identified the Differentially Expressed Genes (DEGs) in the Prefrontal Cortex (PFC), Amygdala (Amg), Choroid plexus (CP), and midbrain of COVID-19 patients. We employed QIAGEN Ingenuity Pathway Analysis (IPA) to identify the key signaling pathways, upstream regulators, and biological functions in the different brain areas of the COVID-19 patients. The canonical pathway analysis revealed the activation pathogen induced cytokine storm signaling pathway, S100 Family Signaling Pathway, IL-6 Signaling, neuroinflammation signaling pathway and neuropathic pain signaling in dorsal horn neurons in the studied brain regions. IPA's network builder was employed to construct a network map between ethanol (EtOH) and pain related entities including pain, neuropathic pain, and inflammatory pain. Simulation activation of EtOH, mimicking alcohol consumption was found to inhibit pain. To study the influence of COVID-19, we overlayed the DEGs from the PFC, Amg, CP, and midbrain onto these networks, mimicking alcohol consumption during SARS-CoV-2 infection. These resulted in the upregulation of neuropathic pain in the Amg, midbrain, CP as well as inflammatory pain in the PFC and CP. Here, we provide novel insights into the signaling pathways associated with chronic pain in COVID-19 patients. We found area specific changes in the modulation of pain in COVID-19 patients. Our results suggests that alcohol consumption directly inhibits pain (analgesic), however, alcohol consumption during the COVID-19 exaggerates impaired cytokine signaling, neuroinflammation and neuropathic pain signaling in the CNS and results in the development of chronic pain.

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**Topic:** D.02. Somatosensation – Pain

**Support:** Versus Arthritis UK Research Award grant n. 21961

**Title:** Imbalance of pro-resolving lipid mediator synthesis in macrophages exacerbates persistent inflammatory pain

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**Abstract:** Pain is a persistent symptom of rheumatoid arthritis, even when joint inflammation is controlled by anti-rheumatic drug treatment. To study mechanisms of arthritis pain, we utilise the K/BxN serum transfer (ST) model of inflammatory arthritis in which mice display ankle joint swelling and allodynia that peak at day 5 K/BxN ST, however, whilst joint swelling resolves by day 25 K/BxN ST, allodynia persists. We previously showed that at day 25 K/BxN ST dorsal root ganglia (DRG) accumulate M1-like proinflammatory macrophages ($MHCII^+$), and down-regulate pro-resolving lipid mediator Maresin 1 (MaR1) levels. Since MaR1 administration attenuates persistent allodynia we suggested that an imbalance of pro-resolving mechanisms within DRG contributes to persistent nociceptive signalling in inflammatory arthritis. Here, we deactivated lipid mediator synthesis in macrophages by silencing ALOX12/15 expression and therefore generating $CX3CR1^{Cre}$:12/15-$LOX^{flox/flox}$ mice and monitored for 25 days the progression of K/BxN ST- induced allodynia (in males and females; n=6-8/sex). We performed von Frey test in $CX3CR1^{Cre}$:12/15-LOX wild type (WT) and $CX3CR1^{Cre}$:12/15-$LOX^{flox/flox}$ (KO) and found that K/BxN ST- induced allodynia was exacerbated in KO compared to WT at day 25 (50% PWT: from 0.41 ± 0.2 g in WT to 0.11 ± 0.06 g in KO). At this timepoint, we compared DRG macrophage phenotype in KO and WT ($CD45^+F4/80^+$ cells) by flow cytometry and observed higher numbers of $MHCII^+$ macrophages (6775 ± 404 in KO vs 4245 ± 205 in WT) but less numerous $MHCII^+MertK^+$ macrophages in KO (860 ± 124 in KO vs 1365 ± 186 in WT). Since the presence of MerTK in macrophages is associated with pro-resolving mechanisms like efferocytosis, this data indicates that pro-resolving mechanisms are likely impaired in KO. Consistently with this, we detected lower MaR1 levels in KO compared to WT K/BxN ST DRG by ELISA (from 26 ± 4.4 pg/mL in WT to 9.6 ± 2.6 pg/mL in KO). We next characterised macrophage efferocytosis of neutrophils using bone marrow derived macrophages (BMDMs) isolated from WT and KO incubated with or without MaR1 (100 nM). We observed that i) neutrophils efferocytosis was impaired in KO derived BMDMs (from 26.5 ± 0.5% in WT to 19.2 ± 1.7% in KO), which expressed lower level of MertK (3898 ± 818 MFI units) than WT (5717 ± 442.0 MFI units),ii) MaR1 incubation increased MertK levels in KO BMDMs (5084 ± 481 MFI units) and restored efferocytic function to WT levels (24.94 ± 1.6%). Our data suggest that impairment of pro-resolving lipid mediator synthesis in macrophages results in macrophage polarization towards a pro-inflammatory phenotype that facilitates nociceptive signalling in DRG under inflammatory pain conditions.

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**Presentation Number:** NANO35.07

**Topic:** D.02. Somatosensation – Pain

**Title:** Spatial RNA Sequencing of Human Dorsal Root Ganglion and Spinal Cord Tissue Enables Discovery of New Druggable Targets for Chronic Pain

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**Abstract:** Chronic pain is a highly prevalent public health problem, but the development of new and effective analgesics has been particularly hindered by a failure in translating preclinical

animal research. The pain pathways involving the dorsal root ganglion (DRG) and spinal cord are crucial for our sensory system's ability to perceive and respond to painful stimuli. During chronic pain, changes in this system can lead to the persistence and amplification of pain signals. It is important to understand these changes in order to identify targets that will lead to better therapies. Despite substantial progress in our mechanistic understanding of nociceptive processing, species-dependent differences in the molecular phenotypes of nociceptors as well as limited access to human nervous tissues have impeded the identification and validation of novel, disease-specific therapeutics. And while high-throughput sequencing technologies, such as Illumina's NextSeq2000, have enabled and accelerated a more comprehensive elucidation of genetic and transcriptomic landscapes, the financial burden associated with sequencing remains prohibitively high. To overcome these challenges, we adopted a spatial sequencing approach to enable a comprehensive view of the transcriptional profile of human DRG and spinal cord at virtually single-cell resolution. Herein, we present an assessment of the recently introduced Ultima Genomics UG100 sequencing platform, which promises reduced operational costs while maintaining high resolution and fidelity. We used the 10x Visium Spatial Transcriptomics technology to prepare libraries from eight lumbar DRG and eight spinal cord tissues obtained from organ donors and then processed the libraries on both the NextSeq2000 and UG100 platforms. The objectives of our investigation were twofold: (1) to evaluate the cost efficiency of the UG100 platform relative to the widely adopted Illumina NextSeq2000 and (2) to understand the impact of UG100's extended read length on gene detection efficacy. Standard downstream processing was performed, resulting in approximately 100 million reads per library after quality control. We observed highly similar spatial clustering and a pseudo bulk Spearman correlation of >0.98 between the samples sequenced on the two platforms, thereby validating the fidelity of the UG100 platform. As expected, we identified distinct and detailed molecular profiles of both neuronal and non-neuronal clusters. This research has enabled us to identify within specific cell populations several novel, human-relevant targets that will serve as promising leads in the development of new and effective pain medications.

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**Topic:** D.02. Somatosensation – Pain

**Support:** Intramural Research Program of the National Heart, Lung, and Blood Institute, National Institutes of Health (HL005702-16)

**Title:** NGF-TrkA-PI3K signaling leads to sensory hypersensitivity in diabetic small fiber neuropathy

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**Abstract:** Diabetic patients often develop nerve damage and dysfunction in the skin, leading to burning or shooting pain called small fiber neuropathy. Patients with diabetic small fiber

neuropathy have abnormal hypersensitivity in sensory nerves in the skin. In addition to functional abnormalities, patients develop degeneration of axon terminals and vascular abnormalities in the skin. Although the symptoms of diabetic small fiber neuropathy are well characterized, the mechanism of etiology and therapeutic strategies remain elusive due to the lack of appropriate *in vivo* mouse models to examine sensory function and morphological defects in peripheral tissues. We combine *in vivo* live calcium imaging of the ear skin explant from *Pirt-GCaMP3* calcium indicator mice and high-resolution whole-mount imaging of the control and diet-induced obesity (DIO) ear skin. The DIO model leads to pre-type 2 diabetic phenotypes such as weight gain and high blood glucose levels from 18 weeks-of-age onward. The ear skins from the DIO mice at 18 weeks-of-age do not show any functional or morphological changes in sensory axons and blood vessels/capillaries. In contrast, the ear skins from the DIO mice at 22 weeks-of-age show hypersensitivity in response to capsaicin, a ligand for transient receptor potential vanilloid subtype 1 (TRPV1) channel, without any severe axon degeneration or blood vessel/capillary abnormality. At 30 weeks-of-age, the DIO skins show a significant reduction of sensory axon terminals in the epidermis, indicating that sensory axons undergo severe degeneration. Additionally, a vascular leakage marker, PLVAP, is detectable in endothelial cells of blood vessels/capillaries. These results clearly demonstrate that sensory hypersensitivity occurs prior to sensory axon degeneration and blood vessel/capillary abnormalities in the DIO skin. At a mechanistic level, the enhanced expression of nerve growth factor (NGF) is detectable in the epidermis of the DIO skin, which contains Tropomyosin receptor kinase $A^+$ ($TrkA^+$) sensory axon terminals. Interestingly, a short-term treatment of the DIO skin explant with anti-NGF neutralized antibody results in the suppression of capsaicin-mediated hypersensitivity. Moreover, Wortmannin as a selective inhibitor of phosphatidylinositol 3-kinase (PI3K) can suppress capsaicin-mediated hypersensitivity in the DIO skin. These results suggest that NGF-TrkA-PI3K signaling is important for sensory hypersensitivity through an increased sensitization of TRPV1. Taken together, a modulation of local NGF-TrkA-PI3K signaling could be an effective therapeutic strategy for diabetic small fiber neuropathy.

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**Presentation Number:** NANO35.09

**Topic:** D.02. Somatosensation – Pain

**Support:** NIH NINDS R01NS103974

**Title:** Understanding Post-transcriptional Mechanisms of Neuropathic Pain

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**Abstract:** Neuropathic pain is a chronic condition which can arise following damage to the somatosensory system and often involves both hyperalgesia and allodynia. The molecular mechanisms of neuropathic pain remain incompletely understood but require enduring alterations in specific gene programs and protein synthesis affecting neuronal signaling and excitability. Non-coding RNA regulatory pathways offer a post-transcriptional mechanism to coordinate programs of protein synthesis to produce long-term altered physiological responses. We investigate non-coding RNA and RNA-binding protein regulatory pathways in impacting

hyperalgesia and neuropathic pain using the mouse spared nerve injury (SNI) model. Nerve injury alters the expression of many miRNAs, including the highly conserved let-7 family miRNAs, which repress pro-growth mRNAs and are implicated in axon growth, neuronal plasticity, and brain circuit development. The Lin28 RNA binding protein can prevent maturation of let-7 precursor RNAs; consequently, increased Lin28 signaling can promote pro-growth gene expression by relieving let-7 mediated gene repression. The regulation and potential roles of Lin28/let-7 pathway in neuropathic pain remain largely unexplored. In preliminary data, we find that Lin28a loss of function in some, but not other, sensory neuron populations can result in a deficit in mechanical hypersensitivity post-injury. In the SNI mouse model, we evaluate molecular mechanisms with multiple approaches including a sensitive RNA imaging assay, RNAscope *in situ* hybridization (ISH), to allow mapping of the spatiotemporal patterns and cell type specificity of changes. We find that Lin28 mRNAs are elevated in classes of injured neurons relative to uninjured neurons in dorsal root ganglia (DRG) which are ipsilateral at early, 3 days, timepoints post SNI surgery. In contrast, Lin28 mRNAs are highly elevated following a more protracted time course post-surgery in the axonal compartment of injured neuronal processes. The spatiotemporal differences in Lin28 mRNA responses across compartments of the nervous system following nerve injury could indicate altered trafficking or local stabilization. Translation of Lin28 mRNA in neuronal processes, functional roles of the local Lin28/let-7 pool, and a genome-wide approach to determining the downstream mRNA targets of Lin28/let-7 pathway in injured sensory neurons are the subject of ongoing investigations.

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**Topic:** D.02. Somatosensation – Pain

**Support:** R35 DE030045

**Title:** Craniofacial neuropathic injury suppresses synaptic plasticity in the dorsal hippocampus of mice

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**Abstract:** Chronic pain is one of the most significant medical problems affecting almost one-third of the world population. Patients with chronic pain suffer from poor cognitive functions including memory. In patients with chronic back pain or trigeminal neuralgia, show reduced hippocampal volumes. In rodents with chronic pain in spinal regions, cognitive function is altered, which is accompanied by comprehensive changes in synaptic plasticity, connectivity, neurogenesis, and neuroinflammation in the hippocampus. However, mechanistic linkages between craniofacial neuropathic pain and hippocampal plasticity are not well understood. We investigated electrophysiological and neurochemical changes in dorsal hippocampus (dHC) following infraorbital nerve chronic constriction injury (ION-CCI) in mice. Field potential recordings in brain slices containing hippocampus showed that the field excitatory postsynaptic potential slope, population spike amplitude and long-term potentiation were significantly suppressed in dHC of ION-CCI group compared to the sham group. Paired pulse ratio was

significantly reduced in the ION-CCI group compared to the sham group, indicating that craniofacial neuropathy mediates the synaptic alteration through presynaptic mechanisms in the dHC. Next, we performed immunohistochemistry experiments to monitor neuroinflammatory response and neurogenesis using a marker for microglia (ionized calcium binding adapter molecule 1; Iba-1) and neurogenesis (doublecortin; DCX) in the dHC of ION-CCI mice. The number of DCX-positive neurons was significantly reduced in the dentate gyrus, whereas Iba1-expressing microglia were significantly greater in the dHC of ION-CCI group compared to sham group. To estimate the extent of neural activation within the hippocampus in vivo, we adopted Targeted Recombination in Active Populations (TRAP) methods. Adeno-associated virus encoding Cre-dependent mCherry was injected into the CA1 region of FosCreER mice. Two weeks after ION-CCI or sham surgery, tamoxifen injection was followed by facial brushing to TRAP hippocampal neurons. The number of mCherry-positive pyramidal neurons in the CA1 region was significantly lower in ION-CCI compared to sham. Our results showed that craniofacial neuropathic injury 1) reduced hippocampal activation by facial sensory stimuli, 2) suppressed synaptic activity and neuronal activity through presynaptic mechanisms in dHC, and 3) induced the inflammatory responses and suppressed adult neurogenesis in the dentate gyrus of dHC. Taken together, these changes in dHC may contribute to cognitive impairment following craniofacial nerve injury.

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**Topic:** D.02. Somatosensation – Pain

**Support:** NIH R01 NS105725

**Title:** Neuropathic pain in chronic SCI is mediated by CST-targeted spinal interneurons

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**Abstract:** Chronic neuropathic pain is one of the most persistent and debilitating outcomes of spinal cord injury (SCI), affecting up to 80% of individuals living with SCI. Post-injury pain, especially below-level pain, is refractory to clinical treatments due to a limited understanding of the brain-spinal cord circuits that underlie pain signal processing. Increasing evidence suggests that the descending corticospinal tract (CST) plays critical roles in sensory modulation during skilled movements and tactile sensation; however, a direct role for the CST in the development of SCI-associated neuropathic pain is unclear. Here we have found that complete, selective CST transection at the medullary pyramids (bilateral pyramidotomy) leads to hindlimb allodynia in chronically injured adult mice. Furthermore, c-fos immunostaining revealed neuronal hyperexcitability within lumbar deep dorsal horn elicited by innocuous hindlimb stimulation. Transsynaptic, anterograde viral transduction allowed us to identify CST-targeted spinal interneurons (CST-SINs) throughout different spinal laminae. Using intersectional viral transduction, we show that CST-SINs activation within laminae III-V by either chemogenetic or MAP kinase pathway activation induces tactile allodynia similar to chronic pyramidotomy. Allodynia depends on afferent input from the paw as it is temporally attenuated by injection of the local anesthetic bupivacaine into the hindpaw pad. To further elucidate the underlying circuit

mechanisms of chronic neuropathic pain in SCI, we are using *in vivo* multi-photon microscopy to visualize activity and structural changes of CST-SINs in longitudinal studies of chronic injury. These findings shed light on an unrecognized circuit mechanism implicated in SCI-induced neuropathic pain and provide a novel target for therapeutic intervention.

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**Nanosymposium**

**NANO36: Respiratory Rhythm**

**Location:** WCC 147A

**Time:** Monday, November 13, 2023, 8:00 AM - 10:15 AM

**Presentation Number:** NANO36.01

**Topic:** E.07.a. Cellular properties – Interneurons and motor neurons

**Support:** R00HL145004
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**Title:** Cellular and network properties in respiratory rhythm generation: an interdependent perspective

**Authors: *R. S. PHILLIPS**, N. A. BAERTSCH;
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**Abstract:** Inspiratory rhythmogenesis occurs in the preBötzinger complex (preBötC). Despite decades of research, the mechanism(s) of preBötC rhythmogenesis remain unresolved. The contemporary debate revolves around two competing conceptual ideas: the *pacemaker hypothesis* and *burstlet theory*. A small fraction of preBötC neurons exhibit intrinsic bursting, which depends on the voltage-dependent properties of a persistent sodium current ($I_{NaP}$). This inspired the *pacemaker hypothesis*, which posits that these intrinsically bursting 'pacemaker' neurons drive preBötC rhythmogenesis by initiating synchronized network activity. Alternatively, *burstlet theory* proposes that network rhythms are driven by weakly synchronized activity referred to as “burstlets” that result from feed-forward excitatory synaptic interactions among preBötC neurons with tonic spiking activity that emerges prior to inspiratory bursts, termed pre-inspiratory spiking. Central to this debate is the role of $I_{NaP}$. Although $I_{NaP}$ is widely expressed in the preBötC, *burstlet theory* considers it dispensable for rhythmogenesis. In contrast, the pacemaker hypothesis considers $I_{NaP}$-dependent intrinsic bursting essential. Consequently, the role(s) of $I_{NaP}$ and intrinsic bursting in preBötC rhythmogenesis have become conflated—an experimentally intractable problem. Using computational modeling, we find that intrinsic bursting is sensitive to an uncharacterized interaction between spike shape and the voltage-dependent properties of $I_{NaP}$. By exploiting this interaction in simulated preBötC networks, we find that rendering all neurons incapable of intrinsic bursting does not eliminate the rhythmogenic properties of the network. Instead, rhythmic activity continues that depends on network interactions among intrinsically tonic neurons and resembles “burstlets”. However, despite the lack of intrinsic bursting, rhythm generation remains dependent on $I_{NaP}$. When spike

shape variability is introduced, allowing a subset of neurons to exhibit intrinsic bursting, the relative prevalence of intrinsic bursting and pre-inspiratory spiking can shift dramatically in response to simulated changes in oxygenation, development, extracellular potassium, and temperature. These results align with many experimental benchmarks that support both the *pacemaker hypothesis* and *burstlet theory*. We propose a unifying hypothesis where intrinsic bursting and pre-inspiratory spiking are viewed as conditional phenotypes of preBötC neurons, whereas excitatory synaptic interactions and the voltage-dependent properties of $I_{NaP}$, represent interdependent features of preBötC rhythmogenesis.

**Disclosures: R.S. Phillips:** None. **N.A. Baertsch:** None.

**Presentation Number:** NANO36.02

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Title:** Revisiting the Roles of Persistent Sodium Current and $Na^+$-$K^+$ Pump Current in the Mammalian Respiratory Central Pattern Generator

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**Abstract:** A slowly-inactivating persistent $Na^+$ current (time constant ~ 2-10 s) has been proposed by many researchers in the field to be the main player in the generation of the respiratory rhythm in the ventral respiratory column (VRC) of mammals under normal conditions. Due to the exceptionally slow inactivation of this current, any attempts to measure the properties of this current by voltage-clamping require exceptionally long sustained voltage steps. We hypothesize that during long voltage steps like this, the effects of the $Na^+$-$K^+$-ATPase pump current will appear similar to the inactivation of a voltage-gated inward current and that the exceptionally slow inactivation that has been previously measured for persistent $Na^+$ current is generated by the activation of $Na^+$-$K^+$ pump current. Furthermore, we hypothesize that the $Na^+$-$K^+$ pump current plays a key role in the generation of the inspiratory bursting rhythm.We built a simplified model of a respiratory pacemaker neuron consisting of a *non-inactivating* persistent $Na^+$ current ($I_{NaP}$), a fast-inactivating $Na^+$ current, a delayed-rectifyer $K^+$ current and $Na^+$ and $K^+$ leak currents in addition to the $Na^+$-$K^+$ pump current ($I_{Pump}$). This model produces an inspiration-like bursting rhythm with a burst period of 4.8 s, a duty cycle of 0.07, and a mean spike frequency of 112 Hz. We found that the dynamics of $[Na^+]_i$ accounted for both initiation and termination of the inspiration-like bursts in this model cell.We constructed a model of a voltage-clamp experiment in order to see how pump current would present itself if one were attempting to measure the inactivation of persistent $Na^+$ current, despite that $I_{NaP}$ in our model is non-inactivating. All currents were blocked except for $I_{NaP}$ and $I_{Pump}$. Modeling a similar protocol to voltage-clamp experiments done previously, we measured the activation and inactivation curves. As expected, we found the voltage of half-activation ($V_{1/2mNaP}$ = -48.4 mV) and the steepness ($k_{mNaP}$ = -3.2 mV) were very close to the actual parameter values used by the model ($V_{1/2mNaP}$ = -50 mV and $k_{mNaP}$ = -3.5 mV). We found a traditional inactivation curve consistent in important ways with previous measurements in the VRC. We repeated this voltage-clamp procedure after removing pump current from the model, and the inactivation curve was no longer present. We conclude that the combination of a non-inactivating, persistent $Na^+$ current and the

$Na^+$-$K^+$ pump current can produce an inspiration-like rhythm. Furthermore, the slow dynamics of $[Na^+]_i$ controlled by the $Na^+$-$K^+$ pump can account for many experimental results previously attributed to some inactivation process inherent to the persistent $Na^+$ current.

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**Presentation Number:** NANO36.03

**Topic:** E.08. Respiratory Regulation

**Support:** NIH-NINDS Intramural Program

**Title:** Multidimensional structure of the rhythmogenic preBotzinger complex region reconstructed by two-photon laser microscopy and massively parallel deconvolution

**Authors: *N. KOSHIYA**, T. T. JOHN, H. KOIZUMI, Y. CHEN, R. ZHANG, J. C. SMITH; NIH - NINDS, Bethesda, MD

**Abstract:** Inspiratory rhythmic activity is generated in mammals within the preBötzinger complex (preBötC) region of the brainstem. Calcium imaging and electrophysiological studies have indicated that the excitatory neuron population crucial for rhythm generation is distributed within the reticular formation ventral to the nucleus ambiguus semicompacta (NAsc). We do not know, however, the 3D spatial layout of these rhythmic excitatory neurons, their relations to other cell types (+1=4D), and how this spatial organization may reflect their rhythmogenic temporal functions (+1=5D). We are imaging the activity and mapping locations of these neurons in rhythmically active neonatal medullary in vitro slices from a transgenic mouse line expressing the calcium-sensitive protein GCaMP6f in glutamatergic (VgluT2-expressing) neurons. In most preparations, tdTomato was concurrently expressed in VgluT2 neurons. The rhythmically active medullary slice (~400 µm thick) was cut so that the rhythmic neuron population was exposed on the caudal surface, and we systematically imaged the slice through this surface with two-photon laser scanning microscopy. XYT images were acquired [512 pixels (438 µm) square at ~28 fps for 4000 frames] through the depth of the slice (~300 µm from the caudal surface) for a Z stack to obtain an XYTZ hypercube (HC). This HC was acquired along with the hypoglossal nerve activity, a monitor of inspiratory circuit activity in the slice. To dissect the ~1-TB HC, we used NIH's massively parallel high-performance computer Biowulf. HC during the inspiratory phase was extracted and compared to the baseline (expiratory) HC to compute dynamic fluorescence ratios to identify the rhythmically active inspiratory neuron population. Active cells were computationally deconvoluted, segmented, and located in the XYZ coordinate space. Non-glutamatergic neurons in HC were located as tdTomato-negative cells. The set of inspiratory cells located in HC were widely distributed in the region ventromedial to NAsc. The current results indicate that our transgenic mouse line, and our dynamic imaging and parallel computational deconvolution approach should allow a multidimensional reconstruction of the inspiratory preBötC excitatory neuron population and other active neuronal populations.

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**Topic:** E.08. Respiratory Regulation

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**Title:** Inhibition drives latent neural dynamics of the Ventral Respiratory Column into an expiratory attractor to pace breathing

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**Abstract:** The vital motor behavior of breathing is generated, patterned, and maintained by populations of neurons distributed throughout the Ventral Respiratory Column (VRC). These populations exhibit diverse transcriptional profiles and respiratory-related activities. Our recent work shows that the network-wide dynamics of VRC populations follow continuous rotational trajectories in a low-dimensional latent neural space. Importantly, these trajectories target a stable, attractor like region of the latent space that corresponds to the offset of inspiration. Here we test the hypothesis that inspiration off is an attractor in the latent space of VRC activity, and that inhibitory signaling drives the latent trajectory into this inspiratory-off attractor. We record along the rostrocaudal extent of the VRC using high-density Neuropixel probes in-vivo in anesthetized mice while optogenetically stimulating inhibitory (Vgat+) or excitatory (DBX1+) brainstem populations. Stimulation of both excitatory and inhibitory populations cause both widespread increases and decreases of firing rates, irrespective of the population stimulated. Our lab previously showed a phase-dependent effect of inhibitory stimulations: stimulation of inhibitory neurons during expiration slows breathing, while stimulation during inspiration speeds breathing (Baertsch et al. 2018). We find that brainstem wide stimulation of inhibitory neurons drives latent trajectories towards the inspiration-off attractor, regardless of trajectory location at the time of stimulation. Stimulation of excitatory populations, in contrast, directs the trajectories to a different region near the center of the rotation, and only perturbs the latent trajectories during pre-inspiratory periods. Upon cessation of excitatory stimulation, latent trajectories relax to the closest point along the rotational manifold, and the cycle resumes. We further use dynamical systems modeling (recurrent linear dynamical systems - rSLDS Lindermann et al. 2017) to show that the inhibitory stimulations drive latent trajectories into a line attractor like energy minimum. We find that the location in which the latent trajectory enters this line attractor well predicts the time until the next breath. Together, these data elucidate a population dynamics level mechanism in-vivo for respiratory pacing via inhibitory signaling that is encapsulated in simple piecewise linear dynamical systems.

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**Topic:** E.08. Respiratory Regulation

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**Title:** Tachykinin-1-expressing neurons mediate opioid-induced respiratory depression

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**Abstract:** Opioids produce strong analgesic effects; however, their therapeutic benefit is limited by their actions on mu-opioid receptors (MORs) in brainstem regions that control breathing, leading to respiratory depression, a slow and shallow breathing pattern that can result in death during an overdose. Although the neuronal populations mediating these effects remain unknown, previous studies have found that loss of tachykinin-1 (*Tac1*, the gene encoding the neuropeptide substance P) gene expression improves analgesia while reducing respiratory side-effects of opioids. Thus, we propose that *Tac1*-expressing neurons mediate the respiratory depressive effects of opioids and that knocking out MORs from these cells will prevent respiratory depression by the opioid fentanyl. To this aim, we generated transgenic knockout mice that lacked functional MORs (encoded by the *Oprm1* gene) in *Tac1*-expressing neurons (*Tac1-Oprm1*$^{-/-}$) by crossing *Oprm1*$^{fl/fl}$ mice with *Tac1*-Cre (control) mice. Respiratory responses were recorded in awake mice using whole-body plethysmography and respiratory depression was induced by a single intraperitoneal injection of fentanyl (0.3mg/kg). Responses were compared to saline injection. We found that systemic fentanyl injection resulted in a significant decline in respiratory rate in control ($P = 0.0006$) but not *Tac1-Oprm1*$^{-/-}$ mice ($P = 0.0791$), compared to saline. Additionally, compared to saline, tidal volume was significantly increased ($P = 0.0027$) while minute ventilation decreased ($P = 0.0483$) in control mice in response to fentanyl. No significant differences were found in tidal volume ($P = 0.9436$) or minute ventilation ($P = 0.4586$) in *Tac1-Oprm1*$^{-/-}$ mice between saline and fentanyl treatments. Taken together, our results show that the absence of functional MORs in *Tac1*-expressing neurons alleviates respiratory depression by the opioid fentanyl, suggesting that these cells may mediate the respiratory side-effects of opioids.

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**Topic:** E.08. Respiratory Regulation

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**Title:** Dysfunction of dB2 neuron activity causes hypoventilation

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**Abstract:** Breathing originates from complex networks of medullary neurons that generate the respiratory rhythm, provide modulatory input, and monitor tissue gas levels. Genetic perturbations contribute to the onset of respiratory disorders, but the dysfunctional circuits associated with them are frequently unknown. Congenital Central Hypoventilation Syndrome (CCHS) is a life-threatening respiratory disorder that is characterized by pronounced hypoventilation, central apnea and diminished chemoreflexes. Mutations in the transcription factors *PHOX2B* or *LBX1* correlate with CCHS, but the affected neurons responsible for this disease are unknown. Here, I will show that distinct, and previously undescribed, sets of medullary neurons co-expressing both factors (dB2 neurons) account for specific respiratory functions and phenotypes seen in CCHS. Our workuncovers specific subgroups of dB2 neurons with key functions in i) respiratory tidal volumes, ii) the hypercarbic reflex, iii) neonatal respiratory stability, and iv) neonatal survival. These data provide functional evidence for the critical role of medullary dB2 neurons in neonatal respiratory physiology and establish dB2 neuron dysfunction as causative of CCHS.

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CAPES

**Title:** Respiratory responses to hypercapnia/acidosis in the working heart brainstem preparation of mice previously submitted to sustained hypoxia

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**Abstract:** Sustained hypoxia (SH) change $CO_2/[H^+]$ sensitivity of the central chemoreception sensitivity and increase the sympathetic-respiratory coupling in rats. The effects of $CO_2/[H^+]$ on the respiratory pattern of mice submitted to SH were not yet evaluated. Herein we evaluated the pattern of respiratory activity during hypercapnia/acidosis challenge in the *in situ* working heart-brainstem preparation (WHBP) of mice previously submitted to SH. To reach this goal, we evaluated the impact of 24-hour SH exposure on respiratory and autonomic parameters in baseline conditions and in response to central chemoreflex activation. C57BL/6J mice (6-8 weeks old, ~25g) were maintained in normoxia or submitted to SH protocol (24h, $FiO_2$ 0.1). After the protocols, in the *in situ* WHBP we recorded phrenic (PN), abdominal, (AbN), cervical vagus (cVN) and thoracic sympathetic (tSN) nerves activities of control and SH mice. The percentage of $CO_2$ in the perfusate was increased from 5% (baseline) to 7% and then to 10%. All experimental protocols were approved by the institutional ethical committee (CEUA/FMRP-USP #076/2021). The frequency of PN discharge was reduced in both hypercapnic challenges in relation to baseline in control group (n=11; 0.64±0.2 vs 0.80±0.3 vs 1.39±0.4Hz, P=0,0001; P=0,0023). Baseline frequency of PN discharge of SH group (n=12) was lower in relation to

control group [n=11, (0.98±0.3 vs 1.39±0.4Hz, P=0.03), but hypercapnic challenges produced no changes in this parameter of the SH group [n=12, (0.79±0.1 vs 0.80±0.3; 0.67±0.2 vs 0.64±0.2Hz). Baseline incidence of Late-E events (active expiration) of SH group was higher than in the control group (59.9±39.5 vs 0±0%, P<0.0001). Hypercapnia (10 and 7%) produced a significant increase in Late-E incidence in the control (97.1±4.0 vs 69.9±27.8 vs 0.0±0%, P<0.0001) and SH (94.9±10.7 vs 83.2±17.9 vs 59.9±39.5%, P<0.0001) in relation to the respective baseline condition (5%). Hypercapnia also increased the duration of expiration in the control group (1.35±0.8 vs 1.37±0.9 vs 0.59±0.2s, P<0,0001) as well as the duration of cVN post-inspiratory activity (0.93±0.6 vs 1.06±0.8 vs 0.43±0.2s, P=0,0244; P=0,0031), but not in the SH group. The changes in the breathing pattern produced by hypercapnia/acidosis in control mice were similar to that observed in SH mice under baseline condition, and the breathing pattern of SH mice was not affected by different levels of hypercapnia/acidosis. We suggest that the changes in the breathing pattern of SH mice is due to sensitization of central $CO_2/[H^+]$ sensors.

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**Presentation Number:** NANO36.08

**Topic:** E.08. Respiratory Regulation

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**Title:** Opto- and chemogenetic dissection of neural circuitry involved in seizure-induced apnea

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**Abstract:** Sudden Unexpected Death in Epilepsy (SUDEP) is defined as the sudden, unexpected, and unexplained death of a person with epilepsy and accounts for between 8 and 17% of epilepsy-related deaths, rising to 50% for patients with refractory epilepsy. In a mouse model of SUDEP, we have recently shown that death is due to seizure-induced respiratory arrest and tonic respiratory muscle contraction is a possible mechanism of apnea. In the present study, we explore 1) whether tonic activity of the inspiratory rhythm generator in the brainstem and/or 2) upper motor neuron activity in the motor cortex drives ictal apnea. Audiogenic seizures were induced ino mice carrying the human *SCN8A* encephalopathy mutation p.Asn1768Asp (N1768D; "D/+ mice") using a 15 kHz pure tone. Video, electroencephalogram (EEG), electrocardiogram (ECG), and breathing via whole body plethysmography were recorded during seizures. To test the necessity of ictal activity from upper motor neurons in the motor cortex are required for generating ictal apnea, we expressed iDREADD receptors in cortical excitatory neurons of D/+ mice and injected CNO i.p. prior to inducing seizures with a 15 kHz pure. Under control conditions, seizures presented with the usual tonic phase apnea and spike wave discharges (SWDs) in the motor cortex. CNO administration robustly inhibited the SWDs, but the tonic

phase and apnea were not affected. The effect of all doses of CNO on ECoG power was significantly greater than the effect on apnea ($p = 0.0001$, 2-way ANOVA). We implanted fiberoptic ferrules bilaterally into the Bötzinger Complex (BötC) of mice that express Channelrhodopsin2 (ChR2) under the vesicular GABA transporter (VGAT) that were crossed with D/+ mice. The goal of the experiment is to photostimulate BötC during ictal apnea to inhibit tonic inspiratory activity and produce expiration. Seizures were evoked using a 15 kHz pure tone. Trains of light pulses (50 ms pulses, 5 mW of 473 nm light) were evoked repetitively during ictal apnea. However, this did not recover normal breathing rhythm and apnea duration was no different for any photostimulation paradigm versus control ($p = 0.7892$, $F = 0.1747$, One-Way ANOVA). Although breathing was not affected during seizures, the effects on baseline breathing were substantial; for example, inspiration was inhibited for a full 10 s photostimulation train. We found that the core inspiratory oscillator circuitry in the brainstem is likely bypassed to create tonic inspiratory activity. Furthermore, inhibition of cortical upper motor neurons has no effect on apnea. Thus, our interpretation is that other pools of upper motor neurons must drive the tonic inspiratory activity and apnea.

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**Topic:** E.08. Respiratory Regulation

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**Title:** Persistent Dyspnea: Insights from Invasive Human Recordings of Respiratory Related Brain Oscillations during Respiratory Challenges

**Authors:** D. MIKHAEL[1], S. BICKEL[1,2,3], A. D. MEHTA[1,3,2], N. MARKOVITZ[1], H. GREENBERG[4], T. SIMILOWSKI[5], ***J. HERRERO RUBIO**[1,2,3];
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**Abstract:** In the past three years, the world has experienced rapid and unforeseen transformations. One important change has been the astonishing increase in the incidence of respiratory diseases, ranging from acute (e.g., COVID-19 ARDS) to more chronic syndromes related to higher levels of pollutants/allergens (i.e., asthma, COPD) and other factors (i.e., long-term COVID, opioid-induced respiratory depression, obese respiratory disorder). Pulmonary rehab does not always alleviate the symptoms of dyspnea (persistent breathlessness) and current drug treatments that target the brain (e.g., opiates) have side effects such us respiratory depression and dependence. The aim here is to better understand the way the brain processes these ascending respiratory signals. To evaluate this, we recorded respiratory related brain oscillations (RRBOs) in 5 epilepsy patients implanted with intracranial electrodes (iEEG) in cortical and subcortical areas during a task that induced dyspnea (breathlessness). Our preliminary findings revealed new insights into the brain's response to respiratory challenges. When the airways were partially obstructed by the experimental loads, we observed increased

RRBOs in the olfactory and cingulate cortices. On the other hand, the (pre)motor cortex exhibited comparable increases during both load and non-load trials. These observations indicate that the premotor cortex may play a pivotal role in monitoring the current behavioral state ("Am I breathing now?"), while the olfactory and cingulate cortices may encode the current effort ("How hard is it to breathe now?"). These results align with previous non-invasive studies that identified dyspnea-related signals in the motor and cingulate cortices. Moreover, our findings suggest that distinct brain areas are engaged in the motor awareness vs. the effort associated with breathing. This nuanced understanding of how different cortical regions process respiratory sensations holds significant implications for comprehending the underlying mechanisms and management of breathlessness

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**Nanosymposium**

**NANO37: Control of Ingestion**

**Location:** WCC 143

**Time:** Monday, November 13, 2023, 8:00 AM - 9:30 AM

**Presentation Number:** NANO37.01

**Topic:** F.08. Food and Water Intake and Energy Balance

**Support:** HHMI
NIH R01-DK106399

**Title:** Sequential suppression of feeding by oral and visceral feedback to brainstem

**Authors: *D. T. LY**[1], J. OH[3], N. SIVAKUMAR[4], S. SHEHATA[3], N. LA SANTA MEDINA[3], H. HUANG[3], W. FANG[3], C. BARNES[3], Z. LIU[5], N. DUNDAR[3], B. C. JARVIE[3], A. RAVI[3], O. BARNHILL[2], C. LI[3], J. CHOI[3], H. JANG[3], Z. KNIGHT[3];
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**Abstract:** The termination of a meal is controlled by dedicated neural circuits in the caudal brainstem. A key challenge is to understand how these circuits transform the sensory signals generated during feeding into the dynamic control of behavior. The caudal nucleus of the solitary tract (cNTS) is the first site in the brain where meal-related signals are sensed and integrated, but how the cNTS processes ingestive feedback during behavior is unknown. Here we describe the natural dynamics of two key cNTS cell types that promote satiety while awake mice eat a meal. We find unexpectedly that one cell type exhibits time-locked activation during ingestion that is driven by the taste of food. Closed-loop optogenetic manipulations demonstrate that these cells specifically control the duration of seconds-timescale feeding bouts, revealing a mechanism by which gustatory cues feed back to restrain the pace of ingestion. In contrast, we show that intermingled neurons are activated by mechanical feedback from the gut; encode the cumulative

amount of food consumed in the magnitude and duration of their activation; and promote satiety that lasts for tens of minutes. These findings expose an organizational logic for the negative feedback control of ingestion, whereby signals from the mouth and gut engage distinct circuits in the brainstem, which in turn control elements of feeding behavior operating on short and long timescales.

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**Presentation Number:** NANO37.02

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NIH Grant R01DK106476

**Title:** Microglia mediate the central effects of maternal high fat during lactation exposure in offspring

**Authors: *H. MENDOZA-ROMERO**[1,2], M. BEDENBAUGH[2], R. B. SIMERLY[2];
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**Abstract:** Obesity can be caused by environmental influences that occur early in life, specifically over-nutrition, which dictates susceptibility to weight gain through developmental programming. Placing mouse dams on a high fat diet during the lactation period (MHFD-L) negatively affects body weight and perturbs the formation of hypothalamic circuits that contribute to food intake in offspring (Vogt 2014). Agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARH) respond to changes in multiple metabolic signals and distribute neuroendocrine information to other brain regions, such as the paraventricular hypothalamic nucleus (PVH). Establishment of axon terminals from AgRP neurons to their targets occurs during the lactation period and these projections are reduced in MHFD-L offspring. Underlying developmental mechanisms remain largely undefined. Microglia are the resident immune cells of the central nervous system that are involved in refinement of neural connections. Since high fat diet exposure activates microglia in adults, we hypothesized that they are activated in offspring exposed to MHFD-L and mediate development of hypothalamic feeding circuitry. Previously, we reported that microglia actively engulf AgRP terminals in the PVH, and that MHFD-L results in larger microglia with enhanced process length and complexity. To determine if microglia are required for the effects of MHFD-L on AgRP innervation of the PVH, microglia were globally depleted using daily intraperitoneal injections of the colony-stimulating factor 1 receptor inhibitor, PLX5622, during the lactation period. PLX5622 injections reduced the density of microglia in the hypothalamus by approximately 40% and body weight was significantly reduced in MHFD-L mice at weaning compared with that of controls. Body weight remained lower in the PLX5622 treated mice in adulthood and the density of AgRP labeled fibers in the PVH appeared to be partially restored in MHFD-L offspring treated with PLX5622, suggesting that reduction of microglia specifically during the lactation period is sufficient to exert a sustained impact on body weight and AgRP innervation. Together, these findings suggest that microglia are activated by exposure to MHFD-L and may interact

directly with AgRP axons during development to permanently alter their density, with implications for neurodevelopmental programming of metabolic phenotype.

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**Topic:** F.08. Food and Water Intake and Energy Balance

**Support:** DK120869

**Title:** Neurovascular plasticity, a dynamic regulator of homeostatic brain functions.Neurovascular plasticity, a dynamic regulator of homeostatic brain functions.Neurovascular plasticity, a dynamic regulator of homeostatic brain functions.Neurovascular plasticity, a dynamic regulator of homeostatic brain functions.

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**Abstract:** The brain is densely perfused by an intricate network of blood vessels that supports the metabolism of neural cells. The current view is that the neurovascular system is a rigid and static “transportation network” which functions only to deliver signalling molecules and/or nutrients from the periphery to the brain. Still, this view rests on little data as most of the brain’s neurovasculature has been inaccessible to experiment. We have overcome this technical hurdle by using the cutting edge tandem iDISCO+/CLEARMAP. This technology allowed us to generate whole-brain maps of vascular structure in an unbiased manner. In brief, we obtained compelling preliminary data demonstrating, that homeostatic perturbations (in this case metabolic shifts) induce neurovascular plasticity. We detected a reorganization of the cerebral vasculature defined by increased vascularization in regions that control energy balance. Beyond these regions, we also quantified decreased vascular densities in cortical regions (important for cognition) and hypervascularization in the lateral septum (vital for stress control), suggesting that shifts in the nutritional environment have a broad impact on central circuits. Structural changes in the neurovasculature can directly affect its function. A unique feature of the neurovasculature is the presence of a tight blood brain barrier (BBB), except in circumventricular organs (CVO). Intriguingly, upon obesity, the BBB is leaky in sites where neurovascular rewiring exists (both CVO and not). Notably, a leaky BBB could have huge implications for brain physiology. For instance, the new nutritional environment, derived from a chronic exposure to a high caloric diet, could impact the composition of molecules travelling into the brain parenchyma which in turn could engage in new patterns of neuronal activity. Together, our data constitutes the stepping stones of a new niche capable of revisiting the holistic view of brain physiology. On our next set of studies, we will aim at understanding which molecular pathways are responsible for the identified alterations in neurovascular topology/function upon obesity by generating the transcriptomic profile (scRNAseq) of all the different cell types populating the neurovascular unit in health a disease.

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**Title:** The bed nuclei of the stria terminalis melanocortin 3 receptor network has sexually dimorphic effects on feeding and stress but not anxiety-like behaviors

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**Abstract:** While there is substantial evidence that neural systems controlling feeding and stress responses are linked, the organization of underlying circuitry, and potential differences between sexes, remain poorly understood. Our previous work found the melanocortin 3 receptor (MC3R) is ideally positioned to mediate direct communication between feeding and anxiety circuits and may do so differently in males and females. Here, the activity of MC3R neurons located in the bed nuclei of the stria terminalis (BST) during feeding, stress and anxiety-like behavioral paradigms was investigated. First, GCaMP-based fiber photometry was used to record *in vivo* calcium dynamics of $BST^{MC3R}$ neurons. Only females exhibited increased frequency of calcium transients when approaching normal chow or high fat diet food after fasting. Both sexes exhibited increased calcium transients in response to restraint stress with males displaying an enhanced event frequency, compared with females. No changes in calcium transients were found in either sex in response to a novel object, the OFT or the NSFT. Activating $BST^{MC3R}$ neurons chemogenetically caused significant decreases in acute feeding bouts in males; more modest effects were recorded in females. $BST^{MC3R}$ neuronal activation significantly reduced food intake in fasted male, but not female, mice. Moreover, activation of $BST^{MC3R}$ neurons significantly decreased struggling bouts in both sexes during restraint, but increased food intake after restraint stress in males only. $BST^{MC3R}$ neuronal activation did not affect responses in the OFT, EPM or NSFT. To identify possible downstream targets of $BST^{MC3R}$ neurons, anterograde tracing was used. The results indicate that $BST^{MC3R}$ neurons send direct inputs to several areas known to drive feeding and stress responses, including the ACB, LHA, VTA and PAG, however, no apparent sex differences were detected. Finally, the distribution of cells providing direct inputs to $BST^{MC3R}$ neurons was mapped using monosynaptic tracing, lightsheet imaging, and cell registration to the Allen Brain Atlas. Neurons that provide direct inputs to $BST^{MC3R}$ neurons were identified in multiple areas known to convey autonomic, emotional and neuroendocrine signals to the BST, including the MEA, SUBv, PAG and PFC. Preliminary brain-wide quantitative comparisons suggest several regions where sexual dimorphism exists. These results, together with the functional studies outlined above, suggest $BST^{MC3R}$ neurons play a key role in modulating feeding behaviors and responses to stressful events, and that they do so differently in males and females.

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**Title:** Preoptic area leptin receptor (POA-Lepr) neurons mediate temperature-dependent food intake adaptations via intersection with the melanocortin pathway.

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**Abstract:** Ambient temperature is a robust modulator of food intake. However, the hypothalamic mechanisms by which ambient temperature influences food intake are not fully understood. Previous work from our lab has demonstrated that glutamatergic $POA^{Lepr}$ neurons are activated by warm ambient temperature, and chemogenetic activation of these neurons decreases energy expenditure in a temperature dependent manner (Yu et al., 2016). Stimulation of $POA^{Lepr}$ neurons also suppresses food intake, suggesting that POA-Lepr neurons may also drive temperature dependent adjustments of food intake, even though the exact circuits to mediate POA-Lepr suppression of feeding remains unclear. The melanocortin energy-sensing pathway, involving the competing actions of Pro-opiomelanocortin (POMC) and Agouti-Related Protein (AgRP) at melanocortin-4 receptor (MC4R) expressing neurons, plays a central role in maintaining body weight homeostasis, however the relationship between this pathway and thermoregulatory POA circuits has not been fully explored. Here, we hypothesized that warm temperature-suppressed feeding is mediated by warm-activated glutamatergic POA-Lepr neurons that integrate into melanocortin circuitry.
We used a combination of synthetic and physiological POA-Lepr activation with chemogenetics or ambient temperature changes (30°C, 10°C), respectively, to test temperature-dependence of food intake suppression. We show that CNO suppressed food intake robustly at 10°C (when POA-Lepr are physiologically inactive) but was significantly blunted at 30°C (when POA-Lepr physiologically activated). Together these data support that glutamatergic POA-Lepr neurons mediate temperature-dependent food intake. Furthermore, anterograde tracing with reporter-expressing AAV supported POA-Lepr innervation of key regions in the melanocortin pathway, such as the ventral dorsomedial hypothalamus, the arcuate nucleus, and the paraventricular nucleus. Similar to what is seen with MC4R activation (Keen-Rhinehart et al., 2007), POA-Lepr activation results in a profound suppression of food intake during refeeding. Further, MC4R activation with melanotan-II (MTII) also showed temperature-dependent food intake suppression, and combined MTII/CNO treatment has no additional effect on food intake. These data indicate that POA-Lepr neurons may suppress food intake via MC4R activation at warm ambient temperature, even though it remains unknown if this connection is direct or indirect. These hypothalamic circuits potentially represent novel targets for the modulation of appetite.

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**Title:** Functional mapping neural projections from Ventral premammillary neurons to the lateral septum

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**Abstract:** Feeding behavior is dynamically regulated by homeostatic internal hunger drive and emotional states as well as environmental threatening cues. Indeed, recent studies suggest that brain structures known to regulate emotional states and stress behaviors also regulate feeding. Our recent studies demonstrate that the lateral septum (LS), known to regulate anxiety, aggression and memory, functions downstream of the melanocortin pathway and play a bona-fide role in feeding and body weight. To identify additional inputs to the LS, we used retrograde viral tracing and found that the ventral premammillary nucleus (PMv) as one prominent site projecting to the LS. The PMv contains two major groups of neurons marked by the expression of dopamine transporter (DAT) and leptin receptors (LepR), and is involved in mediating social attacking behaviors and endocrine reproduction functions. In this study, we aimed to functionally map the projection from the PMv to the LS. With retrograde AAV tracing, we found that PMv LepR neurons but not DAT neurons provide the major projections to the LSv. By using channelrhodopsin (ChR2) assisted circuit mapping, we identified that $PMV^{LepR}$ neurons provide monosynaptic glutamatergic projections to LSv. In vivo photo-stimulation of ChR2 positive $PMV^{LepR}$-LSv terminals produced negative valence in real-time place preference tests. Interestingly, photo-stimulation protocol either promoted or suppressed feeding following overnight fasting, which depended on the stimulation frequency and duration. $PMV^{LepR}$ projected LSv neurons exhibited an increase in activity in response to various stressful stimuli, as revealed by fiber photometry recordings with GCaMP. Furthermore, feeding reduced $PMV^{LepR}$ -> LSv activity, whereas photo-stimulation of ChR2 positive $PMV^{LepR}$ -> LSv increased its activity. Taken together, our data revealed a novel neural circuit from the PMv to the LS that orchestrates behaviors related to emotional states for feeding regulation.

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**Topic:** F.08. Food and Water Intake and Energy Balance

**Title:** A novel deep cerebellum nuclei neural circuit regulates body weight

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**Abstract:** The deep cerebellar nuclei (DCN) play a crucial role in motor coordination and learning within the cerebellum, but their direct involvement in body weight control is not well-established. The cerebellum, including the DCN, has traditionally been associated with motor functions, but emerging evidence suggests that it may also have non-motor functions, including involvement in cognitive and emotional processes. To this end, we used *ex vivo* electrophysiology to record the activity changes of Purkinje neurons after wheel running. We found that the Purkinje neural activity was reduced by wheel running. Next, we injected retrograde Cre-dependent AAV-Ef1a-DIO-EYFP vectors into the fastigial nucleus (FN) region of Pcp2-Cre/Rosa26-LTL-tdTOMATO mice and found that most Purkinje neurons from cerebellar IV/V lobe send projection to the FN. Further, we directly inject Cre-dependent retrograde AAV-hSyn-DIO-hM3D(Gq)-mCherry or AAV-hSyn-DIO-hM4D(Gi)-mCherry vectors into FN. When blocking the specific neural circuit from $Pcp2^{IV/V}$-FN by CNO, animals showed increased voluntary wheel running with increased energy expenditure when compared to saline injection. However, activation of $Pcp2^{IV/V}$-FN neural circuit caused decreased voluntary wheel running with decreased energy expenditure. Chronic blocking $Pcp2^{IV/V}$ neuronal activity by overexpression of Kir 2.1 channel caused decreased body weight gain when mice are fed with high-fat diet. In summary, these findings provide evidence to support the vital role of the $Pcp2^{IV/V}$-FN neural circuit in the beneficial metabolic effects of voluntary locomotion activity.

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**Nanosymposium**

**NANO38: Opioids: Circuitry, Neurophysiology, and Addiction**

**Location:** WCC 201

**Time:** Monday, November 13, 2023, 8:00 AM - 10:00 AM

**Presentation Number:** NANO38.01

**Topic:** G.09. Drugs of Abuse and Addiction

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**Title:** Sex- and time-dependent dysfunction of cortico-striatal circuits underlying opioid self-administration

**Authors: *L. KOUTAH**;
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**Abstract:** Sex- and time-dependent dysfunction of cortico-striatal circuits underlying opioid self-administrationKoutah L, Ouimet A, Czyz A, Salma Elsherbiny, Hearing MC
Canonical thinking in the addiction field posits that early drug use is controlled and mediated by goal-directed circuits such as the prefrontal cortex. With extended use, drug taking is thought to

become more reliant on cortico-striatal habit-associated circuits, however this has not been empirically demonstrated with opioids. We recently found that self-administration of remifentanil promotes a progressive hypoactive state in the prelimbic cortical region of the mouse PFC (PrLC) that underlies impaired decision-making, develops faster in females, and aligns with an escalation of drug intake. The present study sought to examine whether 1) with increased exposure, the PrLC becomes less involved while anterior dorsolateral striatum (aDLS) control of drug intake increases, 2) if this phenomenon occurs faster in female mice, 3) behavioral disruptions align with temporally distinct plasticity in the PrLC and aDLS, and 4) effects of PrLC or aDLS inhibition are selective for drug rewards. To do so, we virally expressed the inhibitory hM4Di DREADD or mcherry control in the PrLC (AAV8-CaMKII-hM4Di) or aDLS (AAV5-CAG-hM4Di) of male and female C57bl6/j mice. Mice intravenously self-administered (SA) remifentanil (5ug/kg/infusion) or orally administer a liquid reward (50% Ensure) for up to 60 days (2h/day/session), with clozapine-n-oxide administered to all mice (2.0 mg/kg; i.p.) on day 15, 30, 45, or 60 with drug available during all sessions. Initial findings show that inhibition of the PrLC reduced drug intake in females ($n=17$; $p<0.01$) and males ($n= 20$; $p<0.001$) on day 15, whereas intake was reduced in males on day 30 ($n=5$, $p=0.02$) but not females ($n=6$, 0.13). Alternatively, inhibition of the PrLC reduced consumption of Ensure in males ($p<0.001$) but not females on day 14, with no effect at 30 days. In contrast to the PrLC, inhibition of the aDLS does not reduce intake on day 15 in males ($n=7$) or females ($n=8$), with preliminary data highlighting a greater reduction at day 30 in females vs males. These data highlight a time- and sex-specific role for the PrLC and aDLS in driving opioid vs appetitive reward taking. Ongoing studies will examine effects at more protracted timepoints and use slice physiology to identify time- and sex-specific plasticity in these regions.

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**Title:** Adolescent social isolation stress disrupts transcriptional homeostasis in brain reward circuitry, resulting in increased vulnerability to heroin exposure

**Authors: *A. CHENG**[1], B. E. GEORGE[2], C. DELANEY[1], S. KIM[1], A. SANCHEZ[1], S. R. JONES[3], K. R. MECKEL[1];
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**Abstract:** Opioid use disorder is a public health crisis, which results in profound morbidity and mortality. Human literature suggests that individuals who experience chronic psychosocial stress

during adolescence exhibit increased vulnerability to developing substance use disorders during adulthood. Additionally, past studies have demonstrated that rats that underwent chronic adolescent social isolation show increased cocaine and alcohol-seeking behaviors compared to group-housed control rats. Given the rising rates of opioid overdose fatalities following the social isolation stress of the COVID-19 pandemic, the present study sought to investigate the molecular changes behind early social isolation stress as a risk factor for developing opioid use disorder, using a rodent model.
Adolescent Long Evans rats underwent 6 weeks of social isolation or group housing (4/cage), beginning on postnatal day 21 (females) or postnatal day 28 (males). Subjects received a series of escalating doses of heroin as follows: Day 1 (2.5 mg/kg, 3 times daily), Day 2 (2.5 mg/kg, 3 times daily), Day 3 (5 mg/kg, 3 times daily), Day 4 (5 mg/kg, 3 times daily). Subjects were sacrificed 36 hours later. Brains were rapidly dissected and flash frozen. Nucleus Accumbens tissue was dissected, and RNA was isolated for RNA-sequencing analysis. Additional tissue from the medial prefrontal cortex, basolateral amygdala, lateral hypothalamus, and hippocampus was rapidly dissected, and RNA was isolated for quantitative real-time PCR analysis. Studies are underway to determine how adolescent social isolation may disrupt transcriptional homeostasis across brain reward and stress circuitry, potentially leaving socially adolescents vulnerable to developing addiction-like behaviors in adulthood.

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**Title:** Investigating the Role of Opioid Signaling on Innate Immune Activation in HIV-infected Microglia

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**Abstract:** People living with HIV-1 and chronically using opioids have elevated risks of developing HIV Associated Neurological Disorders (HAND) than those not using opioids. HAND is a spectrum of neurocognitive deficits often marked by chronic inflammation in the brain. Therefore, it is essential to understand downstream events of opioid signaling contributing to increased inflammation in the brain. Microglia, innate immune cells in the brain, are key when studying HAND as they are infected by HIV-1, and are regulators of neuroinflammation. Our group has previously shown that HIV-1 infection of induced pluripotent stem cell (iPSC)-derived microglia (iMGs) and cytosolic expression of viral intron-containing RNA (icRNA) triggers

inflammatory responses. Furthermore, HIV-1 infection of iMGs in iPSC-derived human 3D-spheroid cultures consisting of microglia, astrocytes and neurons, promotes neurodegeneration. We hypothesize that HIV-1-infected microglia and induced dysfunctional innate immune responses promote neurodegeneration. Microglia express μ opioid receptor, MOR, though immunomodulatory effects of opioids on HIV-1-infected microglia are unclear. Since previous studies showed that MOR activation elevates inflammatory cytokines production, we sought to determine if MOR signaling synergizes with HIV-1 icRNA-induced innate immune responses to enhance neuroinflammation. We tested this hypothesis in human microglia cell line (CHME3), and iMG cultures. Our results suggest that MOR is expressed in CHMEs and iMGs, and activates ERK phosphorylation upon MOR agonist (DAMGO) treatment. An opioid receptor agonist (DAMGO) or antagonists (naloxone and suboxone) did not impact the establishment of HIV-1 infection in CHMEs and iMGs. However, the expression of HIV-icRNA induced inflammatory cytokines, IL-6 and IL-1β was significantly suppressed while type I interferon responses, namely IFNβ expression, were boosted upon naloxone and suboxone treatment in CHMEs. Transcriptomic analyses revealed that DAMGO promoted inflammatory responses, whereas naloxone downregulated inflammatory cytokines while upregulating interferon signaling pathways in iMGs. Our data suggests that naloxone and suboxone block downstream signaling of MOR which lowers NF-kB induced inflammatory cytokine expression and promotes IRF3 mediated interferon responses. Future studies will assess if opioids enhance inflammatory responses in microglia and promote neuronal cell death in HIV-1-infected 3D-spheroids.

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**Title:** Differential Patterns of Synaptic Plasticity in the Nucleus Accumbens Caused by Continuous and Interrupted Morphine Exposure

**Authors: *E. LEFEVRE**[1], M. T. PISANSKY[2], E. GAUTHIER[2], L. L. BYSTROM[3], P. E. ROTHWELL[2];
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**Abstract:** Opioid exposure and withdrawal both cause adaptations in brain circuits that may contribute to abuse liability. These adaptations vary in magnitude and direction following different patterns of opioid exposure, but few studies have systematically manipulated the pattern of opioid administration while measuring neurobiological impact. In this study, we compared

cellular and synaptic adaptations in the nucleus accumbens shell caused by morphine exposure that was either continuous, or interrupted by daily bouts of naloxone-precipitated withdrawal. At the behavioral level, continuous morphine administration caused psychomotor tolerance, which was reversed when the continuity of morphine action was interrupted. Using ex vivo slice electrophysiology in female and male mice, we investigated how these patterns of morphine administration altered intrinsic excitability and synaptic plasticity of medium spiny neurons (MSNs) expressing the D1 or D2 dopamine receptor. We found that morphine-evoked adaptations at excitatory synapses were predominately conserved between patterns of administration, but there were divergent effects on inhibitory synapses and the subsequent balance between excitatory and inhibitory synaptic input. Overall, our data suggest that continuous morphine administration produces adaptations that dampen the output of D1-MSNs, which are canonically thought to promote reward-related behaviors. Interruption of otherwise continuous morphine exposure does not dampen D1-MSN functional output to the same extent, which may enhance behavioral responses to subsequent opioid exposure. Our findings support the hypothesis that maintaining continuity of opioid administration could be an effective therapeutic strategy to minimize the vulnerability to opioid use disorders.

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**Title:** Cold nociception as a measure of hyperalgesia during spontaneous heroin withdrawal in mice

**Authors: *R. C. N. MARCHETTE**[1], L. E. HASTINGS[2], E. V. FRYE[3], E. R. CARLSON[4], V. CHUONG[5], G. F. KOOB[6], L. F. VENDRUSCOLO[7];
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**Abstract:** Opioids are powerful analgesic drugs that are used clinically to treat pain. However, chronic opioid use causes compensatory neuroadaptations that result in increased pain sensitivity during spontaneous withdrawal, known as opioid withdrawal-induced hyperalgesia (OWIH). In humans, cold nociception tests are common, whereas mechanical and heat stimuli are more commonly used to measure OWIH in preclinical studies. Thus, further characterization is needed for cold nociception stimuli in animal models. Here, we assessed three cold nociception tests - a thermal gradient ring (5-30°C, 5-50°C, 15-40°C, and 25-50°C), a dynamic cold plate (4 to -1°C at -1°C/min, -1 to 4°C at +1°C/min), and a stable cold plate (10°C, 6°C, and 2°C) - to measure hyperalgesia in a mouse protocol of heroin dependence. We measured OWIH between 8 to 24 h

into heroin withdrawal. On the thermal gradient ring, mice in heroin withdrawal preferred warmer temperatures, though these results depended on the ring's temperature range. On the dynamic cold plate, heroin withdrawal increased the number of nociceptive responses, with a temperature ramp from 4 to -1°C yielding the most significant increase. On the stable cold plate, heroin withdrawal increased the number of nociceptive responses, with a plate temperature at 2°C yielding the most significant increase. Among the three tests, the stable cold plate resulted in the most robust change in behavior between heroin-dependent and nondependent mice and had the highest throughput. To pharmacologically characterize the stable cold plate test, we used μ-opioid and nonopioid targeting drugs that have previously been shown to reverse OWIH using mechanical and heat assays of nociception s. The full μ-opioid receptor agonist methadone and the partial μ-opioid receptor agonist buprenorphine decreased OWIH, whereas the preferential μ-opioid receptor antagonist naltrexone increased OWIH. N-methyl-D-aspartate receptor antagonists (ketamine, MK-801), a corticotropin-releasing factor type 1 receptor antagonist (R121919), a $\beta_2$-adrenergic receptor antagonist (butoxamine), and a 5-hydroxytryptamine-3 receptor antagonist (ondansetron) had no effect on OWIH. These data demonstrate that the stable cold plate, set at 2°C, yields a robust, reliable, and concise measure of OWIH that is sensitive to manipulations of the opioid system but may not be sensitive to non opioid nociceptive neuromodulators.

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**Title:** Naturalistically tracking the neurobehavioral markers of goals and habits over the course of inpatient treatment in heroin addiction

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**Abstract:** Heroin and other opiate overdose-related deaths have surpassed 100,000 in 2022, necessitating exploration of neuroscience-based addiction severity and recovery biomarkers. A potential basic-science informed target is the motivational control over drug use that shifts as addiction progresses from goal-directed [model-based, via ventromedial prefrontal cortex (vmPFC) and ventral striatum] to habitual [model-free, via dorsal striatum (DS)]. However, potentially because of the narrow range afforded by lab-based tasks, which are commonly used for the needed longitudinal over-training, the evidence for this shift in humans is scarce. Responding to the need for alternative, naturalistic efforts, here we tracked motivational control using computational methods and the two-step decision task, administered via smartphone over eight weeks in 17 inpatients with heroin use disorder (iHUD; 2 women) and 18 healthy controls (HC, 6 women); this task can distinguish reward-guided decision making as model-free (i.e.,

repeating decisions based solely on reward history) and model-based (i.e., repeating decisions based on a more sophisticated model of task structure). Participants also underwent baseline fMRI scans that estimated the neural processing of drug and nondrug cues. Consistent with previous studies that reported lower model-based choice in alcohol use disorder as associated with relapse, across all sessions, we found only model-free choice in iHUD ($p<.001$) while a hybrid model-based and free strategy was evident in the HC ($p=.015$; similar to patterns in the general population). Trajectory analyses indicated that the overall impairment in model-based choice in iHUD vs. HC ($p=.030$) was most pronounced with minimal treatment engagement in iHUD, such that this impairment was only detectible with fewer sessions completed during inpatient treatment. In contrast, the higher the model-based choice estimates, the higher the vmPFC activity during drug cue exposure in iHUD ($p=.019$), suggesting that improvements in this behavioral measure could confer resilience in the face of salient drug cues and associated craving. Using a cutting-edge approach that intersects computational, naturalistic and longitudinal methods, our results identified a sensitive neurobiological marker of addiction severity and recovery that could inform motivation-based treatment and prevention targets to tackle the ongoing opioid epidemic.

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**Title:** Tabernanthalog reduces motivation for heroin and alcohol in a polydrug use model

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**Abstract:** Background: The potential use of psychedelic drugs as therapeutics for neuropsychiatric disorders has been limited by their hallucinogenic properties. To overcome this limitation, we developed and characterized tabernanthalog (TBG), a novel analogue of the indole alkaloids ibogaine and 5-methoxy-*N*,*N*-dimethyltryptamine with reduced cardiac arrhythmogenic risk and a lack of classical psychedelics-induced sensory alterations. We previously demonstrated that TBG has therapeutic efficacy in a preclinical model of opioid use disorder (OUD) in rats and in a binge model of alcohol drinking in mice. Alcohol is commonly co-used in ~35-50% of individuals with OUD, and yet, preclinical models that recapitulate this comorbidity are lacking. Methodology: Here we employed a polydrug model of heroin and alcohol co-use to screen the therapeutic efficacy of TBG on metrics of both opioid and alcohol seeking in male and female Wistar rats. We first exposed the rats to alcohol (or control sucrose-fade solution) in the home cage, using a two-bottle binge protocol, over a period of one month. Rats were then split into two groups that underwent self-administration training for either intravenous heroin or oral alcohol, so that we could assess the impact of home-cage alcohol exposure on the self-

administration of each substance separately. Thereafter, rats began self-administering both heroin and alcohol in the same sessions. Finally, we tested the effects of TBG on break points for heroin and alcohol in a progressive ratio test, where the number of lever presses required to obtain a single reward increased exponentially. Results and Conclusion: TBG effectively reduced motivation for heroin and alcohol in this test relative to vehicle treatment, indicating TBG is efficacious in animals with a history of heroin and alcohol polydrug use.

**Disclosures: J. Peters:** F. Consulting Fees (e.g., advisory boards); Delix Therapeutics, Inc.. **J.A. Heinsbroek:** None. **G. Giannotti:** None. **J. Bonilla:** None. **D.E. Olson:** 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.; Delix Therapeutics, Inc.. F. Consulting Fees (e.g., advisory boards); Delix Therapeutics, Inc..

**Presentation Number:** NANO38.08

**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** NIDA Grant P01DA047233

**Title:** Transcriptome profiling of the brain's reward circuitry in heroin self-administration identifies a ventral hippocampus gene network related to relapse susceptibility

**Authors: *C. J. BROWNE**[1], R. FUTAMURA[1], L. M. HOLT[1], X. ZHOU[2], A. GODINO[1], A. MINIER-TORIBIO[1], A. RAMAKRISHNAN[1], M. ESTILL[1], O. ISSLER[1], M. SALERY[1], E. M. PARISE[1], V. KONDEV[1], J. GARON[1], B. ZHANG[2], Y. L. HURD[3], L. SHEN[1], E. J. NESTLER[1]; [1]Nash Family Dept. of Neurosci., [2]Dept. of Genet. and Genomic Sci., [3]Dept. of Psychiatry, Icahn Sch. of Med. at Mount Sinai, Friedman Brain Inst., New York, NY

**Abstract:** Opioid use disorder (OUD) exacts a devastating toll on individuals, their families, and the healthcare system. Treatment is made exceptionally difficult by prolonged susceptibility for relapse into compulsive drug-seeking and taking, often triggered by re-exposure to drug-associated cues or the drug itself. Lasting relapse susceptibility is thought to be mediated in part by persistent changes in gene expression within interconnected reward-processing regions of the brain. However, few studies have performed transcriptome-wide analyses throughout brain reward regions following volitional opioid intake. We recently combined heroin self-administration in mice, RNA sequencing (RNA-seq), and advanced bioinformatics approaches to identify transcriptional regulation throughout the reward circuit modeling distinct phases of OUD (Browne et al., *Science Advances*, 2023; PMID: 37294757). Our design enabled comparisons of multiple addiction-relevant outcomes, including first-ever heroin exposure, early withdrawal from chronic use, context-induced drug-seeking, and drug re-exposure after abstinence reflective of relapse-like conditions. Bioinformatic analysis of this rich dataset outlined numerous patterns of differential gene expression in a region- and condition-dependent manner. Ongoing efforts are aimed at identifying gene regulatory systems that contribute to drug craving underlying relapse. Employing multiscale embedded gene co-expression network analysis (MEGENA), we identified gene networks associated with drug-seeking behavior and uncover a particularly prominent role for a ventral hippocampus gene network in relapse-like conditions. Interestingly, this network is enriched with genes involved in epigenetic regulation,

including histone post-translational modifications and chromatin conformation. Using CRISPRa/i-mediated gene regulatory strategies we are targeting key hub genes within this ventral hippocampus gene network to determine its causal role in promoting drug-seeking behavior. Additionally, epigenome profiling of the ventral hippocampus after heroin self-administration is underway to identify how shifts in the epigenetic landscape may alter biological functions that unleash drug-seeking behavior and drive relapse.
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**Nanosymposium**

**NANO39: Circuits and Neural Mechanisms for Social Behavior**

**Location:** WCC 144

**Time:** Monday, November 13, 2023, 8:00 AM - 10:00 AM

**Presentation Number:** NANO39.01

**Topic:** H.06. Social Cognition

**Support:** German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), SFB 1528 – Cognition of Interaction
Leibniz Association: Leibniz Collaborative Excellence grant K265/2019 Neurophysiological mechanisms of primate interactions in dynamic sensorimotor settings
Leibniz Association: Leibniz ScienceCampus Primate Cognition

**Title:** Representation of predicted and observed actions of others during dynamic coordination in macaque premotor cortex

**Authors: *S. MOELLER**[1,3], B. DANN[2], H. SCHERBERGER[2,3,4], S. TREUE[1,4,3], A. GAIL[1,4,3], I. KAGAN[1,3];
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**Abstract:** Elucidating neural mechanisms of social decision-making is a fundamental challenge in cognitive neuroscience. The premotor cortex in primates has been implicated in representing the actions of others, but its role in dynamic social interactions is not clear. We examined neural correlates of dynamic coordination within the premotor cortex of two macaque monkeys during a transparent Bach-Or-Stravinsky (BoS) dyadic decision game. This game rewards coordinated choices but also imposes an inherent conflict about which of the two coordinated options to pick. We showed that under conditions of mutual action visibility - when the game is played on a two-sided transparent touchscreen - macaques learned to follow a human partner who alternated

between blocks of selecting own or macaque's preferred option [1]. Here we recorded from multiple implanted electrode arrays in the ventral and dorsal premotor cortex while monkeys made spatial reaching decisions before, concurrently with, or after their partner's action (a dyadic context, with predicable or unpredictable partner), or without a partner (a solo context). The analysis of firing patterns in individual units revealed that many neurons were modulated by the relative timing of monkey's and partner's actions, and by the task context, suggesting a role for the premotor cortex in representing and updating the state of play during coordinated behavior. Furthermore, some units represented partner's or joint choices. On a population decoding level, when the monkey acted prior to the partner, the partner's action was highly decodable following the partner's movement onset. In some sessions where the monkey was successful in anticipating upcoming actions of a predictable partner, the partner's / joint choice was weakly decodable even before the partner's movement onset. When the partner acted first and the monkey followed, the population activity reflected the partner's choice and/or upcoming monkey choice, even before the monkey's movement. However, during a control task with reward for passively observing actions, the decoding of partner's actions disappeared. Thus, the premotor cortex contains representations not only of observed actions but also of anticipated actions of a partner, as long as these actions provide affordances for the monkey's own choices and expected rewards. These findings offer novel insights into the premotor cortex's role in combining predictions and observations of actions of others with own goals and choices, crucial for dynamic strategic coordination.
[1] Moeller et al (2023) Human and macaque pairs employ different coordination strategies in a transparent decision game. eLife 12, e81641.

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**Topic:** H.06. Social Cognition

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**Title:** Frequency dependent cortex-wide synchronization across brains of socially interacting animals

**Authors: *A. SCAGLIONE**[1,2], J. LUCCHESI[2], A. ALLEGRA MASCARO[3,2], F. S. PAVONE[2,1];
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**Abstract:** Social interactions entail complex behaviors that are crucial for the health and well-being of individuals. However, the neural mechanisms underlying social behaviors remain elusive. This is partly due to the complexity of such interactions and also to the challenges of monitoring neural activity simultaneously across the brains of interacting animals. To address these challenges, we developed a custom-made miniaturized microscope, the “miniscope”. This miniscope is mounted on the heads of GCaMP6f transgenic animals, enabling us to record neural activity from the cortex of awake freely moving mice. We then used the miniscope on pairs of animals (dyads) while they roamed freely in a linear chamber. The chamber was divided by a barrier with a center slit, facilitating social interactions between members of the dyad at the barrier. Our findings indicate that social interaction modulates synchrony among homotopic cortical areas, and these synchronizations occur in two different frequency bands: 1) slow (1-4 Hz) and 2) ultra-slow (< 0.1 Hz). Furthermore, while synchronizations at the ultra-slow frequency band were observed across the entire cortex, synchronizations at the slow frequency band were more pronounced in the somatosensory and visual cortices. These results suggest that different areas of the cortex play distinct roles during social interaction and represent important targets for investigating diseases that affect social interactions.

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**Presentation Number:** NANO39.03

**Topic:** H.06. Social Cognition

**Support:** 1R01MH119430-01 (PI JH)

**Title:** Dyadic Neural Mechanisms for Live Facial Expressions: Emotional Contagion and Spontaneous mimicry

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**Abstract: Introduction**. Emerging interest in naturalistic social behaviors underpins a new science of live face-to-face interaction.1 Recent evidence suggests that live face-to-face interaction activates lateral and dorsal cortex beyond the well-known ventral face-processing systems observed with non-interacting, i.e. simulated, face stimuli.2-4 Here we build on this theoretical framework that proposes an interactive face processing network for live face processing by investigating the neural processes of two dyadic face behaviors: emotional contagion and facial mimicry. **Methods**. We employed a two-person neuroimaging paradigm using functional near-infrared spectroscopy (fNIRS) during live emotive face-processing while also measuring eye-tracking, facial classifications, and subjective ratings of emotional valence and intensity (n = 20 dyads). One dyadic-partner viewed evocative short movie clips and emoted natural facial expressions. The other dyadic-partner viewed the expressive face of the “Movie Watcher”. Task and rest blocks were implemented by alternating clear and opaque timed epochs of a “smart glass” that separated the partners. Dyadic roles of “face-watcher” and “movie watcher” were alternated during the experiment. **Results.** Mean cross-partner correlations of

facial expressions (facial mimicry) measured as the first principal component of 17 facial action units (r = 0.36 +/- 0.11 SEM) and mean cross-partner affect ratings of experienced emotional valence and intensity (r = 0.67 +/- 0.04) were consistent with behavioral observations of both facial mimicry and emotional contagion, respectively. Neural correlates of emotional contagion were based on covariates of partner subjective ratings and included angular gyrus and lateral occipital cortex, whereas, neural correlates of the partner's live facial action units included motor cortex and supramarginal gyrus. **Conclusions.** Findings suggest dissociable neural systems for interactive facial mimicry and emotional contagion. Both of these separate live face-processing systems extend the conventional ventral visual face-processing pathways (based on simulated and non-interactive faces) and advance a framework for live dyadic face processing within the context of naturalistic social behaviors.
[1]Hamilton&Holler,*Phil.Transactions B,* 2023; [2]Hirsch, et al, *PLoS ONE,* 2022; [3]Kelly, et al, Frontiers in Robotics and AI, 2021; [4]Noah, et al., 2020, Frontiers in Human Neuroscience.

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**Presentation Number:** NANO39.04

**Topic:** H.06. Social Cognition

**Support:** NIH Grant R01MH120292

**Title:** Social aggressive behavior modulation by social memory in hippocampal area CA2

**Authors: *A. VILLEGAS**[1], S. A. SIEGELBAUM[2];
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**Abstract:** Memories of familiarized individuals are enriched by shared experiences, while novel social encounters demand cognitive flexibility. The dorsal CA2 sub-region exerts a critical role in social recognition memory and in the promotion of social aggression. Whether social aggression and social memory are linked, as well as the specific encoding of aggressive behaviors by CA2 neurons, remains unknown. To test the link between memory and aggression, we conducted a multi-day resident intruder assay of aggression to investigate how social experience regulates aggressive behavior towards familiar and novel conspecifics. On day 1, resident mice were presented with a novel intruder, followed by repeated presentations of the same intruder from days 2 to 5. On day 6, we introduced a different novel intruder. We found a significant increase in the number and total duration of attacks by the resident mouse towards the novel intruder on day 6, compared to the attacks directed at the familiarized intruder on day 5, indicating that social novelty increases aggressive behavior. To explore how CA2 responds during the interaction between resident and intruder, we expressed the calcium indicator GCaMP6f in CA2 pyramidal neurons and recorded calcium signals using microendoscopy. We used both manual labeling and supervised segmentation of nonaggressive social exploration, social dominance (mounting, chasing, aggressive licking), and biting attack behaviors to characterize CA2 neural population representations. Using a linear support vector machine decoding approach, we found that CA2 population activity accurately decoded exploration versus dominance versus attack behaviors in binary comparisons, both during encounters with familiarized and novel conspecifics, with a trend towards increased decoding of attack with the

novel conspecific on day 6. Finally, we used chemogenetics to acutely inhibit CA2 during the presentation of a novel conspecific (day 6). Mice bilaterally expressing the hM4Di inhibitory receptor demonstrated significantly reduced or absent aggression compared to mice expressing the inert fluorescent marker mCherry, when both groups were injected with the hM4Di ligand CNO 30 min prior to the test. Together, these findings suggest that CA2 integrates social experience to guide future behavior and provides insight into how social memory influences aggressive behavior. Novel encounters trigger increased aggression whereas familiarization leads to stabilized aggression levels.

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**Presentation Number:** NANO39.05

**Topic:** H.06. Social Cognition

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The Simons Collaboration on Plasticity and the Aging Brain

**Title:** An inhibitory circuit-based enhancer of Dyrk1a function reverses Dyrk1a-associated impairment in social behavior

**Authors: *Y.-T. SHIH**[1,2,3,4], J. ALIPIO[1], A. SAHAY[1];
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**Abstract:** Heterozygous mutations in the Dual specificity tyrosine-phosphorylation-regulated kinase 1a Dyrk1a gene define a syndromic form of Autism Spectrum Disorder. The synaptic and circuit mechanisms mediating Dyrk1a functions in social cognition are unclear. Here, we identify a social experience-sensitive mechanism in hippocampal mossy fiber-parvalbumin interneuron (PV IN) synapses by which Dyrk1a recruits feed- forward inhibition of CA3 and CA2 to promote social recognition. We employ genetic epistasis logic to identify a cytoskeletal protein, Ablim3, as a synaptic substrate of Dyrk1a. We demonstrate that Ablim3 downregulation in dentate granule cells of adult hemizygous Dyrk1a mice is sufficient to restore PV IN mediated inhibition of CA3 and CA2 and social recognition. Acute chemogenetic activation of PV INs in CA3/CA2 of adult hemizygous Dyrk1a mice also rescued social recognition. Together, these findings illustrate how targeting Dyrk1a synaptic and circuit substrates as “enhancers of Dyrk1a function” harbors potential to reverse Dyrk1a haploinsufficiency-associated circuit and cognition impairments.

**Disclosures: Y. Shih:** A. Employment/Salary (full or part-time):; Center for Regenerative Medicine, Massachusetts General Hospital, Boston. **J. Alipio:** A. Employment/Salary (full or part-time):; Center for Regenerative Medicine, Massachusetts General Hospital, Boston. **A. Sahay:** None.

**Presentation Number:** NANO39.06

**Topic:** H.06. Social Cognition

**Support:** NIH Grant 5R01NS106983

**Title:** Ventral hippocampal CA2 differentially contributes to social memory and social aggression behaviors

**Authors: *W. SHENG**[1], L. BOYLE[1], A. VILLEGAS[2], S. A. SIEGELBAUM[2];
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**Abstract:** The hippocampus, a brain region critical for episodic memory, is composed of distinct anatomical subregions. Along its transverse axis, hippocampus is divided into the dentate gyrus, CA3, CA2 and CA1 regions. Along its longitudinal axis, hippocampus has been divided into a dorsal portion, which is important for cognitive aspects of memory, and a ventral portion, which has been implicated in emotional behaviors. Recent studies from our laboratory have shown that the dorsal CA2 region is required for social memory and acts to promote social aggression. However, it is controversial as to whether CA2 extends to the ventral portion of the hippocampus. Moreover, the behavioral role of ventral CA2 is unclear. Here we report that CA2 extends along the entire dorsal-ventral extent of the hippocampus with topographically distinct projections and synaptic connectivity. Moreover, we find that silencing ventral CA2 does not impair social memory, assessed by the ability of a subject mouse to discriminate a novel from familiar conspecific. In contrast, ventral CA2 silencing caused a marked decrease in social aggression, similar to the action of silencing dorsal CA2. Thus, ventral CA2 may be required for valence-associated social engagement but not for social novelty detection, suggesting that social memory and social aggression functions may be separable.

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**Topic:** H.06. Social Cognition

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**Title:** Contextual modulation of theta and gamma rhythms: insights into social behavior of adult male mice

**Authors: *A. N. MOHAPATRA**, D. PELES, S. NETSER, S. WAGNER;
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**Abstract:** Social interactions between mammalian conspecifics involves dynamic coupling of multiple brain regions to modulate and control behavioural decisions (Chen & Hong, 2018). Oscillatory neural activity in the theta (**θ**: 4-12 Hz) and gamma (**γ**: 30-80 Hz) frequency bands

has been implicated in cognitive functions and social communication across various species (Harris and Gordon 2015; Kuga et al. 2022). The hypothesis driving this research was that coherent **θ** and **γ** rhythms synchronize neural ensembles across the brain, forming networks that adapt to distinct social contexts. We recorded extracellular electrical activity from 18 brain regions in adult male CD1 mice (n=13, 12-16 weeks old) performing three binary social discrimination tasks (social preference (SP), emotional state preference (EsP), and sex preference (SxP)). The test was divided into two 5 min periods, a baseline period (pre-encounter) and a period of encounter with the stimuli. The stimuli for the SP task were a novel group-housed male mouse and a Lego toy. For the EsP task, isolated (7-14 days) and group-housed male mice were used as stimuli. Finally, for the SxP task, group-housed male and female mice served as stimuli. By using the same type of social stimulus (group-housed male) with varying valence in three tests, the study aimed to link rhythmic neural activity to stimulus identity, valence, or its social context. We found that subjects preferred interacting with one stimulus over the other in all three tasks. However, the change in **θ** and **γ** power varied between the tests. Notably, the **γ** power during interaction bouts, for most regions (14 of 18), was associated with stimulus characteristics like valence or identity. Similar to **θ** and **γ** power, the coherence (Co) in these frequencies between the various brain regions differed between the tests, specifically during the encounter period. Further, we found a strong correlation between the social context and the Co at both frequencies during interaction bouts, which provide discriminative information about the distinct albeit similar social contexts. We used a decision trees models trained on coherence data (n=99 pairs) to classify the social context, valence, and identity. The model trained with **θ**-Co data classified the contexts, i.e. the three tasks, better than chance level (3-class model, % ground truth predicted: SP=40.3, EsP = 38.2, SxP = 44.8, and Chance = 33). Thus, **θ**-Co between the recorded brain area could generate predictions regarding the social context, but not the specific stimulus. Overall, our findings highlight the importance of coordinated rhythmic activity in the social brain for context-dependent social behavior.

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**Topic:** H.06. Social Cognition

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NIH Grant R21HD039937

**Title:** Pharmacologically induced defense-like behaviors elicit similar behaviors in non-injected conspecific social partners indicating socially-mediated threat responses

**Authors: *S. J. WATERS**[1,2], C. TURNER[3], P. A. FORCELLI[3,2], L. MALKOVA[3,2];
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**Abstract:** Socially-mediated fear is not well studied in part because it is often difficult to dissociate socially communicated threats from those experienced by direct exposure to threatening stimuli. Here we evaluate whether pharmacologically induced threat responses can

evoke similar responses in observing partners. Previous research by this lab demonstrated that focal pharmacological activation of the deep and intermediate layers of the superior colliculus (DLSC) in rhesus macaques evoked robust defense-like behaviors (e.g., cowering, escape), not present under baseline conditions and in the absence of any threatening stimuli (DesJardin et al., 2013).In the present study, we examine whether these pharmacologically induced threat responses elicit similar responses in conspecific social partners that did not experience DLSC disinhibition. We re-analyzed archived videotapes of dyadic social interactions between familiar pairs of monkeys where only one of the two monkeys received unilateral activation of the DLSC. Behavioral assessments were conducted by two independent observers and included measures of defense-like behaviors and social behaviors (e.g., cowering, escape, grooming, social contact). Compared to baseline, uninfused partners exhibited cowering and escape-like movements (not present at baseline) and *decreased* grooming behaviors. Although the partners exhibited defense-like behaviors that were similar to those displayed by the infused monkeys, these responses were less robust. As shown previously, disinhibition of the DLSC evoked species-specific threat responses. Here we document that these responses prompt similar responses in conspecific social partners, indicating elicitation of socially-mediated threat responses. These findings suggest that pharmacologically induced threat responses might be useful for future studies on socially-mediated fear.

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**Nanosymposium**

**NANO40: Software Tools, Databases: Scientific and Social Impact**

**Location:** WCC 147B

**Time:** Monday, November 13, 2023, 8:00 AM - 10:45 AM

**Presentation Number:** NANO40.01

**Topic:** I.07. Data Analysis and Statistics

**Support:** Wellcome Trust award 226486/Z/22/Z
NSF IIS 1916518

**Title:** Developing an International Data Governance Framework for Brain Data

**Authors:** K. RAY[1], D. EKE[2], R. CHAVARRIAGA[3], F. TOVAR-MOLL[4], A. O. IHUNWO[5], T. E. NICHOLS[6], A. MACKENZIE[1], M. BROWN[7], ***F. PESTILLI**[1];
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**Abstract:** The growing availability of shared neuroscience data is driving unprecedented research and innovation. As a result of a welcome move toward open sharing of neuroscience data, data are often crossing the legal and national borders from where they originate. Modern

international collaborations and scientific projects bring data governance to the fore with the imminent need for coordination across institutions, countries, laws and cultures. Because of the current absence of an agreed upon International Data Governance Framework (IDGF), stakeholders from academia, industry as well as policy domains are struggling with addressing these needs. Generalized solutions for international data governance for research have not yet been proposed, effectively limiting the ability of researchers to tackle global challenges in neuroscience data sharing.The nature of neuroscience data creates challenges for sharing that are not only technical in nature but also economic, ethical, and legal. Similar to human genomic data, heightened sensitivity with neuroscience data comes, in part, from the connection it has to human identity, identification, and personhood. Thus, effective IDG should be compatible with the open-sharing needs of the neuroscience research community while respecting the diversity of ethics, cultures, and privacy around data sharing across nations.We propose an IDGF that will balance data protection and open science, such that personal information is safeguarded while maximizing the potential for sharing and reuse of data. With the trend towards increased data sharing, data generators/repositories and consumers face additional technical limitations, such as data storage infrastructure, findability of data, as well as accessibility. Yet another critical issue in data sharing using data repositories is the potential risk for human subject re-identification. Our proposed IDG will help clarify the technical, legal, and ethical responsibilities that the scientific community has to balance their obligations to scientific discovery while maintaining a commitment to human privacy. All stakeholders have a responsibility in ensuring that governance mechanisms are considered proactively, and not as an afterthought. Data governance must be agreed upon within and across borders. Thus, inclusive dialogues with different stakeholders from different cultures and disciplinary backgrounds should characterize the development of IDG principles for neuroscience. Once an effective IDGF is agreed upon that facilitates data sharing globally, neuroscience researchers will be better equipped to tackle global challenges in neuroscience and mental health.

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**Presentation Number:** NANO40.02

**Topic:** I.07. Data Analysis and Statistics

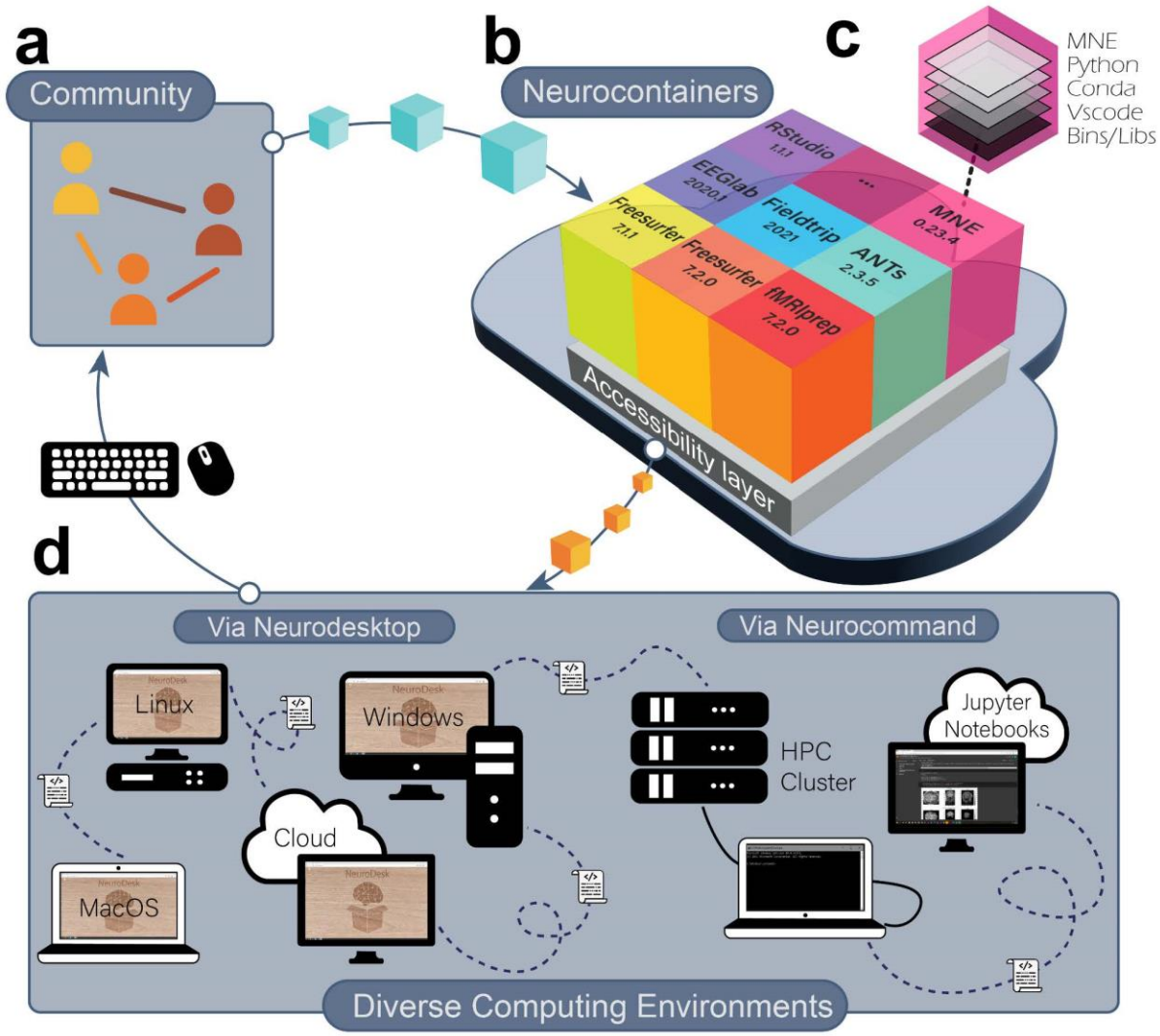
**Support:** Australian Research Data Commons
Oracle for Research
The University of Queensland
National Imaging Facility

**Title:** The Neurodesk platform and its impact on shared datasets and standardized technology in neuroscience research

**Authors: *A. STEWART**, T. DAO, A. NARAYANAN, S. BOLLMANN;
The Univ. of Queensland, St Lucia, Australia

**Abstract:** Neuroimaging data analysis often requires purpose-built software, which can be challenging to install and may produce different results across computing environments. Beyond

being a roadblock to neuroscientists, these issues of accessibility and portability can hamper the reproducibility of neuroimaging data analysis pipelines. The Neurodesk platform provides a sustainable and flexible solution by harnessing software containers to support a comprehensive and growing suite of neuroimaging software (https://www.neurodesk.org/). Neurodesk includes both a browser-accessible virtual desktop environment and a command line interface, mediating access to containerized neuroimaging software libraries from multiple systems; including personal computers, cloud computing, high-performance computers, and Jupyter notebooks. Neurodesk enables various workflows for collaborating on datasets depending on the international data governance model: 1) Neurodesk allows users to bring analysis software to privately stored data, enabling them to execute their pipelines in different countries, institutions and facilities. 2) Neurodesk can also be used for centralized data storage where users from different institutions can access the same data and collaborate on analyses.
This community-driven, open-source platform offers a step towards the common goal of highly accessible, fully reproducible, and portable data analysis pipelines which can be redeployed in perpetuity across computing environments.



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**Presentation Number:** NANO40.03

**Topic:** I.07. Data Analysis and Statistics

**Support:** 1RF1MH117800-01
1R01MH130457-01

**Title:** Neuroscience data governance and human agency: The ethical relevance of lived experience with neurological and psychiatric disabilities

**Authors: *E. KLEIN**;
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**Abstract:** Recent calls for developing international data governance (IDG) in neuroscience (Eke et al. 2022) acknowledge that neuroscientific data raise important ethical issues that need to inform proposed solutions. Progress on IDG will depend on deeper and multicultural understandings of both ethical principles and the range of ethical issues made salient by advances in neuroscience and neurotechnology, including the way that neurotechnologies and neuroscientific data may advance or challenge the exercise of human agency. Whether human agency, the capacity to enact one's intention on the world, is enhanced or undermined by developments in neuroscience is an open question. The voices of people living with or alongside people living with neurological and psychiatric conditions are critical in forging an understanding of how neuroscience data governance does or will affect human agency. We have conducted a series of qualitative ethics engagement studies over the last decade with current and prospective users of implantable neurotechnologies (brain-computer interfaces and deep brain stimulators) who live with a neurological or psychiatric condition (Parkinson disease, multiple sclerosis, depression, obsessive-compulsive disorder, ALS, cognitive impairment, addiction (Boulicault et al. 2023; Klein et al. 2023; Versalovic et al. 2023; Klein et al. 2022; Wexler et al. 2022; Versalovic et al. 2020; Klein et al. 2016). In all of these studies, we have explored stakeholder views on neural data. Pulling from across these studies, this presentation will employ the voices of these varied stakeholders to highlight four dimensions of agency affected by neuroscience data collection and use: responsibility, privacy, authenticity, and trust (Schonau et al. 2021). This work will draw attention to the need, promise, and potential future challenges of taking seriously an imperative to build ethical concepts, like human agency, into the very foundation of neuroscience data governance.

**Disclosures: E. Klein:** None.

**Presentation Number:** NANO40.04

**Topic:** I.07. Data Analysis and Statistics

**Title:** International Research Consortium for the Corpus Callosum and Cerebral Connectivity (IRC5)

**Authors: *L. K. PAUL**[1,2], T. ATTIE-BITACH[3], R. BOOTH[4], W. S. BROWN[5], C. DEPIENNE[6], G. KASPRIAN[7], L. J. RICHARDS[8], E. H. SHERR[9], F. TOVAR-MOLL[10], B. YALCIN[11];
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**Abstract:** The IRC[5] is an international, multidisciplinary effort to promote discoveries leading to understanding the causes, consequences, and effective interventions for corpus callosum dysgenesis (CCD) and associated disorders of cerebral connectivity. CCD is considered a rare disease in the general population, but it is a relatively common neurodevelopmental diagnosis. Functional outcomes of CCD are wide ranging, but only a subset of cases are associated with known etiologies and very little is understood about the relationship between behavior and variations of callosal structure. Consequently, although it can be identified prenatally, a CCD diagnosis currently provides limited prognostic information or direction for targeted intervention. Bringing our collective knowledge and building on the few ongoing long-term in-depth studies of CCD across the world, the IRC[5] formed in 2015 to facilitate collaborative exploration of the relationships between known and novel causes of CCD, neuroanatomic phenotypes, and functional phenotypes across the lifespan. Through this work, the IRC[5] aims to increase understanding into basic mechanisms of cortical development, in addition to guiding advances toward refined diagnosis and ultimately targeted treatments for people with CCD.
This presentation offers lessons learned through formation of IRC[5]: a rare-disease research consortium composed of over 80 basic scientists and clinicians representing 14 countries, 4 continents, and divergent areas of expertise spanning molecular genetics, developmental biology, functional genomics, fetal pathology, neurology, neuroradiology, cognitive neuroscience, neuropsychological assessment, clinical psychology, and psychiatry. First, we examine critical decisions in defining the scientific scope and organizational structure of an international rare-disease research consortium. Next, we describe the process of reaching our key achievements to date (e.g., establishing uniform genetic, neuroimaging and behavioral protocols for coordinated data-acquisition, implementing policies and procedures for data-sharing, convening annual meetings, publishing co-authored papers). Finally, we discuss ongoing challenges related to accommodating regionally-specific ethical standards for human research, communicating across a wide-range of disciplines, navigating logistics of international meetings, maintaining member engagement, facilitating collaboration between members with divergent resources and standards of clinical, and international clinical trials.

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**Presentation Number:** NANO40.05

**Topic:** I.07. Data Analysis and Statistics

**Title:** A market-based governance model for consumer neurotechnologies to ensure innovation of neurotechnologies and safeguard neurodata in the absence of regulation.

**Authors: *R. TRIPLETTE**, D. WHITE;
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**Abstract:** As consumer neurotechnologies enter mainstream commerce, we near an explosion of a massive neurodata market. This presents tremendous opportunities to the medical and commercial sectors, making datasets available that likely hold keys to important questions in neuroscience and mental wellness. However, without proper governance and market guidance, it also presents enormous risks to society. Some of these risks parallel those of previously novel technologies, including privacy and cybersecurity. The implications, though, on individual autonomy are unprecedented. The risks are more acute today than even a year ago with the emergence of generative artificial intelligence and the implications of its use paired with individual and population-scale neurodata.

We propose the utilization of a market-based governance model for consumer neurotechnologies. While there is increased attention of global policymakers on the vast implications of biometric data, most fall short of addressing the unique challenges of neurodata. Indeed, the UK's ICO recently noted the difficulty in how to classify neurodata under existing data laws and the US FTC failed to include neurodata in their May 2023 policy statement on the misuse of consumer biometric data despite a $1.5m privacy breach settlement with Cerebral the year prior. So even while recognizing the need for guidelines in the neurotech market, how to establish such with the "buy in" by commercial stakeholders remains a question.

Building off two decades of experience establishing coalition-based, multi-stakeholder platforms and in consultation with neurodata experts in government, industry and society, we've developed a model that considers personal data empowerment, data sharing, privacy, security, and other emerging issues in the sector. The model provides: (1) a "feedback loop" for industry stakeholders to address emerging concerns in the market; (2) a platform where ethical, stakeholder-based standards can be coded into a database for universal adoption; and (3) a scalable, consumer-focused education arm to build and preserve the sector's social license to operate.

Early efforts at legislative language and regulatory models have failed to account for the impact on the consumer neurotechnology market. Proposed solutions parallel early HIPAA models despite the historical problems of such on commercial innovation and failure to ensure data protection and sharing. We propose to learn from these earlier models, establishing a platform and forum that engages stakeholders in real time and ensures a flourishing and safe future for neurodata.

**Disclosures: R. Triplette:** None. **D. White:** None.

**Presentation Number:** NANO40.06

**Topic:** I.07. Data Analysis and Statistics

**Support:** Krembil Foundation
Discovery Fund
McLaughlin Accelerator Grant in Genomic Medicine

**Title:** Transforming mental health care and brain research with the BrainHealth Databank: A systems approach to enable a learning health system

**Authors:** J. SANTISTEBAN[1], J. YU[1,2], D. ROTENBERG[1], ***S. L. HILL**[1,2,3];
[1]Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Vector Inst. for Artificial Intelligence, Toronto, ON, Canada; [3]Dept. of Psychiatry, Univ. of Toronto, Toronto, ON, Canada

**Abstract:** Mental health care and brain research are crucial components of healthcare systems worldwide, but limitations in current data collection and integration approaches have hampered progress in developing effective treatments and improving patient outcomes. The BrainHealth Databank aims to establish a learning health system for mental health care and research by leveraging digital support for measurement-based care, integration of research measures into care pathways, artificial intelligence, FAIR data, and open science. The initiative's unique contribution lies in its systems approach to designing, deploying, and governing the informatics infrastructure. This approach engages all stakeholders, including clinicians, researchers, privacy and ethics experts, patient and family representatives, and data science and research experts, in a governance structure that ensures seamless integration of research measures into care pathways and harmonization of multidimensional data in an interoperable data representation (FHIR). The BrainHealth Databank at the Centre for Addiction and Mental Health (CAMH, Toronto, Canada) has already accumulated over 7 million data points, including 12,000 patient trajectories through care, and aims to expand to other hospitals and accelerate research linking mental health to physical health conditions. This paper highlights the importance of a systems approach in the BrainHealth Databank initiative, emphasizing how it maximizes the value of the initiative for all stakeholders. We now are extending this approach beyond the hospital, to data federations that can enable learning health systems that span the community, provincial, and pan-Canadian contexts.

**Disclosures: J. Santisteban:** None. **J. Yu:** None. **D. Rotenberg:** None. **S.L. Hill:** None.

**Presentation Number:** NANO40.07

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** Sandia National Laboratories LDRD 151345

**Title:** Language and software tool for large-scale models

**Authors: *F. ROTHGANGER**;
Sandia Natl. Labs., Albuquerque, NM

**Abstract:** Sharing models and data is a prerequisite for progress toward a full understanding of brain function. The Neuroscience Information Framework (NIF) does this for many forms of descriptive data. Interchange languages such as NeuroML/LEMS provide a simulator-agnostic description of models, and repositories such as NeuroML-DB and ModelDB make them searchable and accessible.
However, exchanging models and data is not sufficient. It is also necessary to assemble those shared models into larger functional units, ultimately reaching the level of an entire nervous system. To create abstract descriptions of function, the modeling system must be capable of

crossing all the scale levels, and component models should be expressed in a form suitable for automated analysis.
N2A ("Neurons to Algorithms") is an effort toward these challenging goals. It treats models as data rather than code. This declarative approach describes a model as a set of attributes and equations, without specifying a step-by-step procedure for simulation. It emphasizes the relationships between values within a model and the relationships between models in a larger functional unit.
The declarative approach allows one model to directly extend and modify another, simply by referencing the parent model and declaring new values for specific attributes and equations. A model may also incorporate other models as components, allowing the assembly of arbitrarily deep systems. We will demonstrate an open-source implementation of N2A.

**Disclosures: F. Rothganger:** None.

**Presentation Number:** NANO40.08

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** This project/research was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Institutes of Technology.
European Union Seventh Framework Program (FP7/20072013) under grant agreement no. 604102 (HBP)

**Title:** Exploring the Blue Brain Project's open source single cell electrical modeling suite

**Authors: *A. TUNCEL**, A. JAQUIER, A. ARNAUDON, T. DAMART, H. MARKRAM, W. VAN GEIT;
Blue Brain Project, École polytechnique fédérale de Lausanne (EPFL), Campus Biotech, 1202 Geneva, Switzerland, Geneva, Switzerland

**Abstract:** The Blue Brain Project offers a comprehensive suite of open source software tools aimed at facilitating the process of single-cell model building, validation and analysis.
The Electrophys Feature Extraction Library (eFEL) empowers neuroscientists to automatically extract essential features from time series data obtained from both in vitro and in silico neuronal recordings. By combining trace reading and feature extraction, BluePyEfe simplifies the bulk collection of electrical features from experimental recordings.
For data-driven model parameter optimization, the Blue Brain Python Optimization Library (BluePyOpt) provides an extensible framework. It enables researchers to optimize biophysically detailed electrical models by fitting them to experimental data.
To ensure the comprehensive testing of biophysically detailed electrical models (e-models) in network simulations, BluePyMM facilitates model management. It identifies all possible combinations of morphologies and e-models based on circuit specifications and calculates scores for each combination. Accepted combinations are stored in a database, and a detailed report provides information on the matching results.
BluePyEModel is a workflow system that automates every step of the single-cell e-model building process. It utilizes the Luigi workflow management system to orchestrate the tasks

efficiently and streamline the workflow.
Currentscape is a Python library that simplifies the generation of currentscape figures (Alonso et al., 2019), providing valuable insights into the dynamics of inward and outward ionic currents and voltages over time. With Currentscape, researchers can easily analyze and interpret neuronal data, gaining a deeper understanding of cellular activity.
Once a circuit model is constructed, BlueCelluLab empowers users to simulate and experiment on single cells or small groups of cells within the circuit. It is particularly suited for scripting and statistical analysis on pairs of neurons, lightweight reporting on specific state variables of the models, development of synaptic plasticity rules, dynamic validation of synaptic properties, automation of in-silico whole-cell patching experiments, and scientific and computational debugging.
All these tools are openly available on Github, and are easily installable from the Python package repositories.

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**Presentation Number:** NANO40.09

**Topic:** I.07. Data Analysis and Statistics

**Title:** The FAIR roadmap for neuroscience: practical guidelines, resources, and tools to make neuroscience more open, FAIR, and citable

**Authors: *M. ABRAMS**;
INCF, Karolinska Institutet, Stockholm, Sweden

**Abstract:** The FAIR roadmap project is an ambitious project led by the Council for Training, Science, and Infrastructure (CTSI) of the International Neuroinformatics Coordinating Facility (INCF) that aims to provide a global plan for how to move neuroscience towards a more open, FAIR, and citable discipline. The roadmap is a living document (https://www.incf.org/incf-fair-roadmap) that is intended to serve as a framework for identifying the current gaps, challenges, and opportunities in the landscape of open, FAIR, and citable neuroscience, as well as for coordinating community action to produce practical guidelines and resources that will aid community adoption. To this end, the project has released 3 portfolios on: Principles of FAIR data management, FAIR standards and best practices, and FAIR data repositories and scientific gateways.
The Principles of FAIR data management portfolio is composed of guidelines and multimedia training resources that aim to provide the community with an introduction to the principles of FAIR data management for the entirety of the research data lifecycle. It includes guidelines on How to write a FAIR data management plan, as well as links to open access training courses on topics such as Introduction to FAIR neuroscience. The FAIR standards and best practices portfolio provides the community with an index of robust, well-validated standards and best practices (SBPs) that adhere and support the FAIR principles. A unique feature of the portfolio is that all SBPs indexed have been evaluated against an established set of criteria developed by the INCF Standards and Best Practices Committee that constitutes a 3-step process including expert, community, and committee reviews. All entries in the portfolio contain descriptions of appropriate use cases, links to tools/infrastructures that have implemented the SBP, and links to

implementation tutorials. The FAIR data repositories and scientific gateways portfolio provides the community with guidance in selecting the best infrastructure for their data type, analysis, and sharing needs. All entries in this portfolio have been evaluated using an established set of criteria developed by the INCF Infrastructure Committee. By producing practical guidelines with supporting resources, we aim to increase the discoverability and use of FAIR data management practices by the neuroscience community. AcknowledgementThis work was conducted by the members of the INCF Council for Training, Science, and Infrastructure, the INCF Training and Education Committee, and the INCF Infrastructure Committee.

**Disclosures: M. Abrams:** None.

**Presentation Number:** NANO40.10

**Topic:** I.07. Data Analysis and Statistics

**Support:** NIH 3OT3OD025347

**Title:** Pennsieve:fostering collaborative science through technology and meaningful data sharing

**Authors: *J. B. WAGENAAR**;
Dept. of Biostatistics, Epidemiology and Informatics, Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Researchers spend a disproportionate amount of time developing ad-hoc solutions for scientific data-management, cleaning, and analysis pipelines. We conduct research in siloed, isolated environments and even though we know that significant advances in science will require extensive collaboration and cross-modal data integration, we are often struggling with finding the right tools to make this happen in a meaningful, scalable, and sustainable way. Biomedical Informatics can provide the next generation scientists and clinicians with the tools to cut through these barriers. To enable them to look at all data in context and to provide intuitive, scalable and sustainable mechanisms for data visualization, exploration, discovery, and analysis.The Pennsieve platform is a cloud-based scientific data management platform focused on integrating complex datasets, fostering collaboration and publishing scientific data according to all FAIR principles of data sharing. The platform is developed to enable individual labs, consortiums, or inter-institutional projects to manage, share and curate data in a secure cloud-based environment and to integrate complex metadata associated with scientific files into a high-quality interconnected data ecosystem. Specifically, Pennsieve differentiates itself from other repositories by supporting both large scale file-sharing and integrating this with advanced support for graph-based metadata management. Pennsieve is leveraged by several large scale NIH programs to support data management and publishing such as the NIH SPARC and REJOIN initiatives. The platform is used as the backend for a number of public repositories including the NIH SPARC Portal (https://sparc.science)) and Pennsieve Discover (https://discover.pennsieve.io) repositories. It supports flexible metadata schemas and support a large number of scientific file-formats and modalities.

**Disclosures: J.B. Wagenaar:** None.

**Presentation Number:** NANO40.11

**Topic:** I.07. Data Analysis and Statistics

**Title:** Soft Law Mechanisms for Novel Neurotechnologies

**Authors: *L. TOURNAS**;
Inst. of Neuroethics (IoNx), Atlanta, GA

**Abstract: Soft Law Mechanisms for Novel Neurotechnologies**
Novel neurotechnologies are currently in development to provide innovative treatments for previously difficult to treat neurological diseases, such as paralysis, cerebral palsy, as well as offer new pathways for communication for individuals living with locked in syndrome. They may even provide much needed opportunities for the treatment and management of disorders associated with mental illness and addiction. Additionally, they are being developed as consumer devices for personal focus and for enriched gaming experiences. With all the much-desired potential, it is important to acknowledge these devices are fundamentally digital technologies and will have same complications in managing data governance. It will be critical to both manage risk, while not hampering the duty to bring vital treatment to patients. Here soft law mechanisms may offer tools for both domestic and international management, but much is needed in identified which soft law tools with best align with neurotechnology.
Neurotechnologies do offer look different that other digital technologies as they offer an unprecedented reach into the human mind. This reach often adds to the hype, fear, and attention given to them by media, regulators, NGOs, international organizations, scholars, and potential users themselves. This focus should not be punitive, but rather should aim to both help patients get access to breakthrough technologies, while managing the privacy, individual security, personhood, national security, and ethical considerations.
Here, soft-law mechanisms offer flexibility and agility over traditional hard law and regulation. However, there is much work to be done on identifying which soft-law mechanisms are likely to best support neurotech specifically. Soft law works differently depending on both domestic and international issues, such as growth of industry, liability schemes, product application, national security risks, as well as relationship in international trade and supply chain. This is like most legal or regulatory tools working within emerging technologies. This talk will first give a brief background on soft law and past emerging technologies. Then it will identify specific soft law mechanisms that may well align with the inherent complexities of neurotechnologi

**Disclosures: L. Tournas:** None.

**Nanosymposium**

**NANO41: Synapse Formation and Remodeling**

**Location:** WCC 144

**Time:** Monday, November 13, 2023, 1:00 PM - 4:15 PM

**Presentation Number:** NANO41.01

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NSF 2207383
NSF 2014862

**Title:** Isochronic Development of Cortical Synapses in Primates and Mice

**Authors: *G. WILDENBERG**, H. LI, V. SAMPATHKUMAR, A. SOROKINA, N. KASTHURI;
Univ. of Chicago Dept. of Neurobio., Chicago, IL

**Abstract:** The neotenous, or delayed, development of primate neurons, particularly human ones, is thought to underlie primate-specific abilities like cognition. We tested whether synaptic development follows suit - would synapses, in absolute time, develop slower in longer-lived, highly cognitive species like non-human primates than in shorter lived species with less human like cognitive abilities e.g., the mouse? We report the opposite. Excitatory and inhibitory synapses in mouse and Rhesus macaque cortex form at similar rates and at similar times after birth. Primate excitatory and inhibitory synapses and mouse excitatory synapses also prune in such an isochronic fashion. Mouse inhibitory synapses were the lone exception, which were not pruned and instead continuously added throughout life. The monotony of synaptic development clocks across species with disparate lifespans, experiences, and cognitive abilities argues that such programs are likely orchestrated by genetic events rather than experience. It further argues that if human synaptic neoteny exists, it is likely a quantal, not a gradual, evolutionary adaptation among extant species.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NSF GRFP to AYG-W
Kavli NDI to ACS
NSF 1656592
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NIA R01AG072305

**Title:** Targeted synaptogenesis generates cell-type-specific connectivity in cortical layer 6

**Authors: *A. Y. GUTMAN-WEI**[1], A. C. SPIEGEL[1,3], A. G. CABALINAN[1], K. LEE[1], J. W. ANDERSEN[1], L. A. GOFF[1,3,2], S. P. BROWN[1,3];
[1]Solomon H. Snyder Dept. of Neurosci., [2]Dept. of Genet. Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD

**Abstract:** The function of the mammalian cerebral cortex relies on stereotyped patterns of synaptic connectivity among many neuronal cell types. How cortical neurons identify synaptic partners and form cell-type-specific connections during development is not well understood. One example of synaptic selectivity occurs in layer 6 of sensory cortical areas in mice, where excitatory corticothalamic projection neurons (L6CThNs) rarely synapse onto excitatory neurons that make up the majority of cells in L6 while selectively forming local connections with specific subtypes of inhibitory interneurons. Such synaptic target selectivity could emerge during the initial formation of synapses in a circuit. Alternatively, specificity could arise from initially promiscuous synapse formation, followed by synapse-type-specific elimination. We asked

whether selectivity emerges during the initial formation of L6CThN synapses or via specific elimination using paired whole-cell patch clamp recordings of identified cell types in L6 across the first two postnatal weeks. We found that specific synaptic connectivity is generated during the initial formation of L6CThN synapses. We also did not detect any transient silent synapses lacking AMPA receptors made by L6CThNs on interneurons or nearby L6CThNs, further supporting the role of specific synaptogenesis. By analyzing single-cell RNA sequencing data from postnatal day 6 cortical neurons, we found that L6CThNs expressed Neuropilin 1 (Nrp1), and that its ligand Semaphorin 3A is enriched in inhibitory neurons. Using a conditional knockout of Nrp1 in L6CThNs, we tested whether this pathway is required for L6CThN synaptic targeting, and our results suggest that Nrp1 cKO alone did not alter the probability of connection between L6CThNs and inhibitory neurons. Our results show that targeted initial synaptogenesis underlies the development of the cell-type-specific circuit organization of L6CThNs. Future experiments are required to determine potential molecular guidance mechanisms that may direct synaptic targeting by L6CThNs, and whether the initial establishment of cell-type-specific excitatory connections generalizes to synaptic targeting by other excitatory cell types in the neocortex.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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NIH Grant U24HG009293
HFSP Fellowship LT000127/2016

**Title:** An activity-dependent transcriptional program coupled with coordinated mRNA export drives synaptogenesis during development

**Authors: *C. YEE**[1,2], Y. XIAO[3], H. CHEN[1,2], A. R. REDDY[1], B. R. XU[4,5], T. N. MEDWIG-KINNEY[3], A. BOYLE[6,7], Y. XIANG[4,5], D. Q. MATUS[3], K. SHEN[1,2];
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**Abstract:** Decades of research have been dedicated to understanding the molecular composition, subcellular architecture and membrane trafficking of the synapse. Synapse formation requires rapid and coordinated production of hundreds of synaptic proteins. This process not only involves transcriptional activation, mRNA export and translation, but also requires control by neuronal activity. Our group has previously identified the conserved THO nuclear export complex (THOC) as an important regulator of presynapse development in *C. elegans* and dopaminergic neuron synapse maintenance in mice (Maeder *et al.*, 2019; PMID: 30146163). This study was the first to demonstrate that nuclear export is a critical rate-limiting step for

synaptogenesis and neuronal differentiation. One critical question that was not addressed in this study is how the THO complex selects its mRNA targets.
Using *C. elegans* dopaminergic neurons, we report that synaptic gene expression is controlled by neuronal activity and by two transcription factors (TFs), the AP-1 protein FOS-1/Fos and the zinc finger TF EGL-43/MECOM. Through cell-specific TF-profiling techniques, we find that both EGL-43 and FOS-1 bind directly to promoters of presynaptic genes to activate transcription. Depletion of either TF or mutation of their binding sites on presynaptic loci severely affects presynaptic gene expression *in vivo*. Using endogenous GFP-tagged proteins, we demonstrate that EGL-43 and FOS-1 regulate each other's expression. With a single nucleotide change in the FOS-1 binding site upstream of *egl-43*, we show that enhancing FOS-1 binding is sufficient to dramatically increase expression of EGL-43 and synaptic proteins. We manipulated the activity of dopaminergic neurons bidirectionally to demonstrate that activity regulates FOS-1 expression. These manipulations also impact presynaptic gene expression, suggesting that the EGL-43/FOS-1 program regulates synapse formation during development.
Consistent with mammalian interaction studies, we report that EGL-43 interacts with subunits of THOC. We demonstrate the ability to confer binding of mRNAs to THOC *in vivo* through the insertion of EGL-43 binding sites upstream of housekeeping genes. With this mechanism, EGL-43 provides mRNA target specificity to THOC to facilitate export of presynaptic mRNAs**.** Together, we describe the first evidence of a transcription factor and RNA export machinery directly controlling the expression of functional components of the synapse during development.

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**Presentation Number:** NANO41.04

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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**Title:** Regulation of presynaptic development by a Rap and Rac GTPase signaling cascade

**Authors:** R. LAMB, B. DHAR, A. SAWARDEKAR, M. SCALES, ***S. J. CHERRA, III**; Neurosci., Univ. of Kentucky, Lexington, KY

**Abstract:** The development of presynaptic terminals requires a highly coordinated series of molecular events. Disease-associated mutations indicate that perturbations in synapse development are associated with a variety of neurological disorders. However, the molecular mechanisms that promote synapse development are not completely understood. Here we sought to determine how mutations in a Rap guanine nucleotide exchange factor (GEF) associated with schizophrenia affects synapse development. We used *Caenorhabditis elegans* to dissect how the RapGEF, PXF-1, coordinates the development of presynaptic terminals. We previously found that mutations in *pxf-1* reduce neuromuscular junction function, decrease the intensity of synaptic vesicle markers, and reduce filamentous actin at presynaptic terminals. To determine how PXF-1 regulates the actin cytoskeleton, we investigated whether Rac GTPases were involved in PXF-1 signaling. We found that mutations in *rac-2* produced a reduction in synaptic

vesicle markers and decreased presynaptic actin filaments. Using a constitutively active mutant of RAC-2, we determined that RAC-2 activation was sufficient to restore the intensity of synaptic vesicle markers in *pxf-1* mutants. To determine how PXF-1 modulates RAC-2 signaling, we investigated whether RAP-1, a direct target of PXF-1, influenced synaptic development in *pxf-1* mutants. Using a constitutively active RAP-1 mutant, we observed that activation of RAP-1 was sufficient to restore synaptic development in *pxf-1* mutants. However, RAP-1 activation was not able to restore the intensity of synaptic vesicle markers in *rac-2* mutants. These data suggested that PXF-1 activates RAP-1, which then acts on a downstream effector to stimulate RAC-2 signaling to promote actin filament formation in presynaptic terminals. Overall, this study provides new insights into how RapGEFs modulate synapse development and how their dysfunction could underlie neurological disorders, like schizophrenia.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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**Title:** A cross-species proteomic map reveals neoteny of human synapse development

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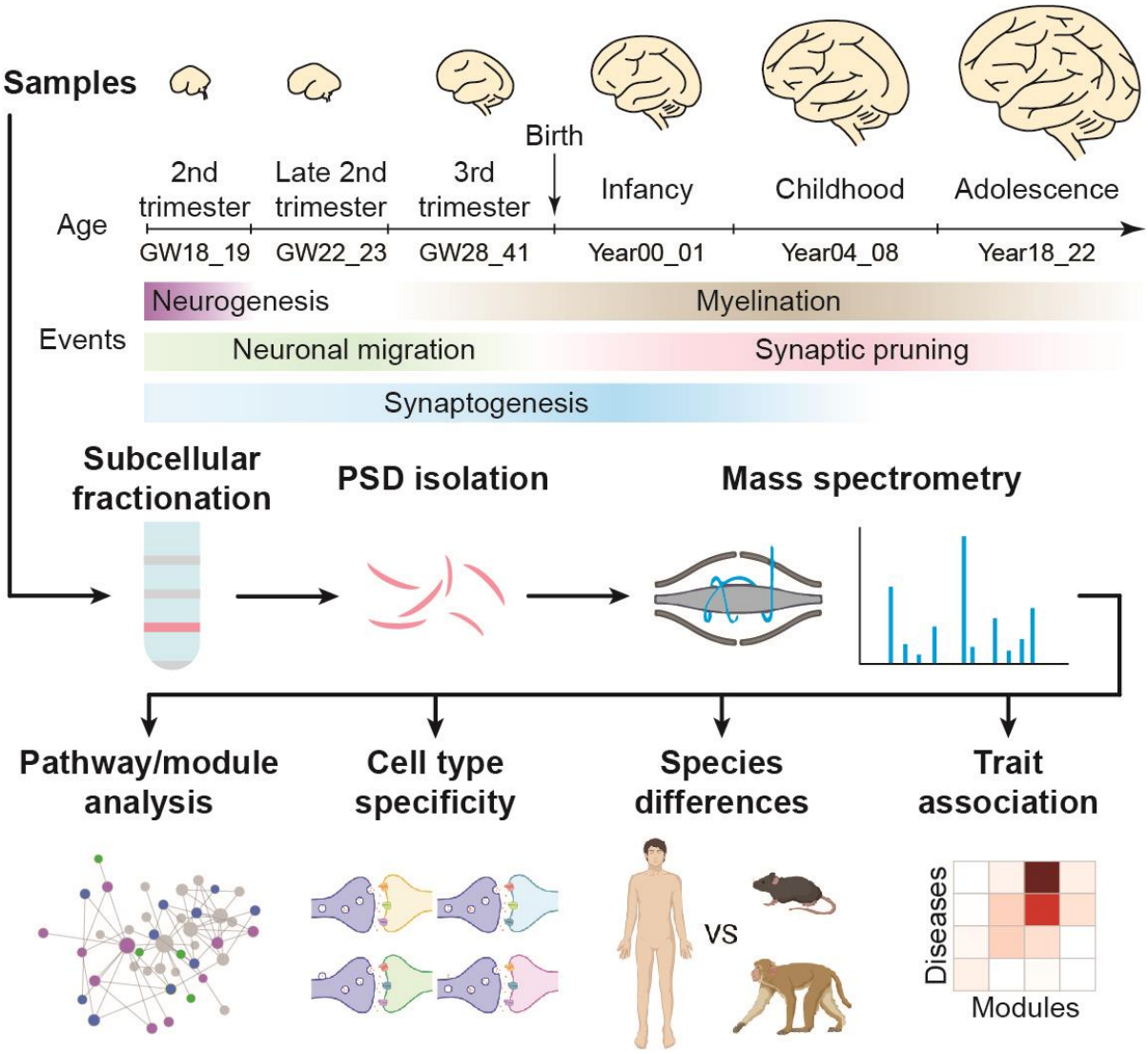
**Abstract:** The molecular mechanisms and evolutionary changes accompanying synapse development are still poorly understood. Here, we generated a cross-species proteomic map of synapse development in the human, macaque, and mouse neocortex. By tracking the changes of >1,000 postsynaptic density (PSD) proteins from midgestation to young adulthood, we found that PSD maturation in humans separates into three major phases that are dominated by distinct pathways. Cross-species comparisons reveal that the human PSD matures about two to three times slower than other species and contains higher levels of Rho guanine nucleotide exchange factors (RhoGEFs) in the perinatal period. Enhancement of the RhoGEF signaling in human neurons delays the morphological maturation of dendritic spines and the functional maturation of synapses, potentially contributing to the neotenic traits of human brain development. In addition, PSD proteins can be divided into four modules that exert stage- and cell type-specific functions, possibly explaining their differential associations with cognitive functions and diseases.

Together, our proteomic map of synapse development provides a blueprint for studying the molecular basis and evolutionary changes of synapse maturation.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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**Title:** Astrocytic ephrin-B1 controls perisomatic inhibitory synapse development by regulating EphB signaling in PV boutons

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**Abstract:** Impaired inhibition and parvalbumin (PV) interneuron hypofunction are thought to underlie the development of hyperactive neuronal networks in neurodevelopmental disorders (NDDs). While previous work has implicated trans-synaptic ephrin/EphB signaling in excitatory synapse development, the role of ephrin/EphB signaling in inhibitory synapse development has not been described. Astrocytes are critical regulators of synapse development; however, few astrocytic proteins contributing to inhibitory synapse development have been identified. Here we show that astrocytic ephrin-B1 (astro-eB1) positively regulates the development of connections between inhibitory PV neurons and CA1 pyramidal cells (PCs) in the hippocampus. Conditional deletion of eB1 from astrocytes during the postnatal (P14-P28) developmental period impaired PV->PC connectivity. We found reduced ErbB4 activation, reduced amplitude of evoked IPSCs and mIPSC in CA1 PCs, increased seizure susceptibility, reduced sociability, and increased repetitive behaviors in mice with postnatal astrocytic eB1 deletion. Overexpression (OE) of astro-eB1 during P14-P28 period using AAV viral approach increased PV->PC connectivity, with OE mice showing an increase in both PV/VGAT positive presynaptic sites and amplitude of evoked IPSCs in CA1 PCs. In mice expressing the excitatory opsin in PV neurons, astro-eB1 OE also increased the amplitude of optically evoked (oe)IPSCs in PCs. We propose that astro-eB1 positively regulates PV->PC connectivity by modulating EphB levels in PV boutons. We find that astro-eB1 deletion increases, but astro-eB1 OE reduces expression of EphB receptors in PV boutons, suggesting that astro-eB1 may regulate PV->PC connectivity by relocating EphB receptors away from PV terminals. Indeed, we found an increase in the number of PV/VGAT presynaptic sites and increased PV fluorescence intensity in the CA1 hippocampus of mice lacking EphB2 in PV cells, indicating that EphB2 may negatively regulate PV activity and PV->PC connectivity through its interaction with neuronal ephrin-B2. Our preliminary results also show increased inhibitory perisomatic innervation in hippocampal primary cell cultures following knockdown of ephrin-B2 with ASO. Altogether, our findings suggest that astro-eB1 positively regulates the establishment of PV perisomatic inhibitory synapses by controlling levels of EphB receptor in PV boutons and possibly through interfering with neuronal ephrin/EphB2 signaling. Our work describes a novel mechanism by which astrocytes regulate PV->PC connectivity and a point of intervention which can be used to correct impaired inhibitory circuits in NDDs.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Title:** Identification of bona fide postsynaptic density E3 ligases and associated proteolytic machinery

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**Abstract: Identification of bona fide postsynaptic density related E3 ligases and associated proteolytic machinery.**
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**AbstractBackground:** Synapses are complex biological assemblies that survive years. How neuronal cells identify and selectively degrade synaptic proteins is an unanswered question so far. Postsynaptic densities (PSD) are highly complex synaptic compartment, for which our understanding of precise proteostasis regulation is unclear. **Methods:** Biochemical isolation of PSD fractions, in conjunction with tandem mass tag (TMT)-based quantitative proteomics approach, revealed presence of multiple E3 ubiquitin ligases and other accessory components of the ubiquitin-proteasome system (UPS) at PSD. Validation of proteomics data were performed using biochemical methods including immunostaining, immunoblots, proteasome isolation and activity assay. Stable isotope labeling with amino acids in cell culture (SILAC)-based approaches and application of selective HECT and WW domain inhibitor drugs helped us identify crucial substrates for the select E3 ligases followed by MS-based identification of ubiquitination sites in the postsynaptic substrate proteins.**Results:** Interestingly, Nedd4 family E3 ligases were identified in two independent TMT experiments. To our surprise, we did not observe autophagy-associated proteins or components in PSD, whereas some small heat shock proteins and molecular chaperones were recovered in synaptosomes and PSD. We confirmed our preliminary observations using western blot and immunofluorescence techniques. We identified several novel substrate proteins for the select Nedd4 family E3 ubiquitin ligases. We confirmed these substrates using online databases, and by confirming their presence in isolated proteasomes from synaptosomal preparations. We also mapped ubiquitination sites of some substrates and confirmed their in vitro ubiquitination in presence of select E3 ligases.**Future Directions:** Interestingly, a large number of the substrate proteins identified are known to be part of one or many pathological conditions, including neurodegeneration and aging. These E3 ligases and their substrates may provide crucial therapeutic candidates in the context of neurodevelopmental disorders and neurodegeneration.
Keywords: Synapse, Postsynaptic density, Proteostasis, Ubiquitin, E3 ubiquitin ligase, Proteasome

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**Title:** The molecular mechanisms underlying synaptic specificity of cortical chandelier cells

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**Abstract:** Inhibitory interneurons (INs) play a pivotal role in shaping and balancing cortical network activity. A variety of IN subtypes that differ in morphology, physiology, gene expression, and connectivity cooperatively achieve this complex task. Distinct subtypes display unique synaptic specificity at different levels such as laminar, cellular, and subcellular scale. However, the intercellular molecular interactions underlying IN synaptic specificity remain largely unknown. Chandelier cells (ChCs) specifically innervate axon initial segments of pyramidal neurons in a specific cortical layer and thus serve as a good model to address the above issue. To begin to understand the molecular mechanisms that dictate IN synaptic specificity, we explored genes encoding cell surface proteins that are preferentially expressed in developing ChCs taking advantage of RNA sequencing-based gene expression comparisons. We identified seven ChC-specific cell surface molecules and found that some of them are essential for ChC synaptic specificity. For example, Immunoglobulin Superfamily member 11 (IgSF11) homophilic adhesion proteins are preferentially expressed in ChCs and their synaptic laminar target and mediate layer specific synapse formation by ChCs through its synapse promoting activity. We also discovered that a cell surface receptor on ChCs that recognize the AIS-enriched cell adhesion molecule is required for their continual synapse formation on AISs. These results suggest that intercellular molecular interactions transmitting distinct signals operate in concert to ensure IN synaptic specificity.

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**Title:** Comparing hippocampal mossy cell ablation vs silencing on the maturation of adult-born dentate granule cells

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**Abstract:** Early survival and circuit integration of adult-born hippocampal dentate granule cells (abDGCs) requires glutamatergic innervation, with hilar mossy cells forming the first functional glutamatergic synapses onto abDGCs. Disruption of hippocampal neurogenesis occurs after brain injury, coincident with loss of the hilar mossy cells. We hypothesized that dysfunction of mossy cells following brain injury contributes to aberrant neurogenesis and integration of abDGCs. We selectively ablated or silenced hilar mossy cells using viral vectors (AAV5-flex-taCasp3, AAV5-flex-TeLC-GFP) in Crlr-Cre (Calcitonin receptor-like receptor) mice. Virus-mediated apoptosis of hilar mossy cells by taCasp3 resulted in extensive (>70%) loss of mossy cell bodies and axonal projections in the inner molecular layer (IML). In contrast, hilar mossy cell bodies and axons were maintained following virus-mediated silencing by TeLC, but axons became beaded in the IML, consistent with block of vesicle release. After mossy cell ablation or silencing, we labeled abDGCs using BrdU or retroviral vectors, and used immunohistochemistry and electrophysiological assays to examine the proliferation, survival, and maturation of abDGCs. Mossy cell loss did not impact proliferation, survival, or morphology of adult-born DGCs, but dendritic outgrowth of abDGCs was accelerated two weeks post-mitosis. Interestingly, despite the loss of mossy cell axons in the IML, dendritic spine density on proximal abDGC dendrites was unaffected. Immunohistochemical staining and electrophysiological assays revealed structural and functional reorganization of the molecular layer after mossy cell ablation, such that middle molecular layer inputs now innervate more proximal abDGC dendrites, potentially compensating for the lack of mossy cell inputs. In contrast, preliminary studies of TeLC silencing of mossy cells revealed modifications in immature DGC density and migration of these cells away from the subgranular zone, without changing the laminar structure of molecular layer inputs. This suggests that functional synaptic innervation at proximal dendritic sites might critically regulate adult neurogenesis, demonstrating the importance of early functional synaptic innervation in the regulation of abDGCs.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

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**Title:** Does Prenatal Opioid Exposure Change Offspring Perineuronal Nets?

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**Abstract:** Opioid use among pregnant women has continuously increased and animal models have identified long-term changes to the offspring brain and behavioral function. Perineuronal

nets (PNNs) surround neurons and regulate plasticity and neuronal growth. Previous research indicates that removal or dysfunction of the PNNs during early development extends the critical period and is linked with cognitive and social behavioral problems during adolescence and adulthood. Prenatal opioid exposure also induces cognitive and social changes in offspring. We know that microglia prune synapses during the critical periods and microglia function may be altered in opioid exposed offspring. Microglia can interact with the extracellular matrix after opioid exposure which may include PNNs, but we do not know whether prenatal opioid exposure directly affects the PNNs. The goal of this study is to identify the changes in the brain responsible for behavioral and cognitive changes seen in prenatal opioid exposed offspring with the hypothesis that the PNNs may play a role. 17 female mice were given morphine (MO) p.o. 0.3mg/ml + 0.2% saccharin through their drinking water, and 16 female mice were given saccharin only, a week prior to mating with DBA males. Maternal MO exposure continued during gestation and lactation until BXD offspring weaned on postnatal day 21(P21). A social interaction test and 5-choice serial reaction time task measured offspring social behavior and executive function. At 12-13 weeks, brain samples were collected from a separate cohort that did not undergo behavioral testing. These brain samples were analyzed by immunohistochemistry using IBA1, WFA and PV to label the microglia, PNNs and parvalbumin neurons. We analyzed parvalbumin cell number, percent of parvalbumin neurons with PNNs, and colocalization of microglia with PNNs and parvalbumin neurons in the prefrontal cortex, amygdala, and nucleus accumbens. We found that mouse dams consumed 50-80 mg/kg of morphine per day. Offspring body temperature, used to assess potential opioid withdrawal, showed no changes across the first week of life and there was also no difference in pup weight on P2 or P19. We did observe an increase in social interaction in the MO offspring coupled with impaired executive functioning. The goal for the mice without behavioral testing is to assess the effects of prenatal MO exposure on PNNs and parvalbumin neurons in the adult brain in regions that are important for controlling social behavior and executive function. Future studies will assess synaptic pruning activities of the microglia and see if changes observed in the PNNs relate to changes in the microglia after prenatal MO exposure.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH Grant HD092369

**Title:** Leptin promotes the development of glutamatergic synapses in the developing hippocampus through the activity of the proteases matrix metalloproteinase 9 and cathepsin B

**Authors: *J. RODRIGUEZ LLAMAS**, C. DILLON, G. WAYMAN;
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**Abstract:** Neurotrophic factors direct the development of the nervous system, and impairments in their function lead to neurological disorders. Therefore, understanding how they regulate brain development is of paramount importance. Leptin is an adipokine that exerts neurotrophic effects on the central nervous system. Leptin is also produced in the hippocampus, where it promotes

synaptogenesis, synaptic plasticity, and neurogenesis, while rodent models with altered leptin signaling exhibit impairments in these processes and defects in hippocampal-related functions, such as impaired learning and spatial memory. Notably, altered leptin signaling is also associated with mental and cognitive disorders in humans, characterized by changes in dendritic spines. Leptin increases the number of dendritic spines and the frequency of mini excitatory postsynaptic currents in developing hippocampal neurons, evidencing an increase in functional glutamatergic synapses. Since morphological changes in dendritic spines are considered the structural basis of learning and memory, leptin's role in spinogenesis may explain the defects observed in rodent models with impaired leptin signaling. Nonetheless, the molecular mechanisms underlying leptin neurotrophic effects are poorly understood.
The endopeptidase matrix metalloproteinase 9 (MMP9), involved in the processing of extracellular matrix and synaptic components, plays an essential role in spine plasticity in the hippocampus, deeply influencing learning and memory. Interestingly, leptin induces the expression and activity of MMP9 in non-neural cells; however, it is unknown whether MMP9 plays a role in leptin neurotrophic effects on hippocampal neurons. Using a combination of cell and molecular biology, biochemistry, and microscopy approaches, we investigated the role of MMP9 in the effects of leptin on glutamatergic synaptic development in the hippocampus. We found that blocking the expression or activity of MMP9 prevented leptin-induced increase in mushroom spines of cultured hippocampal neurons. We also observed that leptin increased MMP9 expression and activity and promoted the exocytosis of MMP9-containing vesicles, causing increased release of MMP9. Finally, we showed that leptin effects also require the MMP9 activating-lysosomal protease cathepsin B, as blocking its expression prevented leptin effects on mushroom dendritic spines. Thus, our results indicate that the proteases MMP9 and cathepsin B are necessary for leptin effects on the structural plasticity of dendritic spines in the developing hippocampus and that leptin signaling regulates MMP9 expression, release, and activity in neurons.

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**Title:** Clustered synapses develop in distinct dendritic domains in the visual cortex before eye opening

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Develop., Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [4]Dept. of Functional Genomics, Ctr. for Neurogenomics and Cognitive Research, VU Univ., Amsterdam, Netherlands

**Abstract:** Synaptic inputs to cortical neurons are highly structured in adult sensory systems, such that synapses representing similar stimulus features are located near each other. This organization of synaptic inputs, called synaptic clustering, is required for high-fidelity signal processing. However, when and how synapse clusters assemble during development remains unclear. Here, we combined *in vivo* whole-cell patch clamp recordings with two-photon calcium imaging of mouse primary visual cortex layer 2/3 neurons, allowing us to map functional synaptic inputs across dendritic arborizations during development. We found that the density of functional synapses and the frequency of synaptic transmission increased significantly between P8 and P13. Interestingly, the observed inter-synapse distance distribution differed significantly from randomized distributions in younger, but not in older dendrites (Kolmogorov-Smirnov tests, n = 6 in each group, p = 0.0035 for younger and p = 0.962 for older dendrites), showing that synapses are spatially organized along developing dendrites. Synaptic inputs also accumulate in functionally separated domains already at the beginning of the second postnatal week. Assessing domain features across development revealed that both the number of synapses per domain (P8-10: 3.2 ± 1.7; P12-13: 6.0 ± 3.2; t-test, p = 0.0007) as well as the density of domains along dendrites (P8-10: 1.4 ± 0.7; P12-13: 2.6 ± 1.0 per 100 μm dendrite; t-test, p = 0.03) approximately doubled. Furthermore, we find that local co-activity within dendritic domains predicts synaptic activity (linear regression, r = 0.37, p = 0.0015), indicating that synapses are sorted into distinct functional dendritic domains through plasticity mechanisms driven by spontaneous network activity. To investigate whether this functional organization into dendritic domains is mirrored by a structural compartmentalization of the dendrite, we implemented an organotypic slice culture system of neonatal (P5) mouse visual cortex. Indeed, the *in vitro* development of dendritic domains matches the *in vivo* situation, indicating that these slice cultures can be used to identify the underlying molecular structure of dendritic domains. Together, these findings show that visual cortex neurons establish functional synapses at a very high rate during the week leading up to eye opening and that developing synapses become organized into functional, spatially distinct domains based on the synchronicity with their neighbors.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH Grant HD092369

**Title:** Determining the role of Apelin in the effects of maternal exercise on hippocampal synaptic development

**Authors: *K. PARKER**, M. JOSTEN, J. RODRIGUEZ LLAMAS, C. DILLON, G. A. WAYMAN;
Washington State Univ., Pullman, WA

**Abstract:** Principal **Topic:** Maternal metabolic health has largely been recognized as a contributor to offspring brain development. Exercise is linked to positive changes in emotion and

cognition including increasing memory and preventing diseases associated with aging. Previous research shows offspring from mothers who exercised during pregnancy also share beneficial changes in metabolism and cognition. The offspring have also been shown to have higher levels of circulating apelin than offspring from controls. Apelin was initially identified as a molecule released during exercise and has recently been implicated in learning and memory as a neurotrophic factor in the hippocampus. We can determine if apelin is required for neurodevelopment by analyzing dendritic spines. Dendritic spines are the post-synaptic components of excitatory synapses. Decreases in the mature form of these spines are associated with various neurodevelopmental disorders. Methods: We hypothesized that apelin is required for the development of dendritic spines through the TrkB/BDNF complex and the associated signaling cascade. Cultured hippocampal neurons from mice pups were transfected with an APJ short-hairpin RNA, the g-protein coupled receptor for apelin, dominant negative forms of TrkB or BDNF, or short-hairpin RNAs for Irisin or PGC1a. These molecules have all been shown to be required for spine growth. For in vivo experiments, apelin was administered to post-natal day (PND) 7 mice 24 hours before extracting the hippocampus. Finally, to examine the effects of exercise by itself on the brain development of the offspring, dames ran 1 hour a day on a treadmill for the duration of the pregnancy and the hippocampus was extracted from the pups on PND 8. Neurons were visualized using DiI and immunohistochemistry and then imaged using a confocal microscope. Results and Conclusions: Apelin administration in vitro and in vivo significantly increases the density of dendritic spines on cultured hippocampal neurons and APJ, TrkB/BDNF, and Irisin/PGC1a are required for this development. In mice, offspring from mothers who exercised during pregnancy have significantly higher densities of hippocampal dendritic spines. Pups from non-exercised litters who received apelin injections also had an increased spine density as compared to saline control. Results indicate that apelin may be a modulator of the changes in neurodevelopment in response to metabolic changes. This research could lead to a therapeutic target to ensure a healthy pregnancy for pregnant individuals who cannot exercise.

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**Nanosymposium**

**NANO42: Presynaptic Mechanisms, Organization, and Structure**

**Location:** WCC 140

**Time:** Monday, November 13, 2023, 1:00 PM - 4:00 PM

**Presentation Number:** NANO42.01

**Topic:** B.04. Synaptic Transmission

**Support:** NIH Grant T32NS091008-07
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**Title:** Role of presynaptic mitochondrial dysfunction in the cellular pathophysiology of psychosis

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**Abstract:** Schizophrenia (SZ) is a complex neurodevelopmental disorder, but imaging, genetic, and postmortem studies implicate aberrant glutamatergic synaptic transmission in the neocortex. The mechanistic underpinnings of SZ likely involve pre- and postsynaptic processes, altered synchronization of excitatory/inhibitory drive, and altered synaptic pruning during adolescence. Synaptic energetics represent another significant but relatively understudied contributor. A disproportionate amount of energy is consumed at presynaptic terminals, where mitochondrial ATP production maintains ion gradients and glutamate loading into vesicles. Prior work demonstrates that in induced pluripotent stem cell (IPSC)-derived neurons from individuals with 22q11.2 deletion syndrome (22q11DS), which carries a 25% risk of SZ, SZ was associated with weaker oxidative phosphorylation (OXPHOS) relative to neurotypical controls and 22q11DS without SZ (-SZ). 22q11DS-SZ was also associated with enhanced expression of mitochondrial biogenesis genes. These findings suggest that most individuals with 22q11DS compensate for mitochondrial weakness by enhancing mitochondrial biogenesis, and those that fail to compensate have a higher risk for developing SZ. But why would weaker OXPHOS increase SZ risk? We hypothesize that 22q11DS is associated with impaired synaptic energetics, that, in concert with polygenetic risk and environmental influences, weaken glutamatergic neurotransmission throughout development to ultimately produce SZ-related symptoms. To test this hypothesis, we are building cell culture, cell transfection, and imaging techniques to study presynaptic energetics in human IPSC-derived cortical excitatory neuron-like cells. Through alterations of constructs originally validated in mice we are now imaging synaptic vesicle cycling with a pH-sensitive GFP variant fused to the luminal domain of human vesicular glutamate transporter 1 (vGpH) and synaptic glutamate release (iGluSNFR3). Electrode stimulation of neuronal spiking, under conditions that either impair mitochondrial ATP production or increase the reliance on this production, are being used to determine whether mitochondrial weakness impairs glutamatergic synaptic release in neurons from the 22q+SZ group. Since synaptic energetics has not previously been imaged in human stem cell derived neurons, this system has tremendous potential for impactful studies of various genetic conditions in which compromise of synaptic energetics has been implicated, including some instances of SZ, autism, and intellectual disability.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Effect of noradrenaline in the ventrolateral preoptic area

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**Abstract:** Neurons in the ventrolateral preoptic (VLPO) area are essential for initiating and maintaining sleep. During wakefulness, the sleep-promoting VLPO galanin ($VLPO^{Gal}$) neurons are thought to be inhibited by noradrenaline (NA) and other wake-promoting neurotransmitters, but the mechanisms through which this occurs are not fully understood. Using *in vivo* and *in vitro* recordings, we investigated how the locus coeruleus (LC) regulates VLPO neurons. We expressed in the VLPO a G-protein-coupled receptor-activation-based NA sensor ($GRAB_{NA}$) and recorded changes in NA levels across the sleep-wake cycle in mice. We found that NA signal in the VLPO is higher during wakefulness than in NREM and REM sleep. We then tested by *in vitro* calcium-imaging and whole-cell patch clamp recordings the effects of NA on $VLPO^{Gal}$ neurons in brain slices. We found that NA directly inhibits the $VLPO^{Gal}$ neurons by post-synaptic alpha-2 adrenergic receptors and indirectly inhibits them by increasing GABAergic synaptic inputs. Optogenetic stimulation of local VLPO GABAergic neurons produced opto-evoked IPSCs in $VLPO^{Gal}$ neurons, indicating a local inhibitory circuit controlling the sleep-active $VLPO^{Gal}$ neurons. NA increased the amplitude of these opto-evoked IPSCs via alpha-1 receptors, demonstrating that NA inhibits $VLPO^{Gal}$ neurons at least in part through activation of this local GABAergic circuit. We previously described that orexin/hypocretin neurons produce wake-promoting responses through their projections to the VLPO. We found that orexin input indirectly inhibits the $VLPO^{Gal}$ neurons via the activation of local GABAergic neurons that express the orexin receptor 2 (Ox2R). In this study, we investigated the effects of orexin on the LC terminals in VLPO. Our hypothesis is that orexin released in the VLPO can activate LC terminals that express Ox1R, and thus presynaptically facilitate NA release. We found in bran slices that stimulation of the $LC^{NA}\rightarrow VLPO^{Gal}$ input evokes release of NA which directly inhibits, by alpha-2 adrenergic receptors, the $VLPO^{Gal}$ neurons. In addition, we found that the release of NA from LC axons in VLPO is increased by orexin. In conclusion, we found that NA is released in VLPO in a state dependent manner. During wakefulness, NA released from the LC axons is enhanced by orexin, contributing to the inhibition of $VLPO^{Gal}$ neurons. In narcolepsy, lack of this orexin signaling is expected to reduce NA release in VLPO, resulting in a diminished inhibition of the $VLPO^{Gal}$ neuron activity which may contribute to the frequent lapses into sleep that are common in narcolepsy.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Fine-scale presynaptic heterogeneity of a brain-wide-projecting individual neuron in the locus coeruleus in vivo

**Authors: *P.-S. XIANG**[1,2], W.-Y. CHEN[1,2], J.-L. DU[1,2];
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**Abstract:** Recent studies have revealed an increasing number of neurons that exhibit long-range projections, targeting multiple brain regions and forming synaptic connections with hundreds of post-synaptic neurons. However, how information encoded by the action potential at the soma is translated to each pre-synaptic site on such complex axonal arborization remains largely unknown. Here, taking advantage of *in vivo* volumetric calcium imaging and whole-cell recording in larval zebrafish, we mapped brain-wide axonal varicosity activity of individual Locus Coeruleus-norepinephrine (LC-NE) neurons. Each individual LC-NE neuron has highly branched axons that project to almost the entire brain. The varicosities, found along the LC-NE axons, are enlarged structures responsible for NE release. We found that somatic action potentials reliably propagate to all axonal branches, but the same somatic action potentials induce heterogeneous activities at varicosities along the axon. When varicosities were distanced within 15 microns or switched into the phasic activity mode, their $Ca^{2+}$ activities were more similar, exhibiting a distance- and activity-dependent spatiotemporal pattern. Furthermore, this heterogeneity is modulated by neuronal activities near the axon. Moreover, Target neurons of LC-NE also showed similar distance-dependent organization as the pre-synaptic sites on the axon. Therefore, our study revealed the distance- and activity-dependent presynaptic heterogeneity of axons in individual neuromodulatory neurons, suggesting the compartment-specific interaction between the NE signaling and its target synapses.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Neurexins are essential for the functional organization of the glycinergic synapse

**Authors:** H.-H. JIANG, R. XU, Z. SU, X. XU, X. NIE, ***F. LUO**;
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**Abstract:** Neurexins are essential presynaptic adhesion molecules that play multifaceted roles in the formation and function of various glutamatergic and GABAergic synapses. However, it remains unknown whether and how neurexins play a major role in the glycinergic synapse. To address these questions, we took advantage of the triple conditional knockout mice to ablate all neurexins using neuronal-specific manipulations. We analyzed the glycinergic synapse formed between the principal neuron in the medial nucleus of the trapezoid body (MNTB) and the principal neuron in the lateral superior olive (LSO), which is vital for computing sound localization. Combining RNAscope, AAV-mediated gene manipulations, optogenetics, and patch-clamp recordings, we showed that the MNTB neurons highly express various isoforms of neurexins. Selective deletion of all neurexins in the MNTB neurons strongly impaired the function but not the formation of the MNTB-LSO glycinergic synapse. Not only the amplitude but also the kinetics of synaptic transmission were disrupted after pan-neurexin ablation. A high

concentration of EGTA, a slow calcium chelator, remarkably blocked synaptic currents in TKO mice but not in control mice, suggesting that pan-neurexin deletion caused a significant change in the tight coupling between voltage-gated calcium channels and synaptic vesicles. Consistent with the reduced glycinergic inputs, both the excitatory-inhibitory ratio and the firing success rate of the LSO neurons significantly increased. Together, our data demonstrated that neurexins act essentially at the glycinergic synapse, further corroborating the universal function of neurexins as a presynaptic organizer in diverse synapses.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Neuronal lipofuscinosis caused by Kufs disease/CLN4 DNAJC5 mutations but not by a CSPα/DNAJC5 deficiency

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**Abstract:** Kufs disease/CLN4 is an autosomal dominant neurodegenerative disorder that affects young adults, caused by mutations in the DNAJC5 gene that encodes the synaptic vesicle co-chaperone Cysteine String Protein α (CSPα/DNAJC5). The Leu115Arg and Leu116Δ mutations in humans are known to independently cause the disease, although the underlying mechanisms are unknown. To investigate the disease mechanisms in vivo, we generated three independent mouse lines overexpressing different versions of CSPα/DNAJC5 under the neuron-specific Thy1 promoter: wild-type (WT), Leu115Arg, and Leu116Δ. Mice expressing mutant CSPα/DNAJC5 are viable and do not show any significant increase in morbidity or mortality. However, we observed the presence of pathological lipofuscinosis in the mutants, indicated by autofluorescent punctate structures labeled with antibodies against ATP synthase subunit C, which were absent in the WT transgenic line. Additionally, transmission electron microscopy revealed intracellular structures resembling granular osmiophilic deposits (GRODs), observed in Kufs disease patients, in the mutants but not in non-transgenic controls or the WT transgenic mice. Notably, conventional, or conditional knockout mice lacking CSPα/DNAJC5 did not exhibit any signs of increased lipofuscinosis or GRODs. Our novel mouse models thus provide a valuable tool to investigate the molecular mechanisms underlying Kufs disease/CLN4. We conclude that DNAJC5 mutations cause neuronal lipofuscinosis through a cell-autonomous gain of a novel but pathological function of CSPα/DNAJC5.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Phenotypic Analysis of Mice Carrying SNAP25 L50S mutation

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**Abstract:** Synaptic vesicle fusion requires proper functioning of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, SNAP25, synaptobrevin-2, and syntaxin-1. Ensuing neurotransmitter release can take place spontaneously, without an external stimulus (spontaneous release), or can be triggered by an action potential, resulting in fast and synchronized synaptic vesicle fusion (evoked release). Mutations in different components of the SNARE machinery can differentially affect evoked and spontaneous release and could underlie neurodevelopmental and neuropsychiatric diseases. Among these, SNAP25 encephalopathies are a group of neurodevelopmental disorders characterized by impaired synaptic function due to mutations in the SNAP25 gene. Among various pathogenic SNAP25 variants, our initial in vitro analysis showed that the L50S amino acid variation largely and selectively results in aberrant spontaneous release and can cause encephalopathy. Here, we generated a new SNAP25-L50S CRISPR-Knock-in mouse model to investigate the behavioral consequences of selective abnormalities in spontaneous release. The heterozygous SNAP25-L50S CRISPR-KI mice

display frequent spontaneous seizures as well as stereotypical movements, abnormal sensorimotor gating, deficits in social interaction, and elevated anxiety. Motor learning and coordination are unaffected although there is a significant decrease in motor strength accompanied by a decrease in myofiber thickness. These results highlight the behavioral phenotypes of dysregulated spontaneous release and suggest that the SNAP25-L50S KI mice may serve as a valuable tool for identifying potential interventions that target spontaneous release.

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**Title:** Resolution of the molecular architecture of active zone proteins at the rod ribbon synapse with 3D-MINFLUX nanoscopy

**Authors: *C. GRABNER**, K. INAMDAR, S. JAKOBS, T. MOSER;
Max Planck Inst. for Multidisciplinary Sci., Goettingen, Germany

**Abstract:** Synaptic transmission relies on presynaptic active zones (AZ) to transduce intracellularly propagated electrical signals into the release of neurotransmitters at the precise time and location. The AZ achieves this by positioning synaptic vesicle (SV) fusion machinery proximal to voltage-gated $Ca^{2+}$ channels ($Ca_V$). In the case of primary sensory neurons in the ear and eye, the AZ is marked by a uniquely large presynaptic density called the synaptic ribbon. In the retina, the synaptic ribbon is necessary for normal visual behavior (Fairless et al., IOVS; 2020). From functional release assays we have proposed that rods form a large RRP of 90 SVs that are within 10 nm from $Ca_V$ channels (Grabner and Moser, eLife; 2021), a finding that approximates the number of docked SVs at the rod AZ. This observation predicts that $Ca_V$ channels are positioned on either side of the AZ, and presumably along with other AZ proteins. In contrast to the wt condition, we found that ribbonless rods were unable to form a large RRP of SVs. To begin addressing whether the ribbonless AZ has malformed SV release sites, we recently developed a novel fixation method referred to as Heat Assisted Rapid Dehydration (HARD) that yielded a thin layer of retinal tissue (< 5 µm thick) on the surface of glass coverslips, which was optimal for 3D-MINFLUX and blinking STORM dyes (Grabner et al., Sci Adv; 2022). Therein we found that $Ca_V$ channels and AZ proteins were arranged in series on both sides of the ribbon. Next, to better image the $Ca_V$ channels, which are densely packed and a challenge to study with STORM dyes, we have utilized conventionally fixed (PFA) retinal slices for DNA-PAINT imaging with 3D-MINFLUX. The emerging results show that DNA-PAINT imaging in slices is optimal for studying $Ca_V$ channels; while the scaffolding protein bassoon, which is more sparsely expressed, can be imaged equally well with DNA-PAINT or STROM strategies. Importantly, we find that $Ca_V$ channel localizations are spaced at ~35 nm from one another (about the width of a SV), in series for the length of the ribbon. This amounts to >1 $Ca_V$

channel per SV release site, which is in support of nano-scale coupling of SVs with $Ca_V$ channels. In the ribbonless rods, the $Ca_V$ channels are expressed broadly across the ribbonless AZ, and bassoon falls into a single, poorly organized track. These findings demonstrate that the ribbon has a strong influence on the molecular topology of $Ca^{2+}$ channels and likely release sites. Further analysis of the results, and examination of additional AZ proteins, will provide a more complete model of the spatial organization of AZ proteins per SV release site.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Dual transmission of acetylcholine and serotonin in the ADF sensory neuron in C. elegans

**Authors: *P. G. CHANABÁ-LÓPEZ**, A. CUENTAS CONDORI, D. A. COLÓN-RAMOS; Dept. of Neurosci., Yale Univ., New Haven, CT

**Abstract:** Dual transmission is the ability of a neuron to transmit two different neurotransmitters. This neuronal mechanism is conserved across metazoans, suggesting that co-transmission is relevant for circuit function. To better understand the functional significance of dual transmission in the nervous system, we are developing *Caenorhabditis elegans* as an *in-vivo* and genetically tractable model to study dual-transmitter neurons. The neuronal transcriptome of *C. elegans* has revealed that about 10% of its neurons have dual transmission potential. One of these neurons, the sensory neuron ADF, expresses the genetic machinery to synthesize and release acetylcholine and serotonin. To monitor cholinergic vesicles, we have developed a tool to label the endogenous acetylcholine vesicular transporter (VAChT/UNC-17) with GFP in ADF. Using this tool, we have confirmed the presence of endogenous cholinergic vesicles in ADF *in-vivo*. The punctate pattern of these vesicles along ADF axons corresponds to the localization of synaptic sites, which have been identified with the active zone marker UNC-13::mScarlet. These observations suggest that cholinergic vesicles are being used in ADF synapses during neuronal communication. To further explore the potential co-transmission of serotonin in addition to acetylcholine in ADF, we are validating an additional tool to label the endogenous serotonin vesicular transporter (VMAT2/CAT-1) with GFP. Preliminary data on VMAT2/CAT-1 labeling at the exogenous level suggest that VMAT2/CAT-1 localizes to several well-known monoaminergic neurons, possibly including ADF. Future analyzes with endogenously tagged VMAT2/CAT-1 will test for the presence of serotonergic vesicles in ADF *in-vivo,* and thus the dynamics of serotonin and acetylcholine co-transmission in this neuron. Lastly, given that co-transmission is currently understudied, our long-term goal is to carry out a genetic screen to identify the genes that regulate transmitter-specific synaptogenesis in dual-transmitter neurons.

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**Topic:** B.04. Synaptic Transmission

**Support:** Natural Science Foundation of China

**Title:** Kinetic dissection of recycling vesicle pool along a close-loop pathway at the Calyx of Held synapses

**Authors: Z. LIU**[1], Y. ZHU[2], Y. HU[4], *J. SUN[3];
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**Abstract:** We studied vesicle recycling under sustained presynaptic stimulation at physiological temperature on calyx of Held synapses. The kinetics of vesicle reuse was revealed by impeding transmitter refilling with folimycin. It was found that about 80% of vesicles in nerve terminals are involved in recycling but they were not homogeneously competent for immediate release. A significant surface pool of vesicles, assayed as an increased membrane capacitance, was detected with different sizes corresponding to different stimulation intensities. We kinetically dissected the recycling vesicle pool as sequentially connected sub-pools, readily priming pool, readily releasable pool, surface pool and post-endocytic pool. The sizes and transition rates among these sub-pools were dynamically regulated by neuronal activity to ensure the efficient synaptic transmission. The depicted kinetic structure of the recycling vesicle pool along a close-loop pathway provided a new insight into the impact of vesicle recycling in stabilizing synaptic transmission and short term plasticity.

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**Topic:** B.04. Synaptic Transmission

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**Title:** Complement protein CD59 mediates GABAergic neurotransmission via SNARE complex but not synaptic pruning

**Authors:** L. WEN, X. YANG, Z. WU, S. FU, Y. ZHAN, Z. CHEN, ***D. BI**, Y. SHEN;
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**Abstract:** Recent exiting research demonstrate that complement proteins mediate neurotransmission and various brain functions in developing, ageing brain and brains with multiple neurodegenerative diseases. Mounting evidence show that this complement-dependent regulation mainly depends on synaptic pruning. In the present study, we have identified a direct regulation of CD59, the endogenous complement system inhibitor, on GABAergic neurotransmission and cognitive functions. Using whole cell patch clamp, molecular biological measurements, and behavior tests, we have found that CD59 knockout mice ($CD59^{-/-}$) exhibit reduced $Ca^{2+}$-dependent mIPSC in hippocampal granule cells and impaired spatial memory. CD59 mediates the formation of SNARE complex, which mediates GABA release from synaptic terminal, by binding to the key component protein VAMP2. Furthermore, CD59 deficiency does

not result in increased synaptic pruning as evidence by unchanged level of complement initiating protein C1q, comparable amounts of inhibitory synapses, and similar levels of microglia engulfment between the hippocampus of $CD59^{-/-}$ mice and wild type mice. Together, these results suggest that the complement system mediates inhibitory neurotransmission and spatial memory by regulating GABA release. This is a novel mechanism by which complement system mediates synaptic and brain functions from the well-recognized synaptic pruning.

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**Topic:** B.05. Synaptic Plasticity

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**Title:** Short term plasticity in hippocampal synapses for different presynaptic activity patterns

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**Abstract:** Short term plasticity (STP) acts as the neural substrate for modulation of synaptic efficiencies over a timescale of milliseconds to several minutes. STP is based on the history of presynaptic activity and involves several molecular players, most importantly presynaptic calcium. We performed *in vitro* experiments on the CA3-CA1 network in acute hippocampal slices from Grik4-cre mice expressing channelrhodopsin specifically in CA3 neurons. We achieved spatio-temporally patterned stimulation of randomly chosen sets of CA3 neurons using optogenetics using patterns composed of 15 micron spots, scaled to match the size of a CA3 cell body. Patterned light stimulation coupled with electrode based field stimulation gave us the ability to carry out STP experiments in different sets of synapses on a CA1 neuron. The CA1 neuron was held in a whole cell patch clamp so as to resolve excitatory and inhibitory synaptic components of the response. We delivered spatial patterns of 5 or 15 spots as paired pulses 50 ms apart. Patterned stimulation was further reduced to constituent single spots to test the summation properties during STP. We saw overall paired pulse facilitation of the second pulse using electrode based field stimulation for excitatory synapses. However, for spatially patterned light stimulation of similar frequency, we observed STP profiles ranging from facilitation to depression for different patterns when excitatory responses were measured. We also observed wide inter-trial variability in the responses for any particular set of synapses. Preliminary analysis showed the difference in STP to be correlated with initial synaptic weights. While stronger synapses depressed, weaker synapses facilitated. Inhibitory synapses showed short term depression of the second pulse for all patterns. Both excitatory and inhibitory responses summed sublinearly when given as patterns. Our work demonstrates the diverse short term properties of CA1 synapses and their role in modulating neuronal output, excitation-inhibition balance and information storage.

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**Title:** α2δ-3 Controls Calcium Channel Abundance at the Presynaptic Active Zones in Homeostatic Plasticity

**Authors:** Y. ZHANG[1], ***T. WANG**[2], Y. CAI[4], M. KUAH[1], S. VICINI[3], T. WANG[5]; [1]Georgetown Univ. Med. Ctr., Washington DC, DC; [2]Dept. of Pharmacol. & Physiol., Georgetown Univ. Med. Ctr., Washington D.C., DC; [3]Georgetown Univ. Med. Ctr., Georgetown Univ. Med. Ctr., Washington, DC; [4]Work, [5]3900 Reservoir Road, Georgetown Univ., Washington, DC

**Abstract:** The homeostatic modulation of synaptic transmission is an evolutionarily conserved neural regulation that stabilizes the nervous system. At the *Drosophila* neuromuscular junction (NMJ), pharmacological inhibition or genetic deletion of the postsynaptic glutamate receptors elicits an increase in presynaptic neurotransmitter release. The enhanced presynaptic release precisely offsets the postsynaptic changes and restores the postsynaptic excitation to the baseline level. This phenomenon is termed Presynaptic Homeostatic Potentiation (PHP). PHP is highly conserved across species and has been observed in both the central and peripheral nervous systems. We previously demonstrated that α2δ-3, an auxiliary subunit of voltage-gated calcium channels, is necessary for both the rapid induction and sustained expression of PHP at the *Drosophila* NMJ. However, the molecular mechanisms underlying α2δ-3-mediated regulation of neurotransmitter release during PHP remain to be elucidated. Here, we combined electrophysiological, confocal imaging, and super-resolution imaging approaches to investigate how α2δ-3 regulates synaptic transmission during PHP. We demonstrated that α2δ-3 controls PHP by regulating the abundance of the calcium channel pore-forming α1 subunit at the presynaptic release sites - active zones. We characterized the function of different structural domains in α2δ-3 and identified the domains that are required for modulating neurotransmitter release and calcium channel abundance for basal synaptic transmission and PHP. Taken together, we provided evidence that α2δ-3 is essential for the trafficking and stabilization of presynaptic calcium channels in homeostatic plasticity.

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**Nanosymposium**

**NANO43: LTP and LTD: Pre- and Postsynaptic Mechanisms**

**Location:** WCC 152B

**Time:** Monday, November 13, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO43.01

**Topic:** B.05. Synaptic Plasticity

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**Title:** All-optical investigation of the role of CaMKII on long-term plasticity in the hippocampus

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**Abstract:** Synaptic plasticity, inducing long-lasting changes in synaptic efficacy and structure, is a major mechanism of information storage in the brain. Calcium–calmodulin-dependent protein kinase II (CaMKII) is one of the most important memory molecules that transform transient synaptic activity events into long-lasting synaptic plasticity through its autophosphorylation feature. Whether CaMKII is essential to induce and/or to maintain synaptic plasticity is still controversial. We took advantage of optogenetic tools to investigate the role of CaMKII in synaptic plasticity, inducing synaptic plasticity and manipulating relevant signaling pathways at the same time. Specifically, we induced spike-timing-dependent plasticity (STDP) at Schaffer collateral synapses in rat hippocampal slice culture by optogenetic stimulation of two neuronal populations expressing spectrally separated channelrhodopsins. The all-optical protocol induced timing-dependent long-term potentiation (tLTP) increased synaptic strength both acutely (about 30 minutes) and more interestingly, chronically (3 days). When we optically inhibited the activity of CaMKIIα while inducing plasticity, a complete blockade of the acute tLTP was observed. Unexpectedly, 3 days later, stimulated neurons received significantly stronger input than their neighbors, a delayed potentiation that appears to be independent of CaMKIIα activity. Coincidently, expression of the immediate early gene *Fos* was also independent of CaMKIIα activity. On the other hand, the direct optical activation of CaMKIIα in the same circuit, accomplished by using a photoactivatable CaMKII, was sufficient for inducing acute functional and structural LTP as well as ultrastructural alterations. However, this CaMKIIα-activation-induced LTP returned to baseline after a couple of days. Together, these data suggest that activity-dependent potentiation of synaptic inputs has two phases: CaMKIIα is necessary and sufficient for the induction of early LTP. A second, CaMKIIα independent mechanism, possibly through the persistent activity of protein kinase Mζ, is responsible for the selective strengthening of inputs days later.

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**Topic:** B.05. Synaptic Plasticity

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**Title:** Distinct functional activation states of CaMKII holoenzymes in intact hippocampal synapses

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**Abstract:** CaMKII is one of the most abundant proteins in the postsynaptic compartment of excitatory synapses and is essential for long-term potentiation (LTP), and for learning and memory. Currently there is no available imaging technique capable of visualizing individual CaMKII holoenzymes in intact synapses. Here we show that individual CaMKII holoenzyme complexes can be identified in 3D scanning transmission EM (STEM) tomography reconstructions in intact hippocampal synapses where APEX2 tagged CaMKII produced contrast in the presence of diaminobenzidine (DAB) and osmication. At the basal state of synaptic activity, in the cytoplasm of postsynaptic terminals, CaMKII holoenzymes are often seen individually with some in clusters; CaMKII complexes are also localized on the extra-synaptic membrane, with a lesser number at the membrane in the core layer of postsynaptic density (PSD). Size analysis of CaMKII holoenzymes suggests that in the basal state the majority of cytoplasmic CaMKII holoenzymes are inactivated. With high K stimulation, the size of CaMKII holoenzymes more than doubled, consistent with their being in fully activated configurations. In synapses with a large active zone, many CaMKII holoenzymes are in proximity to the core structural layer of the PSD, whereas others are in the thickened and widened membrane structures in the PSD, suggesting that CaMKII complexes may either add to core PSD structures with other proteins or CaMKII already in the PSD acting as a scaffold to associate with other added proteins during synaptic activity. In synapses with smaller active zones (length 100-400 nm), prominent electron dense DAB staining is developed from the PSD extending up to 200 nm into the cytoplasm, which corresponds to extensive postsynaptic protein accumulation along with CaMKII in the expanded PSD zone. Tomographic reconstructions show that these electron-dense regions are often featureless due to the lack of stain differentiation in the extensive protein network. However, multiple clearly identifiable structures of diameter ~ 10 nm are sometimes visible, consistent with the size of the hub of the CaMKII complex, which we believe correspond to individual CaMKII complexes in their excited states mingling with PSD proteins located in the PSD pallium, as consistent with the prior immuno-EM studies. Our work demonstrates that not only APEX2-CaMKII can be used to visualize individual CaMKII in synapses, but it can also be utilized as an indicator of CaMKII activity at synapses for electron microscopy. Furthermore, this approach might be applied in future studies to visualize and elucidate CaMKII actions during synaptic activities such as LTP.

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**Presentation Number:** NANO43.03

**Topic:** B.05. Synaptic Plasticity

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**Title:** Obesity dysregulates prelimbic cortex to nucleus accumbens synaptic plasticity

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**Abstract:** Human and animal studies reveal that activity in the prelimbic cortex (PL) and the nucleus accumbens (NAc) is altered in obese states. Yet how precisely how obesity alters PL-NAc circuitry and whether such circuit adaptations persist after weight loss remain unknown. We hypothesized that PL-NAc inputs would be enhanced in obese mice, and that this would reflect increased probability of release in this circuit and dysregulated plasticity mechanisms. *Ex vivo* whole cell patch clamp experiments demonstrated PL-NAc adaptations preferentially enhance synaptic release onto D1-receptor expressing NAc spiny projection neurons ($D1^{SPNs}$) in slices from obese mice relative to lean, and further, this enhanced release persists after weight loss. We then used *in vivo* electrophysiology and optogenetic stimulation to test the hypothesis that long-term synaptic plasticity mechanisms were dysregulated in this pathway in obesity, and saw that a 15 Hz protocol unmasked long term potentiation in slices from lean but not obese mice. This reveals a novel mechanism likely underlying sustained NAc activity during and after obesity and potentially leading to obesity-linked persistent behaviors including increases in hedonic feeding. These experiments may help to hone initiatives such as deep brain stimulation in the NAc for the treatment of obesity.

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**Presentation Number:** NANO43.04

**Topic:** B.05. Synaptic Plasticity

**Support:** NWO KLEIN-1 MACGILLAVRY

**Title:** Mechanisms of postsynaptic nanoscale reorganization during synaptic potentiation

**Authors: *W. J. DROOGERS**, A. K. SERWETA, A. A. MOERKERKEN, L. STEIJVERS, O. K. KLOCK, F. M. BERGER, H. D. MACGILLAVRY;
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**Abstract:** Strengthening of excitatory synapses is pivotal for learning and memory. Long-term potentiation (LTP) of synaptic strength is broadly held to be mediated by an increase in the number of AMPA-type glutamate receptors (AMPARs). However, only AMPARs close to presynaptic release sites are sufficiently activated to contribute to synaptic transmission. It is

therefore predicted that synaptic potentiation not only relies on an increase in the number of receptors but also on the nanoscale reorganization of receptors close to glutamate release sites. Indeed, super-resolution microscopy studies revealed that AMPARs can concentrate in transsynaptic nanodomains, aligned with presynaptic release sites. Thus, unraveling the mechanisms that control AMPAR positioning and clustering within the postsynaptic density (PSD) is critical for a full understanding of synaptic plasticity. Here, we used CRISPR-Cas9 gene editing and single-molecule localization microscopy to test whether synaptic activity induces nanoscale reorganization of endogenous synaptic proteins. We observed an increase in the number and density of subsynaptic nanodomains marked by scaffolding proteins GKAP and PSD95. Monte Carlo simulations predict that this observed reorganization of scaffolding proteins and receptors is sufficient to potentiate postsynaptic currents. Remarkably, knockdown of Shank proteins abolished the reorganization of PSD95 nanodomains. The LTP-induced reorganization was rescued by Shank3 re-expression and depended on the direct interaction between Shank3 and F-actin. In addition, stabilizing the actin cytoskeleton occluded the LTP-induced nanoscale reorganization of the PSD. In summary, synaptic activity-induced PSD reorganization is dependent on Shank-actin interactions and leads to highly dense AMPAR nanodomains.

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**Presentation Number:** NANO43.05

**Topic:** B.05. Synaptic Plasticity

**Support:** NIH Grant 1R01MH119541-01A1

**Title:** Analysis of gene co-expression networks reveal differential recruitment of Gas5 lncRNA interactome by cAMP and mGLUR signaling in hippocampal neurons

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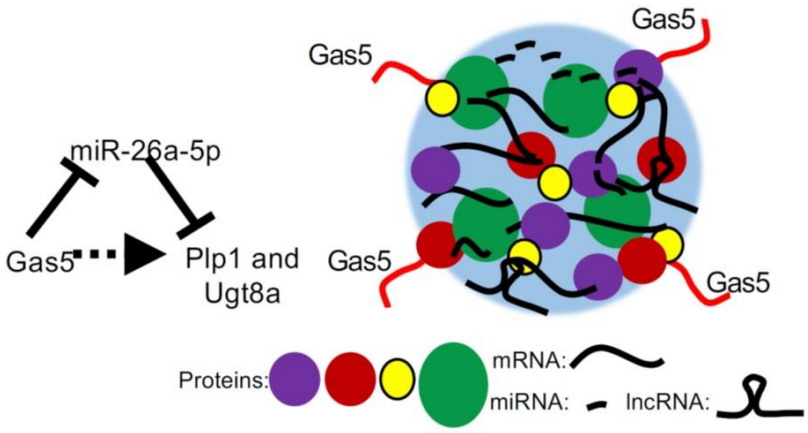
**Abstract:** LTP and LTD are excitatory and inhibitory forms of plasticity involving specific changes in gene transcription that shapes neuronal structural plasticity. Here we describe an unbiased analysis of changes in coding and noncoding transcriptomes induced by the activation of cAMP or mGLUR1/5 signaling in hippocampal neurons. Transcriptomics data showed 583 up and 880 down regulated genes in the cAMP dataset, whereas the mGLUR5 dataset had 201 up and 41 down regulated genes (N=4-6 per group, Padjst=FDR<0.05). Small RNA sequencing showed 32 up and 12 downregulated miRNAs in the cAMP set, 47 up and 57 down regulated miRNAs in the mGLUR5 dataset. We uncovered unique transcriptional signatures, molecular pathways and further constructed multiscale co-expression networks to elucidate distinct co-expression modules and central hub genes between these two pathways. These analyses have led to the identification of lncRNA Gas5, which is specifically upregulated by cAMP (Fold change- 5.99±1.12, Fsk vs DMSO, p<0.05, paired T-test) and interacts with miRNA miR-26a- 5p (Fold change- 1.863±0.42, Fsk vs DMSO, p<0.05, paired T-test) to sequester the degradation of mRNA targets— Plp1 and Ugt8a. miR-26a- 5p directly interacts with Gas5, validated through Luciferase assays and targets GSK-3β. Gas5 also directly interacts with GSK-3β, validated

through RAP and Western Blots. Perturbation of Gas5 changes neuronal arborization, spine dynamics. Electrophysiological measurements with High density Micro electrode Array showed changes in amplitude(Control- 193.96±3.99, Gas5 knockdown- 142.35±4.79 μV), firing frequency (Control- 14.31±0.37, Gas5 knockdown- 9.06±0.57 spikes/sec) and ISI (Control- 46.79±1.77, Gas5 knockdown- 69.49±2.66 millisecond) [One way ANOVA, $p<0.001$, MEA parameters], and neural network behavior (Burst numbers and duration). We find that Gas5 interactome consists of multiple coding, noncoding RNAs and proteins involved in synapse function. To our knowledge, this is the first consolidated study to delineate the unique signaling paradigms governing LTP and LTD.

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**Title:** Corticotropin-releasing factor differentially enhances the synaptic effect of ketamine metabolite (2R,6R) hydroxynorketamine at the hippocampal perforant path and temporoammonic synapses

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**Abstract: Background/Objectives:** Synaptic plasticity resulting in the strengthening of excitatory synapses is likely critical to the rapid onset antidepressant effects of many neuroplastogens including ketamine and its bioactive metabolite (*2R, 6R*) hydroxynorketamine (HNK). In rodent preclinical studies, HNK exerts its rapid and sustained antidepressant effects without undesirable dissociating properties of ketamine and strengthens synaptic transmission in the hippocampus. However, the specific synapses that are potentiated by HNK and mediators of differential synaptic effects, as well as HNKs mechanism of action are not fully understood. **Methods:** Information flow in the hippocampus is through the classical trisynaptic pathway which comprises the entorhinal cortex (EC) perforant path (PP) to granule cell synapse, mossy fiber to CA3 synapse, and Schaffer collateral CA3 (SC) to CA1 synapse. In addition to this is the direct EC to CA1 synapse via the temporoammonic (TA) pathway. We combined electrophysiological recordings of field excitatory postsynaptic potentials and pharmacological approaches in mouse hippocampal slices to investigate the effect of HNK alone and a combination of HNK and the neuropeptide, corticotropin-releasing factor (CRF) on the synaptic transmission at the hippocampal synapses. **Results:** HNK alone potentiated the SC-CA1 synapse. Regardless of the test concentration, HNK did not potentiate PP-GC or TA-CA1 synapses. A combination of CRF and HNK significantly potentiated the TA-CA1 synapse but not the PP-GC synapse. This synergistic synaptic potentiation at TA-CA1 synapses was NMDAR-independent and it was accompanied by a significant decrease in pair pulse ratio indicating a presynaptic mechanism. **Discussion:** These experiments revealed a synapse-specific function of CRF in enhancing the synaptic effect of HNK in the hippocampus, therefore, providing a novel insight into our understanding of the potential synaptic mechanisms by which HNK may exert its rapid onset antidepressant effect. Approaches combining manipulations that increase CRF combined with ketamine may have utility in the treatment of depression.

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**Title:** Mapping memories: pulse-chase labeling reveals AMPA receptor dynamics during memory formation

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**Abstract:** To study mechanisms underlying learning and memory, one would like to map changes in synaptic strength during user-defined time windows in behaving animals. In this study, we present EPSILON (Extracellular Protein Surface Labeling in Neurons), a technique that enables high-resolution mapping of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

receptor (AMPAR) insertion *in vivo*, shedding light on the dynamic nature of memory formation. AMPARs regulate synaptic strength via changes in their density by long-term potentiation (LTP) and long-term depression (LTD). Previous approaches based on pH-sensitive fluorescent proteins have provided valuable insights into AMPAR trafficking at individual synapses. However, limitations in *in vivo* imaging depth and head-fixed behavioral paradigms hindered broad applicability of these tools. EPSILON overcomes these limitations by employing pulse-chase labeling of surface AMPARs with membrane-impermeable fluorescent dyes, followed by *ex vivo* imaging on fixed tissue. This approach enables single-synapse resolution mapping across large brain volumes and multiple regions. We first generated a fusion of GluA1, a subunit of AMPAR, with HaloTag, a self-labeling protein that forms a covalent bond with an external ligand (HaloTag-ligand, HTL). By locally administering a membrane impermeable HTL dye, we saturated the surface-exposed AMPAR population. We then introduced a different-colored HTL dye to selectively label newly surface-exposed AMPARs. High-resolution multicolor imaging of fixed brain slices revealed wide-area maps of AMPAR dynamics across brain regions. We then integrated EPSILON mapping with analysis of cFos expression, a marker of immediate early gene activation associated with engrams. We elucidated the relationship between synapse-level plasticity and cell-level memory encoding in hippocampal CA1 pyramidal cells during contextual fear conditioning (CFC). Strikingly, we observed a robust correlation between synaptic plasticity and cFos expression, highlighting the synaptic basis underlying the association of cFos with memory formation. Moreover, EPSILON mapping revealed sub-cellular spatial patterns of plasticity. We found higher plasticity in perisomatic synapses compared to distal synapses, and we observed local clusters of potentiated spines. The EPSILON technique may be adapted to map the dynamics of other transmembrane proteins, and may find uses in developmental biology, disease modeling, and other related fields.

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**Presentation Number:** NANO43.08

**Topic:** F.08. Food and Water Intake and Energy Balance

**Title:** Mitochondrial fission and reorganization regulates induction of long-term depression

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**Abstract:** Mitochondria are undergoing constant fusion/fission dynamics and provide ATP, calcium buffering etc. to regulate neuronal function and synaptic plasticity. However, the influence between postsynaptic plasticity and dendritic mitochondria reorganization is not fully addressed. We find induction of both chemical induced long-term depression (cLTD) in cultural neurons and electrophysiological LTD in hippocampal slices requires drp1 dependent mitochondrial fission. Inhibition of mitochondrial fission before LTD induction disorganizes dendritic mitochondria and diminishes the loss of spines in cLTD and the decrease of excitatory postsynaptic currents (EPSCs) in electrophysiological LTD. Moreover, increase of dendritic calcium signals and autophosphorylation of the $Ca^{2+}$ calmodulin-dependent protein kinase II (CaMKII) at T286 in LTD was significantly reduced. In summary, we elucidate how

mitochondrial fission in dendrites regulates postsynaptic spines loss and activities through CaMKII autophosphorylation during LTD induction, which providing a novel insight into the mechanism and modulation of long-term plasticity via mitochondria fission.

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**Nanosymposium**

**NANO44: Spatial and Feature-Based Attention**

**Location:** WCC 147B

**Time:** Monday, November 13, 2023, 1:00 PM - 4:15 PM

**Presentation Number:** NANO44.01

**Topic:** D.06. Vision

**Support:** UCSB Academic Senate Grant
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**Title:** Effect of focused and distributed attention on stimulus representations in neural priority maps

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**Abstract:** Computational models suggest that visual attention is directed via activation profiles in neural 'attentional priority maps' spread across retinotopically organized brain regions. Prior work has proposed that these maps independently index the physical salience and behavioral relevance of stimuli, as determined by examining the topography of activation across regions while stimulus features (e.g., contrast) and task demands (e.g., attention) are independently manipulated (e.g., Sprague et al., 2018). Previously, these spatial maps have been quantified by measuring functional MRI while participants attended to one of two stimuli presented in the periphery. Thus, it remains unknown how more diverse modulations of attention—attention to fixation and attention to both stimuli—impact multiple stimulus representations in cortical priority maps. To probe this question, we used a selective attention task in which participants monitored random line stimuli and reported whether the stimuli cohered into a clockwise or counterclockwise spiral. When cued to attend to both stimuli, they reported the direction of the first item to cohere. If cued to fixation, participants maintained attention on a central fixation cross and responded whenever the horizontal or vertical line increased in size. This allows us to better survey the landscape of attentional control states and more concretely assess how attention in different configurations—towards fixation, selective towards one of several stimuli, and distributed across multiple stimuli—changes stimulus representations. Using a spatial inverted encoding model, we were able to reconstruct images of priority maps from retinotopic regions in visual and parietal cortex which contained representations of each visual stimulus. First, we replicated the results that attending to a single stimulus enhanced its representation independent

of stimulus salience (Sprague & Serences, 2013; Itthipuripat et al., 2019) and that attending to one of two stimuli enhances the attended stimulus representation (Sprague et al, 2018). Next, we compared stimulus representations when both stimuli were attended compared to when only a single stimulus was attended. Strikingly, both stimulus representations were enhanced when attended, with no decrease in representation strength compared to focused attention directed to a single stimulus. These results suggest that activation profiles across visual cortex can be thought of as independently indexing whether a location is attended (with a high versus low activation state) and the strength of stimulus drive (e.g., contrast).

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**Presentation Number:** NANO44.02

**Topic:** D.06. Vision

**Support:** Alfred P Sloan Research Fellowship
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**Title:** Prioritization of stimuli across retinotopic cortical regions during visual search

**Authors: *D. THAYER**, T. C. SPRAGUE;
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**Abstract:** Objects in the visual field are attended based on image-computable salience and current behavioral relevance. The combination of salience and relevance is reflected by a feature-agnostic priority map that indexes the most important locations. While attention is ultimately directed based on activation from the aggregate priority map, competition between feature-specific items is reflected within corresponding feature dimension maps (e.g., color or motion map). However, it is unclear how responses within neural feature dimension maps reflect competition between relevant and irrelevant, but salient, items during visual cognitive behaviors. Here, we used a visual search task to evaluate how relevant and salient items compete in neural feature dimension maps and how this competition manifests in an aggregate neural priority map. On each trial, participants were cued to search for a target defined by a specific color or motion direction. Next, a search array was presented which contained 8 colorful moving dot stimuli. All items in the array moved in the same direction and were homogenously colored, except for the target item, which differed solely on the cued feature dimension. Occasionally, one of the non-target items was a salient distractor because it was presented in either a different color or it moved in a different direction from the other items (including the target). We applied a multivariate image reconstruction technique to compute spatial maps from activation patterns in retinotopic regions, including feature-selective regions (putative motion and color maps) and posterior parietal cortex, a candidate aggregate priority map. Both targets and distractors were represented in reconstructed spatial maps. Specifically, neural motion dimension maps preferentially indexed motion-defined salient distractors and motion-cued targets. Furthermore, we identified trials where the salient distractor likely captured attention (slow search RTs) and trials where it was likely ignored (fast RTs). The relative activation of each item corresponded to the behavioral consequences of salient distractor presence during search within motion dimension maps and aggregate priority maps. These results indicate that neural feature

dimension maps are crucial for computing attentional priority and establish potential neural correlates of priority maps during a complex visual cognitive task.

**Disclosures: D. Thayer:** None. **T.C. Sprague:** None.

**Presentation Number:** NANO44.03

**Topic:** D.06. Vision

**Support:** NIH R01-EY027401

**Title:** Spatial organization of top-down attentional signals in visual cortex

**Authors: *E. TÜNÇOK**, M. CARRASCO, J. WINAWER;
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**Abstract:** Covert spatial attention modulates behavioral and neural responses. fMRI studies have reported that voluntary attention increases BOLD amplitude, shifts population receptive fields (pRFs), and alters pRF sizes. Here, we implemented a combined fMRI-psychophysics experiment to concurrently investigate these effects. The experiment was designed to map pRFs while subjects attended a location in anticipation of a target. In every trial, a precue directed participants to either attend to one of four isoeccentric (6°) locations on the cardinal meridians, or to distribute attention across four locations. 300 ms after the precue, a task-irrelevant retinotopic mapping stimulus was presented at one of 48 possible locations. Shortly after the mapping stimulus, low-contrast Gabor patches were briefly presented at each of the four possible target locations. A response cue then instructed participants to discriminate the orientation of one of the four Gabor stimuli. PRF models were fit to the BOLD responses to the mapping stimuli in V1-hV4, V3AB and LO-1. Focal attention improved performance at the cued location and impaired it at uncued locations. In all visual field maps, the BOLD response increased for pRFs near the cued location, and decreased for uncued locations. The magnitude of the BOLD increase was approximately constant, i.e., independent of the mapping stimulus location, indicating a baseline increase rather than a multiplicative gain. The magnitude of the baseline increase was similar across retinotopic maps, but its spatial extent increased from V1 to LO1. pRF centers shifted towards the cued location, with bigger shifts in higher cortical areas. pRF sizes were similar in the focal and distributed attention conditions. Our results indicate that response properties in visual field maps change in anticipation of an attended target, potentially facilitating the subsequent stimulus-evoked activity.

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**Presentation Number:** NANO44.04

**Topic:** D.06. Vision

**Support:** NSF Grant 2050833

**Title:** Traveling waves bridge visual working memory and action

**Authors: *C. LUO**, E. ESTER;
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**Abstract:** Working Memory (WM) is a capacity- and duration-limited system that bridges fleeting sensory phenomena and prospective actions. Contemporary models of WM posit that information is stored in sensory cortical areas, yet it is unclear how information stored in sensory areas is communicated to motor areas that produce WM-guided behaviors. Here, we asked whether this communication is mediated by feedforward cortical traveling waves that are known to contribute to perceptual and memory performance in other contexts. We reanalyzed data from two publicly available studies in which human participants (both sexes) were retrospectively cued to report the orientation of a remembered bar that appeared in the left or right visual field with their left or right hand. Critically, the location of the remembered bar and report hand were fully crossed over trials, which allowed us to measure and quantify directional cortical traveling waves when stimulus location and response hand were congruent (i.e., a left visual field stimulus requiring a left-hand response) or incongruent. During congruent trials, we found strong feedforward theta-band activity linking sensory and motor sites that predicted participants' response onset times and response durations, consistent with a role for feedforward theta in controlling WM-guided behaviors. Critically, this result was more than a feedforward sensory evoked response: an identical analysis applied to EEG data during WM encoding revealed no evidence for feedforward theta activity (or directional activity in any other frequency band). Additionally, analyses of a second data set indicated that feedforward theta-band activity linking sensory with motor areas is related to the generation of an overt response; an identical analysis applied to a pre-cue experiment - which allowed participants to deduce what stimulus to report and what hand to use when responding but required participants to wait several seconds before responding - revealed no evidence for feedforward theta-band activity. Thus, these findings suggest that feedforward traveling waves are a potential mechanism for the production of WM-guided action.

**Disclosures: C. Luo:** None. **E. Ester:** None.

**Presentation Number:** NANO44.05

**Topic:** D.06. Vision

**Support:** NIH R01 EY-016407
NIH R01 EY-033925

**Title:** The spatial tuning of cortical responses during visual memory

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**Abstract:** Neural responses in visual cortex can be elicited by viewing a scene (perception), maintaining a recent percept of that scene (working memory), or retrieving a scene viewed in the past (long-term memory). fMRI studies have shown that the three types of representations share some properties; for example, both forms of memory, like perception, can be retinotopically tuned. However, there has been much less investigation of systematic differences in visual cortex responses during memory vs perception. We used fMRI to quantify spatial tuning in visual cortex during perception, working memory, and long-term memory. During 11.5-second target periods, a peripheral target (7º eccentricity) was either viewed, maintained in working memory following a brief pre-trial exposure, or retrieved from long-term memory, which required pre-

scan associative learning with fixation cues. At the end of each trial, participants made a saccade to the target location. In all three conditions, BOLD responses during the target period in the V1 to LO1 maps were spatially tuned to the stimulus polar angle, confirming retinotopic tuning in memory. As expected, during perception the polar angle tuning became increasingly wide from V1 to LO1, reflecting larger receptive fields. In contrast, during both working memory and long-term memory, tuning width was nearly the same across the seven maps, approximately matched to perception in hV4 to LO1 but wider than perception in V1-V3. This difference in memory-driven responses may reflect a feedback bottleneck, such that tuning in posterior maps cannot be much more precise than it is in the high-level maps that generate or maintain the responses. Finally, we show that the visual cortex responses in memory are relevant for behavior, in that trial-to-trial variability in spatial responses was predictive of the direction of saccade errors to the remembered targets.

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**Presentation Number:** NANO44.06

**Topic:** D.06. Vision

**Support:** NEI R01 EY023915

**Title:** Rethinking simultaneous suppression in visual cortex via compressive spatiotemporal population receptive fields

**Authors: *E. KUPERS**, I. KIM, K. GRILL-SPECTOR;
Stanford Univ., Stanford, CA

**Abstract:** The human visual system is worse at processing multiple stimuli presented simultaneously than sequentially. Likewise, neurons' responses are suppressed when presenting simultaneous vs sequential stimuli in the receptive field—a phenomenon called simultaneous suppression. The prevailing theory suggests that simultaneous suppression is due to competition for neural resources in the receptive field and is modulated by top-down task demands (Desimone & Duncan, 1995). However, the stimulus-driven, computational mechanisms underlying simultaneous suppression are unknown. Here, we leveraged fMRI and a population receptive field (pRF) modeling framework to operationalize what neural computations predict simultaneous suppression at the voxel level. Participants (N=10) viewed colorful squares presented either sequentially (SEQ) or simultaneously (SIM). Crucially, we varied stimulus size and presentation timing for each SEQ-SIM pair. We find that simultaneous suppression occurs in single voxels across multiple visual areas, increases up the visual hierarchy, and varies with both stimulus size and timing. Using participants' independent retinotopy data, we test which of three pRF models best predict voxel's responses: a compressive spatiotemporal model (in visual degrees and milliseconds) recently developed by our lab (Kim, Kupers, Lerma-Usubiaga, Grill-Spector, BioRxiv 2023), a compressive spatial model (Kay et al. 2013), and linear spatial model (Dumoulin & Wandell 2008). Results show that simultaneous suppression is better predicted by compressive spatiotemporal computations rather than spatial computations alone. Inspecting spatiotemporal pRF parameters, we find that increased simultaneous suppression across the visual hierarchy is linked to larger pRF sizes and stronger compressive spatiotemporal nonlinearities. These results necessitate a rethinking of simultaneous suppression as an outcome

of bottom-up compressive spatiotemporal summation within receptive fields, rather than spatial competition governed by top-down processing, and open new opportunities to study visual processing capacity in space and time.

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**Topic:** D.06. Vision

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**Title:** Tuned responses to visual short-term memory load in a frontoparietal hierarchy of topographic maps

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**Abstract:** Visual short-term memory, intentionally remembering image features, relies on interacting sensory and executive processes. Sensory processing represents stimulus content using on tuned neural responses organized in hierarchical networks of topographic maps. How does the brain distribute responses to task demands across neural populations? Using ultra-high field (7T) functional MRI, we examined neural responses to the number of remembered visual items, i.e. the complexity of the remembered images, task difficulty or the load on visual short-term memory. We describe strong changes in neural responses with changes in visual short-term memory load in an extensive network from posterior sensory to anterior executive areas of the human brain. The most anterior responses (in the frontal lobe) monotonically increase in amplitude as visual short-term memory load increases. However, more posterior (parietal and occipital) regions show tuned responses peaking at specific visual short-term memory loads. Within these areas, different recording sites show preferential responses to different visual short-term memory loads, organized in topographic maps where load preferences gradually progress across the cortical surface. These responses are absent when viewing the same stimuli without varying task demands and appear unrelated to response preferences for visual position and numerosity. These results generalize principles of neural tuning, topographic organization, and hierarchical transformations from sensory encoding to the distribution of task demands across neural populations.

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**Title:** Neural dynamics underlying the neurodevelopment of selective attention mechanisms

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**Abstract:** Selective attention plays a crucial role in prioritizing relevant sensory information and filtering out distractions. Several theories of attention have posited that this process involves the fronto-parietal network sending top-down signals to enhance the selectivity of spatial tuning of neural populations in upstream visual areas. However, the developmental trajectory and temporal dynamics of these neural computations as well as their contribution to adult-level selective attention functions remain poorly understood. Here, we investigated these mechanisms by recording EEG from typically developing Thai children (6-12 years), adolescents (13-18 years), and adults (20-33 years) during an attention-cueing Eriksen flanker task. We used the inverted encoding model (IEM) to examine the temporal dynamics of selective visuospatial attention based on alpha band oscillations (8-12Hz) in the EEG data. Our alpha-based IEM analysis yielded two key findings. First we observed an increase in tuning selectivity of the alpha-based spatial reconstructions with higher attention demand (i.e., incongruent vs. congruent stimuli) from ~50-250 ms after the target onset in adults, while no such increase was evident in children or adolescents. Second, the temporal dynamics of alpha-based spatial representations differed across age groups. In children and adolescents, cue-induced increases in tuning selectivity emerged very early (around 50ms after the cue onset) and persisted throughout the cue and stimulus periods. In contrast, adults initially exhibited no tuning selectivity in the alpha-based reconstructions near the cue onset. However, as time progressed, the tuning selectivity of the alpha-based spatial representations increased rapidly until reaching the target onset, followed by a sharp decline after the target onset unlike the alpha-based spatial reconstructions that sustained throughout the entire stimulus period in the younger groups. These findings shed light on the developmental trajectory of selective attention mechanisms and highlight distinct patterns of neural dynamics in the alpha band frequencies that support the neurodevelopment of selective attention.

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**Topic:** D.06. Vision

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**Title:** Retinotopic, not spatiotopic, encoding and decoding of visual field positions throughout human visual cortex

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**Abstract:** We perceive a stable visual world across eye movements, despite the drastic retinal transients these movements produce. To explain vision's spatial stability, it has been suggested that the brain encodes the location of attended visual stimuli in an external, or spatiotopic, reference frame. However, spatiotopy is seemingly at odds with the fundamental retinotopic organization of visual inputs. Here, we probe the spatial reference frame of vision using ultra-high-field (7T) fMRI and single-voxel population receptive field mapping, while independently manipulating both gaze direction and spatial attention. To manipulate spatial attention, participants performed an equally demanding visual task on either a bar stimulus that traversed the visual field, or a small foveated stimulus. To dissociate retinal stimulus position from its real-world position the entire stimulus array was placed at one of three distinct horizontal screen positions in each run. We found that population receptive fields in all cortical visual field maps shift with the gaze, irrespective of how spatial attention is deployed. This pattern of results is consistent with a fully retinotopic reference frame for visual-spatial processing. We next reasoned that a spatiotopic reference frame could conceivably be computed at the level of entire visual areas rather than at the level of individual voxels. To test this we adapted a Bayesian decoding method developed for orientation processing to decode full posteriors over 2D stimulus location from the BOLD response patterns in visual areas. We found that decoded stimulus locations and the associated uncertainty are in line with the retinotopic frame of reference, by shifting with gaze position. Again, this result holds for all visual areas and irrespective of the deployment of spatial attention. We conclude that visual locations are encoded in a retinotopic reference frame throughout the visual hierarchy.

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**Title:** The effects of positive and negative emotions in modulating the spatial scopes of visual selective attention

**Authors: *T. CHAISILPRUNGRAUNG**, K. THANONTIP, S. ITTHIPURIPAT;
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**Abstract:** The ability to selectively process relevant sensory information while filtering out irrelevant distractors is crucial for speedy and efficient sensory encoding. Previous studies have established a strong connection between visual attention and emotion; however, the precise mechanisms by which emotion modulates attention and the susceptibility of this effect to participants' psychological states remain unclear. This study aimed to explore the mechanisms through which emotional valence shapes the spatial extent of visual selective attention. Additionally, we investigated whether this valence-related change in attention depends on inter-subject variability in emotional state. We employed an adapted version of the Eriksen flanker task, where participants observed shape stimuli embedded in a circular array and made judgments about the target shape (bowtie vs. diamond). Critically, the target's location was cued by a preceding face image displaying one of three emotions: happy, angry, and neutral. Prior to the experiment, participants completed a questionnaire (i.e., the Depression, Anxiety, and Stress Scale-21 items or DASS-21) to assess their self-reported levels of negative emotional state. Our findings revealed that emotional valence significantly influenced attention, resulting in a broader spatial scope of processed visual information when positive faces were presented, relative to negative or neutral faces. Moreover, we observed a stronger effect of emotional valence on attention among participants reporting fewer symptoms related to depression, anxiety, or stress. These results shed light on the intricate interactions between selective attention and the affective system, while also highlighting the potential for developing a neural index to measure current emotional states.

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**Topic:** D.06. Vision

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**Title:** The size of visual field maps as a neurobiological and computational constraint on spatial working memory

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**Abstract:** The quality of working memory (e.g., capacity and precision) varies considerably across individuals. Although higher cognitive functions, intelligence, and psychiatric symptoms depend on working memory, the neurobiological basis for individual differences in working

memory remains unknown. It is conceivable that basic properties of the neural populations supporting working memory form the substrate of individual differences. For example, in visual perception, differences in psychophysical thresholds correlate with differences in the surface area of early visual cortex (e.g., Song et al., 2013; Benson et al., 2021). Here we leverage functional neuroimaging (fMRI) and transcranial magnetic stimulation (TMS) to test the hypothesis that the size of visual field maps in frontal and parietal cortex contribute to individual differences in working memory precision. We also use neural network models to identify potential mechanisms that could cause working memory to vary as a function of the size of a neural population.
Using fMRI, we performed population receptive field mapping in human subjects (male and female) to identify retinotopically-organized areas of visual, parietal, and frontal cortex. Subjects also performed a memory-guided saccade task to assess their working memory precision. We then correlated the size of subjects' visual field maps with the average WM error. We found a reliable negative correlation between the size of visual field maps and WM error in three regions: the precentral (sPCS) and intraparietal (IPS) sulci, and V1. We also tested the effect of TMS to sPCS on WM error, finding that subjects with larger sPCS were less impaired by TMS.
We adapted established neural network models of WM (Engel & Wang, 2011) to test why larger network size improves WM precision. We considered three (not mutually exclusive) possibilities: larger size makes the WM representations more resilient to noise; larger size allows better averaging over noise during WM readout; larger size facilitates more precise encoding via finer tuning of units across stimulus space. We found that basic network size effects were best explained by improved readout, with a smaller contribution of resilience to noise. Modeled TMS effects, on the other hand, were primarily explained by increased resilience to noise. Together, our findings provide a mechanistic neurobiological basis for individual differences in WM, which is important for understanding variation in cognitive ability in both healthy and diseased states.

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**Topic:** D.06. Vision

**Title:** Human extrastriate cortex is tiled with somatosensory homunculi

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**Abstract:** Our rich, embodied visual experiences of the world involve integrating information from multiple sensory modalities - yet how the brain brings together multiple sensory reference frames to generate such experiences remains unclear. Recently, it has been demonstrated that BOLD fluctuations throughout the brain can be explained as a function of the activation pattern on the primary visual cortex (V1) topographic map. This class of 'connective field' models allow us to project V1's map of visual space into the rest of the brain and discover previously unknown visual organization. Here, we extend this powerful principle to incorporate both visual and somatosensory topographies by explaining BOLD responses during naturalistic movie-watching as a function of two spatial patterns (Connective fields) on the surfaces of V1 and S1. We show

that responses in the higher levels of the visual hierarchy are characterized by multimodal topographic connectivity: these responses can be explained as a function of spatially specific activation patterns on both the retinotopic and somatosensory homunculus topographies, indicating that somatosensory cortex participates in naturalistic vision. These novel multimodal tuning profiles are in line with known visual category selectivity, for example for faces and manipulable objects. Our findings demonstrate a scale and granularity of multisensory tuning far more extensive than previously assumed. When inspecting their topographic tuning in S1, we find a large band of extrastriate voxels spanning lateral and dorsal visual pathways are tiled with somatosensory homunculi. These results demonstrate the intimate integration of information about visual coordinates and body parts in the brain that likely supports visually guided movements and our rich, embodied experience of the world

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**Topic:** D.06. Vision

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**Title:** Autokinesis reveals a threshold for perception of visual motion

**Authors: Y. LIU**[1], J. TIAN[2], Q. ARSHAD[4], M. ARMAND[3], *A. KHERADMAND[5];
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**Abstract:** In natural viewing conditions, the brain can optimally integrate retinal and extraretinal signals to maintain a stable visual perception. These mechanisms, however, may fail in circumstances where extraction of a motion signal is less viable such as impoverished visual scenes. This can result in a phenomenon known as autokinesis in which one may experience apparent motion of a small visual stimulus in an otherwise completely dark environment. In this study, we examined the effect of autokinesis on visual perception of motion in human observers. We used a novel method with optical tracking in which the visual motion was reported manually by the observer. The effect of autokinesis was measured with viewing of the stimulus when it was stationary in an otherwise completely dark room. The result was then compared with the perceived motion of the visual stimulus when it was moving at different speeds in the dark (baseline speed x $0.16^{o}$/s and speeds 2x, 3x, 4x, and 6x), or when it was moving under light illumination in the presence of visual cues (speed x). We quantified a direction synchrony index with lower values indicating alignment with autokinesis and larger values indicating alignment with the actual direction of motion Experiment results show at lower speeds of motion, the perceived direction of motion was more aligned with the effect of autokinesis, whereas in the light or at higher speeds in the dark, it was more aligned with the actual direction of motion (DSI: -0.53±0.15 at x, -0.31±0.16 at 2x, -0.02±0.09 at 3x, 0.06±0.12 at 4x, 0.04±0.10 at 6x, 0.20±0.07 at x in light). Our data show that autokinesis affects how the moving visual stimulus is perceived and this effect is dependent on the speed of motion. This finding has important implications for understanding how the stability of visual representation in the brain can affect accurate perception of motion signals.

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**Nanosymposium**

**NANO45: Cortical Planning and Execution: Human and Non-Human Primate Neurophysiology**

**Location:** WCC 150

**Time:** Monday, November 13, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO45.01

**Topic:** E.04. Voluntary Movements

**Support:** Michael J. Fox Foundation: Therapeutic Pipeline Program
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**Title:** The Cortical and Subcortical Controls of Gait Initiation in People with Parkinson's Disease

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**Abstract:** Patients with Parkinson’s Disease (PD) often experience postural instability, leading to difficulties with gait initiation and increased falls and reduced quality of life. To develop effective therapies, improved conceptualizing of the **neural circuitry involved in gait initiation, including the effects of dopamine replacement therapy and correlates with task performance and quality must be undertaken.**We examined cortico-pallidal network changes associated with gait initiation in two male PD patients implanted with an investigational bidirectional neural stimulation device (Medtronic Summit RC+S). Local field potentials (LFPs) were streamed from bilateral subdural electrodes over the cortex (premotor and primary motor, or M1, areas) and deep brain stimulation (DBS) leads in the pallidum. Participants performed gait initiation trials on two force plates (one foot on each), with a self-selected stepping foot in response to a visual “go” cue. Cortical and subcortical LFPs were aligned with biomechanics data quantifying postural responses while the patient was in the ON and LOW dopamine medication state while OFF DBS. Spectral power across all canonical frequency bands were averaged during the preparatory (quiet standing to cue), reaction (cue to anticipatory postural adjustment, or APA, onset), and execution (APA onset to stepping foot off force plate) phases. Intrasubject LFP spectral power was analyzed between medication states at all cortical and pallidal areas, using Wilcoxon ranked-sum tests. Regression analysis was also undertaken to investigate possible correlation between average spectral power during phases of the task and the timing and amplitude of postural responses.We found significant power differences shared and patient-specific across alpha (8-12 Hz), beta (13-30 Hz), and broadband gamma (50-150 Hz) frequency bands between the ON and LOW medication states at the cortical and pallidal contacts. Specifically, the globus pallidus internus (GPi) showed greater beta band spectral power under LOW medication during the preparatory and execution phases. M1 exhibited increased broadband gamma power in the ON medication state during the execution phase. By

correlating spectral power with APA metrics, we found significant correlations at the cortical areas for theta (4-8 Hz), alpha, and broadband gamma frequencies impacting both time to and peak APA amplitude.These exploratory results indicate dynamic modulation of pallidal and cortical neural oscillations during gait initiation. This modulation varies with medication state and may be linked to the quality of postural responses, affecting gait initiation quality and safety.

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**Presentation Number:** NANO45.02

**Topic:** E.04. Voluntary Movements

**Support:** Burroughs Wellcome Fund
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**Title:** Cortico-subcortical coordination underlying alternating limb movements in humans

**Authors: *S. KONDAPAVULUR**[1], C. K. STARKWEATHER[1], M. MORRISON[2], J. BALAKID[1], K. LOUIE[1], K. PRESBREY[1], M. YAROSHINSKY[1], P. STARR[1], D. D. WANG[1]; [1]Neurolog. Surgery, [2]Radiology, Univ. of California, San Francisco, San Francisco, CA

**Abstract: Introduction:** Fundamental to the study of human gait is understanding the motor network basis of rhythmic arm swing and stepping. This knowledge is crucial for development of therapeutic interventions to treat gait dysfunctions. Here we present results from the first invasive human study comparing network neural activity during stereotyped upper and lower limb movements.
**Methods:** 3 subjects with Parkinson's disease (PD) and 2 subjects with essential tremor (ET) were included in the study. All patients were treated with clinically indicated deep brain stimulation (DBS) with leads implanted in the subthalamic nucleus (STN, n=1) or globus pallidus internus (GPi, n=2) for PD patients, and ventral intermediate nucleus (VIM, n=2) for ET patients. Temporary 28-contact cortical paddles were placed over the hand knob area of motor cortex, and a NeuroOmega system was used to record field potential data. Subjects completed arm swing and stepping tasks, with accelerometers or Xsens IMU sensors capturing movement. We subsequently analyzed swing- and step-locked local field potential power and phase-amplitude coupling (PAC).
**Results:**We found movement-specific modulation across multiple frequency bands (delta, theta, alpha, beta, and gamma) during swing/step across cortex, GPi, STN, and VIM. For cortical field potentials, significant power modulation in low frequencies were driven by either ipsilateral or contralateral swing or step (effector-hemibody specific), in addition to either step or swing (effector-specific) or left or right movement (hemibody-specific). Subcortical contacts similarly exhibited movement time-locked low frequency band modulation. Compared to an overall trial period, there was decreased beta-gamma PAC during arm swing and stepping relative to theta-gamma PAC. This was found in swing or step in 96% of cortical contacts (5 subjects, n=166), 100% of GPi contacts (2 subjects, n=10), 100% of STN contacts (1 subject, n=3), and 95% of VIM contacts (2 subjects, n=22). Intriguingly, cross-area PAC (cortical low frequency phase-subcortical high gamma amplitude, subcortical low frequency phase-cortical high gamma amplitude) also exhibited a similar theta-beta PAC modulation with movement.

**Conclusion:**Cross-area PAC could be an important method of communication across the motor network. Additionally, both ipsilateral and contralateral representations of arm and leg movement can be found in a single hemisphere. These findings support the idea that cortical-subcortical network oscillations may play important roles in coordinating stereotyped movements across upper and lower limbs on both sides of the body.

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**Presentation Number:** NANO45.03

**Topic:** E.04. Voluntary Movements

**Title:** Non-focal beta rhythm suppression modulates corticomuscular coherence along the motor cortex somatotopy

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**Abstract:** For performing even the simplest of motor behaviors, the human primary motor cortex (M1) must exert stable regulatory control over skeletal muscles in the trunk and the extremities. This precise M1 efference is reflected in the power of the M1 beta (13-30 Hz) rhythm and the magnitude of beta corticomuscular coherence (CMC), with attenuation of beta power and CMC corresponding to activation of the M1. Interestingly, beta power suppression is inversely related to the number of activated muscles in a moving limb and to the exerted motor effort. Contrary to popular interpretations, this suggests that beta power suppression reflects the additive effect of the activation of the somatotopic areas corresponding to a network of muscles involved in performing a given task. Investigating the spatial specificity of the beta rhythm with non-invasive electrophysiology is challenging due to the limited spatial resolution afforded by these techniques. To circumvent this challenge, we investigated how the CMC between the M1 and a specific muscle is affected by movements executed by a different muscle.
To do so, we applied a dual task in which participants ($n$ = 22, 6 female) held an isometric contraction of the right First Dorsal Interosseous (rFDI) muscle at 10% of their maximum contraction force. While holding the contraction, a jittered auditory tone prompted the participants to perform a small internal/external rotation of the right shoulder, resulting in a small movement of the lower arm in the horizontal plane. EEG and EMG from the right FDI were recorded throughout the experiment.
Our results indicate that, compared to the rest period prior to movement onset, executing the shoulder rotation led to significant suppression of the beta power at electrode C3 at timings 0-1 s after movement onset ($t(21) = 5.52$, $p < 0.001$) and 1-2 s after movement onset ($t(21) = 4.21$, $p = 0.001$), had no effect on the beta EMG of the right FDI, and significantly suppressed the CMC between M1 and rFDI at timings 0-1 s after movement onset ($t(21) = 2.79$, $p = 0.049$).
The effect of the executed shoulder movement on the CMC between the left M1 and the rFDI shows that voluntary movement of one muscle induces a suppression of the beta rhythm which spreads to somatotopic areas beyond the ones strictly involved in the executed movement. Therefore, our results caution against interpreting beta power suppression as a strictly focal

phenomenon. Instead, the movement-relevant changes in beta power detected in electrophysiological measures may reflect an aggregation of precise regulatory excitation-inhibition processes along multiple somatotopic regions of M1.

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**Topic:** E.04. Voluntary Movements

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**Title:** Action execution, action observation and action sentence responses in human frontoparietal cortex measured with intracranial recordings

**Authors: *V. BOUGOU**, E. KEIRSE, T. DECRAMER, A. VAN HOYLAND, P. JANSSEN, T. THEYS;
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**Abstract:** The role of the mirror neuron system (MNS) in humans remain elusive, despite multiple functional imaging studies. Translatability of nonhuman primate (NHP) findings from extensive electrophysiological recordings to the human brain is controversial. We performed invasive human recordings using 16-channel epidural electrode grids (Specify 5-6-5, Medtronic) targeting the primary motor cortex (M1) and covering a broad region of the central frontoparietal cortex in three patients with refractory neuropathic facial pain. Our tasks included visually-guided grasping, grasping imagery, observation of real-life grasping, passive fixation of action videos and controls, and silent reading of action sentences. We analyzed 64 channels in the high-gamma frequency range (60-300 Hz) and employed a one-way ANOVA with a sliding window (100 ms, 50% overlap) to compare baseline and stimulus or action - evoked activity, identifying channels with significant responses ($p < 0.05$) in at least 3 consecutive time bins. Our results revealed that 63 channels (98.4%) exhibited significant responses during action execution (visually guided grasping), while 31 (49.2%) channels also showed significant responses during the observation of human action videos (mirror contacts). Among these mirror contacts, 23 channels (74.2%) exhibited significant responses to ellipse movements following the same trajectory, even in the absence of a meaningful action. Furthermore, 48 out of the 63 action execution channels (76.2 %), of which 39.6 % mirror channels, exhibited significant responses while reading action sentences. Additionally, coherence analysis demonstrated significant gamma band coherence between anatomically grouped contacts during real-life action observation. Notably, action observation elicited a pronounced increase in gamma coherence across all areas, with stronger coherence observed for the ‘premotor-M1’, ‘premotor-S1’, and ‘premotor-parietal’ contacts. Interestingly, this pronounced increase was not observed during motor imagery, suggesting differences in functional connectivity underlying different processes. These findings on action execution and action observation in the human frontoparietal cortex, including control stimuli without meaningful action as well as action sentences, shed light on the human MNS and bridge previous findings from NHPs with human imaging studies.

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**Topic:** E.04. Voluntary Movements

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**Title:** A cortical mechanism for eye-head-hand coordination: lateral prefrontal 'gaze' signals encode future head / hand motion during visually guided reach

**Authors: *J. CRAWFORD**[1], V. NACHER[1], P. ABEDI[1], H. ARORA[2], X. YAN[3], H. WANG[3]; [1]Psychology, [2]Kinesiology, [3]VISTA, York Univ., Toronto, ON, Canada

**Abstract:** Most of what we know about sensorimotor neuroscience comes from the study of single effectors, such as eye or hand motion, or relatively stereotypical behaviors such as eye-head coordination for gaze shifts. However, real world behavior requires the adaptive coordination of multiple effectors for different circumstances. For example, eye-head coordination patterns change during reach, with gaze movement followed by enhanced head (and hand) motion toward the reach target (Arora et al., 2019). The high-level cortical mechanisms for such strategies (particularly for head control) are unknown. We investigated this by recording single units from posterior lateral prefrontal cortex (pLPFC; spanning Brodman areas 45, 46, & 8a) while two Rhesus monkeys performed head-unrestrained reaches toward visual targets. Most (374 / 499) task-related neurons showed time-locked gaze-related responses, with some of these cells showing sustained responses during reach. Surprisingly these 'gaze' responses disappeared (35 / 84 neurons) or diminished during controls (head-unrestrained gaze shifts toward the same targets) without reach. We then performed an in-depth spatial analysis on the reach task data by fitting 6 spatial 'models' (future gaze, head, & hand displacement / position) derived from laboratory behavioral measures against spatially tuned response fields derived from pLPFC 'gaze' responses in 253 neurons, recorded during the same trials. This analysis confirmed that these 'gaze' responses were not what they appeared to be: in contrast to the saccade system (Sajad et al. 2015, Sadeh et al. 2015, Bharmauria et al. 2021), gaze displacement models provided the *worst* fits to these data. Instead, spatially tuned pLPFC 'gaze' responses preferentially coded skeletomotor motion, specifically future head (49% neurons) or hand (33%) models, with this balance shifting toward reach coding later in the task. This is an important demonstration that signal timing does not always reflect spatial tuning in the same neurons. We conclude that most pLPFC 'gaze' responses are not involved in gaze control, but rather reflect gaze inputs that trigger complex head-hand repertoires: in other words, a high-level neural mechanism for eye-head-hand coordination.

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**Title:** Spatial microstructure of motor cortical neural dynamics

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**Abstract:** Recent advances in silicon electrode technology enable dense, simultaneous sampling of many neurons. As significant computational and theoretical work has begun to understand the computations performed via neural population dynamics, these tools open experimental avenues to explore how these dynamics are organized within neural circuits. We recorded in macaque primary motor and dorsal premotor cortices using Neuropixels and collected a dataset comprising 6,990 neurons (36 sessions, 2 monkeys). We analyzed neural responses during a reaching task to investigate the spatial, laminar, and synaptic organization of neural populations engaged in motor control. We revisited a long-held view that the motor cortex exhibits columnar architecture organized by shared preferred movement directions (PDs). In contrast with this view, we found that spatial proximity was not predictive of similarity in PD. More generally, nearby neuron pairs did not exhibit increased PSTH correlation or a reduced angle between GPFA loading vectors. We further verified this lack of spatial micro-organization using a mutual information metric relating individual neurons with their spatial nearest neighbor, and comparing this statistic against synthetic cortical models with columnar or spatially clustered organization. Similarly, spatially intermingled neural activity was also observed when the arm was mechanically perturbed during reaching, resulting in sensory errors that evoked corrective feedback responses in the motor cortex. We used current source density to coarsely divide neurons into superficial and deep cells and found that responses to visual task cues and proprioceptive errors (when perturbing the arm mid-reach) appeared earlier in superficial cells than deep cells. We then used a representational similarity metric to assess whether superficial neurons contained response features not found in deeper neurons. This analysis identified transient periods after task cues and mechanical perturbations where local response features emerge in superficial cells before becoming distributed throughout the full population. Collectively, these analyses depict highly heterogeneous, spatially intermingled neuronal responses throughout the motor cortex. Visual and proprioceptive task inputs evoke local response features first in superficial neurons and then may rapidly spread through recurrent connections. These inputs then initiate and mold pattern-generating dynamics which are finely, spatially intermingled in the cortical population.

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**Topic:** E.04. Voluntary Movements

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**Title:** Reliability of a TMS-derived threshold matrix of corticomotor function

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**Abstract:** Conventional transcranial magnetic stimulation (TMS) experiments typically investigate primary motor cortex excitability by examining suprathreshold motor evoked potentials (MEPs). This approach may overlook subliminal levels of corticomotoneuronal (CM) activity that could provide information about the integrity of the CM tract after neurological injuries such as stroke. The present study examined the reliability of metrics derived from a novel compositional analysis of MEPs acquired using single-pulse TMS. The compositional analysis permits evaluation of subthreshold, subliminal, and suprathreshold activation measures within a framework we have termed a Threshold Matrix. Twenty-three adults participated in two experimental sessions (13 females; mean age: 68 years). Stimulus-response (S-R) curves and threshold matrices were constructed from MEP amplitudes obtained from four upper limb muscles bilaterally. Stimulation intensities ranged from 30-100 % maximum stimulator output (%MSO), increasing in 10% increments and including 65 %MSO. For a given stimulation intensity and muscle, the percentage of trials that resulted in a MEP ≥50 μV was calculated (i.e., persistence) and entered into a threshold matrix. Subthreshold, subliminal, and suprathreshold excitability states were categorised based on MEP persistence. Intraclass correlation coefficients (ICC) were calculated to assess the reliability of S-R curve slopes and threshold matrix components. Bayesian paired t-tests were used to compare measures obtained from the dominant (D) versus non-dominant (ND) hemispheres. A Bayesian correlational analysis explored associations between subliminal excitability and resting motor threshold (RMT). The sub- and supra-threshold components of the threshold matrix showed good-excellent reliability respectively (D subthreshold ICC = 0.89; ND subthreshold ICC = 0.81; D suprathreshold ICC = 0.91; ND suprathreshold ICC = 0.94). By contrast, subliminal responses showed poor reliability, presumably due to a floor effect (D subliminal ICC = 0.41; ND subliminal ICC = 0.00). No differences in threshold matrix composition were found between D and ND hemispheres (suprathreshold $BF_{01}$ = 4.41; subthreshold $BF_{01}$ = 2.73; subliminal $BF_{01}$ = 0.94). There was no association between subliminal responses and RMT (D r = 0.30, $BF_{01}$ = 1.7; ND r = 0.34, $BF_{01}$ = 1.5). A threshold matrix can reliably analyse sub- and supra-threshold responses in neurologically healthy older adults. Further studies are required to evaluate the threshold matrix, particularly subliminal responses, in assessing the CM pathway after neurological injury.

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**Nanosymposium**

**NANO46: Network Activity, Inhibitory Control and Disorders of Executive Functions**

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**Presentation Number:** NANO46.01

**Topic:** H.04. Executive Functions

**Support:** TL1TR001431

**Title:** Unitaryneural correlates of executive control in pediatric transdiagnostic psychopathology

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**Abstract: Introduction**. Childhood psychiatric symptoms are highly comorbid which is partially explained by deficits in executive control. Here, we study parcel-level integration with functional networks in two fMRI executive tasks in order to identify a common neural correlate of executive control and test its association with psychopathology in children with diverse psychiatric problems.
**Methods**. We studied 94 children [27 F/66 M/1 NB; mean age (SD)=11.3 years (1.67)] with diverse diagnoses including ADHD (n=39) and ASD (n=34). We extracted a latent general factor of psychopathology ("p" factor) from parent-report Child Behavior Checklist syndrome scores. Subjects completed two fMRI probes, the NBack Task tapping working memory and the Stop Signal Task tapping motor inhibition. Following preprocessing and denoising steps, we extracted time-series from parcels in the 7 Network Schaefer 400 parcellation atlas and constructed pairwise functional connectivity (FC) matrices reflecting overall connectivity. To identify the most integrative parcels, group average matrices were iteratively thresholded from highest 20% to 5% of connections, and participation coefficient (PC), a measure of cross-network connectivity, was summed across iterations. Parcels with >90$^{th}$ percentile PC in both tasks were selected for analysis (29 total). As an initial exploration, we tested the similarity across tasks of each parcels' FC and its relationship to "p" factor.
**Results**. The 29 selected parcels, which in both tasks were highly integrative across networks, primarily came from the dorsal attention and salience networks. A multiple regression model with "p" factor as dependent variable revealed three parcels where their FC similarity across tasks significantly predicted "p" factor. These parcels were anatomically overlapping with FEF (Dorsal Attention-affiliated; B=5.48, p=0.03) and dACC (Salience-affiliated; B=9.35, p=0.007), where more severe psychopathology was associated with greater similarity, and with dorsal medial PFC (Default Mode-affiliated; B=-5.19, p=0.007), where more severe psychopathology was associated with less similarity.
**Discussion**. Our results identify 29 parcels with highest cross-network FC, or integration, in two fMRI tasks. Similarity in FC across tasks in three parcels was associated with "p" factor, indicating that severe psychopathology was linked to more similar patterns of FC in FEF and dACC regions, and to less similar patterns of FC in a dorsal medial PFC region. We believe this is a first step in identifying clinically-relevant neural correlates of controlled behavior which may partially explain psychiatric comorbidity.

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**Topic:** H.04. Executive Functions

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**Title:** Ketamine anesthesia induces coordinated multiarea gamma bursts and disruption of sensory processing in macaques

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**Abstract:** Ketamine is a World Health Organization essential medicine that has broad clinical significance, including its long history as an anesthetic, its crucial value in low resource clinics, and its more recently established antidepressant properties. Ketamine's primary molecular effect is N-methyl-D-aspartate receptor (NMDAR) inhibition. NMDARs are broadly expressed throughout the central nervous system and have a key role in neuronal signaling. Despite ketamine's inhibitory effect on a single-cell level, high-dose ketamine anesthesia has been shown to cause cortical excitation. This effect has a paradoxical relationship with prevalent theories of unconsciousness associated with decreased neural complexity. In the frontal cortex, distinct bursts of high frequency gamma oscillations separated by prominent slow waves have been identified as a signature of anesthetic doses of ketamine in humans and non-human primates. However, the mechanism linking gamma bursts to behavioral loss of consciousness are not well understood. We hypothesized that "gamma bursts" reflect a systemic restructuring of neuronal communication, which disrupts sensory processing and ultimately leads to loss of consciousness. We recorded bilateral, multi-area, laminar intracranial electrophysiology in 6 rhesus macaques following 10-20 mg/kg IM ketamine boluses. We quantified multiarea neuronal dynamics, including both local field potential oscillations and single unit activity, by extending our previously published hidden Markov Model approach for quantifying ketamine electrophysiology. We found that ketamine-induced gamma bursts were highly coordinated across hemispheres, across cognitive, sensory, and associative cortices, and across cortical layers. Gamma burst dynamics were time-locked to neuronal up and down states: most neurons were strongly inhibited during the slow waves that occur between gamma bursts. High dose ketamine disrupted behavioral responsiveness and sensory processing measured by auditory and somatosensory evoked potentials in the frontal cortex. Sensory evoked activity recovered when cross-area gamma burst coordination decreased, over an hour before the return of behavioral responsiveness. Our results indicate that ketamine-induced gamma bursts reflect a rigidly structured neuronal dynamic that disrupts the transfer of information across cortical areas. Gamma bursts are associated with behavioral unresponsiveness and loss of sensory processing, and thus characterize ketamine induced loss of consciousness.

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**Presentation Number:** NANO46.03

**Topic:** H.04. Executive Functions

**Title:** Neural flexibility profiles of infant intrabrain networks encode cognitive flexibility on population and individual levels

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**Abstract:** The ability to flexibly adapt behavior in response to changing environmental demands is a crucial aspect of healthy cognitive development. The social environment plays a pivotal role in shaping emerging executive function skills, including cognitive flexibility. However, the neural processes that facilitate flexible shifts in cognition from moment-to-moment remain poorly understood. Here, we use dyadic-EEG to investigate neural processes involved in infant cognitive flexibility (CF) during an interactive attention set-shifting task. We address whether the hierarchical community organization of neural networks, and their temporal evolution within and between brains is related to infant CF. By adopting resolution-limit-free community detection for complex systems, we compute fine-grained neural flexibility profiles that capture the temporal and topological organization of dynamic networks, and assess their capacities at population and individual levels.
Infants ($N$=33, $n_{female}$ = 23, $M_{age}$ = 489 days), and their mothers participated in an object play-based CF task. Mothers scaffolded infants' attentional shift from a high-salience (e.g. shape) to a low-salience dimension (e.g. compressibility). From concurrent EEG recordings, peak power, peak frequency, weighted phase lag index, and neural flexibility profiles were employed for Theta band. Inter- and intrabrain multilayer networks were partitioned using the constant potts model. By quantifying changes in a node's community membership over time and community densities, flexibility profiles were generated. To classify low and high CF, five prominent machine learning methods were rigorously assessed.
On a population level, using FDR-corrected linear mixed analysis, we found that infants' CF was significantly related to the neural flexibility profiles of infants' intra-brain networks at parietal locations, with higher CF being linked to higher neural flexibility. On an individual level, predictive performance of kfold cross-validation revealed that infants' neural flexibility consistently outperformed all other measures. Only infants' flexibility profiles showed a significantly higher ROC AUC compared to prediction on randomly perturbed labels. Findings were robust against residualizing for age. Again, parietal locations showed highest feature importance.
In summary, both inference on population level and prediction on individual level revealed robust relations between infants' neural flexibility profiles and CF. These findings provide

empirical evidence that dynamic changes in early brain network organization may support emerging cognitive flexibility skills.

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**Topic:** H.04. Executive Functions

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**Title:** A call to rethink the cognitive benefits of physical exercise: An umbrella review of randomized controlled trials

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**Abstract:** The positive effect of regular physical exercise at the cognitive level in the healthy population is nowadays taken for granted. In fact, a myriad of systematic reviews and meta-analyses report a strong connection between physical exercise and improved cognitive function across the lifespan. Here, we assess the causal evidence supporting this relationship in the healthy population, using an umbrella review of meta-analyses limited to randomized controlled trials (RCTs). Despite the majority of the 24 meta-analyses, we examined reporting an overall positive effect, our assessment uncovered several issues, including limited statistical power in the primary RCTs, selective inclusion of studies, publication bias, and significant variation in preprocessing methods and analytical decisions. Furthermore, our Bayesian meta-analysis of all the primary RCTs included in the revised meta-analyses revealed that the exercise-related benefits were relatively modest in all age groups, from children to the elderly ($d = 0.22$). Notably, these small benefits diminished significantly after accounting for key factors such as active control groups and baseline differences ($d = 0.13$) and became negligible when adjusting for publication bias ($d = 0.05$). These findings emphasize the need for caution when making claims and recommendations about the cognitive benefits of regular physical exercise in the healthy population. It is crucial to accumulate more reliable causal evidence before drawing definitive conclusions in this area.

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**Topic:** H.04. Executive Functions

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**Title:** Impaired inhibitory control in a mouse model of C9orf72 behavioral variant frontotemporal dementia

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**Abstract:** Frontotemporal dementia (FTD) is a leading cause of presenile dementia characterized by progressive atrophy of the frontal and/or temporal lobes, with onset most commonly occurring in middle-age. The most prevalent genetic cause of FTD is a $G_4C_2$ repeat expansion in the first intron of *C9orf72*, which encodes a guanylate nucleotide exchange factor, leading to aberrant RNA production and translation. Gain of toxic function due to dipeptide repeat (DPR) proteins translated from repeat RNAs has emerged as a major pathogenic mechanism. Arginine containing DPR proteins, including poly(GR), have been shown to be especially toxic. Among various forms of FTD, the most prevalent is behavioral variant (bvFTD), where marked changes in personality occur including loss of empathy, reduced sociability, and diminished inhibitory control, a cardinal feature. Inhibitory control is an executive function that refers to the ability to suppress one's behavior, thoughts, and emotions and is regulated by the prefrontal cortex, which is damaged in bvFTD. However, the neuropathological mechanisms underlying behavioral deficits seen in bvFTD are largely unknown. In this exploratory study, we used an inducible tetracycline-based system to express 80 repeats of GR in forebrain CaMKII-containing neurons (CaMKII;$GR_{80}$) that accumulate in an age-dependent manner. We developed an automated operant-based impulsive choice assay to assess loss of inhibitory control in our bvFTD mouse model. Food-restricted mice learned to press one of two levers: a smaller-sooner (SS) lever delivering 10-20ul of 10% sucrose water immediately or a larger-later (LL) lever delivering 60-100ul of the same solution at increasing delays between the lever press and delivery. Choosing the LL lever more frequently indicates decreased impulsive choice and exhibition of self-control. Compared to littermate controls, 12-month-old middle-aged male and female CaMKII;$GR_{80}$ mice display increased impulsive choice, while having intact learning, motor, and sensory control, suggesting impaired inhibitory control. To assess the neural pathways and circuits involved during self-control in this task, we performed an activity-dependent c-Fos mapping assay co-labeled with several cell specific markers and identified several prefrontal cortical regions differentially activated during self-control. Ongoing experiments aim to delineate the underlying neural mechanisms and restore impulsive choice tendencies in mutant mice by targeting the neuropathological deficits in identified prefrontal circuits and cell types.

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**Title:** Examining motor evidence for the pause then cancel model of action inhibition

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**Abstract:** Presented with a salient stop cue, humans can rapidly terminate already initiated actions. Much interest has centered around identifying the neural mechanisms enabling this ability. Recent research has indicated that neural signatures of action stopping occur during at least two distinct time points suggesting that multiple inhibitory mechanisms may subserve inhibition. However, the necessity of multi-stage models of action inhibition has been questioned with some still arguing for a single global-suppressive mechanism account. To differentiate global motor suppression from other potential inhibitory sources, the present experiment leverages single-pulse TMS to measure net corticospinal excitability from a muscle when it is totally irrelevant to an action stopping task (global suppression) and when it is a primary effector in the task (suppression from multiple sources). We predicted that a specific two-stage account, the pause-then-cancel model, would be supported by additional CSE suppression emerging at later stimulation times in the task-relevant muscle. Although we did not find evidence for additional late suppression from the task-relevant muscle when comparing successful Stop trials to an active baseline condition, we did find evidence for this when comparing successful Stop and Go trials. Also, whereas CSE was highly correlated across early stimulation times when the muscle was relevant vs. irrelevant to the task, Bayes factor indicated statistical independence at the late stimulation time. In sum, the results were largely consistent with the pause-then-cancel model as a two-stage account of stopping. Finally, we discuss our follow-up work aimed at addressing some limitations of the current study.

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**Topic:** H.04. Executive Functions

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**Title:** Interrelated effects of birth weight, parental socioeconomic status, handedness and brain volume on ACC cortical folds patterns

**Authors: J. MATHAN**, M. MAXIMINO PINHEIRO, G. REZENDE, L. LE STANC, M. IRIS, N. POIREL, C. OPPENHEIM, O. HOUDÉ, G. BORST, *A. CACHIA;
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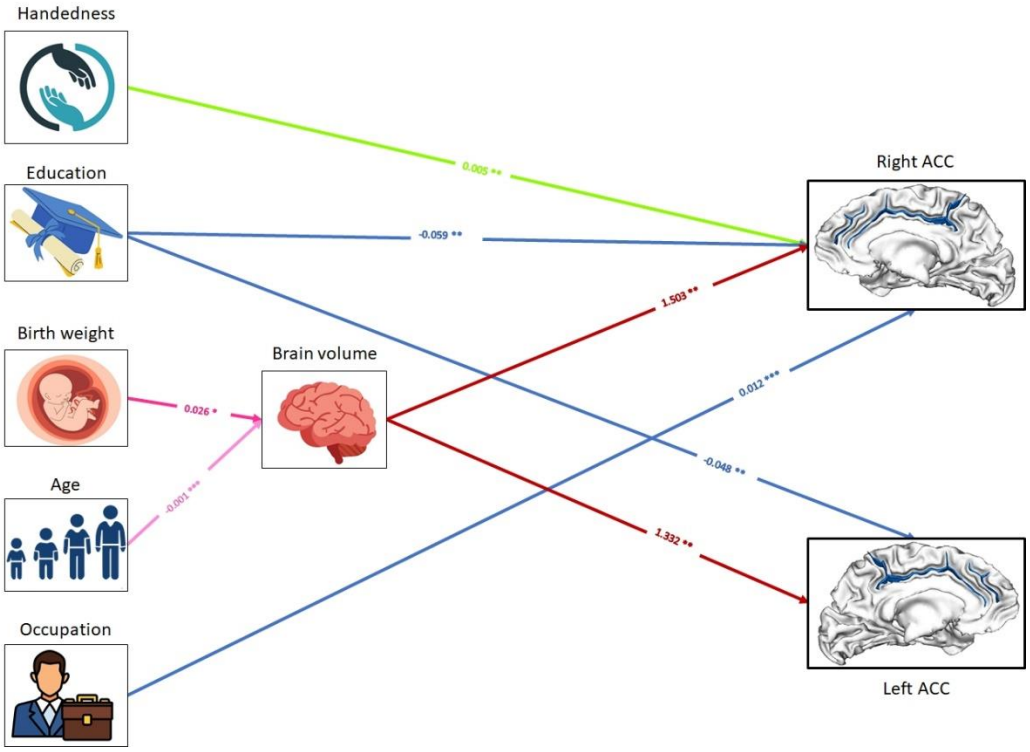
**Abstract:** Growing evidence supports that prenatal processes play an important role for cognitive ability. Such findings have driven the search for brain features in postnatal life that could serve as a proxy for fetal events. A very interesting candidate is the sulcal pattern, namely the qualitative spatial organization of the cortical folds. Indeed, sulcal patterns are determined before birth and are stable across lifespan. Sulcal patterns therefore offer a window on the fetal constraints of specific brain areas on cognitive abilities. Several theories have been proposed to explain the formation of the sulcal patterns, but the exact factors that contribute to their variability are still a topic of intense debates. Because socioeconomic status (SES) affects prenatal development and birth weight (BW) and that prenatal development along with the total grey matter volume (GMV) and handedness influence sulcal patterns, we aimed at unraveling the interrelations of these different factors. In this context, 88 children (9-10 years) and 90

adolescents (16-17 years), were recruited. We focused on the Anterior Cingulate Sulcus (ACC), with two distinct sulcal patterns that were repeatedly associated with executive functions and several psychiatric disorders. The 3D reconstructions of cortical folds from anatomical MRI were used to visually classify the ACC into two sulcal patterns, "single type" or "double parallel type". Parental SES was indexed by the highest education degrees and occupation status of the two parents. We then fitted a multivariate structural equation model. The analysis revealed that the ACC sulcal pattern depends on complex interactions between the different factors. Our findings provide the first evidence that the sulcal pattern is determined by a complex interaction of several early neurodevelopmental and transgenerational factors. Our study therefore provides new insights into the intergenerational transmission of cognitive inequality.

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**Nanosymposium**

**NANO47: Distributed Mechanisms for Working Memory**

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**Topic:** H.05. Working Memory

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**Title:** Dedicated representational roles for sensory and anterior regions during working memory load

**Authors: *T. B. CHRISTOPHEL**[1], V. CHOPURIAN[2], J. P. SEABRA[2];
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**Abstract:** Working memory is believed to rely on a large number of neural stores, distributed across the cortical sheet but is limited to representing as few as three or four items. This limited capacity is likely the result of (1) the allocation of working memory items to different stores, (2) interference within and between these regions, and (3) the region-inherent forms of neural codes used to retain features. In a series of experiments, we demonstrate that different cortical regions fulfil independent representational roles during working memory.
First, we asked how load affects working memory storage in different cortical regions. In each trial, participants (n = 81, average age = 25) had to memorize two sequentially presented items, either two visual items, two auditory items or one of each. Crucially, this means that overall working memory load was constant at two items, but unisensory load varied between one and two items. We analyzed fMRI data using support vector machine classifiers to continuously reconstruct memorized orientations throughout the delay period. In visual cortex (but not anterior regions), we observed a decreased decoding accuracy with increased unisensory working memory load. We further find an increase in information for more recent items in FEF, but not in visual cortex. Consistently, memory recall performance was better at lower loads and when a more recent item was recalled. Information in all three regions correlated with behavioral performance. Our results suggest change in the division of labor between visual and anterior regions with varying load.
In the second experiment, we investigated whether cortical codes during the working memory reflect abstract encoding strategies and how these representations change over time. Subjects (n = 40, average age = 26, two MRI sessions per subject) performed a standard orientation and location delayed recall task. We used multivariate cross-condition modelling (CrossMANOVA) to differentiate shared and separable components of orientation and location coding. In particular, we used data from the location task to train an encoding model aiming to predict neural responses in the orientation task. We trained two separate models, one using the upper location stimuli and one using the lower location stimuli. We found that locations on the upper half of the visual field are more suitable to predict orientation representations during working memory. This upper visual field bias is more pronounced in anterior regions and late into the delay. These results explain how the stronger involvement of anterior regions during higher loads lead to more degraded recall.

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**Presentation Number:** NANO47.02

**Topic:** H.05. Working Memory

**Title:** Decoding spatial location from aperiodic and alpha oscillatory activity in working memory

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**Abstract:** Recent studies support an emerging view that alpha oscillations coordinate the underlying population codes that retain the content of visual working memory (WM), with non-spatial feature storage bound to location irrespective of the task relevance of location. For example, work by Foster and colleagues demonstrated that alpha oscillatory activity tracks the specific spatial location of WM items (Foster et al., 2016), even when spatial position is task-irrelevant (Foster et al., 2017). Although there is significant evidence supporting alterations in narrowband alpha power during WM, oscillations frequently manifest in bursts amidst consistent non-oscillatory aperiodic activity (Jones, 2016; Cole & Voytek, 2019). Conventional spectral analyses, such as narrowband filtering within a predetermined frequency range, may mistakenly interpret dynamic and task-related modifications in this aperiodic activity as changes in oscillatory activity, even in the absence of actual oscillations (Donoghue, T., Haller, M., Peterson, E.J., et al., 2020). Through parameterization of both aperiodic and alpha oscillatory activity, this work aims to disentangle the roles of aperiodic and alpha oscillatory activity in visual WM. Using an open EEG dataset from Foster and colleagues (2016), we first reproduced the authors' decoding of spatial location during WM maintenance from total alpha power using an inverted encoding model (IEM). To adjudicate whether this decoding of spatial location during WM from total alpha power is supported by aperiodic and/or alpha oscillatory activity, we trained separate IEMs to decode spatial location from each. Alpha oscillatory power showed high decoding performance during the delay period, confirming its role in maintaining the spatial location of the stimulus in WM. In contrast, decoding from aperiodic activity was highest during stimulus presentation and retrieval, suggesting aperiodic activity encodes the spatial location during presentation and then retrieves it from WM. The differential decoding time courses of spatial WM for aperiodic and alpha oscillatory activity imply novel, unique contributions of aperiodic and alpha oscillatory activity to the encoding, maintenance, and readout of stimulus feature representations during visual WM.

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**Topic:** H.05. Working Memory

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**Title:** EEG correlates of active removal from working memory

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**Abstract:** The removal of no-longer-needed information from visual working memory (WM) is important for the functioning of WM, and can be flexibly accomplished with different mechanisms. Previously, with an "ABC-retrocuing" WM task, we have shown that simply withdrawing one's attention from the to-be-removed memorandum (i.e., "passive removal") results in that item exerting an attractive serial bias, whereas "active removal" results in it exerting a repulsive serial bias (Shan and Postle, 2022). In the current study, we recorded EEG signals while subjects performed the ABC-retrocuing task to investigate neural correlates of active removal vs. passive removal, and found two noteworthy effects. The first, in the ERP to

the “drop” cue, may reflect the operation of the active removal mechanism itself: 300 ms following cue onset, at central electrodes, the negative-going component of the ERP was significantly greater in the active-removal condition relative to the passive-removal condition. The second, later in the trial, revealed a downstream consequence of active removal: the response in the theta band to a task-irrelevant visual “ping” was reduced in active-removal relative to passive-removal trials, most notably at right posterior electrodes. These results suggest that active removal from visual WM may be accomplished by the suppression of perceptual representations via a phasic top-down mechanism, a trace of which persists for at least several seconds in the form of decreased excitability in the visual cortical circuits that had represented that item. This residual consequence of active removal may explain the repulsive serial bias that is observed on the subsequent trial.

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**Topic:** H.05. Working Memory

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**Title:** Anterior hippocampal involvement in multisensory spatial and temporal working memory

**Authors: *V. TRIPATHI**[1], D. BEELER[1], R. MARSHALL[1], M. DUNNE[1], T. M. MORIN[2], C. E. STERN[1], D. C. SOMERS[1];
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**Abstract:** Evidence from both animals and humans has suggested that working memory is a distributed process, that includes not only the prefrontal cortex but also the hippocampus and related medial temporal lobe regions (Lee and Kesner, 2003; Gilbert and Kesner 2006, Manns et al., 2010; Stern et al., 2001; Ranganath and D’Esposito 2001; Schon et al., 2004; Newmark et al., 2013; Schon et al., 2016). In earlier studies, we demonstrated the presence of interleaved auditory and visual sensory-biased working memory regions within the prefrontal cortex with visual regions more responsive to spatial information and auditory regions more sensitive to temporal information (Michalka et al., 2015; Noyce et. al. 2017). Here, we report results from a multisensory working memory (visual, auditory, and tactile) fMRI experiment using human subjects (n=17) while they performed two distinct working memory tasks. Stimuli included visual Gabor patches, auditory warbles, and tactile vibrations using an MR-compatible vibrotactile digit stimulator. Tasks included a 2-back working memory task designed to localize sensory-biased working memory regions and a change detection task in which subjects were required to detect changes in either the spatial or temporal order of target stimuli. Each task consisted of working memory and sensorimotor control blocks for the three sensory modalities within each run. We ran a GLM analysis using FSL with active vs sensorimotor control contrasts for each of the three modalities. Our preliminary analyses suggest that the anterior hippocampus responded preferentially to the spatial and temporal working memory conditions across the three modalities but not during the 2-back working memory task. The activations were bilateral for auditory and visual blocks but left dominant for tactile blocks, consistent with the unilateral

stimulation of the right hand of the subjects. Our study adds to a growing body of evidence suggesting functional subdivisions along the long axis of the hippocampus.

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**Topic:** H.05. Working Memory

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**Title:** Its not just about acts! Both rule and motor representations are equally present in Primary Motor cortex, but only when rules switch

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**Abstract:** Rule representations governing the selection of the correct response are a key aspect of cognitive control. These representations are believed to be a feature of higher transmodal regions like the multiple-demand regions, and not of the primary motor cortex (M1). While past studies have found rule information in the activity patterns of primary motor regions, these have been interpreted as resulting from top-down control of transmodal regions on the M1. Representations in the primary motor regions are regarded primarily as motor in nature. To test this assumption, we investigated if the neural activities of the M1 carry more information about motor acts or about the rule being used to select those acts.We used dissimilarity of activity-patterns as a measure of information content. If neurons of a region represent rules, then different rules will evoke dissimilar patterns of activity, and the extent of dissimilarity will depend on the magnitude of this representation.Participants (n=29) executed a rule-switching fMRI task. A trial could involve one of two rules conveyed by the color of the number-stimuli frame: blue - classify the number as even/odd, green - classify it as <10/>10.Responses were made by the right index or middle finger. Rules were randomly selected. Trials either had the same rule as the preceding trial (repeat trials) or had a different rule from the preceding trial (switch trials).We first localized the set of primary motor cortical voxels where index and middle finger responses generated different patterns of activity such that the identity of the motor act could be decoded from them.We measured 'motor-dissimilarity' as the mathematical distance between activity-patterns produced by different motor acts generated using the same rules and 'rule-dissimilarity' as the distance between patterns produced by identical motor acts generated using different rules.If the activity of these primary motor cortical voxels contained more motor information than rule information then motor-dissimilarity will be higher than rule-dissimilarity and vice-versa.We found that motor-dissimilarity was higher only on repeat trials wherein the rules being used had already been established. On switch trials wherein new rules were being established, rule-dissimilarity was as high as motor dissimilarity. Crucially, motor dissimilarity on switch trials was lower than on repeat trials, suggesting that establishing new rules decreased the amount of motor-related information in the patterns of motor cortical activity. Our results

suggest that rule information in the primary motor cortex can be as high as motor information when new rules are being established.

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**Title:** Persistent stimulus representations in working memory as a potential neural mechanism for behavioral flexibility

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**Abstract:** People can flexibly manipulate levels of information in working memory to accommodate various task needs. Theories of flexible control posit two potential mechanisms to achieve behavioral flexibility: forming low-dimensional abstract representations over stimulus representations to enable knowledge generalization, or, preserving copies of high-dimensional stimulus representations to facilitate flexible transformation. Here we tested how the two mechanisms were implemented at the whole-brain level in a visual working memory task with nested levels of control demands. During functional magnetic resonance imaging (n = 24), participants switched between memorizing an orientation (maintenance) and categorizing an orientation following learned rules (categorization) on a trial-by-trial basis, with categorization rules being flexibly switched between blocks. We tracked representations of stimulus and abstract category information combining multivariate encoding and decoding approaches, representational similarity analysis, and linear mixed effects modeling. Converging evidence from complimentary approaches demonstrated increased stimulus representation in frontal cortex in categorization, which exclusively predicted behavioral performance. Early visual cortex, on the other hand, demonstrated decreased stimulus representation and surprisingly stimulus-independent category representation in categorization. To explore the potential neuronal mechanism underlying this observation, we trained two-module recurrent neural network (RNN) models using the same flexible task design, with early module corresponding to visual cortex and late module corresponding to frontal cortex. We found that the network replicated human behavior when stimulus information was required for output in addition to category information: with increased stimulus representation in the late module and category representation in the early but not late module. When only category information was required for output, the late module represented category information with little stimulus information. Altogether, these findings provide both empirical and computational evidence for ‘meta-flexibility,’ with persistent stimulus representation as a possible ‘lazier’ coding scheme for the brain to accommodate the ever-changing environment.

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**Title:** Neurophysiological evidence for activity silent dynamic processes as neuronal correlates of human working memory

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**Abstract:** Auditory working memory (WM), the ability to maintain and manipulate minutely acquired sound information, is critical for goal directed behavior and communication. The neuronal processes that enable WM remain intensely debated, within and beyond the auditory domain. The long-standing theory that WM maintenance is based on persistent delay activity has been challenged by a hypothesis proposing that maintenance is instead supported by activity silent dynamic processes. Here, we utilized non-invasive and invasive human recordings and machine learning techniques to elucidate how the human brain maintains non-verbal auditory WM.

In a series of experiments, we used retro-cue paradigms with spectrotemporally modulated broadband or "ripple" sounds as memoranda, to discourage non-auditory maintenance strategies. The maintenance period and the number of memorized items changed according to the needs of each experiment.

Using magnetoencephalography (MEG, n=20), we found that WM content can be decoded from long-range synchronization patterns of neuronal oscillations between auditory and frontoparietal cortices. In auditory cortices, WM content was decodable from high-frequency (60-120 Hz) gamma oscillations after the presentation of a task-irrelevant "impulse sound", in line with earlier findings of "activity silent" maintenance mechanisms in the visual domain.

To enable more detailed focus on neuronal mechanisms in humans, we collected intracranial stereo-EEG recordings (n=12) from participants during our WM task. In most participants, auditory WM content could be decoded from broadband high frequency activity (HFA), a putative correlate of increased multiunit activity. However, we have not found persistent increase

of HFA power or of the event related potentials in the invasive contacts with significant decoding results. However, consistent with our MEG data the decoding accuracy increased in many contacts after the presentation of an irrelevant “impulse sound”.
Our MEG and sEEG findings led us to investigate whether these activity silent processes could be reinforced during maintenance. To this aim, we conducted a TMS-EEG study (n=20). Here, we sent an excitatory single TMS pulse to the posterior STG in the brain in the middle of the maintenance period. In a control experiment, the TMS coil was 2.5 cm away from the scalp to diminish its cortical effects. Our results show an elevated decoding accuracy after the TMS pulse compared to the control condition.
Our results conjointly point towards an activity silent, dynamic, and anatomically distributed process as neuronal correlate to WM maintenance.

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**Topic:** H.05. Working Memory

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**Title:** Temporal expectation triggers removal of irrelevant information from working memory that leads to forgetting

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**Abstract:** The activation of representations in working memory fluctuates over time due to multiple factors including expectations about their task relevance. How does the waxing and waning of items in working memory affect their long term retention? In the current experiment, we manipulated temporal expectations about items held in working memory and then later probed long-term recognition memory for these items. We recorded EEG while participants (N =20) maintained a face and a scene image in each trial. After a variable delay, they were tested on one of these images using a rapid serial visual presentation (RSVP) probe sequence. The task relevance of memoranda was manipulated in two ways. First, scene images were tested twice as often as face images. Second, if a face image was tested in a trial, this always occurred after a short 1-sec delay. Otherwise, the scene image would be tested 3 sec later. We expected participants to switch from prioritizing the face memory to prioritizing the scene memory during the longer delay window. We hypothesized that reducing the priority of an item in working

memory could reduce its subsequent accessibility in long-term memory. To test this idea, we included a surprise long-term memory test at the end of the experiment to assess recognition memory for face images that had been prioritized and then deprioritized in working memory (i.e., face images from long-delay trials when the scene image was tested). We then linked these long-term memory outcomes to temporal profiles of face and scene representations from the preceding working memory task. Using multivariate EEG analyses, we found different profiles of working memory representations for faces that were later remembered vs. faces that were later forgotten. Forgetting of a face image was associated with a marked decrease in neural evidence for the face representation in working memory after it became deprioritized. The decreased evidence of faces during the later delay window was replicated in analyses that ruled out encoding failures. These results confirm that working memory representations fluctuate based on temporal expectations of task relevance. The suppression of no longer relevant representations from working memory can weaken that item's memory trace in long-term memory.

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**Title:** Probing spatiotemporal neural dynamics of working memory reactivation

**Authors: *J. LI**[1], L. LIU[2], H. LUO[1];
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**Abstract:** Working memory (WM) is posited to rely on short-term neural plasticity (STP) and neural reactivations. Motivated by this time-based STP principle, our previous work developed a purely bottom-up, behavioral "dynamic perturbation" approach to manipulate the recency effect in sequence WM. However, two questions remain unanswered. First, direct neural evidence for the dynamic perturbation approach is lacking. Second, the brain regions involved in WM reactivation during maintenance are also unknown. We employed an impulse-response approach combined with magnetoencephalography (MEG) recordings to address the questions. Participants were instructed to retain a sequence of two gratings in WM and later recall their orientations. During retention, we applied the "dynamic perturbation" approach by presenting flickering probes with different temporal associations with luminance sequences. In order to examine whether neural reactivation profiles are altered by memory manipulation, we presented a neutral impulse (PING stimulus) after the perturbation period. We also performed source localization analysis of brain activities to identify brain regions involved in WM reactivations. First, "dynamic perturbation" modifies the multi-item neural reactivation profiles after the PING stimulus, offering direct neural evidence of memory manipulation. Specifically, for random luminance conditions that would not interfere with recency effect, the neutral PING triggers a backward memory reactivation profile. By contract, the synchronous luminance condition reveals a disrupted neural index of recency effect, consistent with behavioral results.

Furthermore, source localization analysis demonstrates dissociated brain regions for WM encoding and reactivation stages, with the frontoparietal region for encoding and the medial temporal lobe (MTL) for memory reactivation during retention. In summary, our findings constitute novel neural evidence for the effectiveness of STP-based "dynamic perturbation" in manipulating WM. Importantly, WM encoding and reactivation engage different neural networks, i.e., WM information is retained in parietal and frontal regions and tends to be reactivated through the engagement of the hippocampus-related medial temporal cortex, implying an intertwined link between WM and episodic memory.

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**Title:** Distractor Resistance and Anticipation in Working Memory: Oscillatory Correlates from Primate Prefrontal Local Field Potentials and Whole-Brain Electroencephalography

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**Abstract:** Neural oscillations in different frequency bands have been linked to different cognitive functions. In particular, it is postulated that theta band (~4Hz) reflects working memory maintenance of task-relevant information, while alpha band (~10Hz) supports that maintenance through gating/inhibiting task-irrelevant stimuli (distractions). These observations have been made at the fine spatial scale of intracranial local field potentials (LFPs) in animals, and also, separately, at the large spatial scale of non-invasive electroencephalography (EEG) in humans. These parallel observations across spatial scales raise the question of whether these oscillatory signatures in LFPs and EEG reflect a consistent computational process, or rather different components of working memory maintenance in the presence of distractions that happen to have similar frequency characteristics. Our study aimed to delineate the functional roles of theta and alpha band oscillations across neural scales in relation to distractor anticipation during working memory maintenance in non-human primates. We simultaneously recorded LFPs from the lateral prefrontal cortex (LPFC) and scalp EEG signals from monkeys performing a memory-guided saccade task. Distractors randomly flashed at a predetermined time during the maintenance interval, activating the brain differentially and yielding distinct neural patterns in LPFC LFPs and scalp EEGs. From EEG, we identified that theta-band oscillation was prominent throughout the brain, enabling decoding of information, surprisingly, with or without the presence of distractors. These findings suggest that anticipation of distractors shapes brain activity to enhance the representation of remembered information (recall). In prefrontal LFPs, we observed that theta-band and alpha-band oscillations played distinct roles in memory

maintenance and distraction anticipation. Theta-band activity exhibited stronger decoding performance for contralateral items when distractors were present, while decoding failed without distractors. Conversely, alpha-band activity facilitated decoding of ipsilateral items in the absence of distractors, consistently observed across subjects. Notably, alpha-band activity acted as a gating mechanism for the visual hemifield ipsilateral to the information location, anticipating and preparing for the presence of distractors. These results provide insights into the differential engagement of theta and alpha band oscillations in distractor resistance and anticipation, shedding light on the neural mechanisms underlying working memory processes in the presence of distractors.

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**Topic:** H.05. Working Memory

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**Title:** Distinct Rotation Dynamics Between Prefrontal and Parietal Cortex Encoding Working Memory

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**Abstract:** The dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex (PPC) have been actively involved in behavioral tasks requiring working memory (WM). The response of a neuronal population to stimuli can be encoded into an activity subspace, and their geometrical transformations are informative about the nature of the representation of stimulus and task information. We measured the geometrical representations of stimuli at the neuronal population level to investigate how neurons in PFC and PPC represent spatial locations of stimuli held in memory. We collected neurophysiological recordings from 2783 PFC neurons and 1336 PPC neurons from eight adult monkeys in two tasks, the oculomotor delayed response (ODR) task, and the match-stay, nonmatch-go (MSNG) task. To measure the information encoding capacity for PFC and PPC, we calculated the deviation of neuronal responses from the baseline activity (average in the cue period) in a reduced space (defined by the first three principal components). We found a more considerable change in PFC than PPC neurons, and the results were consistent during different task epochs (cue period: PFC 6.65±0.51 vs. PPC 3.31± 0.54; delay period: PFC, 7.18±0.47 vs. PPC, 2.69±0.33). Moreover, the representation in the reduced PCA space also shows that PFC has a much more substantial deviation from baseline activity than PPC. We further investigated the representation of neural activity subspace in ODR task between the cue and delay period in PFC and PPC and found only a slight rotation in both areas. This result was consistent in four monkeys. Interestingly, the representation of both PFC and PPC neural activity subspace in the more complicated MSNG task, during the cue and delay period showed much more significant rotation. Our results thus provided clear evidence demonstrating the encoding

properties in a high dimensional space of PFC and PPC, which could further our understanding of PFC and PPC functionality, especially in neuronal population encoding capacity.

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**Topic:** H.05. Working Memory

**Support:** R01 EB028154-01

**Title:** Task-specific neural modules for spatial working memory in the frontal cortex

**Authors: *J. PARK**, C. D. HOLMES, L. H. SNYDER;
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**Abstract:** Many tasks require spatial working memory (Baddeley 2017) and indeed, mnemonic neural representations have been revealed in many of these tasks (e.g., Constantinidis et al. 2004). However, it remains unclear whether spatial working memory is supported by a single module or if different tasks engage different modules. In this study, monkeys memorized spatial locations that indicated, in separate blocks of trials, either where to look (memory-guided saccade task) or where not to look (non-match to sample task). We identified and compared the neuronal subspace underlying memory activity in the frontal cortex in each task (compare Hasegawa et al. 2014).
First, we asked whether mnemonic information is carried by the same cells and in a similar manner in the two tasks. To answer this, we used targeted dimensionality reduction (TDR; Mante et al. 2013) to estimate denoised regression coefficients for target direction in each task. These coefficients form a mnemonic direction axis within the neural subspace. We tested 3 possibilities: 1) The two mnemonic axes are not significantly different, indicating that the same cells carry mnemonic information in the two tasks and use a similar encoding scheme. 2) The axes are orthogonal, suggesting that mnemonic information is carried by different cells in each task. 3) The axes have an intermediate relationship, significantly different from one another yet not fully orthogonal, suggesting that the mnemonic networks in the two tasks are composed of partially overlapping sets of cells and/or encode mnemonic information independently. We observed that the target direction axes were similar and well-correlated between the tasks during a visual period (150-350 ms from target onset). However, during a memory period (300-600 ms from target offset), the mnemonic direction axes were neither well-correlated nor fully orthogonal (possibility 3). Second, a mnemonic information decoder based on a demixed principle component analysis (dPCA; Kobak et al. 2016) did better when tested on a “left-out” trial from the task on which it was trained compared to a trial from the other task. Third, using either TDR or dPCA, we found a static shift in population activity by task that persisted throughout each task block, even during a period of fixation prior to the appearance of the mnemonic stimulus. These findings all suggest that the neuronal circuits involved in spatial working memory differ across tasks. This in turn suggests that working memory is not subserved by a single dedicated module in the brain. Instead, different circuits are engaged in different contexts.

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**Nanosymposium**

**NANO48: Human Memory: Neurophysiology, Neuromodulation and Closed-Loop Stimulation**

**Location:** WCC 143

**Time:** Monday, November 13, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO48.01

**Topic:** H.07. Long-Term Memory

**Title:** Optimized individual network-targeted stimulation locations yield more robust effects

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**Abstract:** We previously reported that multiple consecutive days of individualized hippocampal-network targeted (HNT) noninvasive stimulation significantly improved hippocampal-dependent associative memory recall (Wang et al., 2014, Science). It has since been reported that this effect failed to replicate (Hendrikse et al, 2020, Cortex), despite several methodological discrepancies (e.g., fewer sessions of stimulation; more variable sample age) and other successful attempts (Kim et al., 2018; Nilakantan et al. 2019; Freedberg et al., 2019; Gao et al., 2021). Recently, Cash and colleagues (Cash et al., 2022, Brain Stimulation) reanalyzed the Hendrikse et al., dataset, in order to determine "optimized" stimulation targets. They found that the memory effect reported in Wang et al., was negatively correlated with distance between the actual and optimized stimulation target. In other words, the closer the actual target was to the optimal target, the more likely the Wang et al., memory effect was replicated. Here, we used Cash et al methods to determine the "optimal" stimulation sites in the Wang et al., data. The actual stimulation locations used in the Wang et al., data were on average, ~12mm (range = 4-20mm) away from the "optimal" stimulation locations. Similar to the findings reported in Cash et al., we also find that the closer the actual and optimal locations, the stronger the memory effect. Our results contribute to growing evidence that optimizing the individualization techniques for target determination is crucial for causing robust effects via noninvasive brain stimulation.

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**Topic:** H.07. Long-Term Memory

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**Title:** Causal role of frontoparietal theta connectivity in the prioritization of internal representations: a dual-coil TMS study

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[3]Univ. of North Carolina, Univ. of North Carolina, Chapel Hill, NC

**Abstract:** Cognitive control is the ability to guide behavior towards abstract goals. In order to enact goal-directed behavior, internal representations must be maintained and manipulated. Our previous study investigated the role of neural oscillations during the processing of a retrospective cue that signals the prioritization of an internal representation held in working memory. We found that frontal and parietal cortex exhibit coherent theta frequency (4-8 Hz) neural oscillations immediately following the presentation of the retrospective cue. This activity that was not present with an uninformative neutral cue. In order to evaluate the causal role of coherent theta oscillations in the frontoparietal network, we delivered theta-frequency rhythmic transcranial magnetic stimulation (TMS) simultaneously to both frontal and parietal cortex during the processing of a retrospective cue. Using a baseline EEG session, we individualized theta TMS to be the frequency with peak functional connectivity between frontal and parietal electrodes. The regions targeted by dual-coil rhythmic TMS were also personalized. Using a baseline functional MRI session, we chose the region of the anterior middle frontal gyrus that showed increased activity for the retrospective cue relative to the uninformative neutral cue. Using a task-based functional connectivity analysis, we selected the region of the superior parietal lobule with peak connectivity to the prefrontal region. Rhythmic TMS was delivered either simultaneously to both regions (in-phase) or with a 180-degree phase delay (anti-phase). As a control condition, arrhythmic stimulation was delivered in-phase to both region or delivered as two independent trains that were matched for duration and number of pulses. As an additional control, we delivered TMS in the alpha-band (8-12 Hz). As hypothesized, we found that in-phase theta TMS to the frontoparietal network decreased response time relative to anti-phase theta TMS and there was no impact to behavior from arrhythmic or alpha TMS. These findings provide causal evidence that the frontoparietal network orchestrates the prioritization of internal representations via coherent theta-frequency oscillations.

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**Presentation Number:** NANO48.03

**Topic:** H.07. Long-Term Memory

**Title:** Closed-loop optimization of network-targeted stimulation for single-trial experimental paradigms

**Authors: *P. F. AGRES**, R. B. BAUDO, J. E. KRAGEL, J. L. VOSS;
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**Abstract:** Participants that do not exhibit changes in behavior following network-targeted transcranial magnetic stimulation (TMS) are sometimes categorized as “non-responders”. Such individual differences in efficacy may result from heterogeneity in functional properties of brain

regions or in brain network organization. Hippocampal theta rhythms are important for episodic memory function. Theta-burst stimulation (TBS) is thought to mimic the endogenous theta rhythm of the hippocampus, and in the case of stimulation targeting the hippocampal network, TBS has been shown to have greater impact on hippocampal-dependent memory performance than stimulation applied at other rhythms. However, individual differences in response magnitude are observed for TBS and may be due to differences in endogenous theta rhythms across participants. It is challenging to address this possibility because of the difficulty of measuring hippocampal theta rhythms noninvasively. We thus set out to develop a closed-loop optimization method to determine optimal stimulation rhythms and/or locations that yield effects on hippocampal-dependent memory at the level of individual experimental trials. To provide initial validation of this method, we conducted a preliminary closed-loop optimization experiment using the motor-evoked potential (MEP) as a clear behavioral response with an independently verifiable optimal neural target. We used neuroadaptive Bayesian optimization, a machine learning framework in which experimental conditions are informed by brain and behavioral data from prior trials. Participants underwent structural MRI to identify motor cortex for neuronavigated stimulation, which was performed under the control of a robotic TMS system. We performed an automated, adaptive protocol to find the coil position and stimulation output to achieve the MEP threshold as measured with electromyography. MEP responses were recorded and used to update the coil position and stimulation intensity for the subsequent trial via Bayesian Adaptive Direct Search. Our results suggest this closed-loop stimulation paradigm may be generalized to other experimental designs where single-trial behavioral responses are recorded, such as for trial-level episodic memory performance for applications to hippocampal-network targeted stimulation. This individualized optimization approach may be particularly useful for studying groups with higher levels of heterogeneity in responses to stimulation and higher variability in brain network functional organization, such as older adults experiencing age-related memory impairments.

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**Topic:** H.07. Long-Term Memory

**Title:** Real time influence of episodic memory retrieval on the procedural network

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**Abstract:** The hippocampal-centered episodic and caudate-centered procedural memory networks interact to support memory, but the biological dynamics of this relationship are unclear. fMRI studies suggests that engagement of one network suppresses the other on a trial-by-trial timescale. However, MRI cannot capture these interactions in real time due to the slow time course of the hemodynamic response. Here, we sought to examine these interactions more precisely by using transcranial magnetic stimulation (TMS) and electromyography (EMG). We measured procedural memory network excitability during episodic memory retrieval by delivering single-pulse TMS to the motor cortex, which is densely connected with the procedural

memory network, and measuring ensuing motor-evoked potentials (MEPs). We predicted that episodic memory retrieval would suppress the excitability of the procedural memory network only during the period of maximum memory retrieval elaboration, which is between 500 and 1500 ms after the onset of an episodic memory cue. Participants completed four blocks of a behavioral task. Each block included a study and response phase. During the study phase, twelve arbitrary pairs of words were shown to participants. To manipulate retrieval demands, half of these pairs were shown three times (easy condition) and the remaining pairs were shown once (hard condition). During the subsequent response phase, participants experienced trials with memory and perceptual task demands. For memory trials, participants were shown the first word from each pair presented during the study phase and instructed to recall the second one. For perceptual trials, participants were shown a colored word and were instructed to decide which of six colors it resembled most. To equate the effort required during the two tasks, the difficulty of perceptual trials was anchored to the accuracy of the memory trials using an adaptive algorithm. MEPs were normalized to baseline MEPs acquired before task performance during quiet rest. Our preliminary results suggest that memory but not perceptual demands significantly reduced MEP amplitude, but only during the period of maximum memory retrieval elaboration: 500-1500 ms after cue onset. These data suggest that the suppressive effects of the episodic memory network retrieval on the procedural memory network occur within a sub-second timescale rather than broadly across seconds or minutes.

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**Presentation Number:** NANO48.05

**Topic:** H.07. Long-Term Memory

**Support:** 1ZIANS002977

**Title:** State-dependency of TMS-induced changes in memory and its neural correlates

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**Abstract:** Episodic memory allows us to reflect on the past, make decisions about the future, and form a learned identity. It depends on the hippocampus as well as on the distributed set of regions which form the hippocampal-cortical network (HCN). We and others have shown that transcranial magnetic stimulation (TMS) targeted to this network via resting-state hippocampal fMRI connectivity can modulate HCN activity and connectivity, improving memory in healthy adults. However, connectivity of this network is altered by engagement in memory tasks and it remains unknown whether and how these changes in network state may alter the response to stimulation. Here we used simultaneous TMS, EEG, and cognitive testing to investigate how task phase influences the effect of TMS on memory processing. Participants (n=32) were shown objects in one of four locations and were later asked to recall the objects and their locations. Trains of theta-burst TMS were delivered either prior to or immediately after visual stimulus onset to a subject-specific HCN-node in lateral parietal cortex or to a control site at the vertex.

Effects of state-dependent (before vs. during-trial) HCN-targeted stimulation, relative to vertex, were investigated on memory performance and EEG signatures of successful encoding. HCN-targeted TMS enhanced item memory performance when delivered with the trial stimulus. Additionally, during successful encoding trials, memory-state HCN-targeted stimulation was associated with changes in the late positive evoked potential and theta oscillatory power, two canonical signatures of successful memory. Together, this suggests that engagement in encoding makes the HCN-network more susceptible to stimulation. Further, TMS modulates memory performance in a network-specific, task-dependent manner, arguing for controlling neural and behavioral state at the time of TMS delivery in future studies.

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**Presentation Number:** NANO48.06

**Topic:** H.07. Long-Term Memory

**Support:** NIH Grant NS113804

**Title:** Theta synchronized stimulation modulates the hippocampal network in humans

**Authors: *J. E. KRAGEL**[1], N. P. ISSA[1], H. A. HAIDER[1], J. TAO[1], S. WU[1], P. WARNKE[1], S. SCHUELE[2], J. ROSENOW[2], J. F. DISTERHOFT[2], A. WIDGE[3], J. L. VOSS[1];
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**Abstract:** Theta oscillations organize memory processing across cortico-hippocampal networks to support memory function. The phase of theta dictates the flow of information to and from the hippocampus, with sensory inputs optimally timed to arrive at the trough of hippocampal theta. Modulation of hippocampal activity timed to the ongoing theta rhythm would provide a framework to causally assess a behavioral role of theta and develop stimulation-based techniques to restore memory function. However, phase-locked stimulation of hippocampal networks and resulting neurophysiological changes have not been characterized in humans. Here, we used a phase-locking algorithm to drive direct electrical stimulation (DES) of the lateral temporal cortex, a node of the hippocampal network. Network-targeted stimulation was timed to the trough of invasively recorded hippocampal theta oscillations or at random phases of theta in a phase-blind control condition (N = 8 participants total). We assessed the effects of theta-synchronized stimulation on the hippocampal network using single-pulse electrical stimulation and measures of inter-regional synchrony. After phase-locked DES, single-pulse evoked potentials in the hippocampus increased in amplitude ($p$ = .01, permutation tests). Phase-locked stimulation increased theta synchrony compared to a pre-stimulation control (4 - 8 Hz, all $p$ < .04, permutation tests, N = 2 participants). After separating stimulation-evoked and endogenous activity, we found theta-synchronized stimulation increased theta power throughout the treatment interval. Taken together, these findings suggest that theta-phase synchronized stimulation is an effective means to modulate the hippocampal network, providing a pathway to modulate hippocampal-dependent behaviors.

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**Presentation Number:** NANO48.07

**Topic:** H.07. Long-Term Memory

**Title:** Efficacy of hippocampal indirectly targeted stimulation (HITS) for recollection improvement: A systematic review of transcranial electromagnetic stimulation experiments

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**Abstract:** Hippocampal indirectly targeted stimulation (HITS) is an experimental approach whereby network connectivity metrics are used to select locations for brain stimulation intended to produce trans-synaptic effects on hippocampal function. Although HITS can be accomplished using a variety of invasive and noninvasive stimulation methods, the majority of experiments have used noninvasive transcranial electromagnetic stimulation (TMS). To address the question of whether HITS via TMS reliably influences hippocampal-dependent recollection memory, we conducted a systematic review following PRISMA guidelines. We identified 32 published experiments satisfying our search criteria. Of these, all but three reported significant positive (i.e., enhancement of behavior) effects of HITS on recollection, with the majority having standardized effect sizes in the medium to large range. One of the null-effect publications (Hendrikse et al. 2020) was subsequently reanalyzed by the same research group (Cash et al. 2022) and reported to have had problems with data quality that, when corrected, led to a positive association between HITS and recollection. Several of the published positive findings were in clinical samples with memory impairment, including mild cognitive impairment, mild to moderate Alzheimer's dementia, and schizophrenia, suggesting that the beneficial effects of HITS for recollection can occur in these conditions. Further analysis suggested that HITS could be accomplished via different neocortical and cerebellar cortical stimulation targets and that the effect-size magnitude was associated with the number of stimulation sessions and with the stimulation rhythm, although the independent effects of these factors have not been fully dissociated in published studies. These findings indicate that HITS with TMS is a highly replicable method for robustly influencing hippocampal-dependent recollection memory.

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**Topic:** H.07. Long-Term Memory

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**Title:** Functional and anatomical connectivity mediate the mnemonic effects of closed-loop brain stimulation

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**Abstract:** Direct electrical stimulation of the brain, when applied in a closed-loop manner, can lead to improvements in behavioral function, including memory (Ezzyat et al., 2018) and mood (Scangos et al., 2021a). In these approaches, brain states control the timing of stimulation or the choice of parameters, which can account for variability in stimulation's neural and behavioral effects based on brain state at the time of delivery (Basu et al., 2021; Bradley et al., 2022; Scangos et al., 2021b). Although closed-loop algorithms provide greater control over stimulation's physiological and behavioral effects, variability in outcomes remains a primary challenge. Here, we evaluate the hypothesis that stimulation's behavioral and physiological effects depend on the anatomical and functional network properties of the stimulation target. Closed-loop stimulation was delivered via intracranially-implanted electrodes as neurosurgical patients studied and recalled word lists. Multivariate classifiers, trained to predict momentary lapses in memory function, triggered stimulation of the lateral temporal cortex (LTC) during the study phase of the task. We found that closed-loop stimulation of LTC improved memory relative to random stimulation and produced the largest memory improvements when delivered to targets near white matter pathways. The functional connectivity profile of the stimulation target predicted how stimulation affected low-frequency activity in downstream areas; downstream modulation of low-frequency activity was also correlated with the change in memory performance. These data reveal how anatomical and functional properties of a stimulation target mediate stimulation's physiological and behavioral effects, provide further evidence that closed-loop LTC stimulation can improve episodic memory, and suggest strategies for stimulation targeting for effective neuromodulation.

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**Presentation Number:** NANO48.09

**Topic:** H.07. Long-Term Memory

**Support:** NSF-GRFP Grant #1324585

**Title:** Features of human hippocampal sharp-wave ripples during sleep as biomarkers for long-term memory functioning

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**Abstract:** The hippocampus is a crucial node within the episodic memory network. Synchronized neurophysiological activity called hippocampal sharp-wave ripples (SWR) are well established to play a unique role in episodic memory encoding, consolidation, and recall. Less explored is how features of SWRs are altered in patients that have impaired hippocampal

structure and clinical memory deficits. Such a neurophysiological biomarker of hippocampal integrity could have potential clinical implications in characterizing memory impairment. To address these questions we analyzed intracranial electroencephalography recorded directly from hippocampi of epilepsy patients undergoing seizure onset localization prior to intervention. This method provides a unique temporal and spatial perspective on potential electrophysiological biomarkers of memory not accessible with other methods. We detected SWRs during NREM sleep and quantified their features (amplitude, duration, and rate) in patients with left mesial temporal sclerosis and bilateral hippocampal implants (N = 4), as well as patients with seizure onsets outside of the hippocampus (N = 8). We compared results from the MTS hippocampus to healthy hippocampus within-subjects and across subjects. We also correlated SWR features with performance in the Rey Auditory Verbal Learning Test (RAVLT). We found SWR features specific to patients with left MTS and a higher degree of variability among patients with seizure foci not localized to the hippocampus. Overall amplitudes of SWRs were significantly smaller and duration significantly longer in the left sclerotic hippocampus compared to the healthy right hippocampus, across the group and within most subjects (3 of 4), specific to the MTS group. Interestingly, longer SWR duration in the left hippocampus was moderately associated with improved memory performance in delayed recall scores in the RAVLT. Additional exploratory analyses provided insight into the factors contributing to potential reorganization of functional memory networks and ripple rate synchronization across bilateral hippocampi. In summary, this study emphasizes the importance of considering hippocampal laterality when studying SWR activity in sleep and wake states in patients with left MTS, as there were significant differences both within subjects and within-group. The study also reported novel findings related to the relationship between SWR features and performance on the Rey Auditory Verbal Learning Test (RAVLT). Taken together, the results of this study ideally provide the ground-work to establish a neurophysiological biomarker for hippocampal integrity in humans.

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**Nanosymposium**

**NANO49: Network Models**

**Location:** WCC 152A

**Time:** Monday, November 13, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO49.01

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NSF 2207891
NIH RF1MH125933

**Title:** Models of large-scale neuronal population activity with hierarchical, principal-component, and global-coupling constraints

**Authors: *A. ABBASI**[1], A. NANDA[2], J. MANLEY[3], A. VAZIRI[3], M. RUBINOV[2];
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**Abstract: Background.** A major challenge in systems neuroscience is the need to test new discoveries against benchmark models of fundamental existing knowledge. In practice, such tests require the ability to quantify statistics of benchmark-model null distributions. In turn, the complexity of benchmark models typically necessitates to estimate these statistics from simulated benchmark-model data.
**Methods.** Here, we developed nullspace-sampling based methods to simulate benchmark-model data with three specific features: hierarchical modular organization of population activity, low-dimensional structure of principal component analysis, and coupling of individual neurons to the global population mean. We benchmarked the performance of these models on two-photon calcium imaging of neuronal activity across large parts of the mouse cortex during visual and tactile stimulation. We calculated three types of evaluations for empirical and model data: correlations between all cell pairs, tuning of each cell to visual and tactile stimuli, and manifold structure of the data extracted using UMAP.
**Results.** Our findings indicated that our models successfully explained several aspects of the empirical data (Figure 1). Specifically, the population-coupling model moderately explained correlations between every cell pair (mean [95% confidence intervals], r = 0.471 [0.470,0.472]). Furthermore, this model, as well as other models largely captured cell tuning to visual and whisker stimuli (r = 0.672 [0.655,0.689]). Finally, all three models successfully preserved the first component of the UMAP manifold structure (r = 0.882 [0.876,0.888]).
**Conclusions.** Our framework can efficiently generate synthetic data with strong and biologically interpretable constraints. Our results demonstrate that these constraints capture important properties of emergent activity of wide interest to systems neuroscience. Collectively, our methods and results facilitate the principled modeling of large-scale and complex datasets of neuronal population activity.

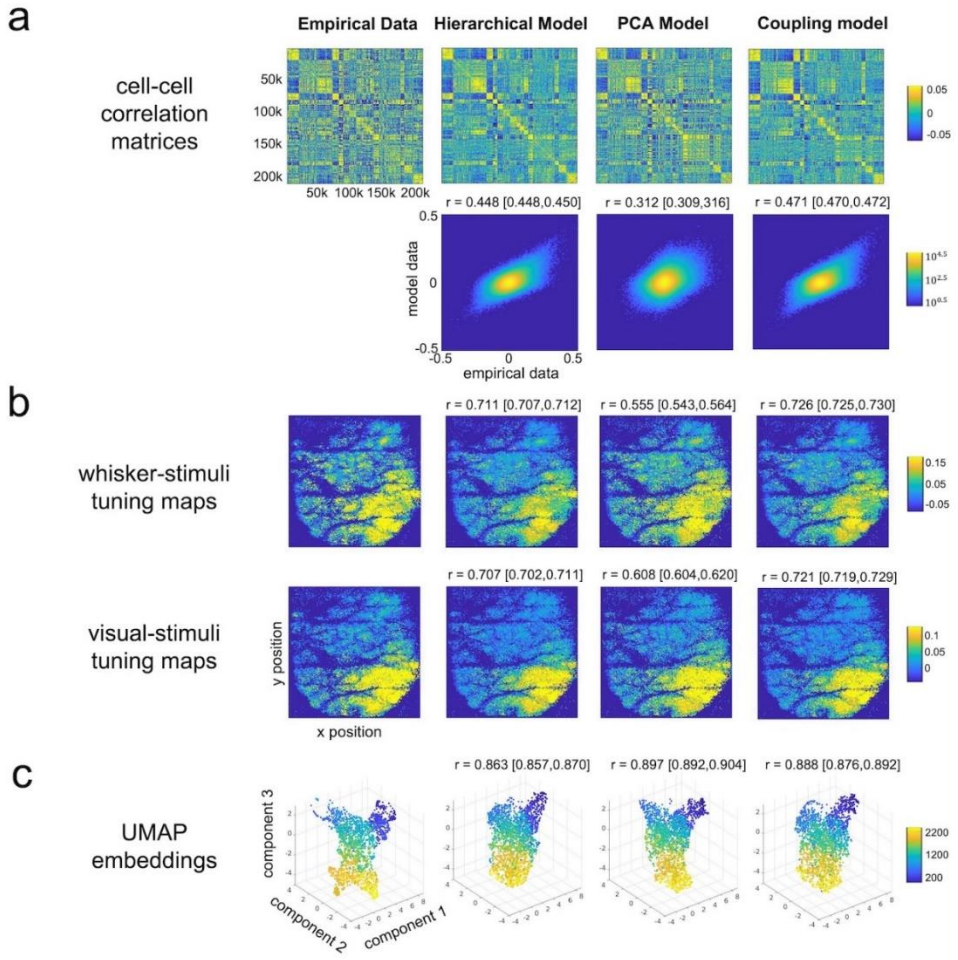


**Figure 1.** Benchmarks for hierarchical, principal-component, and global coupling models.

a. Top: cell-to-cell correlation matrices for empirical and model data. Bottom: two-dimensional histograms of scatter plots between empirical and model cell-cell correlation matrices. Brighter colors denote higher point densities. r values denote correlation coefficients between pairs of matrices.
b. Whisker and visual tuning maps for empirical and model data. Colors denote correlation coefficients to whisker stimuli (top) or visual (top) stimuli. r values denote correlation coefficients between empirical and model maps.
c. UMAP embeddings for empirical and model data. r values denote mean correlation coefficients between the first empirical and model UMAP components.

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**Presentation Number:** NANO49.02

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** HFSP Grant RGP 0019-2018

**Title:** Neurowiz: a platform for large-scale neuron simulation

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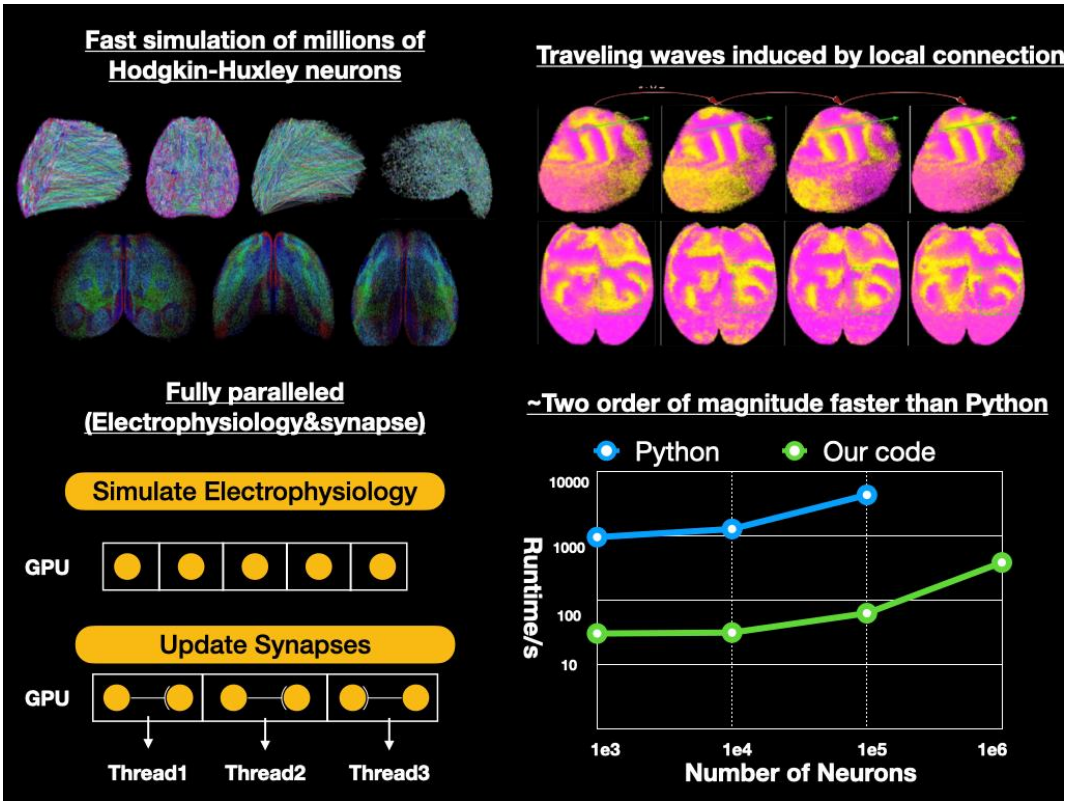
**Abstract:** Direct simulations of large neuronal networks offer a more precise understanding of network behavior compared to simplified models based on firing rate or mean-field approximation. However, conducting such simulations is challenging and resource-intensive. Existing computational platforms like NEURON, GENESIS, and GeNN have taken distinct approaches to address those challenges, yet they still lack essential functions such as fast visualization, connectivity building, and tools like EEG that closely resemble experimental setups.

Now, we introduce NeuroWiz, a novel computational platform capable of simulating millions of neurons in near real time, incorporating various utilities and employing modern visualization techniques. NeuroWiz capitalizes on the parallelism of GPUs to simultaneously handle electrophysiology, synaptic coupling, and visualization. Moreover, NeuroWiz offers a range of utilities to simulate EEG/FMRI measurements, ensuring closer resemblance to experiments. Neuron positions are utilized by NeuroWiz for building connectivity and visualization.

By incorporating realistic connectivity patterns, NeuroWiz accurately replicates phenomena such as traveling waves in the cortex and slow-wave activity during NREM sleep. These behaviors are modulated by adjusting global and local connectivity, as well as the ionic current for each neuron. The platform also enables the computation of paracrine signals that diffuse through the extracellular environment. Overall, NeuroWiz serves as a comprehensive platform for assimilating extensive single-cell data, performing state-of-the-art computations, and visualizing cellular activity.



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**Presentation Number:** NANO49.03

**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** Studying the Neocortex By Comparing two Biologically Detailed Models: Human vs Rodent

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**Abstract:** The human neocortex is the brain area that differentiates us most from other species to a great extent by imparting unique cognitive skills, such as spoken language. Even though Ramon y Cajal was already studying the neocortex in the 19th century, very little is known regarding some of its basic structural characteristics. It has been previously shown that the use of morphologically detailed computational models and simulations can help speed up the process of unraveling the mystery of brain circuits. In this approach, most of the sparse data available within the community is combined and integrated into a coherent model. By comparing computational models for different species we can highlight functional differences between them, find their anatomical or physiological basis and thus improve our understanding of cortical function. Consequently, in this study we have built a detailed model of a human cortical microcircuit following the approach used in Markram et al., 2015 to build a rat microcircuit. We generated new original data on human X, Y and Z and combined it with data in the literature to parameterize the model. We also developed various strategies to overcome the missing data, such as generalizing or adapting data from other species. The goal of this study is to unravel human cortical structural and functional characteristics by comparing the human with the previously built rodent models. We found that the human cortex is less dense in terms of cell bodies than the rodent cortex. Human cells have more complex branching, but lower bouton densities than rodent cells. However, the number of connections between cell types is similar. We characterized the implications this has for the topological structure of connectivity in terms of robustness, degree distributions, symmetry, and related measurements. We also found that human synapses, between pyramidal cells, are stronger and with higher release probability than the ones from rodents.

**Disclosures: N. Barros-Zulaica:** A. Employment/Salary (full or part-time):; EPFL, BBP. Other; This study was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Inst. **V. Sood:** None. **P. Rai:** None. **L. Kanari:** None. **A. Arnaudon:** None. **N. Barros-Zulaica:** A. Employment/Salary (full or part-time):; EPFL. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Blue Brain Project. Other; This study was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Inst. **Y. Shi:** None. **W. Van Geit:** None. **M. Zbili:** None. **M. Reva:** None. **T. Damart:** None. **D. Mange:** None. **R. Ranjan:** None. **R. Perin:** None. **M. Pezzoli:** None. **J. DeFelipe:** None. **R. Benavides-Piccione:** None. **L. Alonso Nanclares:** None. **C.P. De Kock:** None. **E. Mertens:** None. **I. Segev:** None. **H. Markram:** None. **M.W. Reimann:** None.

**Presentation Number:** NANO49.04

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NIH/NIDCD R01DC016950

**Title:** Variability of transient synchronizations and treatment-independent recovery in post-stroke aphasia

**Authors: *I. FALCONER**[1,2], A. BILLOT[2], M. VARKANITSA[2], N. CARVALHO[2], N. JHINGAN[3], S. KIRAN[2];
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**Abstract:** Aphasia, a language disorder commonly resulting from stroke, exhibits varying extents and rates of recovery, and the mechanisms supporting language recovery are poorly understood. Our previous work investigating dynamic functional connectivity (dFC) using functional MRI has revealed that greater temporal variability (TV) of transient synchronizations in the language network (LN) is associated with (1) increased response to treatment and (2) treatment-induced changes in LN topology. We propose that transient synchronizations enhance communication between regions, facilitating activity dependent plasticity, and that increased TV further facilitates reorganization by creating a greater diversity of connectivity configurations. This study aims to extend these findings to recovery, independent of a specific aphasia therapy, by testing the hypothesis that high TV is associated with milder aphasia in patients with greater lesion size (LS) - after accounting for months post-onset (MPO) - due to a greater extent of recovery.
We analyzed dFC in 19 patients with chronic post-stroke aphasia computed from resting state functional MRI. Structural scans were also collected and used to generate lesion maps which were used for masking lesioned voxels. After preprocessing with SPM12, mean time series per region of interest (ROI) were computed using the CONN toolbox and AAL3 atlas. Sliding window dFC and TV were calculated for bilateral language ROIs using custom MATLAB scripts. Aphasia severity was assessed using the Western Aphasia Battery - Revised Aphasia Quotient (WAB AQ). A multiple linear regression model used to test the hypothesized effect of

TV included TV, LS, MPO, and an interaction of TV and LS as predictors of WAB AQ. The full model was significant ($p = 0.0018$) explaining 59.4% of the variance in WAB AQ. A significant interaction effect between TV and LS ($\beta = 15.6$, $p = 0.011$) indicated that, for larger lesions only, higher TV was strongly associated with milder aphasia (e.g., for a patient with a LS at the 75$^{th}$ percentile, the predicted WAB AQ increased by 16 points for every standard deviation increase in TV).

Our results showed that patients with large lesions and high TV had aphasia severity similar to that of patients with smaller lesions, potentially reflecting greater recovery than those with large lesions and low TV, whose aphasia remained more severe. This aligns with previous findings and supports the hypothesis that higher TV facilitates functional reorganization underlying language recovery. Future research should investigate factors influencing interindividual differences in TV and whether modulating TV enhances treatment benefits.

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**Presentation Number:** NANO49.05

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NSF NCS-FR 1926781

**Title:** The aging brain becomes functionally more segregated

**Authors: *R. RAZBAN**, B. ANTAL, K. DILL, L. R. MUJICA-PARODI;
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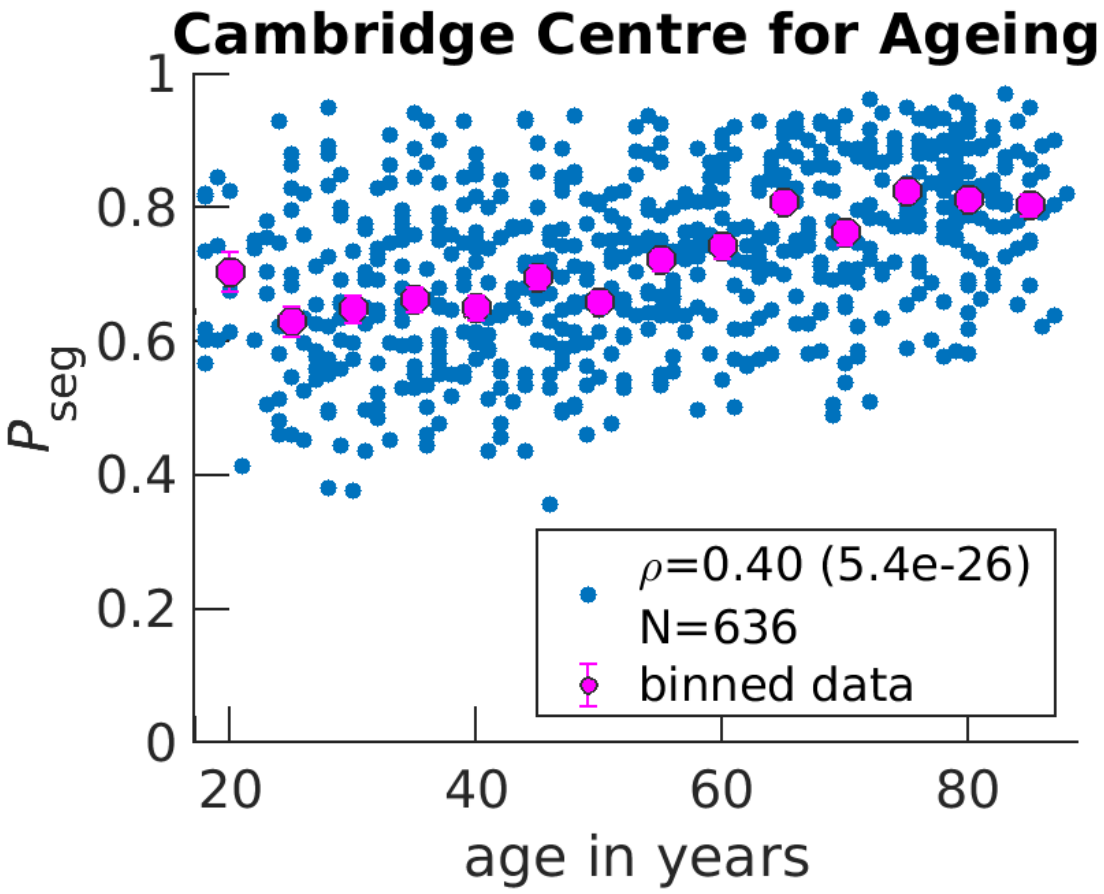
**Abstract:** The brain is under conflicting constraints to optimally function while minimizing metabolic energy costs. How the brain resolves this trade-off as it ages remains unclear. The integration-segregation framework, which reduces brain dynamics into two states: global (integrated) and local (segregated) signaling, has proven insightful in capturing this trade-off. Here, we map integration and segregation to order and disorder states from the Ising model in physics to calculate state probabilities, $P_{int}$ and $P_{seg}$. Across functional MRI scans from the Cambridge Centre for Ageing, UK Biobank and Human Connectome Project, we find that the aging brain exhibits more segregated signaling. Younger individuals exhibit brain dynamics closer to optimal $P_{int} = P_{seg} = 1/2$; while older individuals, closer to random ($P_{int} = 0$ and $P_{seg} = 1$). Through simulations backed up by structural and diffusion MRI data from the UK Biobank, we demonstrate that degeneration of axon signaling could underlie global network reorganization.




**Disclosures: R. Razban:** None. **B. Antal:** None. **K. Dill:** None. **L.R. Mujica-Parodi:** None.

**Presentation Number:** NANO49.06

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NIMH Grant 1R21MH132240-01

**Title:** Resting state networks embed anatomically reliable nonlinear dynamics

**Authors: *R. CHEN**[1], M. SINGH[1], T. S. BRAVER[2], S. CHING[1];
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**Abstract:** Analysis of whole-brain functional connectivity (FC) is commonplace in functional neuroimaging and has generated many insights into cognition and psychiatric disorders. However, the mechanistic underpinnings of FC and the causal dynamic relationships between brain areas are still not well-understood. In particular, it remains hotly debated as to whether FC is best characterized as a unimodal stationary process sampled over time, or instead, in terms of a nonlinear dynamical system that generates non-trivial fluctuations in brain-wide covariation, as is assumed by short-time windowed analysis such as dynamic FC (dFC). Here, we provide evidence for the latter, by constructing whole-brain dynamical systems models from individual resting-state fMRI (rfMRI) recordings in the Human Connectome Project using the Mesoscopic

Individualized NeuroDynamic (MINDy) platform. MINDy models consist of hundreds of neural masses connected by fully trainable weights, with parameters fit on an individual basis, thus enabling generative predictions regarding the activity of a person's brain. Our prior work validated that MINDy models are reliable in parameter space and recapitulate key statistical properties of resting state brain activity, including FC and dFC. Interestingly, we found here that MINDy models were consistently nonlinear in each individual, with a diversity of non-trivial attractor dynamics observed, including multiple fixed points and limit cycles. However, when projected into anatomical space, these attractors mapped onto a more limited set of canonical resting state networks, which were reliable at the individual level. Further, by creating convex combinations of models, we were able to induce several bifurcations resulting in the full spectrum of dynamics found via fitting. This suggests that the brain at rest traverses a quite diverse set of dynamics, which generate several distinct but anatomically overlapping attractor landscapes. Importantly, we demonstrated that such a property does not emerge in models fit to surrogate data with matched covariance (FC) and power spectral density. These findings suggest that treating rfMRI as a unimodal stationary process (conventional FC) may miss critical attractor properties and structure within the resting brain that may be better captured through neural dynamical modeling and analysis approaches. The results provide new insights into the intrinsic spatiotemporal organization of brain networks, and suggest opportunities for future brain-phenotype analyses derived from MINDy-based dynamical characterization of rfMRI data.

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**Presentation Number:** NANO49.07

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** Research Foundation - Flanders 11K1924N

**Title:** Responders and non-responders of DBS for OCD: differences in frontal activation patterns

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**Abstract:** Introduction: Obsessive-compulsive disorder (OCD) is a debilitating psychiatric condition that affects 2-3% of the population. Pharmacological treatment and cognitive behavioral therapy may alleviate symptoms. Deep brain stimulation (DBS) is emerging for treatment-resistant OCD (TR-OCD). Objective: to investigate response and non-response to DBS treatment for TR-OCD. Methods: A finite element model of the electrical field and volume of tissue activated (VTA) model were built, and structural connectivity was mapped using the Lead DBS software. Co-regisitration of the preoperative MRI to the postoperative CT and normalization were done using Advanced Normalization Tools (Avants, 2008). Brainshift correction was applied using a coarse mask (Schönecker, 2008). Electrodes were segmented using the refined TRAC/CORE method. Electrode segmentation was manually optimized. Electrodes were visualized in the MNI space together with a OCD reponse tract atlas (Li, 2020). A finite element model of the electrical field was constructed using the SimBio/FieldTrip method based on the stimulation current on the active electrodes. Conductivity of the brain tissue was assumed 0.14S/m. A threshold of 0.2V/mm was used to calculate the VTA surrounding the

active electrodes. The VTA was used as a seed while mapping the structural connectivity, based on a structural group connectome of 32 subjects from the Human Connectome Project (MGH-USC HCP 32, Horn, 2017). We report on the first results of one responder and one non-responder. Our current long-term follow-up clinical dataset consists of an additional 14 responders and 10 non-responders. Results: Preliminary results comparing one responder and one non-responder show a different pattern in the structural connectivity map. The responder showed more bilateral frontal connectivity. Conclusions: An individualized structural connectivity map might assist in analyzing response to DBS treatment for TR-OCD.

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**Presentation Number:** NANO49.08

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NIH Grant R01-MH117807

**Title:** Uncovering Altered Developmental Patterns in Structural Connectivity for Autism Spectrum Disorder through Deep Generative Modeling of Normative Development

**Authors: *R. SHEN**[1], B. TUNÇ[3], Y. OSMANLıOğLU[4], D. PARKER[2], D. AUNAPU[2], I. WINGERT[2], B. YERYS[3], R. VERMA[2];
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**Abstract:** The evolution of structural connectivity during development involves intricate biological processes, disruptions in which are the hallmark of several disorders, including autism spectrum disorder (ASD). To characterize the normative developmental patterns and detect deviations therein, we propose a novel deep generative model which employs a variational auto-encoder with biologically meaningful wiring constraints to infer the latent embedding of structural connectivity at different ages, as shown in Fig.1. This approach allows us to 1) obtain latent features and map normative variation for typical developing controls (TDCs), 2) estimate brain age for each subject, 3) generate brain networks that mimic observed ones, and 4) detect individual deviation from the normative model to derive an overall deviation score. We assessed the model's performance in two datasets: PNC (TDC=968, age 8-12) and CAR (TDC=196, ASD=229, age 6-19). We compared our model to 13 classic generative models and 5 machine/deep learning models. Our model consistently achieved the best performance with smallest discrepancy between generated and observed data, and the lowest MAE & RMSE between estimated brain age and chronological age. The gap between brain and chronological age is significantly correlated with autistic traits (Spearman $R = -0.29$, $p < 0.01$) and IQ (Pearson $R = 0.20$, $p < 0.01$). We observe substantial variability in regional differences among autistics, indicating notable heterogeneity in our sample. Generally, youth with more autistic traits exhibit larger brain deviations across more brain regions. The visual (right medial visual cortex), default mode (bilateral prefrontal cortex), dorsal attention (bilateral frontal eye fields), somatomotor (right pericentral cortex), and executive control networks (bilateral lateral prefrontal cortex) are most frequently associated with autism. Overall, our model is effective in characterizing

normative and altered developmental brain trajectories, and brain deviation is a promising subject-specific marker of autism.

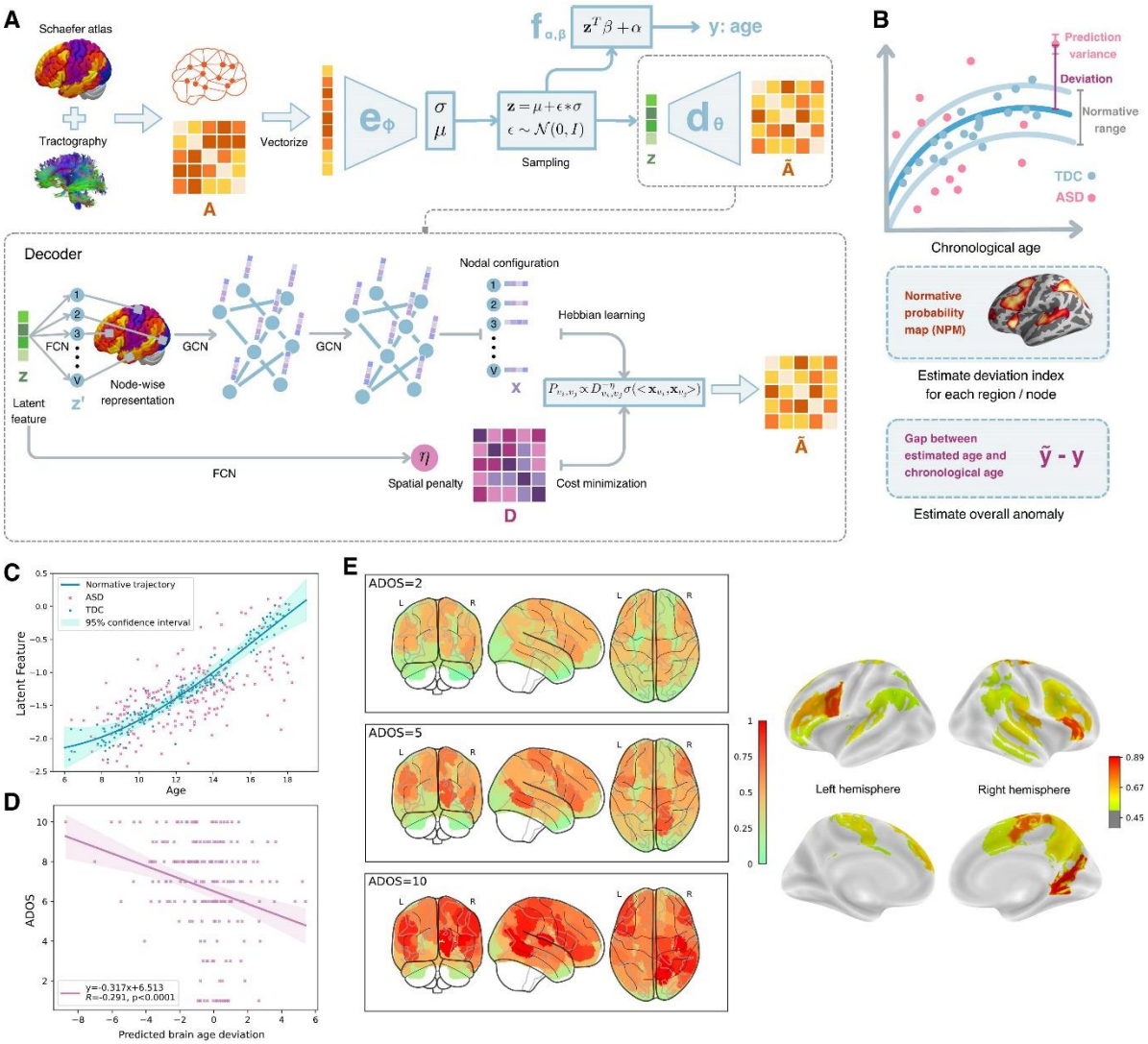


Fig.1 Normative modeling of structural connectivity with a constrained variational auto-encoder to detect atypical developmental patterns. A) Architecture of deep generative model that explicitly integrates two biological meaningful wiring constraints while taking computational advantages of the graph-based variational auto-encoder. B) Gaussian process regression is used to model normative trajectory between chronological age and the latent feature of each region. For each subject, Z score of their regional deviation from the typical range can be quantified to obtain a normative probability map (NPM). The brain age gap is used as the proxy for the overall anomaly. C) Illustration of normative modeling for a latent feature using ASD and TDC samples from CAR. D) The brain age gap captured by our model significantly correlate with ADOS. E) Averaged NPMs across subjects with an ADOS score of 2, 4, 10 (left) and across all ASD samples (right).

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**Presentation Number:** NANO49.09

**Topic:** I.06. Computation, Modeling, and Simulation

**Title:** Network discovery using information theory: From molecular to functional networks.

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**Abstract:** Reconstruction of molecular networks allows structural and functional characterization of neurons, tissues, and organs. A network analytical approach to statistical correlations in molecular data can provide new insights into the organizational principles of molecular networks, helping with e.g. target discovery and pathway analysis. We, therefore, developed the "Toolbox for Information-Theoretic Analysis of Networks (TITAN)", an open-source toolbox in Google Colab, Python, Jupyter Notebook, MATLAB, and Octave for network analysis, reconstruction, and visualization.
TITAN reconstructs gene co-expression networks from transcriptional data using mutual information to quantify connectivity and variation of information to estimate the weight of connectivity. TITAN controls for indirect correlations in the dataset, implements bias correction and an automated threshold for network identification, and offers quantitative mapping of the sample size requirement to confidence in estimations. The toolbox also includes functions to reveal the topological structure of correlations in biological systems. We demonstrate the accuracy of the resulting networks first using synthetic data, then by recreating five gene co-expression networks in S. cerevisiae that were previously identified experimentally. We exemplify the utility of TITAN by reconstructing the sex-dependent house-keeping gene networks in the primary somatosensory cortex of the rodent, identifying constitutively expressed gene networks, using a publicly available dataset (Kole et al, 2017; PMID: PMC5965344). TITAN is a general tool for network reconstruction. It could be used on any large, high-dimensional dataset to identify patterns, with the shortcoming of dramatically increased computation time in very large networks. As a proof of principle, by taking advantage of the Cell Atlas made available by the Allen Brain Institute, we show that TITAN could be used to compute the similarity of stimulus representations in single neurons, identify functional subclasses of neurons, and reduce dimensionality in neural representations to unravel stimulus selectivity of neurons.

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**Nanosymposium**

**NANO50: Computational Tools for Microscopy, Imaging and Behavior**

**Location:** WCC 201

**Time:** Monday, November 13, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO50.01

**Topic:** I.07. Data Analysis and Statistics

**Support:** CIHR Grant PJT178059

**Title:** A deep learning pipeline for 3D mapping of neuronal activity in tera-voxel light sheet microscopy data

**Authors: *A. ATTARPOUR**[1,3], S. PATEL[3], M. ROZAK[1,3], T. QI[4], N. K. LAL[4], J. MCLAURIN[6,2], L. YE[4,5], B. STEFANOVIC[1,3], M. GOUBRAN[1,3];
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**Abstract:** Motivation: Mapping neuronal subpopulation-specific activity is critical to understanding brain network dynamics underlying behavior and cognition. Advances in light sheet microscopy (LSM) and tissue clearing have enabled high-fidelity imaging in intact tissue; producing large 3D datasets. However, current computational pipelines rely on either 2D-based techniques or traditional algorithms which require manual parameter tuning, confounding quantitative analysis of 3D neuronal volumetric changes brain-wide. Methods: To map neuronal activity in whole-brain tera-voxel LSM data, we developed 3D deep learning models based on state-of-the-art (SOTA) vision transformers (UNETR). The models were trained on LSM data of optically-cleared brains from 18 healthy transgenic mice (TRAP2-Ai9) with the cFos promoter (10/3/5 animals for training/validation/testing). A series of semi-automatic image processing steps with the expert intervention was utilized to generate silver ground truth (GT) data. We divided each dataset into smaller 3D patches ($96^3$ *voxels* $=\sim 0.35^3$ *mm*) and selected subsets of 45,600 patches for training. To quantify the confidence/uncertainty and generalizability in the models' predictions, each model's output was obtained by averaging 50 models' predictions using the Monte-Carlo dropout technique. We evaluated the models using both test and unseen datasets, employing the Dice coefficient/F1-Score, Precision, Sensitivity, and Hasusdorff distance. Finally, we compared the performance of our models against the SOTA cell-detection and segmentation algorithms Cellfinder and Ilastik. Results: The Monte-Carlo dropout technique improved the precision and performance of our DL model. Our UNETR model outperformed Ilastik and Cellfinder on all evaluation metrics (pvalue<0.0001), resulting in an F1-score of 0.77±0.08, compared to 0.55±0.15 for Cellfinder and a Dice coefficient of 0.76±0.07 compared to 0.29±0.06 for Ilastik. Conclusions: We present a novel robust 3D DL ensemble network to map neuronal somas in whole-brain LSM data, surpassing current detection and segmentation algorithms. Future work includes integrating our models with registration pipelines and implementing advanced statistical algorithms for neuronal subpopulation-specific analyses of LSM data.

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**Presentation Number:** NANO50.02

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NIH U01-MH117072

**Title:** A generalist approach for volumetric cell phenotyping

**Authors: *X. GU**[1], C. ZHAO[2], J. PARK[2,3,4,5], S. CHOI[4], Y. TIAN[4], W. GUAN[4], C. SU-ARCARO[2], K. CHUNG[6,2,3,4];
[1]Electrical Engin. and Computer Sci., [2]the Picower Inst. for Learning and Memory, [3]Inst. for Med. Engin. and Sci., [4]Chem. Engin., [5]Ctr. for Nanomedicine, [6]Brain and Cognitive Sci., MIT, Cambridge, MA

**Abstract:** Characterizing the spatial organization of diverse cell types is essential for understanding brain function and dysfunction. Recent advances in volumetric tissue processing

and imaging technologies have enabled rapid visualization of cells labeled with a wide range of molecular markers. However, accurately detecting individual cells in such large-scale datasets and classifying them based on their labeling patterns remains challenging. While deep learning-based methods have been widely employed, they often rely on extensive training data specific to certain cell types and time-consuming manual annotations, which are susceptible to human biases and errors. Moreover, existing methods have struggled to effectively handle 3D datasets due to the increased morphological diversity captured and the limited availability of training data. To overcome these challenges, we have developed a novel approach termed Generalist 3D Cell Phenotyping (GCP), which enables scalable and precise cell detection in large-scale 3D brain datasets. The GCP approach combines a generally trained neural network-based model with a non-learning-based cell phenotype recognition algorithm. This hybrid approach enables rapid and accurate cell mapping, independent of image resolution, labeling patterns, or tissue processing techniques. Furthermore, GCP eliminates the need for model retraining while maintaining high cell detection accuracy. Our experimental results demonstrate the efficacy and robustness of GCP in correctly detecting multiple cell types across various resolutions and labeling techniques in multiplexed imaging datasets. With the inclusion of a user-friendly, browser-based interface, we envision that the broader scientific community will readily adopt GCP for a wide range of applications without requiring specialized computational knowledge.

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**Presentation Number:** NANO50.03

**Topic:** I.07. Data Analysis and Statistics

**Support:** National Institute of Mental Health Intramural Research Program (ZIC-MH002968)

**Title:** A Functional Mixed Models Framework for Analysis of Fiber Photometry Experiments

**Authors: *G. LOEWINGER**[1], F. PEREIRA[2];
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**Abstract:** Fiber photometry has become a popular technique to measure neural activity in vivo, but common analysis strategies can reduce detection of subtle effects because they coarsen information by 1) condensing *within-trial* signals into summary measures, and 2) discarding trial-level information by averaging *across trials*. To fill this gap, we propose a novel functional mixed effects model framework for photometry analysis which i) enables hypothesis testing of experimental variable effects at *every point in the trial*, and ii) uses signals from every trial and animal in the analysis. In essence, the approach allows one to compare the "shapes" and magnitude of signals across conditions while accounting for between-animal differences in those "shapes." Our framework produces a series of plots that visualizes covariate effect estimates and statistical significance at each trial time-point, thereby simplifying analysis and reporting. Through reanalyzing published data, we show the methodology reveals significant effects obscured by standard methods and we demonstrate how to pose scientific questions about complex experimental designs within our framework. We conduct simulation experiments on

realistic synthetic data, which show our methodology yields improved statistical power over standard photometry analysis approaches and achieves nominal coverage with 95% confidence intervals. Finally, we provide an open-source R package implementing our framework.

**Disclosures: G. Loewinger:** None. **F. Pereira:** None.

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**Topic:** I.07. Data Analysis and Statistics

**Support:** NIH Grant R21 DA052419
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NIH Grant R24 MH114793

**Title:** A modular framework for standardizing analysis of large-scale whole brain microscopy data

**Authors: *I. VASYLIEVA**[1,3], M. SMITH[3], K. HE[3], T. LING[7], E. ARAVIND[4], M. JACOBY[5], W. KLIMSTRA[5], R. W. LOGAN[8,4], Z. FREYBERG[2,6], A. WATSON[3];
[1]Cell Biol., [2]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [3]Ctr. for Biologic Imaging, [4]Dept. of Psychiatry, [5]Dept. of Immunol., [6]Dept. of Cell Biol., Univ. Of Pittsburgh, Pittsburgh, PA; [7]Carnegie Mellon Univ., Pittsburgh, PA; [8]Univ. of Massachusetts Chan Med. Sch., Worcester, MA

**Abstract:** Modern microscopy techniques generate large and diverse neuroimaging data, such as multi-terabyte whole brain images. Standardizing the storage format for diverse data types, and creating tools that improve data usability would significantly increase impact within the community. Although FAIR (Findability, Accessibility, Interoperability, Reusability) principles are a guidance to both storing data and designing analysis workflows, there are no widely adopted data formats and tools for analyzing whole brain microscopy data. Experimenting with analysis on large data volumes can be very time- and resource-consuming. We propose adopting practices which use one standard workflow per data type or analysis type, and provide a modular framework to modify workflows, reusing the components. Whole brain imaging data exhibits numerous similarities, and the goal of the analysis is often to identify labeled brain cells and the circuits they form. The cells could be labeled for example in a similar way due to viral infection, disease-specific markers, or activated in response to an addictive substance etc., which offers opportunities for using modular reusable pipelines. Instead of creating narrowly tailored new tools, we strive to reuse existing community derived tools, making them more modular, scalable and easy to use. We embarked on an effort to harmonize community derived tools and combine them into a single modular framework, leveraging containerization tools and workflow languages to make the tools compatible with different computational platforms. We created a scalable analysis pipeline that combines conventional image analysis and deep learning methods to extract soma locations, detect neuronal projections, and localize them in the common coordinate framework space. In this way, we convert multiscale multi-terabyte whole brain images into more manageable high-level data indexed, searchable and visualizable as a web based interactive dashboard. The dashboard is general enough to allow investigators exploring relationships between any variables in their data in an unbiased manner. We showcase

application of the pipeline to diverse problems: (i) describe progression of the viral encephalitis infection in mouse brain over time and brain regions, (ii) understand relevant mouse brain regions involved in the response to opioids and (iii) reconstruct the dopamine neurons circuitry. By generating easily searchable, comparable, and visualizable high-level data representations, our pipeline empowers researchers to access and utilize the data efficiently, thereby advancing our understanding of brain function as well as disease.

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**Presentation Number:** NANO50.05

**Topic:** I.07. Data Analysis and Statistics

**Title:** A multimodal digital atlas of the tree shrew (Tupaia belangeri) brain

**Authors: *M. ARCARO**[1], K. T. HITCHENS[2], J. WEKSELBLATT[3];
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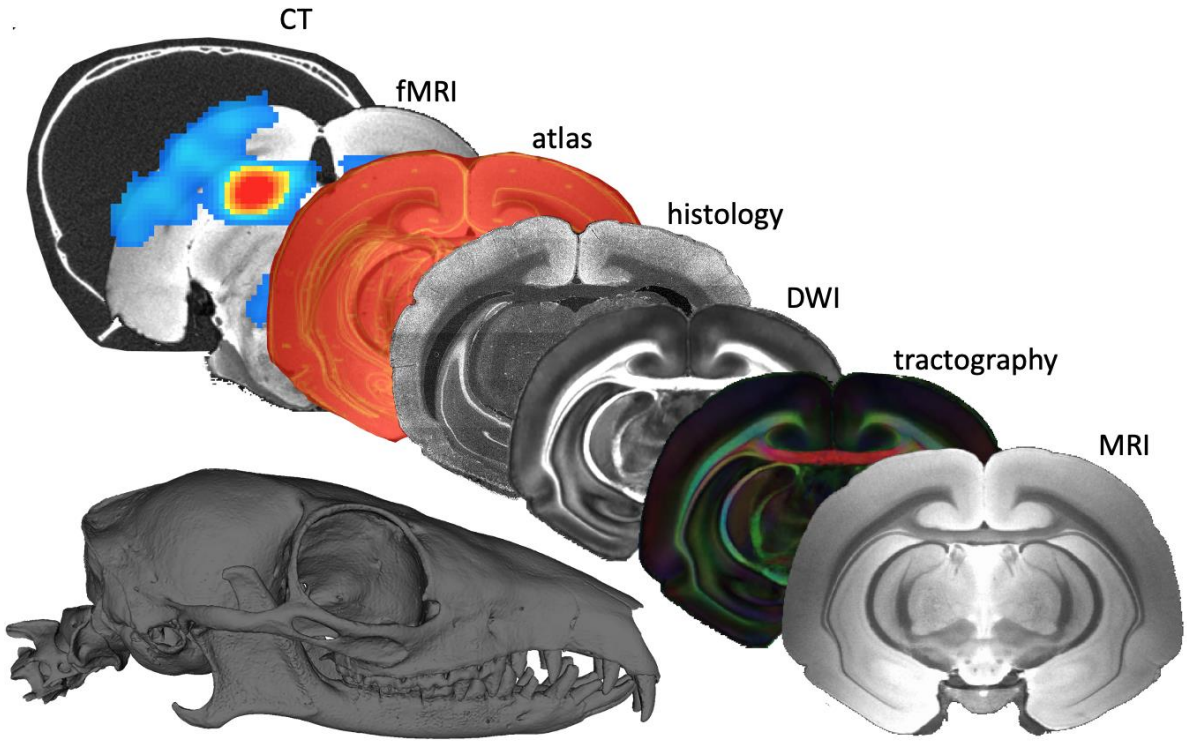
**Abstract:** Digital brain atlases have been vital resources for neuroscientific investigations involving humans and widely used animal models, such as primates and rodents. These atlases provide essential tools for precise surgical targeting, localization of neural measurements, and cross-subject and cross-dataset comparisons. Here, we present a digital brain atlas of the northern tree shew (*Tupaia belangeri*), a close living relative to primates. Our atlas was constructed using *ex vivo* T2-weighted anatomical MRI (50 $\mu m^3$), diffusion MRI (80 $\mu m^3$), and CT skeletal imaging (40 $\mu m^3$). MR imaging was performed on an 11.7T Bruker scanner with the use of gadolinium contrast agent to facilitate accelerated image acquisition. Diffusion data were acquired with 60 directions across 3 shells (b=1000, 2000, 3000 or 3500 s/mm$^2$). CT imaging was performed on an MI Labs U-CT. The atlas includes individual subject data and population-averaged templates for T2-weighted images of the brain and head, skull CT reconstructions, diffusion tensor reconstructions, and multiple diffusion contrasts including fractional anisotropy and mean diffusivity. The atlas incorporates data from 10 adults aged 1.5-4 years. From the anatomical MRIs, we segmented grey and white matter and generated models of the cortical surface that facilitate data visualization and laminar analyses. Major white matter tracts were identified using deterministic diffusion tractography. To aid in the identification of individual brain structures, we digitized and registered the Zhou and Ni book atlas (2016) to the template. Ongoing work aims to expand the atlas by including angiography, functional ultrasound, whole-brain fMRI, and 3D reconstructions of slice histology. This atlas provides the scientific community with a comprehensive platform that facilitates neuroscientific investigations into the structure and function of the tree shrew brain. Researchers can leverage this platform to bridge data across subjects, methodologies, and spatial scales, thereby promoting the integration of findings into a unified reference space.




**Disclosures: M. Arcaro:** None. **K.T. Hitchens:** None. **J. Wekselblatt:** None.

**Presentation Number:** NANO50.06

**Topic:** I.07. Data Analysis and Statistics

**Support:** National Defense Science and Engineering Graduate Fellowship
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Simons Collaboration on the Global Brain
NEI R01-EY022933
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**Title:** Stimpack: precise and flexible generation of realistic stimuli for neuroscience

**Authors: *M. CHOI**[1], J. B. MELANDER[2], S. G. HERBST[3], L. BREZOVEC[2], S. DRUCKMANN[2], S. A. BACCUS[2], T. CLANDININ[2], M. H. TURNER[2];
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**Abstract:** A central goal of neuroscience is to understand how animals engage with the natural world. Therefore, a deep understanding of the brain requires the use of stimuli that reflect the natural world statistically and interactively. On the other hand, efficient and precise parameterization of stimuli can make analyses more tractable. Generating stimuli that are both realistic and precisely parameterizable, however, can be technically challenging. For this reason, current software solutions for stimulus generation often prioritize one or the other goal.
We thus present Stimpack, a powerful Python software suite designed to facilitate precise, yet

flexible, delivery of multisensory stimuli that are both realistic and precisely parameterizable. Sensory objects and environments rendered in Stimpack can follow dynamics that are defined flexibly, such that they can even follow recorded trajectories of freely behaving animals. That, combined with Stimpack's ability to render stimuli in closed-loop interaction with the experimental animal's real-time behavior, enables the study of animal interactions with realistic objects. Furthermore, the virtual sensory environments can be replicated flexibly in different laboratory settings. For example, the visual module can render both 2D and 3D objects on an arbitrary number and arrangement of screens, and compensates for visual distortions caused by variable distances between points on the stimulus canvas and the subject's eye. This flexibility enables reproducibility as well as comparative studies across systems.
We also present our applications of Stimpack in our investigation of how internal states and sensory priors shape visual perception in fruit flies and in our study of how naturalistic visual stimuli are encoded in the visual cortex of the mouse.
Stimpack, available on https://github.com/ClandininLab/stimpack, is a comprehensive software suite that facilitates stimulus delivery in systems neuroscience experiments. In addition to existing stimulus modules, Stimpack provides a modular framework that allows researchers to create stimulus modules for different sensory modalities that can interact with each other. With expressive, Pythonic definitions of dynamic sensory stimuli, closed-loop interactions between the sensory world and the animal's behavior, and flexible rendering of sensory environments across different experimental setups, Stimpack opens up new possibilities for novel and reproducible studies of how animals and their brains perceive and interact with the world.

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**Presentation Number:** NANO50.07

**Topic:** I.07. Data Analysis and Statistics

**Support:** Woodruff Funds Synergy Awards

**Title:** Nexus: An interoperable and distributed platform for optimal closed-loop experiment design

**Authors:** Y. ZHU[1], S. HOSSEIN[2], P. SARIKHANI[2], P. GU[2], S. BETTERS[2], S. LIU[2], R. TWEEDY[2], P. KATHIRAVLU[2], T. PAN[2], M. TREADWAY[2], ***B. MAHMOUDI**[2];
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**Abstract: Introduction**: Closed-loop optimal experiment design is an emerging approach that offers increased sampling efficiency and the ability to reach targeted brain states in fewer steps. However, implementing adaptive experiment designs using real-time closed-loop pipelines and AI algorithms like Bayesian optimization can be challenging for experimentalists due to infrastructure requirements and limited technical expertise. Moreover, the need to swap and test different algorithms in various experiment settings poses an obstacle for algorithm development, requiring specific interfaces for each individual setup. **Methodology**: To address these problems, we present NEXUS, an interoperable, scalable and distributed platform for real-time closed-loop experiment design. The NEXUS platform can operate remotely from an experiment setup to implement distributed closed-loop workflows. A workflow containing multiple algorithm blocks

is invoked on demand through file write signals. The output experimental parameters are then sent to the behavioral task computer for the next trial. The platform leverages our previous workflow orchestration framework and employs Docker containers for each algorithm block. Each algorithm block has a separate host-agnostic execution environment and is wrapped by a simple shell script, conforming to a standardized input/ output pattern defined by the platform. This containerized framework allows easy adoption of existing algorithms into the platform, deployment in different host servers, and scalability through deploying multiple workflow instances. **Results**: We validated the functionality of the platform by conducting pilot closed-loop fMRI neuro-behavioral experiments using Bayesian optimization to maximize dACC activation by optimizing the reward and the effort parameters of a behavioral task. Our preliminary results demonstrate that closing the loop with the NEXUS platform introduces an overhead of 5.93±0.50s (mean±std) in addition to the running time of the algorithm blocks. This overhead, which includes file transfer checks and input backups, remains well below the typical inter-trial interval for behavioral tasks. **Conclusion**: Our platform shows promising potential for AI-driven closed-loop fMRI experiment design, as it enables researchers to efficiently orchestrate and test different algorithms in diverse experimental settings. Future work will involve expanding the study to include more subjects, developing analytic modules and growing the platform's algorithm libraries to broaden the applications and improve the user experience.

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**Presentation Number:** NANO50.08

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NINDS R01(RNS118648A)

**Title:** Supervised Dynamical Components Analysis identifies neural subspaces predictive of behavioral subspaces

**Authors: *K. ZHUANG**[1,4,2], J. YEUNG[3,4], J. BAK[4,2,6], K. BOUCHARD[1,4,2,5];
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**Abstract:** The brain evolved to produce adaptive behaviors in ethologically relevant contexts. Advances in recording technologies allow simultaneous measurement of many neurons. However, relating neural population dynamics to the dynamics of ethological behaviors remains a challenge. In particular, ethological behaviors (e.g., vocalizations) are typically complex and can be described by many features, but not all behavioral features may be equally important from a neural perspective, even if those features have high variance. This motivates unsupervised analysis methods to identify neural components that are causally related to behavioral components. Such methods would advance understanding of how neural systems generate behaviors, but are currently nascent.
To address this gap, we developed supervised Dynamical Components Analysis (sDCA). sDCA

is a linear dimensionality reduction method that identifies subspaces of neural and behavioral dynamics with maximal predictive information between the past neural and future behavior. Compared with supervised techniques such as Prefered Subspace Identification (PSID), sDCA performs unsupervised dimensionality reduction on both neural and behavioral dynamics, extracting low dimensional representations while preserving key dynamical features of both. We validated in synthetic data that sDCA correctly identified the behavioral dimensions and recovered the relative importance of the contributing neurons (not the case for PSID). We applied sDCA to the canonical setting of macaque motor cortex recording during an arm reaching task. sDCA determined the true behavioral dimensions from added extraneous variables and found the same reduced behavior dimensionality (D = 3) across a range of neural projection dimensions. Decoding performance of reach, velocity, and acceleration was on par with PSID. Finally, we examined human electrocorticography recording from the sensorimotor cortex during speech production. We found that sDCA identified behavioral subspaces for specific consonants, despite having lower acoustic variance, and further identified the relevant neural subspaces for those sounds with a causal phase offset of ~100 ms. Together, these results demonstrate that sDCA is an interpretable, unsupervised method for elucidating complex brain-behavioral relationships.

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**Topic:** I.06. Computation, Modeling, and Simulation

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**Title:** Carlsim 6: an open source library for large-scale, biologically detailed spiking neural network simulation

**Authors: *R. K. BAIN**[1], K. CHEN[1], L. NIEDERMEIER[3], J. XING[1], J. L. KRICHMAR[1,2]; [1]Cognitive Sci., [2]Computer Sci., Univ. of California, Irvine, Irvine, CA; [3]Niedermeier Consulting, Zurich, Switzerland

**Abstract:** Spiking Neural Networks (SNNs) implement the biological properties of neurons and synapses that are crucial for modeling dynamic neural networks and express adaptive behavior. CARLsim 6 was developed as an open-source software package to address the challenges of managing computational burden while performing biologically accurate simulations. CARLsim provides essential features for learning and memory in SNNs, leveraging parallel computing (e.g. GPUs) to improve performance. CARLsim supports SNN simulations across different scales, ranging from single neurons with detailed anatomical structures using multi-compartment models to large-scale networks comprising millions of neurons and billions of synapses. It also offers monitoring network activity, such as membrane potentials, spiking activity, and connection weights. The recorded data can be analyzed using the analysis toolbox that accompanies CARLsim.
CARLsim is a mature and thoroughly tested software framework that is now on its 6th major release. With the integration of CMake, CARLsim 6 has a more streamlined installation process

and is compatible with Linux, Windows, and MacOS operating systems. CARLsim 6 provides further flexibility in incorporating long-term and short-term synaptic plasticity. Additionally, CARLsim 6 incorporates evolutionary computation packages that enable parameter tuning and network optimization. Recognizing the dynamic network properties brought about by neuromodulation, CARLsim 6 now supports multiple neuromodulators for simulating neural excitability and synaptic plasticity. This allows for rapid few-shot learning, network rewiring, and neural activity modulation. Moreover, CARLsim 6 supports changes in axonal delay during runtime, enabling axonal plasticity. This feature has been effectively employed in path planning, combining axonal plasticity and path learning through eligibility traces.
Despite the addition of these features, CARLsim 6 maintains its computational efficiency. Researchers worldwide have adopted CARLsim due to its fast execution and comprehensive support for biologically plausible simulations. The software remains under active development and continues to be open-source at https://github.com/UCI-CARL/CARLsim6, fostering collaboration and innovation in both the neuroscience and machine learning communities.

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**Nanosymposium**

**NANO51: Neurodevelopmental Disorders: Diversity in Causes and Phenotypes**

**Location:** WCC 201

**Time:** Tuesday, November 14, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO51.01

**Topic:** A.07. Developmental Disorders

**Support:** NIH NINDS K99 NS112415
NCI Intramural Research Program

**Title:** Investigating the immediate consequences of rapid MeCP2 protein degradation

**Authors: *L. D. BOXER**[1], S. SU[1], R. SAKR[1], T. MASUDA[1], T. WHITWAM[2], A. SILBERFELD[2], W. RENTHAL[2], B. NABET[3], M. GREENBERG[2];
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**Abstract:** Rett syndrome is a neurodevelopmental disorder caused by mutations in the methyl-DNA-binding protein MeCP2. The function of MeCP2 has puzzled researchers for years because the severe disease symptoms associated with MeCP2 mutations are accompanied by only subtle molecular and cellular changes. These include small-magnitude changes in gene expression (both up- and down-regulation), some changes in mRNA splicing, slight changes in chromatin compaction, and a slight decrease in total cellular RNA levels and nuclear size. We recently found that the genes transcriptionally upregulated by MeCP2 mutations in human and mouse brain tissue tend to have high levels of CA DNA methylation (mCA), a neuron-specific modification bound by MeCP2, suggesting that MeCP2 may directly repress high-mCA genes.

However, these findings and most other studies of MeCP2 function were in the context of constitutive loss of MeCP2, so the changes observed likely represent a mix of primary and secondary effects. Thus, it is currently unknown which changes are primary consequences of MeCP2 mutations and which are secondary consequences of neurological impairment. To distinguish the primary and secondary consequences of MeCP2 loss, we generated a mouse line with a degradation tag (dTAG) on MeCP2, which enables rapid and specific MeCP2 protein degradation upon treatment with the small molecule dTAG-13. We are using this system to identify the molecular and cellular changes that occur immediately after acute MeCP2 loss and characterize the subsequent order of events. Importantly, we chose the rapid protein degradation approach over a genetic approach because the MeCP2 protein has a very long half-life (~2 weeks). Our experiments show that the MeCP2 protein can be degraded within 30 minutes of dTAG-13 treatment in neurons cultured from the MeCP2-dTAG mice. Furthermore, we developed an approach to degrade MeCP2 within 2 hours of dTAG-13 injection in vivo in the adult mouse brain. We performed RNA-seq from the adult mouse hippocampus at time points between 3 and 72 hours after injection of dTAG-13 or a vehicle control and identified differentially expressed genes at each time point. The genes differentially expressed after MeCP2 degradation tend to have high mCA levels, suggesting they are direct MeCP2 targets, and there is some overlap with the genes changed in constitutive MeCP2 knock-out mice. In ongoing work, we are characterizing other molecular and cellular changes after MeCP2 degradation, including nuclear size, chromatin compaction, and histone modifications. These experiments highlight primary molecular targets of MeCP2 and potentially inform discovery of treatments for Rett syndrome.

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**Presentation Number:** NANO51.02

**Topic:** A.07. Developmental Disorders

**Support:** NIH R01 NS 113140

**Title:** Glial targeted glutaminase inhibition leads to improvement in Rett syndrome phenotype in adult Mecp2 Het mice.

**Authors: *P. VYAS**[1], E. L. WILKINSON[2,3], A. FOWLER[1], N. SAH[1], E. S. KHOURY[1], J. LIU[1], A. SHARMA[2], S. GUPTA[4], A. BEDNER[1], P. MAJER[5], T. TICHY[5], A. G. THOMAS[6], B. S. SLUSHER[6], R. RAIS[6], R. M. KANNAN[2,3], S. KANNAN[1];
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**Abstract:** Rett Syndrome is a progressive neurodevelopmental disorder caused by mutations in gene encoding for methyl-CpG binding protein 2 (MeCP2). Although therapies are being explored for symptomatic management, no treatment is available to modify the course of disease progression. Recent work with MeCP2 deficient mouse models demonstrated a major role for glutamatergic pathways, specifically microglial-produced glutamate, due to upregulation of

glutaminase in Rett pathology.The glutaminase inhibitor, 6-diazo-5-oxo-L-norleucine (DON) inhibits glutamine-utilizing reactions, however it is associated with systemic toxicities. We have used hydroxyl PAMAM dendrimers (~4 nm, non-toxic) that target the activated glia and astrocytes in the Mecp2-deficient mice but not in the WT mice upon systemic administration. We have used DON TTM020, which is a prodrug of DON synthesized specifically for conjugation with the PAMAM dendrimer. We tested if systemic targeted monotherapy with dendrimer conjugated DON TTM020 (D-DON TTM020) results in microglial glutaminase inhibition and improvement in behavior phenotype in Mecp2-KO and Het mice, while eliminating peripheral toxicity of free DON. To determine the optimal dose of D-DON TTM020, we first treated Cx3CR1-GFP$^{/+}$ Mecp2 KO male mice (5-6-week-old) with a single 0.3 mg/kg or 1 mg/kg intraperitoneal dose of D-DON TTM020 and isolated the microglia from the brain 24 hours post-treatment. Selective inhibition of glutaminase was noted in the isolated microglia indicating microglial targeting by D-DON TTM020 with the 1mg/kg dose as most effective. Based on this, we next treated the WT and symptomatic Mecp2$^{-/+}$ Het mice biweekly for 8 weeks with saline or 1 mg/Kg ip of DON or D-DON TTM020, and their neurobehavioral phenotype was examined pre and post-treatment. Mecp2-Het mice treated with D-DON TTM020 showed improvement in neurobehavioral scores, particularly paw-clench, while DON and saline treated groups did not. D-DON TTM020 restored long-term retrieval of conditioned fear memory and improved the cue-response during fear extinction post-treatment. Other assessments are ongoing to access the behavioral and quantitative electroencephalographic changes in D-DON TTM020 treated versus untreated animals, and subsequent impact of therapy on neuronal pathology in MeCP2 Het mice. Our study provides evidence that glial-targeted glutaminase inhibition may be a potential therapeutic avenue for treating glutamate dysregulation in Rett syndrome.

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**Presentation Number:** NANO51.03

**Topic:** A.07. Developmental Disorders

**Title:** Dysfunctional modulation of primary visual cortex from anterior cingulate cortex in a mouse model of Fragile X syndrome

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**Abstract:** Attention deficit is one of the most prominent and disabling symptoms in Fragile X Syndrome (FXS). Hypersensitivity to sensory stimuli contributes to attention difficulties by overwhelming and/or distracting affected individuals, which disrupts activities of daily living at

home and learning at school. For example, reading in a café, while ignoring irrelevant sounds or lights, can be very challenging to an individual with FXS. Currently there is a considerable gap in our understanding of the neural mechanisms that contribute to overcoming distractors. To investigate the neural mechanisms of distractor susceptibility, we designed a distractor task and found that, compared to wild-type (WT) mice, *Fmr1* knockout (*Fmr1* KO), a well-established animal model of FXS mice, were unable to tune out distractors. In addition, vasoactive intestinal polypeptide (VIP) interneurons in primary visual cortex (V1) were less modulated by visual stimuli and distractor presentation in *Fmr1* KO mice especially during incorrect responses.Top-down modulation from frontal brain regions influences sensory guided behaviors and drives selective attention and learning (Miller and Cohen, 2001). Prior studies have demonstrated that long-range inputs from one frontal cortical region - anterior cingulate cortex (ACC) - are involved in increasing cortical response to behaviorally relevant information and attenuating responses to extraneous inputs (Zhang et al, 2014; Fiser et al., 2016; Norman et al., 2021). In mice, projections from ACC heavily innervate V1 and influence local visual processing (Zhang et al, 2016). We propose that a dysfunction in afferent inputs from ACC to V1 may contribute to an inability to tune out sensory distractors and selectively attend to behaviorally relevant stimuli in *Fmr1* KO mice. Our in vivo two-photon calcium imaging of ACC axon terminals in V1 indicates that these terminals are more active in early trials of the distractor task than later trials in WT mice. During passive viewing of visual stimuli, our preliminary data shows hypoactivity in ACC ◊ V1 in *Fmr1* KO mice. We hypothesize that these terminals will be less active in *Fmr1* KO mice in the early phase of the distractor task. In addition, we hypothesize that enhancing ACC to V1 inputs using a chemogenetic or optogenetic approach will rescue modulation of cortical VIP cells, visual discrimination performance, and susceptibility to distractors in *Fmr1* KO mice. Identifying disruptions in these long-range inputs to V1 will provide a much-needed mechanistic understanding of distractor susceptibility in a range of neurodevelopmental disorders.

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**Title:** Targeting adenosine $A_{2A}$ receptor in FXS-patient derived human cortical organoids and cortical culture for animal free drug discovery and repositioning

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**Abstract:** Fragile X Syndrome (FXS) is a heritable cognitive impairment caused by expanded CGG repeats in the FMR1 gene, leading to the loss of fragile X mental retardation protein (FMRP). The absence of FMRP results in excessive protein synthesis in dendrites and synapses,

causing hyperactivity, autism, and seizures. Recent research suggests an association between FXS and over-activation of metabotropic glutamate 5 receptor (mGlu5R)-mediated signaling, which is linked to adenosine $A_{2A}$ receptors ($A_{2A}R$). However, specific pharmacotherapies for FXS have not been approved due to limited understanding of its pathophysiology and differences between human and animal models.Our study aims to characterize FXS comprehensively using induced pluripotent stem cells (iPSCs) derived from patients. We successfully generated 3D cortical brain organoids and 2D cortical cultures from iPSCs (>4 batches for each iPSC line), which displayed neurodevelopmental alterations seen in FXS at molecular, cellular and functional level. Specifically, we observed increased neuronal excitability in FXS neurons during network maturation. To explore potential treatments, we investigated the effects of Istradefylline (KW6002), an $A_{2A}$ receptor antagonist used in Parkinson's treatment, in our model systems. KW6002 has shown promising results in improving synaptic and cognitive abnormalities in Fmr1 knockout mice. Using iPSC-derived 2D cultures and 3D organoids, we evaluated the impact of KW6002 treatment at molecular, cellular, and functional levels.Our findings revealed that KW6002 treatment reduced spontaneous network activity, restoring normal neuronal excitability in FXS. Additionally, the treatment influenced gene expression in FXS cultures, effectively restoring the expression levels of differentiation-related genes comparable to the control group. These findings underscore the potential of humanized models and 3D brain organoids in discovering treatments for Fragile X Syndrome and other neurodevelopmental disorders.

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**Topic:** A.07. Developmental Disorders

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**Title:** Fyn kinase inhibition rescues molecular and behavioral phenotypes in a mouse model of Fragile X syndrome

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**Abstract:** Silencing of the Fmr1 gene and loss of its gene product, Fragile X Messenger Ribonucleoprotein (FMRP), results in fragile X syndrome (FXS) characterized by intellectual disability, anxiety, language deficits, hyperactivity, and disrupted sleep. FMRP is a widely expressed RNA-binding protein essential for proper development and maintenance of synapses. We previously showed that glutamatergic synapses encode information from extracellular inputs using highly interconnected and dynamic protein interaction networks (PINs). These PINs undergo widespread reorganization following synaptic activity, thus allowing cells to distinguish between signaling inputs and generate coordinated cellular response. Here, we investigated how FMRP deficiency disrupts signal transduction through the glutamatergic synapse PIN. We used a

novel proteomic technique, quantitative multiplex co-immunoprecipitation, to characterize genotype- and activity-dependent rearrangements in a targeted synaptic PIN in cultured cortical neurons or acute cortical slices from P7, P17 and P60 FMR1$^{-/y}$ and wild-type (WT) littermate controls. We demonstrated that stimulation of discrete glutamate receptors (NMDAR and mGluR1/5) results in a coordinated, input-specific rearrangement of the targeted PIN, and that the basal state of the PIN in FMR1$^{-/y}$ mice suggests tonic mGluR5 hyperactivity. Activity-dependent PIN rearrangements are disrupted in FMR1$^{-/y}$ mice following activation of mGluR5, but not NMDA receptors. In addition, we found that many interactions involving the Src family kinase (SFK) Fyn were already activated in basal state and not changed after mGluR1/5 stimulation. Therefore, we tested whether targeting Fyn in FMR1$^{-/y}$ mice can modify disease phenotypes. We showed that inhibition of hyperactive Fyn signaling by saracatinib (SCB), an FDA-phase-2 SFK inhibitor, normalizes elevated basal protein synthesis in acute cortical slices from P21 FMR1$^{-/y}$ mice. SCB treatment (5mg/kg by oral gavage) beginning after weaning and lasting for 7 days prior to behavioral testing resulted in improved novel object recognition memory and social behavior in FMR1$^{-/y}$ mice. However, SCB treatment did not normalize the PIN to a 'WT-like' state *in vitro* or *in vivo*, but rather modified it by inducing extensive changes in protein complexes containing Shank 3, NMDARs and Fyn. Overall, this study shows that identifying and targeting abnormal nodes of the PIN can suggest potential targets for disease-modifying drugs, but behavioral normalization does not correlate with PIN normalization.

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**Topic:** A.07. Developmental Disorders

**Title:** The Fragile X messenger ribonucleoprotein 1 (FMRP) regulates the proliferation and differentiation of neural precursor cells

**Authors: *O. DIONNE**, S. SABATIÉ, M. AVINO, B. LAURENT, F. CORBIN;
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**Abstract:** Fragile X Syndrome (FXS), the most common hereditary cause of intellectual disability and autism spectrum disorder, is caused by the lack of expression of Fragile X messenger ribonucleoprotein 1 (FMRP). FMRP is a widely expressed RNA-binding protein that plays a key role in the regulation of protein synthesis and the absence of its expression has been linked to abnormal synaptic transmission and improper dendritic spines morphogenesis. However, our current understanding of FXS physiopathology remains limited since most research investigations have been conducted with animal models. To overcome this issue, we studied FXS etiology using induced pluripotent stem cells (iPSCs) derived from FXS patients. We investigated the neurodevelopmental characteristics of the disease by i) evaluating the commitment of iPSCs towards neuronal differentiation in absence of FMRP expression. ii) assessing the neural progenitor cell (NPC) proliferation and differentiation. We showed that FXS-derived NPC exhibited dysregulated cell signaling, protein synthesis, proliferation, and cell fate upon differentiation. Multi-omics analyses of FXS NPC and neurons revealed key molecular insights into how the loss of FMRP expression leads to these phenotypes. We seek to further investigate the potential contribution of these mechanisms to FXS pathophysiology and evaluate their modulation as a potential therapeutic avenue for FXS.

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**Topic:** A.07. Developmental Disorders

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Tetra Therapeutics

**Title:** Peak Alpha Frequency as a Potential Physiological Biomarker for Assessing Cognitive Effects of BPN14770 in Fragile X Syndrome: Insights from a Phase 2 Clinical Trial

**Authors: *J. NORRIS**[1], E. BERRY-KRAVIS[3], L. ETHRIDGE[2];
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**Abstract:** Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by a CGG trinucleotide expansion on the 5' untranslated region of the FMR1 gene and characterized by intellectual disability, anxiety, and difficulties with executive function. A recent phase 2 clinical trial assessing BPN14770, a first-in-class phosphodiesterase 4D inhibitor, in adult males with FXS demonstrated cognitive improvements in domains related to language and caregiver reports of both daily functioning and language. However, a priori secondary physiological measures from electroencephalography (EEG) demonstrated only marginal efficacy. Given the richness of EEG datasets and the significant clinical outcomes, a secondary analysis of the EEG data was conducted to explore potential efficacy in the resting state EEG data. Participants were 30 males (age 18-41 years, M = 31.63, SD = 7.32) with FXS participating in a single-center, phase 2 clinical trial assessing the efficacy and safety of BPN14770. The clinical trial was randomized, double-blinded, placebo-controlled, and utilized a two-period crossover design. Participants (n=19 with complete datasets) completed 3 minutes of resting EEG at baseline, cross-over, and end of trial. EEG variable combinations, which included canonical frequency bands, peak alpha frequency (PAF), and burst metrics, were investigated using a multiclass Naïve Bayes Classifier, a machine learning classification algorithm, with bootstrapped cross-validation, to determine which exploratory variables, if any, differentiated drug from placebo. PAF emerged as a potential biomarker demonstrating BPN14770 efficacy. All PAF variables (frontal, occipital, and whole head PAF) for baseline, drug and placebo showed an area under curve = .64, consistent with carryover effects from the original trial. BPN14770 vs. baseline area under the curve= .76). Drug difference from baseline was significantly different from 0, $t(18) = 3.53$, $p = .001$, $d = .81$), with placebo intermediate, consistent with carry-over. At baseline, whole head PAF was positively correlated with Visual Analog Scales Daily Functioning subscale scores ($r = .49$, $p = .015$), a measure which showed improvements with BPN14770.Given the relationship between PAF and cognitive function among typically developed adults, PAF represents a successful physiological measure of BPN14770 efficacy supporting cognitive shifts noted in the original clinical trial analyses. Improvements in PAF with BPN14770 treatment for those with FXS suggests BPN14770 may enhance processing speed within the alpha range, a frequency band often reported to have low power in individuals with FXS.

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**Presentation Number:** NANO51.08

**Topic:** A.07. Developmental Disorders

**Support:** NARSAD 30044

**Title:** The Effects of Down Syndrome on Cilia Formation and Function

**Authors:** R. STANDER[1], G. J. GALEOTOS[2], ***K. HSIAO**[3];
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**Abstract:** Over one percent of babies born in the United States have Down Syndrome. Individuals with Down syndrome are more likely to have cerebellar hypoplasia than those without the disorder. The exact cause of cerebellar hypoplasia in Down syndrome is not fully understood, but it is thought to be due to defective primary cilia that affect signaling pathways such as calcium and sonic hedgehog (SHH) signaling. Our study examined ciliary deficits in an animal model for Down syndrome, DS-TcMAC. DS-Tc-MAC is a transchromosomic mouse line containing a freely and stably segregating long arm of human chromosome 21 (HSA21q) that can be attributed to its association with Down's syndrome. In cerebellar sections from the DS-TcMAC mice, we found the number of primary cilia was decreased in both the Purkinje cell and granular cell layers. Next, we established and optimized a procedure to isolate fibroblasts from an ear biopsy. We observed that Pericentrin, a protein that plays a critical role in cell division and development, is increased in the DS-TcMAC fibroblast, and promoting ciliation in these cells reduced Pericentrin significantly to normal level. This suggests that reduced primary cilia formation within the cerebellum could lead to the disruption of Pericentrin function, which could contribute to cerebellar hypoplasia associated with Down Syndrome.

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**Title:** Rcan1 rescue of sleep impairments in Down syndrome model mice

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**Abstract:** Approximately 60% of individuals with Down syndrome (DS) have sleep abnormalities independent of breathing obstruction. Previously, we demonstrated increased wakefulness and decreased NREM sleep in the *Dp(16)1Yey/+* DS model mouse (Dp16). In this study, we determined whether increased RCAN1 levels mediate sleep disruption in Dp16 mice. Reviewers blind to mouse genetic make-up characterized sleep architecture and electroencephalogram (EEG) patterns in male and female mice from young (aged 3-4 mos.) and aged Dp16 (aged 9-12 mos.) mice with genetically restored disomic levels (Dp16 *Rcan1*$^{2N}$) of *Rcan1*. ANOVA indicated that young Dp16 and Dp16 *Rcan1*$^{2N}$ mice did not differ in sleep architecture or EEG spectral characteristics from wild-type (WT) mice. However, the sleep deficits manifest in aged Dp16 mice are partially rescued by *Rcan1* dosage correction. Aged Dp16 mice exhibit significantly diminished mean EEG total power across different states and activity phases. In contrast, WT and Dp16 *Rcan1*$^{2N}$ mice show no age-related differences across states and stages. Aged Dp16 mice diverged from aged WT mice across multiple wake and sleep frequency bands during dark and light phases. In contrast, EEG characteristics of aged Dp16 *Rcan1*$^{2N}$ differed only modestly from those of WT mice. We observed some differences between male and female mice in sleep architecture and EEG patterns. Examination of parvalbumin-positive (PV+) neurons in the hippocampus, the reticular nucleus of the thalamus (RT), and the medial septum (MS) of the forebrain revealed significantly fewer PV+ neurons in the hippocampus and RT, and no differences in the MS of Dp16 mice. Restoration of *Rcan1* gene levels significantly affected PV+ neuron numbers in the RT, but not hippocampus, of Dp16 *Rcan1*$^{2N}$ animals. Combined, our data demonstrate that restoring *Rcan1* gene levels mitigates some sleep architecture disruptions and EEG oscillation differences observed in aged Dp16 mice.

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**Topic:** A.07. Developmental Disorders

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**Title:** Overexpression of Down syndrome cell adhesion molecule (DSCAM) contributes to altered neural development in Down syndrome

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**Abstract:** Down syndrome results from the triplication of human chromosome 21 (HSA21) and is the leading cause of intellectual disability. Down syndrome cell adhesion molecule (*DSCAM*) is located on HSA21 and overexpressed in Down syndrome. DSCAM is a receptor for netrin-1, a guidance cue that regulates axonal pathfinding during development. Thus, DSCAM is important for neural wiring in the developing brain. However, much about the contribution of increased expression of DSCAM to intellectual disability, an invariable phenotype of Down syndrome, remains elusive. Using cellular, molecular, and behavioral approaches that employ a *Dscam* gain-of-function mouse model and Down syndrome human induced pluripotent stem cell (hiPSC)-derived cortical neurons, here we investigate how DSCAM overexpression contributes to altered neural development in Down syndrome. Analysis of morphological parameters, including axon length, number of primary branches, total neurite length, soma area, and number of dendrites, revealed impaired morphological development in cultured mouse embryonic hippocampal neurons overexpressing DSCAM compared to their wild-type uterine-mates. Live cell imaging using the Dunn chamber axon guidance assay showed that netrin-1-mediated attractive turning is also lost in these mouse hippocampal neurons overexpressing DSCAM. Down syndrome hiPSC-derived cortical neurons show a similar phenotype: a significant reduction in the length of the longest neurite, number of branches, total neurite length, soma area, and a loss of netrin-1-mediated attractive turning. Knockdown of *DSCAM* in Down syndrome hiPSC-derived neurons rescues some neuronal phenotypes, including axon length, number of primary branches, and total neurite length, and partially rescues deficits in netrin-1-mediated axon guidance. Interestingly, DSCAM overexpression also impairs interhemispheric connectivity, including the hippocampal commissure, in P0 mouse brains. In line with this data, DSCAM overexpression leads to learning deficits in male and female adult mice and reduced anxiety-like behavior in male mice. Taken together, these results suggest that *DSCAM* plays an essential role in the development of neuronal networks, and its overexpression may contribute to intellectual disability in Down syndrome.

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**Topic:** A.07. Developmental Disorders

**Title:** Gray versus white matter: distinct structural brain changes in Williams syndrome

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**Abstract: BACKGROUND:** Williams syndrome (WS) is a rare genetic disorder caused by hemideletion of about 25 genes at chromosomal locus 7q11.23 and is typically characterized by increased social drive and marked difficulty with visuospatial construction. Decreased total brain volume in people with WS compared to typically developing individuals has been reported. To better understand how white and gray matter volumes specifically are altered in WS, we tested for between-group differences in the percentage of total brain volume composed of white and gray matter in children with WS and typically developing children (TDs).
**METHOD:** T1 weighted MRI images were collected on a 3T scanner from 23 individuals with WS (mean age = 10.2±3.6, 17 females) and 20 TDs (mean age = 11.4±3.2, 12 females). Freesurfer7 was used to process the images and provide measures of volume for total brain, cortical gray matter, and cerebral white matter. We then used R's lm to fit three multiple linear regression models analyzing group differences between individuals with WS and TDs, while accounting for age and sex. The first model assessed total brain volume. The other two models assessed group differences in the proportions of cortical gray and white matter volume in relation to total brain volume.
**RESULTS:** Consistent with prior reports, individuals with WS had reduced total brain volume compared to TD children ($p=2.85e-08$). There were distinct alterations in white and gray matter volumes such that, relative to TDs, individuals with WS had a higher percentage of gray matter volume composing total brain size ($p=0.005$) and a lower percentage of white matter volume composing total brain size ($p<0.001$).
**CONCLUSION:** We confirmed findings from previous research showing that individuals with WS have smaller total brain sizes, and further demonstrated that the relative proportion of cortical gray and white matter are differentially affected in WS. Future work will test for relationships between these measures and the behavioral and cognitive profile observed in individuals with WS.

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**Topic:** A.07. Developmental Disorders

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**Title:** Adaptive behavior deficits in 3q29 deletion syndrome

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**Abstract: Background**3q29 deletion syndrome (3q29del) is associated with a significantly increased risk for neurodevelopmental and neuropsychiatric phenotypes. Mild to moderate intellectual disability (ID) is common in this population, and previous work by our team identified substantial deficits in adaptive behavior. However, the full profile of adaptive function in 3q29del has not been described, nor has it been compared to other genomic syndromes associated with elevated risk for neurodevelopmental and neuropsychiatric phenotypes.**Methods**Individuals with 3q29del (n=32, 62.5% male) were evaluated using the Vineland Adaptive Behavior Scales, Third Edition, Comprehensive Parent/Caregiver Form (Vineland-3). We explored the relationship between adaptive behavior and cognitive function, executive function, and neurodevelopmental and neuropsychiatric comorbidities in our 3q29del study sample, and we compared subjects with 3q29del to published data on Fragile X syndrome, 22q11.2 deletion syndrome, and 16p11.2 deletion and duplication syndromes.**Results**Individuals with 3q29del had global deficits in adaptive behavior that were not driven by specific weaknesses in any given domain. Individual neurodevelopmental and neuropsychiatric diagnoses had a small effect on adaptive behavior, and the cumulative number of comorbid diagnoses was significantly negatively associated with Vineland-3 performance. Both cognitive ability and executive function were significantly associated with adaptive behavior, and executive function was a better predictor of Vineland-3 performance than cognitive ability.
Finally, the severity of adaptive behavior deficits in 3q29del was distinct from previously published data on comparable genomic disorders.**Conclusions**Individuals with 3q29del have significant deficits in adaptive behavior, affecting all domains assessed by the Vineland-3. Executive function is a better predictor of adaptive behavior than cognitive ability in this population and suggests that interventions targeting executive function may be an effective therapeutic strategy.

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**Nanosymposium**

**NANO52: Microglia, Oxidative Stress, and Blood-Brain Barrier**

**Location:** WCC 147A

**Time:** Tuesday, November 14, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO52.01

**Topic:** B.09. Glial Mechanisms

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**Title:** The Role of Perivascular Macrophages and Microglia in the Maintenance of Resting Basal Cerebrovascular Tone

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**Abstract:** Microglia are yolk-sac derived CNS resident macrophages long recognized for their roles in synaptic pruning, clearance of neurotoxic substances- such as amyloid-β in Alzheimer's Disease- and cerebrovascular development. Despite knowledge of the latter, their role in the regulation of cerebral blood flow (CBF) has long been overlooked. Using laser-speckle contrast imaging, we recently demonstrated that myeloid cell depletion using PLX3397 resulted in impaired vessel dilation upon hypercapnic challenge. Given that 1) laser-speckle contrast imaging only measures blood flow in cerebral vessels just below the pial surface, 2) the cerebral myeloid cell population includes border-associated and perivascular macrophages (BAMs/PVMs) recently shown to also modulate CBF and 3) our new data showing PLX3397 depletion of BAMs, PVMs and microglia- the definitive role of microglia in CBF regulation at all blood vessel types needs further clarification. Using two-photon imaging through a cranial window, our data shows a significant reduction in capillary diameter at four days post PLX3397 ablation, despite not all post-ablation measurements being vasoconstrictions. This was not the case for arterioles or venules. An analysis of PVM distribution confirms that PVMs largely reside at arterioles and venules, thus suggesting that this capillary phenotype is indeed due to loss of microglia. Interestingly, preliminary data further suggests that this phenotype is only specific to males but not females, thus potentially showing a sex-specific role of microglia regulation in the maintenance of capillary basal tone. To definitely determine the role of PVMs in cerebral blood flow regulation, future studies will utilize clodronate liposomes to specifically deplete border-associated and perivascular macrophages, with vascular volume and diameter measured via two-photon imaging through a cranial window. Together, these results will shed light on the specific role of PVMs versus microglia in the regulation of resting basal cerebrovascular tone at all levels of the vascular tree.

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**Title:** Region specific regulation of microglia phagocytic states by neuronal Sirpα

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**Abstract:** Microglia utilize their phagocytic activity to prune redundant synapses and refine neural circuits during precise developmental periods and in diverse neurological diseeases. However, the neuronal signals that control this phagocytic clockwork remain largely undefined. We have shown that neuronal signal regulatory protein alpha (SIRPα) is a permissive cue for microglial phagocytosis in the developing murine retina and in retinorecipient targets in the brain. Removal of neuronal, but not microglial, SIRPα reduced microglial phagocytosis, increased synaptic number, and impaired circuit function. Conversely, prolonging neuronal SIRPα expression in retina extended developmental microglial phagocytosis. These outcomes depended on the interaction of presynaptic SIRPα with post-synaptic CD47. Global CD47 deficiency modestly increased microglial phagocytosis, while CD47 overexpression in retina reduced it. This effect was rescued by co-expression of neuronal SIRPα or co-deletion of neuronal SIRPα and CD47. Using RAIN-STORM nanoscopic imaging, we further show that Sirpα levels vary at single synapses to instruct engulfment outcomes in the retina and in retinorecipient areas of the brain. These data indicate that neuronal SIRPα regulated microglial phagocytosis by limiting access of microglial SIRPα to neuronal CD47. This discovery may aid our understanding of synapse loss in neurological diseases.

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**Title:** Microglial P2Y12 signaling modulates microglial morphology and injury response in a region specific manner

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**Abstract:** Synaptic plasticity allows the central nervous system (CNS) to incorporate new sensory experiences and information, and its disruption is associated with many neurological and psychiatric disorders. Much recent work has focused on the contribution of non-neuronal CNS cells, especially microglia, to synaptic plasticity. Though classically thought of in their immune capacities, microglia are vital to many homeostatic processes, including synaptic plasticity of

nascent and adult neuronal networks. Despite the emerging consensus that microglial dynamics are critical to brain function during physiological as well as pathological conditions, it is unclear whether these microglial roles and their underlying mechanisms are universal or differ between brain regions. There is a growing body evidence to suggest microglia exhibit a high degree of regional specialization; existing on a continuum from homeostatic (cortex) to immune vigilant (cerebellum) even in the absence of pathological stimuli. Indeed, cerebellar microglia exhibit unique transcriptional and epigenetic profiles, and distinct functional properties, such as being more phagocytic, morphologically less ramified and less densely distributed. As a consequence, cerebellar microglia survey less of parenchyma than cortical microglia, but compensate for this by undergoing frequent somatic translocations under homeostatic conditions, a phenomenon not observed in cortex. Despite such differences, cerebellar microglia maintain common microglial functions. One molecular pathway of great interest to cortical microglial mediated synaptic plasticity is purinergic signaling through the P2Y12 receptor, which has been shown to be critically involved in microglial roles in synaptic remodeling and rapid chemotaxis to sites of injury. We used a combination of histology and time-lapse *in vivo* imaging, to visualize cerebellar microglial surveillance, and injury response while microglial P2Y12 signaling was manipulated. We report an increase in microglial ramification in P2Y12 deficient animals, unlike what has been reported in cortical microglia. We found that P2Y12 deficiency did not alter the response of cerebellar microglia to focal injury, again differing from what has been reported in cortical microglia. We further investigated alternative, P2Y12 independent, damage signaling pathways in cerebellar microglia. These findings indicate a cerebellum specific role for P2Y12 signaling in governing cerebellar microglial dynamic responses in both homeostatic and pathological conditions.

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**Topic:** B.09. Glial Mechanisms

**Support:** K08NS114170

**Title:** Satellite microglia: loss of neuronal regulation after traumatic brain injury and role of P2Y12receptors

**Authors:** A. FEICHTENBINER, K. SYTSMA, R. O'BOYLE, C. RANSOM, ***A. NOLAN**; Univ. of Washington, Seattle, WA

**Abstract:** Microglia, the primary mediators of innate immune activation in the brain, are increasingly recognized as key modulators of neuronal activity. Prolonged activation of the innate immune system can impede repair in TBI, and it is not understood how microglia’s impact on neuronal activity might contribute or protect. One microglial subtype that may be critical in the regulation of neuronal excitability is the perineuronal satellite microglia (Sat-MG). These microglia are juxtaposed adjacent to neurons with their soma and processes entwined around the neuronal cell body. To understand how these microglia modify neuronal excitability and change after injury, we utilized patch clamp recordings, immunohistochemistry, and confocal imaging. We found an increase in the numbers of Sat-MG in the orbitofrontal cortex in both a murine

model of TBI that is associated with network hyperexcitability and deficits in reversal learning moths after TBI, as well as human tissues from donors with a history of chronic TBI, compared to controls. Our data, utilizing whole cell recordings in transgenic mice with GFP-labeled microglia (Tmem119-EGFP), also indicate that Sat-MG suppress neuronal excitability, in control mice, but lose this ability in chronic TBI with an associated decrease in expression of P2Y12 receptors, which appears more selective for the satellite microglial subtype. Furthermore, preliminary data suggests acute treatment with a P2Y12R antagonist could reverse the Sat-MG associated reduction in excitability and increase network excitability, supporting our hypothesis that reduction of P2Y12Rs may contribute to loss of neuronal regulation and cognitive dysfunction after TBI.

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**Title:** Microglial impairment as a novel basis for hypothalamic dysfunction in Prader-Willi Syndrome

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**Abstract:** Prader-Willi Syndrome (PWS) is a rare neurodevelopmental disease caused by a deletion at the paternal copy of chromosome 15q-q13, of which two variants are known: type I (PWS T1) deletion lacks an extrachromosomal segment compared to type II (PWS T2). PWS symptoms are of hypothalamic etiology, with PWS T1 subjects showing more severe phenotypic traits (i.e., cognitive impairment), yet the neurobiological underpinning to this is unknown. We performed RNA sequencing of hypothalamus from individuals with PWS T1 and PWS T2 deletions and characterized glia and neurons of the hypothalamus and hippocampus in the postmortem human brain tissues of PWS T1 and T2 deletions individuals. We identified three T1 deletion and seven T2 deletion individuals and compared them to matched controls (n=32). We found predominantly downregulated genes in PWS T1 in comparison to TWS T2, including genes that are involved in immunity, cytoskeleton structure and synaptic function. Strikingly, microglia in PWS T1 displayed marked cytoplasmic fragmentation with an enlarged microglial phagocytic compartment, but decreased expression of lysosomal protease, indicating defective phagolysosome activity. These dysmorphic microglia in PWS T1 are associated with disruption

of fornix integrity and reduction of hypothalamic synaptophysin expression. By contrast, we found increased hypothalamic microglial numbers in T2 compared to the controls, and unaltered white matter microstructure and synaptophysin expression. Whereas the reduction of hypothalamic neuropeptidergic neurons was observed in both PWS T1 and T2. We conclude that PWS T1 deletion-induced microglial morpho-functional impairment leads to disturbed neuronal communication, which may underlie the greater phenotypic severity of these individuals compared to PWS T2.

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**Title:** Quantitative and spatially resolved functional analysis of microtubule dynamics and orientation as activation markers in primary microglia

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**Abstract:** Microtubules are dynamic cytoskeletal components essential for diverse cellular processes. Understanding the regulation and functional implications of microtubule dynamics is crucial for elucidating fundamental cellular mechanisms. However, functional live imaging of microtubules in primary microglia can be challenging, given their intrinsic resistance to manipulation. Microglia, the brain's innate immune cells, are vital for preserving a healthy brain

environment. Under homeostatic conditions, microglia have a branched shape and continuously monitor their environment. However, if triggered by inflammation or injury, microglia undergo notable alterations in gene expression and morphology usually referred to as “microglia activation”. Due to their innate responsiveness to external activating stimuli, we here explore optimized culturing conditions and imaging techniques to minimize activation *in vitro*. Simultaneously, we demonstrate how this approach enables the analysis of microglia microtubules, selecting dynamics and orientation parameters to be used as activation markers in both fixed and live primary microglia cells. We describe how to isolate cortical primary microglia from C57BL6/J pups (postnatal day 0-2), preparing suitable imaging conditions, and employing fluorescent microscopy techniques to visualize and analyze microtubule behavior. Our findings demonstrate that primary microglia are refractive to lentiviral delivery of typical microtubule-end fluorescent markers (<1% of EB3-EGFP$^{+}$ cells), particularly when challenged with LPS-IFNγ to steer microglia toward a classically activated reactivity state (N=3). Therefore, we present alternative strategies for i) tracking the length changes of microtubules in live imaging experiments using SiR-tubulin labeling, to quantify dynamics parameters (N=4) and ii) determining microtubule orientation by measuring the fluorescence intensity gradient of plus-end binding proteins (EB1) signal in relation to the cell center in fixed samples. Both these analysis approaches show consistent differences between microglia challenged either with LPS-IFNγ or IL-4 to recapitulate activated and alternatively activated reactive states, respectively. Given the important contribution of microglia in neurodegenerative and neurodevelopmental disorders, this study offers valuable insights into the intricate mechanisms underlying microtubule dynamics in primary microglia, facilitating a better understanding of their functional roles and providing valuable biomarkers to be translated into clinical research.

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**Topic:** C.01. Brain Wellness and Aging

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**Title:** Long-lived mitochondrial proteins in mammalian brains - what are they and why do they exist?

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**Abstract:** Continuous replenishment of mitochondria is essential for maintaining high-quality organelles throughout the lifespan of post-mitotic neurons. Age-related impairments in mitochondrial renewal contribute to progressive neuronal. Accordingly, average half-life of mitochondrial proteins is estimated at less than 3 weeks. However, a discrete subset of the mitochondrial proteome persists for at least 4 months in mammalian brains. These mitochondrial

long-lived proteins, or mt-LLPs are enriched at cristae invaginations and include OxPhos, MICOS, mt-DNA proteins, chaperones, and cytochrome C. Cross-linking MS-based quantitative analysis revealed spatial restriction of mt-LLPS and co-preservation within same cristae. Since cristae stability is intimately coupled to mitochondrial function, I propose that mt-LLPs could play a previously unrecognized role in the long-term stabilization of mitochondrial ultrastructure, which in turn might be imperative for mitochondrial fitness. My ongoing research efforts are centered on (1) the investigation of molecular quality-control mechanisms governing the exceptional persistence of mt-LLPs, (2) the spatio-temporal distribution of mt-LLP within neuronal mitochondrial networks, and (3) their persistence in the context of cristae stability and remodeling. Insights from these experiments will provide with a new understanding of the role of mt-LLPs in neurons, and could lead to novel targets for potential therapeutic interventions for neurological disorders.

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**Topic:** C.01. Brain Wellness and Aging

**Title:** Schizophrenia in the context of 22q11.2 deletion syndrome is associated with lysosomal and mitophagy dysfunction in iPSCs-derived neurons.

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**Abstract:** Energy metabolism is essential to many aspects of neuronal development and function. Limited bioenergetics may affect the developing brain and alter critical processes leading to neurodevelopmental diseases. Among them, schizophrenia (Sz) is a chronic and severe neuropsychiatric disorder that appears in late adolescence. The 22q11.2 deletion syndrome (22q11DS) is a relatively common microdeletion with a wide spectrum of clinical manifestations, and it is the strongest known genetic risk factor for Sz. Indeed, 25-30% of 22q11.2 carriers develop Sz. Despite considerable research, mainly in mouse models, the mechanism by which the haploinsufficiency for the 45 protein-encoding genes whitin the deleted region causes Sz remains unclear. We focused on mitochondrial dysfunction, since at least 6 of the deleted genes encode for mitochondrial-localizing proteins. Our team reported decreased activity of the mitochondrial oxidative phosphorylation complexes in iPSC-derived neurons (iNs) from 22q11DS patients diagnosed with Sz (22q+Sz) compared to healthy control, and to iNs from 22q11DS patients without psychosis (22q-Sz). Remarkably, iNs from the 22q-Sz group showed increased expression of genes associated to mitochondrial biogenesis (qPCR) and turnover (RNAseq), suggesting that, in 22q11DS, Sz risk may be associated with a decreased turnover of defective mitochondria. Focusing on mitophagy, the quantification of the lysosomal marker LC3b (fluorescence microscopy) and a fluorogenic proteases' substrate internalized by the organelle, showed a reduced lysosomal activity in 22q+Sz iNs compared to both healthy controls and 22q-Sz. Furthermore, live imaging of iNs show that the compromised lysosomal activity depended on intraluminal lysosomal alkalinization, which may be linked to an imbalance between mitochondrial ATP production and sustaining the V-ATPase activity. Additionally, a

reduced mitochondria-lysosome interaction was observed in iNs from 22q+Sz compared to 22q-Sz and healthy controls, reinforcing the hypothesis that mitophagy failure, due to lysosomal dysfunction, is an important contribution to the development of energy failure psychosis in the 22q11DS background. Since compounds aimed to enhance and restore lysosomal-mediated mitophagy are available, we suggest that lysosomes could represent a potential pharmacological target to treat, or eventually prevent, psychosis in 22q11DS patients.

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**Presentation Number:** NANO52.09

**Topic:** C.01. Brain Wellness and Aging

**Support:** R35 GM127122

**Title:** Nocturnin's role in oxidative stress mediated neurodegeneration

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**Abstract:** Oxidative stress, an imbalance of reactive oxygen species (ROS) and antioxidants, is a central focus in neurodegenerative disorders such as Parkinson's Disease (PD) due to its strong correlation with neuronal cell death. Our lab has identified Nocturnin, an NADPH phosphatase, as a putative regulator of oxidative stress. NADPH is a powerful reductant for regenerating antioxidants, such as glutathione (GSH), to lower ROS. Overexpression of Nocturnin in HEK293 cells results in significant decreases in cellular viability following ROS challenge. In addition, Nocturnin is increased in Parkinson's patients and increases dyskinesia in L-DOPA treated patients. The extent of Nocturnin's mechanistic role in disrupting oxidative homeostasis and causing cell death, and whether this plays a role in development of PD is not yet known. I hypothesize that Nocturnin has a role in neurodegeneration in PD by lowering NADPH levels which exacerbates oxidative stress conditions and therefore a loss of Nocturnin will be protective. To characterize how changes in Nocturnin expression affects cellular health, I measured markers of oxidative stress such as viability, GSH, and ROS levels in WT and Nocturnin knockdown (KD) CAD neurons in basal and oxidative stress conditions. Cell viability and GSH levels are increased in Nocturnin KD neurons while ROS levels are significantly decreased in oxidative stress conditions. Additionally in ongoing work, we have found that loss of Nocturnin in a Parkinsonian mouse model with an overexpression of mutant alpha-synuclein results in increased numbers of dopaminergic neurons. Together these data support the hypothesis that a loss of Nocturnin protects these cells from cellular stress, through increases in antioxidant levels, which may rescue neurodegeneration *in vivo.*

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**Topic:** C.01. Brain Wellness and Aging

**Support:** NIH Grant R00 AG054748
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American Parkinson's Disease Association Research Grant

**Title:** Causes and effects of somatic mutation accumulation in the aging human brain

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**Abstract:** Somatic mutations accumulate in cells of the human body during aging because the genome is under constant assault from endogenous and environmental mutagens. Somatic mutations are dangerous because they represent permanent changes to the genetic code. Thus, each time a somatic mutation occurs, the probability of critical gene regulatory networks being perturbed increases. Somatic mutations are particularly hazardous to the human brain, where most post-mitotic neurons cannot be replaced during life. Our previous work showed that gene expression levels and somatic mutation rates were linked, with highly expressed genes showing higher somatic mutation rates than repressed genes. To explore the relationship between single-cell gene expression and somatic mutation rates, we applied single-nucleus RNA sequencing (snRNA-seq) and single-cell whole-genome sequencing (scWGS) to a cohort of 13 neurotypical postmortem donors ranging in age from infancy to over 100 years old. We analyzed over 290,000 single nuclei by snRNA-seq and 59 neurons by scWGS. We quantified changes in the expression of core metabolic genes and cell identity genes during aging across several brain cell types during aging, defined changes in DNA repair gene expression that correlate with specific patterns of somatic mutation, and identified relationships between gene length, somatic mutation burden, and expression changes during brain aging. Thus, a two-way relationship between gene transcription and somatic mutation shapes the molecular landscape of the human brain during aging.

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**Presentation Number:** NANO52.11

**Topic:** F.05. Brain Blood Flow, Metabolism, and Homeostasis

**Title:** NX210c drug candidate peptide strengthens the blood-brain barrier

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**Abstract:** Most of CNS diseases or injuries imply blood-brain barrier (BBB) alterations that contribute to disease progression and functional impairments. Here, we screened the effect of a subcommissural organ-spondin-derived peptide (NX210c), known to promote recovery in several neurological disorders, on BBB integrity in several cellular models. To do so, NX210c effects were tested on mouse endothelial cell (EC) monolayers using bEnd.3 cell line and on two

triculture human models containing EC, astrocytes and pericytes, under static (transwell) or 3D dynamic (on-chip with shear stress) conditions. EC were treated with NX210c at 1 μM, 10 μM or 100 μM, or its vehicle (cell culture water) for up to 72h. As read-outs, tight junction protein levels (WB or ICC), transendothelial electrical resistance (TEER), and/or permeability to FITC-dextran were evaluated. In mouse EC monolayers, NX210c induced a transient increase in occludin levels after 24h of treatment (+ 37% at 100 μM, $p=0.0112$, $n=8$). Claudin-5 levels were increased after 24h with NX210c (+43% at 100 μM, $p=0.0002$, $n=10$), and this effect was maintained for at least 72h (+19% at 100 μM, $p=0.0416$, $n=7$). Accordingly, NX210c decreased by half the permeability of EC monolayers to a 40 kDa-FITC-Dextran after 24h and 72h of treatment ($p<0.05$, $n=3$). In addition, TEER was increased in presence of NX210c from 24h post-exposure and up to 72h (+31% at 100 μM, $p<0.0001$, $n=18$). In a human static BBB model, NX210c increased by 30% the TEER at 100 μM from 72h post-exposure and up to 5 days ($p<0.01$, $n=6$). NX210c also increased TEER in a more complex human 3D dynamic BBB model at 100 μM after only 4h of treatment ($p<0.05$, $n=4$), which was associated with a reduced permeability of a 4 kDa-FITC-dextran ($p<0.05$, $n=4$). We are currently deciphering the mechanism of action behind the modulatory effect of NX210c on claudin-5 and occludin and the subsequent strengthening of the BBB. Through this property, NX210c may represent a breakthrough treatment for neurological diseases. Finally, a clinical phase 1b evaluating the safety, tolerability and pharmacodynamic effects (including BBB integrity) of multiple ascending doses of NX210c in elderly healthy volunteers is ongoing.

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**Presentation Number:** NANO52.12

**Topic:** F.05. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NMSS Grand RG-1507-04951

**Title:** Exploring the Relationship between Meningeal Lymphatic Vessels, Glymphatic Function, and Cognition: A Multi-Modal Neuroimaging and Neuropsychological Study in Humans

**Authors: D. SIVAKOLUNDU**[1], M. ZUPPICHINI[3], K. WEST[4], S. VENKATARAMAN[6], K. SINGH[2], J. SPENCE[4], A. MAHAJAN[2], S. GAUTHIER[7], T. NGUYEN[7], *B. RYPMA[5]; [1]Neurol., [2]Radiology, Yale Univ., New Haven, CT; [3]Psychology, Univ. of Michigan, Ann Arbor, MI; [4]Ctr. for BrainHealth, [5]Univ. of Texas At Dallas, Dallas, TX; [6]Fac. of Med. and Hlth. Sci., McGill Univ., Montreal, QC, Canada; [7]Weill Cornell Univ., New York, NY

**Abstract:** The glymphatic system and meningeal lymphatic vessels (mLV) play crucial roles in clearing waste from the brain and draining cerebrospinal fluid (CSF). The relationship between the glymphatic system and mLVs is not fully understood. Previous research has linked glymphatic system changes to various neurological disorders and systemic illnesses that feature cognitive impairment, but the mechanisms underlying such impairment remain unclear. Rodent studies suggest that impaired mLV function is associated with cognitive impairment, yet no studies have explored this relationship in humans. The present study aims to investigate phenotypic changes in mLVs, age and sex-related variations in CSF dynamics, relationships between CSF production, mLV characteristics, and glymphatic function, and the impact of brain

lymphatic function on processing speed and cognitive abilities. This study involved 27 healthy controls recruited from the Dallas-Fort Worth area. Various MRI scans were performed to acquire data, including $T_2$-FLAIR, $T_1$-MPRAGE, SWI, and Diffusion Tensor Imaging (DTI). mLVs along the sagittal sinus were visualized, segmented, and characterized. The glymphatic system was evaluated by measuring diffusivity along the perivascular space (ie, using DTI-ALPS). Choroid plexus volumes and brain volumes were estimated. Neuropsychological tests were conducted to assess cognitive domains. As mLV volume increased, thickness ($p<0.001$) and surface area ($p<0.001$) also increased, showing greater variability at larger volumes. Glymphatic function decreased with age, $r_s(24)=-0.62$, $p<0.001$, while mLV ($r_s(24)=0.37$, $p=0.06$) and choroid plexus volumes ($r_s(26)=0.55$, $p=0.003$) increased. Males had higher mLV volume than females ($M_{male}=18.1cm^3$, $M_{female}=18.1cm^3$, $W=111$, $p=0.03$), but no sex differences were observed in glymphatic function, and choroid plexus volume. We found that mLV volume increased as glymphatic function decreased (ß=-0.60, $p<0.001$), independent of age, and a lymphatic latent variable predicted processing speed (ß=-0.27, $p=0.04$), indicating that brain lymphatics play an important role in determining processing speed in humans. Our findings suggest that, with increasing age, glymphatic function declines, which in turn leads to increases in mLV volume. We also observed increased variability in the phenotypic characteristics of mLVs as their volume increased, indicating dynamic structural changes in response to declining glymphatic clearance. These changes in the brain lymphatic system contribute to the variability in processing speed seen among healthy adults that may have implications for higher-order cognition.

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**Nanosymposium**

**NANO53: TDP-43, Tau, and Synuclein Proteinopathies: Mechanisms and Therapeutics**

**Location:** WCC 207A

**Time:** Tuesday, November 14, 2023, 8:00 AM - 11:30 AM

**Presentation Number:** NANO53.01

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

**Support:** SERB-CRG grant

**Title:** The interplay of charge-neutralizing Post-translational Modifications modulates the aggregation behavior of amyloidogenic proteins in Neurodegenerative Diseases

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**Abstract:** The “protein aggregation” continues to be the dominant hypothesis for Neurodegenerative diseases (NDs) such as Alzheimer’s disease (AD) and Parkinson’s disease

(PD). However, identifying the specific molecular species or event that could trigger aggregation in vivo continues to be challenging. The absence of a bonafide pathological molecular species against which aggregation inhibitors could be developed is problematic, as the non-specific targeting leads to the clearance or inhibition of the natural function of the physiological forms of such proteins. The spurt of data from cryo-EM structures of fibrils and proteomics studies on soluble and insoluble fractions from patients have indicated that different sets and frequencies of PTMs are present in a disease-specific manner. Thus we need to urgently decode the role of PTMs in a site-specific manner individually as well as combinatorially. We have previously identified carbamylation as one of the PTMs that can drastically alter the aggregation propensity locally and globally in tau and α-synuclein. We have extended the studies to TDP-43 and N-terminal huntingtin, with similar outcomes. We have additionally utilized differentially modified small peptides (6-30 residues) derived from aggregation-prone as well as lysine/serine/threonine-rich regions of amyloidogenic proteins, to understand the combinatorial effect of charge-neutralizing PTMs, namely acetylation, carbamylation, and phosphorylation (pseudo charge neutralization). We have demonstrated that while charge-neutralizing PTMs (acetylation and carbamylation) generally enhance the aggregation propensity and can cause aggregation of unsuspecting sequences (carbamylation being a more potent factor than acetylation), there appears to be a pattern but no universal rule. Also, the length of the model peptide appears to significantly impact the overall outcome, with shorter sequences typically being more amyloidogenic upon modification but longer ones playing a protective role. In general, we have noticed a reverse effect with phosphorylated sequences as it is only pseudo charge neutralization working globally. We have also established that, if not all, most of the model peptides exhibit seeding and recruitment of unmodified sequences. These observations have further been substantiated with MD simulations. These findings hint at a very prominent role of PTMs in a site-specific and combinatorial manner. We are additionally attempting to create full-length proteins with differentially decorated PTMs. We hope to identify a few combinations that could recruit other sequences and act as the trigger for amyloidogenesis.

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**Presentation Number:** NANO53.02

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

**Support:** Research Foundation—Flanders (FWO)
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NIH

**Title:** Increased levels of TMEM106B leads to severe lysosomal dysfunction in a novel TMEM106B overexpression mouse model

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**Abstract:** Genetic variation in Transmembrane protein 106B (TMEM106B) is known to influence the risk and presentation in several neurodegenerative diseases and modifies healthy aging. Yet, the precise function of TMEM106B is undetermined and it remains unclear how TMEM106B modulates disease risk. Current evidence suggests that the risk allele is associated with higher levels of TMEM106B. As a lysosomal transmembrane protein, TMEM106B regulates several aspects of lysosomal function and proper TMEM106B levels are crucial for lysosomal health.
To study the effect of increased TMEM106B levels, we generated Cre-inducible transgenic mice expressing human wild-type TMEM106B (TMEM106B) under the control of the CAG promoter. To generate mice which express TMEM106B in all epiblast-derived tissues, the mice were crossed with mice expressing Cre recombinase under the Meox2 promoter (JacksonLab). We show that our model stably expresses the transgene resulting in increased TMEM106B RNA and protein levels, unlike a previous model that failed to induce overexpression due to the tight regulation of Tmem106b levels. Detailed characterization of this model at multiple time points is currently ongoing.
We show that embryonic fibroblasts derived from this model are filled with vacuole-like enlarged lysosomes, which are not acidic, and show increased levels of Lamp1 and Progranulin. This indicates that the increase in TMEM106B levels leads to severe lysosomal dysfunction. Preliminary results show a suggestive reduction in neuronal markers, which may indicate that increased levels of TMEM106B induces neuronal loss. Furthermore, bulk RNA sequencing on brain tissue from 15month old animals shows the downregulation of several genes associated with neuronal plasticity, learning, and memory. Follow-up of this data and behavioral testing of this model is currently ongoing.
In conclusion, we created a novel TMEM106B overexpression model and show that overexpression of TMEM106B induces severe lysosomal dysfunction which may contribute to neurodegeneration *in vivo*. This model holds the potential to identify key pathways that are misregulated which may underly the disease-modifying effect and supports the hypothesis that TMEM106B modulates the resilience or vulnerability of the brain to neurodegeneration and aging.

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**Presentation Number:** NANO53.03

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

**Support:** NIH Grant NS128031

**Title:** Progranulin insufficiency and TDP-43 overexpression interact to worsen phenotypes in a mouse model of Frontotemporal Dementia

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HERSKOWITZ, A. ARRANT;
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**Abstract:** Heterozygous, loss-of-function mutations in progranulin (*GRN*), most of which result in progranulin haploinsufficiency, are a major cause of Frontotemporal Dementia (FTD). FTD-*GRN* patients commonly present with behavioral changes such as social withdrawal, apathy, disinhibition, and compulsivity. These patients also develop TDP-43 pathology, characterized by nuclear clearing of TDP-43, TDP-43 mislocalization to the cytoplasm, and formation of TDP-43 inclusions. *Grn*$^{+/-}$ mice are a genetic model of FTD-*GRN* and model the behavioral symptoms of FTD, exhibiting age dependent deficits in social dominance, fear conditioning, and sociability. However, *Grn*$^{+/-}$ mice do not develop TDP-43 pathology. *Grn*$^{-/-}$ mice develop some TDP-43 inclusions at advanced ages, but model the complete loss of progranulin that causes Neuronal Ceroid Lipofuscinosis. To better model FTD-*GRN*, we crossed *Grn*$^{+/-}$ mice with a human TDP-43 transgenic mouse line (TAR4 line). Homozygous human TDP-43 transgenic mice (hTDP++) develop TDP-43 mislocalization and inclusions, resulting in severe motor deficits that are fatal by 30 days of age. Hemizygous human TDP-43 transgenic mice (hTDP+) have a mild phenotype and normal lifespan, developing subtle motor deficits and gliosis by 12 months of age. To determine if progranulin insufficiency exacerbates development of TDP-43 pathology, we crossed *Grn*$^{+/-}$ mice with hTDP++ mice. We found that the *Grn*$^{+/-}$:hTDP++ mice had significantly more CD68 and GFAP immunoreactivity than *Grn*$^{+/+}$:hTDP++ mice. Ongoing studies are investigating if TDP-43 mislocalization and aggregation is also worse in the *Grn*$^{+/-}$:hTDP++ mice. Behavioral changes are a key feature of FTD-*GRN*, so we investigated behavior in the *Grn*:hTDP mice. Due to the early mortality of hTDP++ mice, we utilized hTDP+ mice to generate the cross. *Grn*$^{+/-}$:hTDP+ mice had more severe social dominance deficits that either *Grn*$^{+/-}$:hTDP- mice or *Grn*$^{+/+}$:hTDP+ mice in the tube test, an assay dependent on the medial prefrontal cortex (mPFC). This low social dominance phenotype occurred without worsened TDP-43 pathology or signs of lysosomal dysfunction and inflammation. To elucidate the mechanisms of behavior abnormalities, we utilized mRNA sequencing of the mPFC and analyzed dendritic branching and dendritic spine morphology of mPFC neurons.

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**Presentation Number:** NANO53.04

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

**Support:** National Institute of Neurological Disorders and Stroke (NS085770)
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**Title:** Exosomes isolated from human cortex with TDP-43 proteinopathy, TDP-43 transgenic mice and media from cultured human microglia contain human TDP-43

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**Abstract:** Exosomes are extracellular vesicles produced in the endosomal compartment and released by most eukaryotic cells. They are the smallest extracellular vesicle and have been implicated in cell-to-cell communication under normal conditions and propagation of pathology in neurodegenerative disorders. In particular, microglia-derived exosomes have been implicated in pathologic tau propagation in Alzheimer's disease. It is not known whether exosomes, including those derived from microglia, contribute to propagation of pathology in other neurodegenerative disorders. As a first step in this process, we isolated and characterized exosomes from various sources, with the eventual aim of investigating their involvement in propagation of TDP-43 proteinopathy in frontotemporal lobar degeneration (FTLD-TDP) Frozen postmortem human tissue from the middle frontal gyrus of four participants with FTLD-TDP and one cognitively-normal control, frozen fontal cortex from two wild-type and three human TDP-43 transgenic C57/Bl6 mice, and pooled media from primary human microglia cultures with exosome depleted serum in media were used and exosomes were prepared employing established high speed ultracentrifugation and fractionation protocols. Western blot analyses were used to determine the presence of exosome markers flotillin-1 and CD63, microglia marker CD68, and human TDP-43 in isolated exosomes. Successful isolation of exosomes was confirmed through electron microscopy. All isolated exosome preparations contained the exosome markers flotillin-1 and CD63. Exosomes isolated from cultured human microglia and human brain contained CD68. Human TDP-43 was present in exosomes isolated from frozen human brain with FTLD pathology, human microglia cultures, and TDP-34 transgenic mice. Weak bands were detected in wild-type mice, most likely due to partial homology of TDP-34 protein in the mouse and human. In conclusion, exosomes can be successfully isolated from various sources of brain tissue and cells, a proportion originating from microglia, and they contain human TDP-43. It remains to be seen whether exosomes isolated from FTLD-TDP or TDP-34 transgenic mice can seed or propagate pathology when injected into wild-type or transgenic mice.

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**Presentation Number:** NANO53.05

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Support:** 21H00446 from Ministry of Education, Culture, Sports, Science, and Technology, Japan

**Title:** Utility of a newly establishes tauopathy mouse model using Rosa26 knock-in stratagy

**Authors: *N. SAHARA**[1,2], R. YANAI[2], M. SHIMOJO[2], T. MINAMIHISAMATSU[2], M. HIGUCHI[2];
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**Abstract:** Animal modeling plays a critical role in elucidating the causal mechanisms of neurodegeneration and facilitating the development of therapeutic interventions for

neurodegenerative disorders. Tauopathy stands as a prominent pathological characteristic of neurodegenerative diseases. To induce tauopathy and observe disease-related phenotypes, previous studies have employed transgenic models that overexpress mutated human tau, which is associated with several mutations in the tau gene identified in FTDP-17. Here, we employed the knock-in (KI) strategy to insert the P301L mutated 1N4R human tau cDNA, along with the tetracycline response element (TRE) promoter, into the Rosa26 safe harbor gene. By crossing the KI mouse with CaMKII-tetracycline transactivator (tTA) transgenic mice under C57BL/6J background, we generated a mouse model that expresses human tau. The homozygous Rosa26-KI tau expressing (hereafter referred to as rTKhomo) exhibited age-dependent tau accumulation in a regional-specific manner. Utilizing CUBIC tissue cleaning and immunostaining protocols for whole brain imaging, we identified the distribution of tau deposits along the extra-hippocampal projection from the hippocampal CA1-subicular connections to brain regions, including prefrontal cortex, retrosplenial cortex, basolateral amygdala, lateral entorhinal cortex, and medial entorhinal cortex. Furthermore, the number of neurons in the hippocampal CA1 pyramidal layer was significantly reduced in aged rTKhomo mice compared to control mice. In the hippocampal CA1 area, the presence of microglial activation and phagocytic cells correlated with tau deposition. These findings suggest a close association between tau-induced neuronal loss and the neuroinflammation. Overall, our newly established tauopathy mouse model successfully recapitulates key features of tauopathy and provides a valuable platform for investigating the regulatory mechanisms underlying tau-induced neurodegeneration and neuroinflammation.

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

**Support:** NINDS Grant NS085770
ADRC Grant from NIA AG072977

**Title:** Age-dependent expression and accumulation of TDP-43 in a conditional wild-type human TDP-43 transgenic mouse model on the C57BL/6 genetic background

**Authors: Y. ANTEZANA**[1], A. CHIODINI[2], *A. BAHRAMI[3], I. AYALA[2], K. SADLEIR[2], R. J. VASSAR[4], H. DONG[5], M.-M. MESULAM[6], C. GEULA[7];
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**Abstract:** Frontotemporal lobar degeneration (FTLD) is among the most prevalent dementias of early onset. Pathologically, FTLD presents with tauopathy or TAR DNA-binding protein 43 (TDP-43) proteinopathy. A biallelic mouse model of FTLD was produced on a mixed FVB/129SVE background overexpressing wild-type human TDP-43 (hTDP-43) employing tetracycline transactivator (tTA) which activates the hTDP-43 gene, placed downstream of the tetracycline response element (TRE). We backcrossed the tTA and hTDP-43 transgenic mice to

the C57BL/6 background and investigated age-dependent expression and accumulation of TDP-43 in cortical neurons. TDP-43 expression was turned on at 21 days postpartum by taking animals off doxycycline in diet. Mouse brain tissue from bigenic and wild-type animals harvested at 14 days through 24 months of expression of human TDP-43 was analyzed using Immunohistochemistry with an antibody against wild-type human TDP-43. After 14 days of expression, lightly stained human TDP-34-positive cortical neuronal nuclei were visible in bigenic animals, whereas no staining was observed in wild-type animals. The number and staining intensity of nuclear TDP-43 immunoreactivity gradually increased and was at a peak at 24 months of expression. We also observed gradual age-dependent accumulation of cytoplasmic TDP-43 in some cortical neurons. At the peak of expression and accumulation, positive neurons were most abundant in superficial and deep cortical layers and were primarily present in frontal and temporal cortical regions. Few and small punctate TDP-43 immunoreactive inclusion-like structures were visible in cortical neurons at 10 months of expression and thereafter. Large Iba1 immunoreactive microglia were observed in areas with high TDP-43 nuclear and cytoplasmic immunoreactivity. In summary, our findings reveal a gradual increase in human TDP-43 in neurons with age, accompanied with large (activated) microglia. Greater expression and accumulation of TDP-43 in frontal and temporal cortices are consistent with the pattern of atrophy seen in FTLD.

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**Presentation Number:** NANO53.07

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Support:** NIH grant R01AG0066211
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**Title:** Photoconvertible fluorescent protein-tagged tau expression in C. elegans neurons as a model of tau proteostasis

**Authors: *M. HAN**[1], A. SAXTON[2], H. CURREY[2], S. WALDHERR[2], N. LIACHKO[2], B. KRAEMER[2];
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**Abstract:** Aging-related neurodegenerative diseases pathologically characterized by aggregation of the microtubule-associated protein tau have become known as tauopathies. The free-living nematode *Caenorhabditis elegans* serves as a powerful model organism for the study of tauopathy disease mechanisms due to its facile genetics, short lifespan, convenience of live animal imaging, thoroughly documented neuro-connectome, and well established functional assays. Historically, most transgenic *C. elegans* neurodegenerative disease models utilized multicopy integration of the disease protein to elicit a phenotype, but moderating the level of transgenic expression proved difficult. To address this issue, we compared conventional integrated multi-copy transgenic array and single-copy strains generated by recombinase-mediated cassette exchange expressing pan-neuronal photoconvertible protein Dendra2 fused to wild-type human tau. Multicopy Dendra2::tau strains displaying a wide range of disease

phenotypes can be ameliorated by known genetic suppressors of tauopathy. While single-copy Dendra2::tau strains lack distinguishable disease phenotypes, they can be utilized to identify new enhancers of tau. Multicopy Dendra2::tau allows immediate tau visualization and enables optical pulse-chase experiments to measure tau turnover *in vivo*. Preliminary pulse-chase experiments in multicopy strains reveal neuronal expressed Dendra2::tau half-life of 9.056hrs, compared to the Dendra2-only half-life of 5.617hrs, indicating that the fusion of tau to Dendra2 delays turnover of the protein. Relative half-life of Dendra2::tau compared to Dendra2 alone could be used to compare turnover rates of tau under different conditions without the need for expensive and labor intensive pulse labeling-based biochemical approaches. In summary, we present single- and multicopy Dendra2::tau models as a novel tool for investigating the molecular genetic mechanisms of tau proteostasis.

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**Topic:** C.06. Neuromuscular Diseases

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**Title:** Developing RNA therapeutics for TDP-43 proteinopathy in ALS/FTD

**Authors: *K. E. COPLEY**, A. SMIRNOV, J. SHORTER;
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**Abstract:** Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases that exist on the same clinical spectrum. ALS and FTD share a common molecular pathology, wherein TAR DNA-binding protein 43 (TDP-43) mislocalizes from the nucleus to form cytoplasmic aggregates in 97% of ALS cases and approximately half of FTD cases. TDP-43 is an essential RNA-binding protein with critical RNA processing functions, including regulating splicing and RNA stability. Previous work has shown that RNA can solubilize TDP-43. My research explores whether short RNA sequences called ‘bait RNAs’ can serve as therapeutics for ALS/FTD patients. I test this hypothesis via *in vitro* assays utilizing bait RNAs to prevent aggregation of purified recombinant TDP-43. I find that bait RNAs work in a sequence-specific manner to prevent aggregation of wild-type TDP-43 and of disease-relevant variants of TDP-43, including disease-causing missense mutations and post-translational modification mimetics. A subset of disease-relevant variants displays altered dose responses compared to wild-type TDP-43. Utilizing *in vitro* binding assays, I find that while a threshold of binding is required for a bait RNA to potently prevent TDP-43 aggregation, above this threshold

other factors besides binding affinity impact prevention efficacy. From my work, I conclude that a subset of disease-linked TDP-43 variants interact with RNA differently compared to wild-type TDP-43. My work indicates that bait RNAs are a promising therapeutic approach for ALS/FTD that may ameliorate both loss- and gain-of-function toxicity resulting from TDP-43 pathology.

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**Presentation Number:** NANO53.09

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Support:** NIH Grant R21 AG069475
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**Title:** Acute tau antibody treatment restores neuronal function and prevents microglial activation in tauopathy mice as analyzed in vivo by two-photon imaging

**Authors: *A. AVILA**[1,2,3], Y. LIN[2,3], M. WALKER[3], E. M. SIGURDSSON[2,3,4];
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**Abstract:** Brain aggregates of the tau protein is a hallmark of Alzheimer's disease and other tauopathies. Several tau immunotherapies are in clinical trials but their mechanism of action is unclear. Microglia are the resident phagocytes of the brain but not much is known about how they interact with cells accumulating tau or with tau-antibody complexes. While reactive microglia are found in close proximity to tau aggregates, microglia away from tau lesions do not display such an activated phenotype. Microglia are highly dynamic and their motility is modulated by neuronal activity. However, both functional neuronal deficits and structural dynamics of microglia have not been well explored in the course of tau pathology or during tau immunotherapy. In this study, by using two-photon in vivo imaging in head-restrained mice attached to a free-floating treadmill, we visualized neuronal activity and structural microglia dynamics in the motor cortex during tau pathology progression and following tau antibody treatment. To achieve that, we crossed Thy-1$^{GCaMP6}$, Cx3cr1$^{GFP}$, and Cx3cr1$^{CreER}$:tdTomato$^{flox}$ mice with PS19 tauopathy mice. Neurons in young control Thy-1$^{GCaMP6}$:nTg mice (1-6 months) increased their somatic activity during running periods as expected, but their tauopathy littermates Thy-1$^{GCaMP6}$:PS19$^{+/-}$ did not ($p<0.0001$). Importantly, acute tau immunotherapy restored neuronal function after two 100 µg i.v. injections of anti-Asp421 tau antibody in Thy-1$^{GCaMP6}$:PS19$^{+/-}$ mice (5-6 months, $p=0.0002$), whereas control IgG1 antibody had no effect. In addition, tau pathology is evident in 2-3 months old Cx3cr1$^{GFP/+}$:PS19$^{+/-}$ mice, and is associated with reduced complexity of microglial processes ($p<0.0001$) but no change in microglial soma morphology, compared to control littermates Cx3cr1$^{GFP/+}$:nTg. In 7 months old Cx3cr1$^{CreER/+}$:tdTomato$^{flox/+}$:PS19$^{+/-}$ mice, their microglia soma was larger and in a more activated state than in their control littermates ($p<0.0001$). However, that inflammatory

phenotype was no longer evident in those tauopathy mice following the same acute tau antibody treatment paradigm that restored neuronal function. Our current findings indicate that functional deficits in cortical neurons associated with tau pathology start early in PS19 mice, and are initially associated with reduced complexity in microglial processes without structural changes in microglia soma, which are seen in older animals. This suggests that neuronal deficits linked to tau accumulation precede microglial activation. Also, our data indicate that acute tau antibody treatment restores neuronal function and prevents microglial activation in tauopathy mice

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**Presentation Number:** NANO53.10

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Support:** Intramural Research Program of the National Institutes of Health, National Institute on Aging grant (1ZIAAG000936)

**Title:** Redirecting microglia phenotype via inhibition of NFAT1 ameliorates deficits in mouse model of synucleinopathies

**Authors: *C. KIM**[1], M. IBA[1], Y. LEE[2], L. HORAN-PORTELANCE[1], K. CHANG[1], R. RISSMAN[3], S. YOU[2], S.-J. LEE[4], M. COOKSON[1], E. MASLIAH[1];
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**Abstract:** Abnormal deposition of alpha-synuclein (a-syn) and neuroinflammation are key features of synucleinopathies. We recently demonstrated that leucine-rich repeat kinase 2 (LRRK2) and nuclear factor of activated T-cells 1 (NFAT1) modulates microglial neurotoxic neuroinflammation in synucleinopathies. Therefore, we hypothesized that targeting NFAT1 might be a novel therapeutic strategy for synucleinopathies. To this aim, we utilized 11R-VIVIT, a NFAT1 inhibitory peptide, in in vivo and in vitro synucleinopathy models, to evaluate the neuroprotective effects of NFAT1 inhibition. Due to chronic disease condition, microglia in the synucleinopathy mouse model were excessively activated. However, NFAT1 inhibition decreased microglial neuroinflammation, thereby ameliorating neurodegeneration, a-syn neuropathology, and behavioral deficits in vivo. Furthermore, in vivo microglial transcriptomic analysis revealed that inhibition of NFAT1 restored motility and phagocytosis abilities of the cells in the model. Thus, we propose that by redirecting excessively-activated microglia to healthy active microglia, NFAT1 inhibition represents a promising novel therapeutic strategy for synucleinopathies.

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**Presentation Number:** NANO53.11

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

**Title:** Lysosomal dysfunction modulates myeloid cell state and immune responses

**Authors: *L. TEJWANI**, A. RANA, E. W. SUN, G. A. FITZGERALD, G. LUNKES DE MELO, C. HA, D. TATARAKIS, L. SARRAFHA, M. J. SIMON, T. SANDMANN, G. DI PAOLO;
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**Abstract:** Macrophages and microglia are myeloid cells that play critical roles in the surveillance of the local environment of the tissues in which they reside. The ability of these phagocytes to perform key functions is contingent on their capacity to sense extracellular cues and mount responses that involve chemotaxis, proliferation, cytokine secretion, and phagocytosis of various cargos for lysosomal clearance. Our overarching hypothesis is that lysosomal degradation of phagocytic cargoes is critical for the resolution of cellular/tissue damage, as well as of inflammation, and that failure to accomplish this step affects myeloid cell states and immune responses. To test this hypothesis, we examined the consequences of disrupting lysosomal function on myeloid cell state, using *GRN* deficiency as a model of primary lysosomal dysfunction. *GRN* is highly expressed in these cells and encodes progranulin, a frontotemporal dementia-linked protein critical for the regulation of lysosomal degradative processes. We first performed single-nucleus RNA-sequencing of purified microglia from aged wild-type and *Grn* knockout (KO) mice, defining the diverse subpopulations that emerge with disease in this context. Next, we characterized primary microglia and macrophages from *Grn* KO mice cultured in the absence of other cell types, which revealed robust cell autonomous transcriptional and functional alterations resulting from *Grn* deficiency. To further examine how specific forms of lysosomal dysfunction impact myeloid cell state, we induced pharmacological and genetic perturbations of various lysosomal properties, which uncovered a robust response of myeloid cells to lysosomal deacidification that largely overlaps with that observed in *Grn* KO myeloid cells. Finally, we identified putative transcription factors that mediate the transcriptional remodeling of myeloid cells in response to lysosomal stress. Overall, these data reveal a novel link between lysosomal health and myeloid cell transcriptional and functional states.

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THERAPEUTICS INC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DENALI THERAPEUTICS INC. **D. Tatarakis:** A. Employment/Salary (full or part-time):; DENALI THERAPEUTICS INC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DENALI THERAPEUTICS INC. **L. Sarrafha:** A. Employment/Salary (full or part-time):; DENALI THERAPEUTICS INC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DENALI THERAPEUTICS INC. **M.J. Simon:** A. Employment/Salary (full or part-time):; DENALI THERAPEUTICS INC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DENALI THERAPEUTICS INC. **T. Sandmann:** A. Employment/Salary (full or part-time):; DENALI THERAPEUTICS INC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DENALI THERAPEUTICS INC. **G. Di Paolo:** A. Employment/Salary (full or part-time):; DENALI THERAPEUTICS INC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DENALI THERAPEUTICS INC..

**Presentation Number:** NANO53.12

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

**Support:** Alz Association AARG-22-923548

**Title:** Biodegradable Paclitaxel-Conjugate for Alzheimer's Disease Treatment

**Authors: *D. CROSS**, S. LI, A. JENSEN, M. MONSON, M. GAMBLES, J. WANG, R. ALJASSIMI, S. MINOSHIMA, J. KOPECEK, J. YANG;
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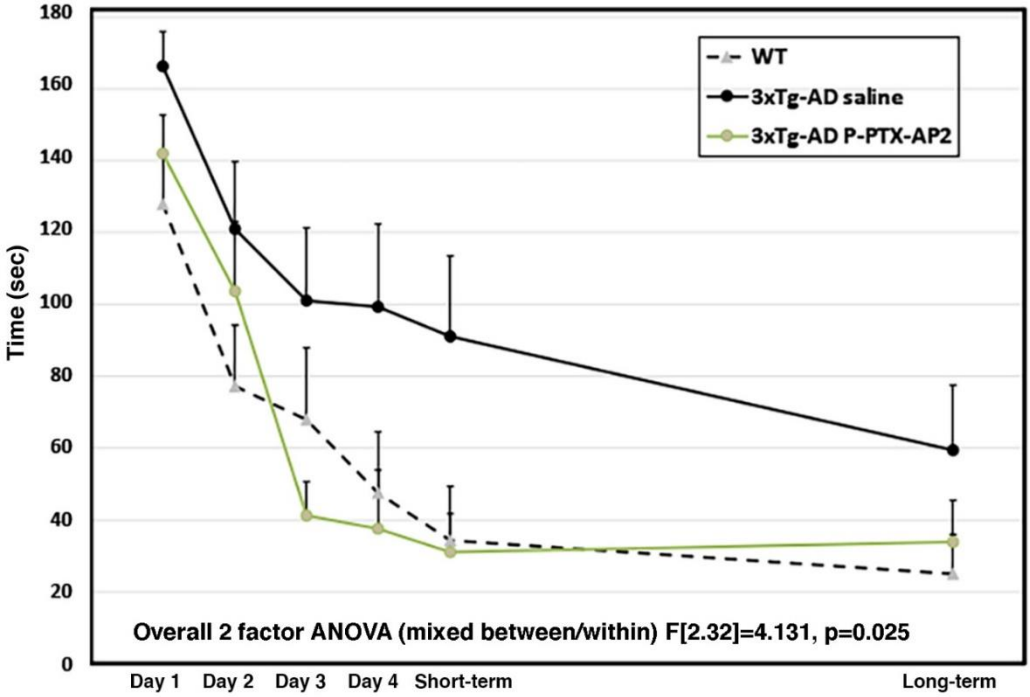
**Abstract:** Alzheimer’s disease (AD) treatments focusing on amyloid removal have found only modest cognitive improvement. Previously we showed that treatment with low doses of intranasal paclitaxel (PTX), a microtubule-stabilizing agent, altered AD phenotypic features in 3xTg-AD mice. Since PTX does not cross the blood brain barrier (BBB) we developed a novel PTX-conjugate, P-PTX-AP2, an HPMA (*N*-(2-hydroxypropyl)methacrylamide) copolymer-paclitaxel conjugate containing Angiopep-2 for brain delivery of PTX. Neurotoxicity of free PTX and P-PTX-AP2 was tested in SH-SY5Y cells. Cognition was evaluated in vivo in 13 mo. 3xTg-AD mice after 4 treatments at two week intervals with P-PTX-AP2 using a radial water tread maze. IC50 doses (24 h) in SH-SY5Y cells were 143.5 nM and 36.9 nM, for P-PTX-AP2 and PTX respectively. A two-factor between/within subjects (group by days) ANOVA revealed a significant differences between groups ($F[2,32]=4.131$, $p= 0.025$), and group by days interaction ($F[2,32]=3.823$ $p\leq0.032$). A Helmert *a priori* individual comparisons test confirmed saline-treated 3xTg-AD mice (n=11) performed significantly worse than the P-PTX-AP2-treated (n=12) and WT (n=12) ($p\leq0.007$) and were not significantly different from WT ($p\leq0.921$). Our findings suggest that microtubule stabilization via PTX is a promising therapeutic target for AD, and that the use of Angiopep-2 peptide and polymer-drug conjugate platform technology could improve its BBB permeability and therapeutic benefits for these disorders.

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**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Support:** NIH grants R21 AG059391
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**Title:** Single-domain antibody-based protein degrader for synucleinopathies

**Authors: *Y. JIANG**[1,2], Y. LIN[2], C. JI[2], R. PAN[3], X.-P. KONG[3], E. E. CONGDON[2], E. M. SIGURDSSON[2,4];
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**Abstract:** Synucleinopathies are neurodegenerative disorders characterized by accumulation of α-synuclein (α-syn) in the brain. Currently, there are no curative treatments available for these diseases. Antibody-based therapies that target α-syn aim to inhibit its aggregation and enhance degradation. However, these therapies face challenges in crossing the blood-brain barrier. In this study, we developed a single-domain antibody (sdAb)-based protein degrader 2D8-PEG4-T by conjugating 2D8 sdAb and thalidomide (T) with different length polyethylene glycol (PEG) linkers. Binding experiments confirmed that the conjugation did not affect the sdAb's affinity for

α-syn. 2D8-PEG4-T targets both α-syn and Cereblon (CRBN), a substrate-receptor for the E3-ubiquitin ligase CRL4CRBN, thereby inducing α-syn ubiquitination and proteasomal degradation. In a primary neuronal model, 2D8-PEG4-T prevented α-syn-induced toxicity and reduced intracellular α-syn levels via activation of both lysosomal and proteasomal degradation pathways. This led to superior efficacy compared to the parent unmodified 2D8 sdAb, which mainly degraded α-syn through the lysosome pathway. Next, we evaluated their therapeutic efficacy in the M83 synucleinopathy mouse model (n=17). The brain α-syn burden was first assessed in these mice immediately following an i.v. injection of a different 2D10 sdAb, which can accurately predict α-syn burden in intact animals when linked to a near-infrared tag using an In Vivo Imaging System (IVIS, Jiang Y et al Sci Adv May 10, 2023). The mice were assigned to treatment groups with similar average α-syn burden, received 3 i.v. sdAb injections (molar equivalent of 100 μg of 2D8) 3 days apart, and were re-imaged 3 days later. 2D8-PEG4-T significantly reduced (81%, $p = 0.0049$) α-syn brain signal compared to the PBS control group. In western blots of brain fractions, insoluble total and phospho-serine 129 (pS129) α-syn was reduced by 59-69% by 2D8 ($p<0.01$), compared to controls, whereas 2D8-PEG4-T was more efficacious (89-93% reduction, $p<0.0001$). Furthermore, 2D8-PEG4-T decreased soluble total and pS129 α-syn levels by 70% ($p = 0.0072$) and 90% ($p = 0.0001$), compared to the PBS group, whereas 2D8 did not significantly alter soluble α-syn. Our findings indicate that 2D8-PEG4-T enhances proteasomal degradation of α-syn, in addition to maintaining 2D8's lysosomal clearance. Its improved α-syn clearance, compared to unmodified 2D8 sdAb in vitro and in vivo models, supports its therapeutic promise for synucleinopathies. Small sdAbs with better brain entry than whole antibodies and enhanced potency may improve the clinical benefits of antibody-based therapies.

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**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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DVT-14-322623
MCDN-15-368711
AARF-19-617868

**Title:** Cis proline directed proteotoxicity in the early development and therapy of traumatic brain injury and vascular dementia

**Authors: *C. QIU**[1], O. ALBAYRAM[3], A. KONDO[4], B. WANG[5], N. KIM[6], K. ARAI[7], E. H. LO[2], X. ZHOU[8], K. LU[8];
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PLEASANT, SC; [4]Sumitomo Dainippon Pharma, Osaka City, Osaka, Japan; [5]Vizgen, Cambridge, MA; [6]Beth Israel Deaconess Med. Ctr., Boston, MA; [7]Massachusetts Gen. Hosp., Charlestown, MA; [8]Div. of Translational Therapeut. Dept. of Med., Beth Israel Deaconess Cancer Ctr. Harvard Med. Sch., Boston, MA

**Abstract:** Compelling evidence supports vascular contributions to cognitive impairment and dementia (VCID) including Alzheimer's disease (AD), but the underlying pathogenic mechanisms and treatments are not fully understood. Cis P tau is an early driver of neurodegeneration resulting from traumatic brain injury, but its role in VCID remains unclear. Here we found robust cis P tau despite no tau tangles in patients with VCID and in mice modeling key aspects of clinical VCID, likely due to the inhibition of its isomerase Pin1 by DAPK1. Elimination of cis P tau in VCID mice using cis targeted immunotherapy, brain specific Pin1 overexpression or DAPK1 knockout effectively rescues VCID like neurodegeneration and cognitive impairment in executive function. Furthermore, single cell RNA sequencing revealed that young VCID mice display diverse cortical celltype specific transcriptomic changes resembling old patients with AD, and the vast majority of these global changes were recovered by cis targeted immunotherapy. Moreover, purified soluble cis P tau was sufficient to induce progressive neurodegeneration and brain dysfunction by causing axonopathy and conserved transcriptomic signature found in VCID mice and patients with AD with early pathologies. Thus, cis P tau might play a major role in mediating VCID and antibody targeting it may be useful for early diagnosis, prevention and treatment of cognitive impairment and dementia after neurovascular insults.

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**Nanosymposium**

**NANO54: Olfaction: Circuits and Behavior**

**Location:** WCC 152B

**Time:** Tuesday, November 14, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO54.01

**Topic:** D.04. The Chemical Senses

**Support:** Simons Foundation SCGB 543003
CAREER Award 1845137
New Innovator Award DP2NS116768

**Title:** Turning associated neurons RIV, SMB, and SAA gates sensorimotor response in C. elegans upstream of neuron AVA

**Authors: *S. KUMAR**[1], A. SHARMA[2], A. TRAN[1], A. LEIFER[1,2];
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**Abstract:** The nervous system of an organism plays an important role in responding to sensory cues in the environment by incorporating the animal's current behavior state. However, the

underlying neural mechanism behind this sensorimotor response is still unknown. To answer this question of how current behavior affects the processing of sensory stimuli, we studied the nematode *C. elegans* and its response to gentle touch stimuli. When presented with a gentle touch stimulus while moving forward, a worm responds by reversing back. However, when the touch stimulus is delivered to the worm amid a turn, the probability of reversal is significantly reduced [1, 2]. To understand the underlying circuit mechanism, we optogenetically activated the downstream neurons in the mechanosensory processing pathway using a closed-loop targeted optogenetic delivery system developed by our group [2]. We systematically activated the downstream neurons while the worm was either moving forward or executing a turn. These measurements showed that the probability of reversal upon optogenetic stimulation of neurons AIZ, AIB, RIM, and AVE is significantly lower during turns than during the forward movement. However, upon activation of neuron AVA, the worm is equally likely to reverse irrespective of whether the worm is moving forward or turning. This shows that inhibitory signals during turns are integrated somewhere upstream of neuron AVA [3]. Next, we investigated the source of this inhibition. Wang et al. [4], have previously shown and we have independently confirmed that activity in turning neurons RIV, SMB, and SAA inhibits reversals [3]. We found that inhibiting the turning neurons RIV, SMB, and SAA and simultaneously activating touch neurons during turns restored the probability of reversal to that of during forward locomotion [3]. This further validates that these turning neurons are the source of sensorimotor suppression during turns. We then probed the receptors that are required for this behavior state dependent mechanosensory processing. Our investigation showed that mutant worms lacking the acetylcholine receptor LGC-47, have a similar probability of mechanosensory evoked reversals while the worm is moving forward or turning. This is consistent with a model in which neurons RIV, SMB, and SAA inhibit reversals during turns through LGC-47.
References: 1) Liu et al., 2018, eLife 2) Liu et al., 2022, PLoS Biology 3) Kumar et al., 2023, arXiv 4) Wang et al., 2022, eLife

**Disclosures: S. Kumar:** None. **A. Sharma:** None. **A. Tran:** None. **A. Leifer:** None.

**Presentation Number:** NANO54.02

**Topic:** D.04. The Chemical Senses

**Support:** NIH R01 NS126334

**Title:** Sexual dimorphism of whole-brain responses to a broad chemical space

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**Abstract:** Sexually dimorphic brain circuits play a critical role in shaping sex-specific behaviors in many species. These circuits integrate sensory inputs and modulate neural activity in response to external cues, ultimately leading to sex-specific behaviors. C. elegans males and hermaphrodites exhibit differences in their responses to environmental cues, such as food availability and pheromones. Previous studies suggest that the structural and functional differences in the nervous system play a crucial role in mediating sexually dimorphic behavior.

Recently obtained male connectome data revealed substantial differences in neural wiring patterns between the sexes; however, the full extent of how functional connectivity and neuronal activity contribute to sexually dimorphic behavior remains unclear. In this study, we developed a novel system to record whole-brain neuronal activity in both male and hermaphrodite C. elegans while presenting them with a diverse set of external sensory cues. Our system utilizes a modified confocal microscope with single-cell resolution, capable of imaging the worm's nervous system at up to 10 volumes per second. We designed and fabricated microfluidic devices tailored to both sexes, enabling simultaneous imaging of neuronal activity in nearly all nervous system neurons. Through these devices, we sequentially delivered 10 different chemosensory stimuli, including gustatory, olfactory, and nociceptive cues, to the animals. To evaluate variability in state-dependent responses, we repeated the sequence three times and observed the animals' responses for over 30 minutes. With this comprehensive approach, we discovered a substantial set of previously unknown dimorphisms. Our system determined stimulus-evoked responses in sensory, inter-, and motor neurons, recapitulating previous findings such as sexually dimorphic responses of the ADF-mediated food/pheromone pathway. We additionally found many novel sexually dimorphic responses with significant differences observed in the shape, amplitude, and kinetics of hermaphrodite versus male neural activity. Nevertheless, on a global scale, we found pairwise correlations between individual neurons to be largely similar between sexes, indicating the potential for a large degree of conserved functional connectivity.

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**Presentation Number:** NANO54.03

**Topic:** D.04. The Chemical Senses

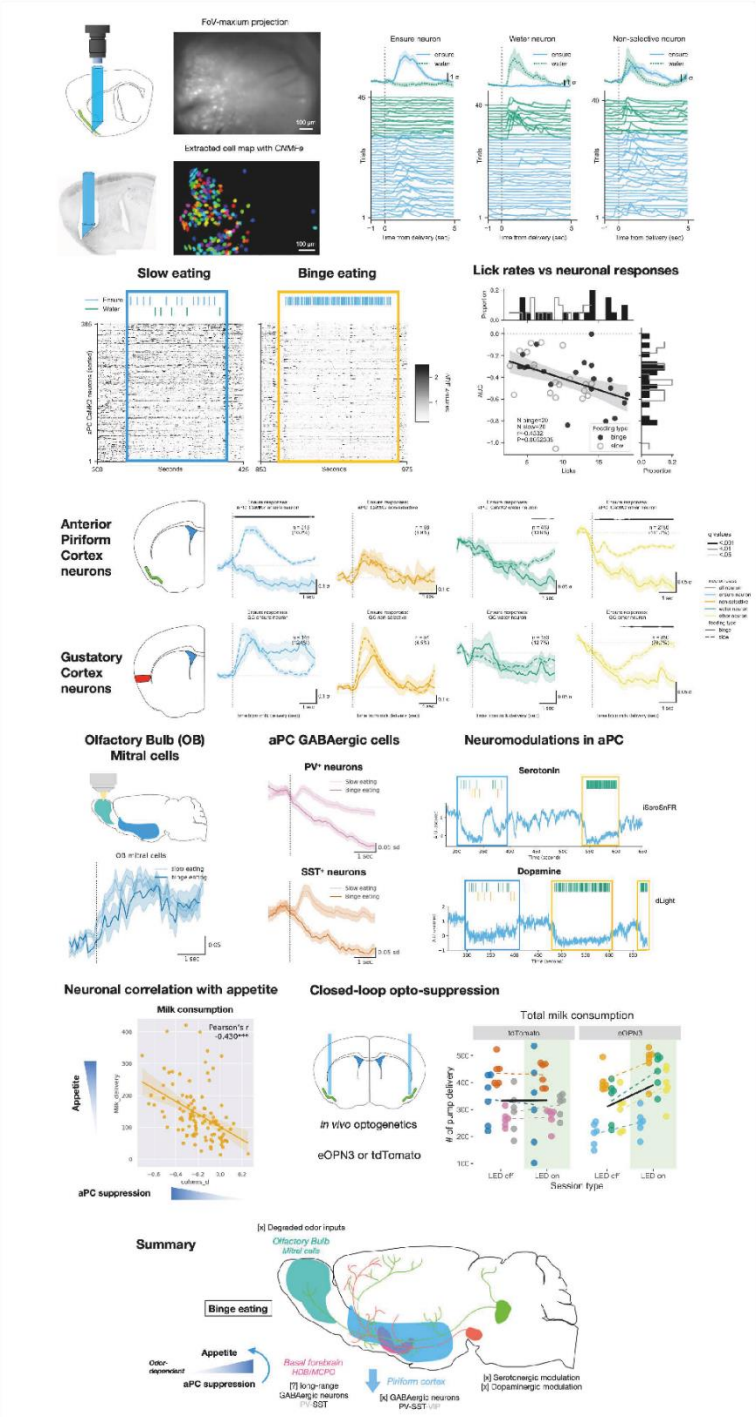
**Support:** DFG - 458236353

**Title:** Binge eating-induced olfactory cortex suppression promotes feeding

**Authors: *H. LO**[1,3], A. SCHOENHERR[1], M. L. TANTIRIGAMA[2,4,6], L. MORENO VELASQUEZ[1], L. FAISS[1,7], B. ROST[1,7], M. E. LARKUM[4], B. JUDKEWITZ[1,3,2], K. STUMPENHORST[5], M. RIVALAN[2], Y. WINTER[5], D. SCHMITZ[1,3,2,7], F. W. JOHENNING[1,3]; [1]Neurosci. Res. Ctr., [2]NeuroCure Cluster of Excellence, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Einstein Ctr. for Neurosciences Berlin, Berlin, Germany; [4]Inst. für Biologie, [5]Cognitive Neurobio., Humboldt Univ. zu Berlin, Berlin, Germany; [6]Present address: Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, Norwegian Univ. of Sci. and Technol. (NTNU), Trondheim, Norway; [7]Ctr. for Neurodegenerative Dis. (DZNE), Berlin, Germany

**Abstract:** Appropriate feeding behavior is the foundation of maintaining homeostasis. Elevated eating speed (binge eating) is a common trait of eating disorders, and it is associated with obesity. It is also known that flavor perception regulates feeding. However, the effects of eating speed on sensory feedback from flavor perception remain unknown. We developed a liquid food delivery system that enables Ensure consumption at different eating speeds. Using miniscopes for *in vivo* calcium imaging in freely foraging mice, we identified distinct neuronal responses in the anterior olfactory (piriform) cortex (aPC) upon slow and binge eating; we observed specific excitatory flavor responses during slow eating but unspecific activity suppression upon binge

eating. We found a positive correlation between lick rate and aPC suppression, suggesting this suppression may originate from behavioral differences. This binge-induced suppression is only observed in aPC, while neuronal responses in the gustatory cortex remain similar in both slow and binge eating. Odor inputs from olfactory bulb mitral cells remain stable upon binge eating, suggesting the suppression is not inherited from upstream elements of the olfactory pathway. Neither local inhibitory circuits in aPC nor inhibitory effects of serotonergic and dopaminergic modulations in aPC mediate suppression. We found that the strength of binge-induced suppression in the aPC predicts mice's total Ensure consumption. Mimicry of this observation by optogenetically suppressing aPC neurons upon binge eating in closed-loop experiments promotes consumption. Taken together, our results reveal behavioral state-dependent flavor representation in aPC neurons. We demonstrate a direct link between the binge-induced suppression of aPC flavor representation and consummatory behavior. This circuitry may contribute to binge-induced overeating due to reduced sensory feedback of food items, providing new insights into flavor perception, consumption, and satiation.




**Disclosures: H. Lo:** None. **A. Schoenherr:** None. **M.L. Tantirigama:** None. **L. Moreno Velasquez:** None. **L. Faiss:** None. **B. Rost:** None. **M.E. Larkum:** None. **B. Judkewitz:** None. **K. Stumpenhorst:** None. **M. Rivalan:** None. **Y. Winter:** None. **D. Schmitz:** None. **F.W. Johenning:** None.

**Presentation Number:** NANO54.04

**Topic:** D.04. The Chemical Senses

**Support:** NIA Grant AG- 049937A

**Title:** Circuit Specific Projections Of Basal Forebrain GABAergic Neurons To The Olfactory Bulb

**Authors: J. ZEGERS-DELGADO**[1], K. RAJENDRAN[1], A. NARASIMHAN[2], P. S. VILLAR[3], *R. C. ARANEDA[1];
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**Abstract:** Early olfactory processing is flexibly adjusted by feedback projections from multiple brain regions that convey the animal behavioral state. Among these areas, the basal forebrain (BF), which plays an important role during olfactory learning and attention, contains varied populations of cells including GABAergic and cholinergic neurons that innervate the olfactory bulb (OB) and regulate its neural output. While the functional roles for cholinergic modulation in the OB has been proposed, less is known about the role of BF inhibition to the OB in odor processing. Here, we use viral tracing and electrophysiology in male and female mice acute brain slices to investigate the cell-type diversity of the BF GABAergic output neurons and their innervation by the piriform cortex. Our whole brain imaging experiments show that most top-down GABAergic projections to the bulb concentrate in a BF nucleus called magnocellular preoptic area (MCPO), with a small number of neurons found in nearby areas. The MCPO contains at least two non-overlapping populations of neurons, characterized by the expression of the cellular markers somatostatin (Sst) and calretinin (Cr) which differentially target the glomerular and inframitral layers of the bulb, respectively. We demonstrate that fast glutamatergic inputs from the piriform cortex into the BF elicit responses in Sst but not Cr neurons. These results suggest a high degree of circuit specialization among the BF neuromodulatory neurons that project to the OB. Furthermore, our results provide support for the existence of a long-range feedback loop that can recruit BF GABAergic cells through direct PC glutamatergic inputs to drive fast feedback inhibition to the OB upon odor stimulation.

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**Topic:** D.04. The Chemical Senses

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University of California Hellman Fellowship
DoD NDSEG Fellowship

**Title:** Control of innate olfactory valence by segregated cortical amygdala circuits

**Authors: *J. R. HOWE**[1], C. L. CHAN[1], D. LEE[1], M. BLANQUART[1], J. ZHANG[1], A. M. ZADINA[2], F. MILLS[3], K. TYE[3], C. M. ROOT[4];

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**Abstract:** All animals perform innate behaviors, displaying stereotyped responses to numerous specific evolutionarily relevant stimuli in the absence of prior learning or experience. Specific odorants have innate valence, consistently evoking opposing appetitive or aversive responses. Though innate valence has long been identified as a central feature of olfaction, the underlying neural circuits have not been identified, and disparate theories have attempted to bridge this gap. Here, we examine and characterize the neural substrate underlying these stereotyped olfactory valence responses, identifying a divergent, segregated organization that specifically and selectively controls innate olfactory valence responses. First, we show a topographic organization to valence behaviors along the anterior-posterior axis in the posterolateral cortical amygdala (plCoA), a region previously implicated in innate olfactory valence, where photostimulation of the anterior region induces negative valence responses, while posterior stimulation induces positive valence responses. We then comprehensively identified all cell types in plCoA using single-cell multi-omic sequencing, finding a hardwired anteroposterior cell type gradient, where anterior glutamatergic neurons preferentially express Slc17a6 and posterior neurons express Slc17a7. While activation of these respective cell types recapitulates appetitive and aversive valence behaviors, inhibition reveals only partial necessity for valence responses to innately appetitive or aversive olfactory stimuli. We next identified topographically organized circuits and their relationship to cortical amygdala cell types, where anterior neurons preferentially project to medial amygdala, and posterior neurons preferentially project to nucleus accumbens, which are respectively sufficient and necessary for negative and positive olfactory valence. Together, these data advance our understanding of how the olfactory system generates stereotypic, predetermined attractive and aversive olfactory behaviors, and supports a model where distinct, hardwired, topographically distributed plCoA populations direct innate olfactory valence responses by signaling to divergent valence-specific targets, linking upstream olfactory identity to downstream valence behaviors.

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**Topic:** D.04. The Chemical Senses

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**Title:** Social odor memory mediated by beta band oscillations

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**Abstract:** Recognition of familiar individuals in rodents can be mediated through the sense of smell. We have previously shown that oxytocin functionally activates olfactory networks such as the anterior olfactory nucleus (AON) and modulates odor representations in the olfactory bulb (OB) and AON. Olfactory cues can trigger release of oxytocin, and be sufficient to recognize familiar conspecifics at a distance. Neural recordings in the anterior olfactory nucleus showed

that familiarity is encoded by reinforced neural representations and renders these representations more distinct from other, non encoded odors. Our recordings also show increase beta range oscillations in response to the familiar odor. We here use our computational model of olfactory bulb (OB) and anterior olfactory nucleus (AON) to explore how these observations can arise from known neural structures in the olfactory system and from know effects of oxytocin on OB and AON neural function. Our simulations show that implementation of Hebbian learning on association fibers in the AON lead to the experimentally observed reinforcement of familiar odor representation and renders these more distinct from novel odors. In addition, increased connectivity between pyramidal cells in the AON leads to synchronization of pyramidal cell odor responses in the dynamic range of beta oscillations. Beta oscillations in this model emerge from pyramidal cell spike rate adaptation, the time constant of this adaptation, when cells are strongly connected leads to beta range oscillations. Because these emerge from Hebbian learning, they are restricted to the familiar odor and thus can signal memory for an encoded odor.

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**Topic:** D.04. The Chemical Senses

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**Title:** Cortical feedback into the olfactory bulb flexibly multiplexes stimulus identity and reward contingency in a task with rule reversal

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**Abstract:** While animals readily adjust their behavior to adapt to relevant changes in the environment, the neural pathways enabling these changes remain unclear. Mice excel in discriminating odorants in complex sensory conditions, yet little is known about (**1**) how changes in stimulus contingency modify odor representations and; (**2**) how updating of odor representations is causally related to behavioral adjustments. The piriform cortex receives inputs from the olfactory bulb, as well as top-down input from association areas including the orbitofrontal and medial prefrontal cortex. Furthermore, the anterior part of the piriform cortex (aPCx) sends dense feedback to the bulb and shapes specifically the activity of mitral cells (MCs)[1,2], one of the two output channels of the olfactory bulb. Therefore, the piriform cortex is ideally positioned to integrate sensory input and contingency information and shape bulb output according to behavioral needs[3].To investigate the role of cortical-bulbar feedback in supporting flexible behavior, we trained water-deprived mice in a Go/No-Go rule reversal task guided by olfactory and auditory cues. Within the same session, stimulus-reward contingencies were

reversed across blocks of contiguous trials. In parallel, we monitored the piriform cortex-to-bulb feedback (GCaMP) using multiphoton microscopy. Cortical feedback activity triggered by the odor, as well as the sound cue, preceded the behavioral reporting (licking) and mirrored the reversals in stimulus-reward contingency throughout each session. A given stimulus triggered similar cortical feedback activity in blocks of trials of the same contingency rule, and dissimilar representations in blocks of the opposite reward contingency, revealing attractor-like behavior in the piriform-to-bulb neural dynamics. Re-shaping of individual bouton responses to the same sensory cue occurred within seconds of the rule reversals and was tightly correlated with changes in behavioral performance. Multilayer perceptron classifiers trained to decode behavioral contingency rapidly increased their performance in conjunction with the cue delivery and before the animal's decision. Optogenetic perturbation of the cortical feedback within the bulb (Jaws) disrupted the behavioral performance.Our results indicate that the piriform-to-olfactory bulb feedback multiplexes stimulus identity and reward contingency signals, and is rapidly re-formatted according to changes in the behavioral context. In ongoing experiments, we analyze the interplay between bulb feedforward and cortical feedback signals in supporting behavioral flexibility.

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**Presentation Number:** NANO54.08

**Topic:** D.04. The Chemical Senses

**Support:** NIH Grant P30AI117970
NSF IOS-1355034
Howard University College of Medicine

**Title:** Cannabinoid receptor-mediated synaptic signaling and neural plasticity in central olfactory neurons

**Authors: *T. HEINBOCKEL**, Z.-J. WANG;
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**Abstract:** Our studies aim to understand integrative and computational mechanisms that allow main olfactory bulb neurons to respond to afferent input and synaptic or feedback signals. The endocannabinoid (eCB) signaling system has been functionally implicated in many brain regions but our understanding of the role of cannabinoid receptor type 1 (CB1R) in olfactory processing remains limited. Endocannabinoids are known to mediate retrograde signaling at synapses in several brain regions through a form of short-term neural plasticity. Endocannabinoids are released from depolarized principal neurons and rapidly diffuse to presynaptic inhibitory interneurons to transiently reduce presynaptic firing and neurotransmitter (GABA) release (Depolarization-Induced Suppression of Inhibition, DSI). We study the function of the endocannabinoid system in regulating neural activity at synapses in the main olfactory bulb, the first central relay station in the brain for the processing of olfactory information coming from the nose. Our experimental approach uses electrophysiological recording techniques, specifically whole cell patch clamp recordings. Previously, using anatomical approaches, we showed that

CB1R is present in periglomerular processes of a GAD65-positive population of interneurons but not in mitral cells, key output neurons. We detected eCBs in the mouse main olfactory bulb as well as the expression of CB1R and other genes associated with the cannabinoid signaling system. Output neurons such as mitral cells and tufted cells in the olfactory bulb are computational elements in brain circuits that integrate incoming signals with membrane properties to generate behaviorally relevant synaptic output. Our data support the notion that retrograde signaling is present in neural circuits involving mitral and tufted cells. Mitral and tufted cells release endocannabinoids and, through retrograde signaling, inhibit presynaptic interneurons such as periglomerular cells, which controls the GABA release of these presynaptic neurons. This, in turn, allows mitral and tufted cells to temporarily regulate their synaptic input and relieve them from synaptic inhibition. Endocannabinoids function as retrograde messengers to regulate neural signaling and mediate plasticity at olfactory bulb synapses with potential effects on olfactory threshold and behavior.

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**Presentation Number:** NANO54.09

**Topic:** D.04. The Chemical Senses

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**Title:** Essential oils as repellents in house crickets, Acheta domesticus

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Biol. Sci., Towson Univ., Towson, MD

**Abstract:** Animals use their chemical senses (smell and taste) for orientation, food selection, and mate finding. Insects have a well-developed sense of smell (olfaction) which is often indicated by their distinct antennae. To smell and detect environmental cues, insects use their antennal sensory organs or sensilla. These sensilla contain receptor neurons which encode and process olfactory stimuli. Such stimuli can trigger behaviors, such as orientation toward food and mating partners or avoidance of predators. House crickets, *Acheta domesticus*, make excellent model systems in neuroscience as they, like other insects, have moderately complex nervous systems and exhibit a rich behavioral repertoire. House crickets are generally considered to be pests and are not welcome in human dwellings. Due to their omnivorous feeding preference, they can potentially contaminate food sources with their fecal matter. In this study, the objective was to determine the behavior elicited by several essential oils. These essential oils were previously reported to evoke repellent behavior or movement away from the odorant source in some insect species. In our experiments, we tested a panel of essential oils on house cricket behavior. We hypothesized that some of the essential oils would act as repellents in house crickets as they did in several other insect species. Our results indicate that house crickets respond with repellent behavior to some, but not all, essential oils tested. This suggests that selected essential oils can be used as repellents for house crickets. In future studies, we will determine which specific chemical derivatives are responsible for the deterrent behavioral effects in house crickets.

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**Presentation Number:** NANO54.10

**Topic:** D.04. The Chemical Senses

**Support:** NIH (NIDCD) 1R01DC02084201

**Title:** Sensorimotor prediction errors in the mouse olfactory cortex

**Authors: *M. A. M. DUSSAUZE**[1,2], P. GUPTA[1], U. LIVNEH[1], D. ALBEANU[1];
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**Abstract:** During behavior, sensation and action operate in closed-loop. Movements shape sensory input and sensory inputs guide motor commands. Through experience, the brain may learn the reciprocal relationship between sensory inputs and movements to build internal models that accurately predict the sensory consequences of upcoming actions (sensorimotor predictions). In vertebrates, olfaction is intrinsically linked to motor action through sniffing and, as for other sensory modalities, via body movements. However, most studies have probed olfactory processing during passive odor sampling. Even when studying odor-guided navigation, the effect of movements on olfactory representations has been rarely analyzed.
We hypothesized that, in closed-loop olfaction, mice predict the sensory consequences of their actions (the next most probable odor input). Movement-related predictions of expected odor input get compared with current odor input within the olfactory cortex to represent olfacto-motor prediction errors. To test these hypotheses, we developed a behavioral task where head-fixed mice are trained to steer the left-right location of an odor source by controlling a lever with their forepaws. In this manner, 1) we link a precise motor action to well-defined sensory expectations (odor location) and 2) subsequently violate the learned expectations via online sensory feedback perturbations in expert animals.
Strikingly, mice readily counter brief sensorimotor perturbations, by making precise corrective movements that provide us a read-out of their individually learned sensorimotor predictions. Importantly, odor-driven responses in cortical neurons are re-shaped by olfacto-motor expectations. Using chronic recordings during behavior, we found that transient perturbations often trigger stronger responses than those evoked by any other task variable. Our results suggest that the olfactory cortex computes sensorimotor prediction errors by integrating sensory information with movement-related predictions, presumably relayed via top-down feedback. Using cell-type analysis and activity manipulations, we further aim to identify the circuit elements that facilitate the comparison of olfactory inputs with predictions.

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**Presentation Number:** NANO54.11

**Topic:** D.04. The Chemical Senses

**Support:** NIH R01 MH113924
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**Title:** Adult-neurogenesis allows for representational stability and flexibility in early olfactory system

**Authors: *Z. CHEN**[1], K. PADMANABHAN[2];
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**Abstract:** Adult-neurogenesis throughout animal's lifetime continuously replaces the largest class of neurons to the main olfactory bulb, resulting in a constant restructuring of the circuit architecture of early olfactory system. While neurogenesis has been proposed as a mechanism for plasticity and learning, the continuous turnover of cells and an ever-changing network architecture raises the fundamental question of how the brain maintains perceptual and representational stability when the earliest sensory circuits are so inherently unstable. Using a detailed spiking network model, we identified how adult neurogenesis can support both representational stability and plasticity. In the main olfactory bulb, adult-neurogenesis modulates the individual cell responses, acting to support gain control while preserves the representations of odors at a population level. By contrast, in olfactory piriform cortex, both individual cell responses and population representations change progressively due to the plasticity of adult-neurogenesis in the bulb. This gives rise to the representational drift in cortex. These findings present a novel function for adult-neurogenesis, by which the two seemingly opposing computations, perceptual stability, and ongoing plasticity, are achieved simultaneously by early olfactory system.

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**Presentation Number:** NANO54.12

**Topic:** D.04. The Chemical Senses

**Support:** 1K99-DC019668

**Title:** Unveiling the hidden layers: how astrocyte epigenetics encode sense of smell

**Authors: *D. SARDAR**[1], Y.-T. CHENG[1], J. WOO[1], D.-J. CHOI[1], Z.-F. LEE[1], W. KWON[1], H.-C. CHEN[1], B. LOZZI[1], A. CERVANTES[1], K. RAJENDRAN[1], T.-W. HUANG[1], A. JAIN[1], B. ARENKIEL[1], I. MAZE[2], B. DENEEN[1];
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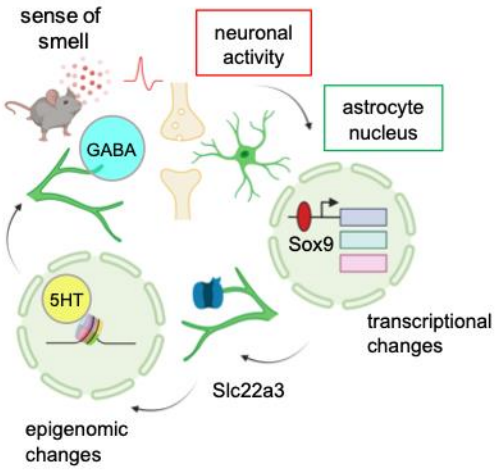
**Abstract:** Astrocytes are non-neuronal cells widely distributed in the brain and astrocyte-neuron communication play critical roles in modulation of behavior. Sensory stimuli like odors activate neurons to induce gene expression changes that facilitate sensory processing. While such gene regulation has been described in neurons, whether similar transcriptomic changes also occur in astrocytes are unknown. Here, using a chemogenetic mouse model of neuronal activation, we show astrocytes indeed undergo robust gene expression changes in response to neuronal activity and these changes are largely distinct from neurons. Using an astrocyte transcription factor-based screen, we further identified an activity-dependent neuromodulator transporter Slc22a3 in astrocytes of the olfactory bulb, the brain region responsible for processing smells. Since odor stimuli activate neurons in the olfactory bulb, olfaction represents a natural model to investigate the effects of neuronal activation on astrocytes. Therefore, using olfactory processing in combination with a Slc22a3 conditional knockout mouse model and viral vector delivery, we

determined astrocyte-specific and olfactory bulb region-specific function of Slc22a3. We show that Slc22a3 maintains astrocyte serotonin in the olfactory bulb, and loss of Slc22a3 leads to impaired sense of smell. This is mediated through a novel epigenetic mechanism of histone serotonylation, wherein serotonin is directly incorporated into histones to control gene expression. Our results further show that histone serotonylation epigenetics regulate GABA biosynthetic genes, and loss of Slc22a3 leads to loss of tonic GABA release from olfactory bulb astrocytes. Ultimately, such serotonin mediated epigenetic control of GABA tone by astrocytes leads to olfactory processing deficits. This work defines for the first-time an astrocyte epigenetic pathway that influences animal behavior. Taken together, these results uncover new epigenetic mechanisms of how astrocytes encode the sense of smell.

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**Nanosymposium**

**NANO55: Glia and Vasculature in Alzheimer's Disease**

**Location:** WCC 143

**Time:** Tuesday, November 14, 2023, 8:00 AM - 10:30 AM

**Presentation Number:** NANO55.01

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

**Support:** F32 NS117649
R01 AG062738

**Title:** Loss of perivascular fibroblasts in cerebral amyloid angiopathy impairs structural integrity of brain arterioles

**Authors: *S. K. BONNEY**, M. J. SOSA, M. KILBY, M. B. PETERSEN, A. Y. SHIH;
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**Abstract:** Tortuous arterioles and impaired vasomotion are hallmarks of the small vessel disease, cerebral amyloid angiopathy (CAA), and likely exacerbate the accumulation of amyloid-beta along the vascular wall. Mural cells and perivascular fibroblasts (PVFs) reside within the vascular wall, supporting vessel integrity, reactivity, and function. In CAA, smooth muscle cell (SMC) coverage is reduced along arterioles, impairing vasomotor oscillations and ultimately affecting clearance of amyloid-beta from the vascular wall. However, it is unknown if and how PVFs contribute to CAA pathology. By studying CAA-PVF reporter mice (Tg-SwDI; Col1a1-GFP), we find that PVF numbers are reduced along arterioles in the cortex and hippocampus at 6-months of age. The appearance of parenchymal amyloid-beta plaques occurs around 6-months of age whereas vascular amyloid-beta begins around 9-months of age in the cortex and hippocampus. In contrast, SMC coverage assessed by immunohistochemistry for alpha-SMA is maintained until late stages of CAA (20-24 months). This suggests that PVF loss is an early event in CAA, potentially due to presence of parenchymal amyloid-beta plaques and preceding CAA and SMC loss. *In vivo* two photon imaging of 12-month-old CAA-mural cell and PVF reporter mice (Tg-SwDI; PdgfrβCre-tdTomato), revealed that arterioles are more tortuous, and this correlates significantly with a reduction in PVF density. Further, direct optical ablation of PVFs along arterioles in healthy, young PVF reporter mice increases arteriole tortuosity. These findings support a model in which PVF loss may contribute to progression of CAA resulting in increased arteriolar tortuosity and altered vasomotion ultimately impairing clearance of amyloid-beta through perivascular routes. The mechanism by which the arteriole wall is perturbed during PVF loss is under investigation but may involve alterations to the vascular wall basement membrane or impaired PVF-mural cell interactions. Finally, the basis of PVF vulnerability to amyloid-beta remains unclear and requires further investigation in model systems with varied amyloid-beta pathology.

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**Presentation Number:** NANO55.02

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

**Support:** NIH NIA P20AG068077
Harvey Family Endowment

**Title:** Vascular Integrity, Autophagy and Alzheimer's Disease

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**Abstract:** Our work is focused on understanding vascular and autophagy factors as contributors to Alzheimer's (AD) and cerebrovascular diseases in underrepresented groups in New Mexico. We have collected 265 brains in a UNM Brain Bank, with consent, from our ethnically diverse population, with 61% Hispanics. Hispanics have a high incidence of mixed dementias. White matter changes from vascular injury alone may present with cognitive impairment, and when

coupled with protein processing defects may potentiate harm. To study this, we identified biomarkers of small vessel disease and/or autophagy genes (ATG) to apply to brains in our Brain Bank. For ATG, we used machine learning to identify other unknown ATG, are developing a comprehensive annotated ATG list and using the list to scan genomic databases for allelic variations associated with AD. Our data show that human primary microglia elevate ATG expression when exposed to human paired helical filaments. For vascular markers, we selected two matrix-metalloproteinases, MMP1 and 10, that appear in CSF of subjects with mixed but not pure AD dementias. We performed immunohistochemistry for MMP1 and 10 on 9 cases +/- AD taken from our archival post-mortem brain bank. We scored AD cases in histopathology with either Bielschowsky silver for Amyloid/Braak/CERAD (ABC) on superior and medial temporal gyrus, inferior parietal lobule (IPL), and middle frontal gyrus or with p-tau IHC on IPL, and examined cerebral vessels for evidence of vascular disease and/or microhemorrhages. MMP1 stained arterial smooth muscle and pericytes and was not visible in venous structures. In some sections, capillaries stained only part-way along their length, suggesting that pericytes do not uniformly surround them. MMP10 stained perivascular macrophages, more prevalent in cases with vascular disease. We further developed double chromogen staining for identification of cell expressers and will also stain for ATG proteins associated with AD to determine cell of origin. We are developing new MR-pathology techniques to correlate abnormalities found by ante-mortem imaging in life with post-mortem cellular and molecular pathologies. Our preliminary conclusions are that MMPs appear in the CSF from vascular origins, that MMP1 is useful for arterial smooth muscle and pericyte integrity, and that MMP10 will inform on perivascular inflammation and neuronal injury in histopathology of VCID and AD. Further, we have identified a subset of ATG carrying AD-associated allelic variations which are differentially expressed in AD.

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**Title:** Cardiovascular disease increases aggregation in both heart and brain, with protein profiles similar to Alzheimer’s disease

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**Abstract:** Cardiovascular disease (CVD) is the leading cause of death in the world and in the United States. Reduced cardiac output due to CVD has been previously associated with decreases in systemic and cerebral perfusion. Cerebral hypoperfusion increases the risk of Alzheimer’s disease, through accumulation of aggregates that feature Aβ amyloid and hyperphosphorylated tau. Protein aggregation is a shared hallmark of aging and

neurodegenerative diseases. We previously reported elevated aggregation in hearts of hypertensive and aged mice, and now find that aggregation is induced in hearts and brains of male C57BL6 mice after transient ligation of the left coronary artery, mimicking myocardial infarction (MI). We also show increased Endoplasmic Reticulum (ER) stress in both heart and brain of these mice after MI, including elevation of proteins involved in protein homeostasis: CAND1, SerpinH1, NEDD4 and UCP1. Knockdown of RNAs encoding these proteins reduced aggregation in a neuroblastoma cell line (SY5Y-$APP_{Sw}$). We carried out aggregate crosslinking studies in brain tissues of control (AMC), Alzheimer's (AD) and heart disease (HtD) individuals using a click chemistry protocol. AD brains had elevated aggregate interactions relative to AMC. Interestingly, although HtD aggregates have complexity (interaction levels) similar to AMC, aggregation of critical proteins involved in neurodegeneration pathways such as those comprising the Ubiquitin Proteasome System (UPS), mitochondria, and ER are present in HtD and AD aggregates at similar levels, but not in AMC. This suggests that heart disease might predispose proteins in the brain to aggregate, leading to conditions favoring cognitive decline, eventually leading to Alzheimer's disease.

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

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USUHS

**Title:** Engineering a pH-sensitive Mouse Transferrin Receptor Binding Nanobody for Blood Brain Barrier Transcytosis of Macromolecular Cargo

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**Abstract:** Background: The blood brain barrier limits entry of macromolecular diagnostic and therapeutic cargos. Blood brain barrier transcytosis via receptor mediated transport systems, such as the transferrin receptor, can be used to carry macromolecular cargos with variable efficiency. Transcytosis involves trafficking through acidified intracellular vesicles, but it is not known whether pH-dependent unbinding of transport shuttles can be used to improve blood brain barrier transport efficiency.Methods: A mouse transferrin receptor binding nanobody, NIH-mTfR-M1, was engineered to confer greater unbinding at pH 5.5 vs 7.4 by introducing multiple histidine mutations. The histidine mutant nanobodies were coupled to neurotensin for *in vivo* functional blood brain barrier transcytosis testing via central neurotensin-mediated hypothermia in wild-type mice. Multi-nanobody constructs including the mutant $M1_{R56H, P96H, Y102H}$ and two copies of the P2X7 receptor-binding 13A7 nanobody were produced to test proof-of-concept macromolecular cargo transport *in vivo* using quantitatively verified capillary depleted brain lysates and *in situ* histology. Results: The most effective histidine mutant, $M1_{R56H, P96H, Y102H}$ -neurotensin, caused >8°C hypothermia after 25 nmol/kg intravenous injection. Levels of the heterotrimeric construct $M1_{R56H, P96H, Y102H}$ -13A7-13A7 in capillary depleted brain lysates

peaked at 1 hour and were 60% retained at 8 hours. A control construct with no brain targets was only 15% retained at 8 hours. Addition of the albumin-binding Nb80 nanobody to make $M1_{R56H, P96H, Y102H}$ -13A7-13A7-Nb80 extended blood half-life from 21 minutes to 2.6 hours. At 30-60 minutes, biotinylated $M1_{R56H, P96H, Y102H}$ -13A7-13A7-Nb80 was visualized in capillaries using *in situ* histochemistry, whereas at 2-16 hours it was detected in diffuse hippocampal and cortical cellular structures. Levels of $M1_{R56H, P96H, Y102H}$-13A7-13A7-Nb80 reached more than 3.5 percent injected dose/gram of brain tissue after 30 nmol/kg intravenous injection. However, higher injected concentrations did not result in higher brain levels, compatible with saturation and an apparent substrate inhibitory effect. Conclusion: The pH-sensitive mouse transferrin receptor binding nanobody $M1_{R56H, P96H, Y102H}$ may be a useful tool for rapid and efficient modular transport of diagnostic and therapeutic macromolecular cargos across the blood brain barrier in mouse models. Additional development will be required to determine whether this nanobody-based shuttle system will be useful for imaging and fast-acting therapeutic applications.

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**Title:** Blood brain barrier (BBB) disruption precedes brain atrophy in APOE3 and APOE4 mice crossed with a transgenic mouse model of tauopathy (P301S) in an APOE isoform-dependent manner

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**Abstract:** Early blood-brain barrier (BBB) breakdown contributes to cognitive impairment in Alzheimer's disease (AD). The E4 allele of the *APOE* gene, *APOE4*, the major susceptibility factor for AD, compared to *APOE3* that carries lower risk for AD, exerts substantial cerebrovascular toxicity including BBB breakdown both in humans and in *APOE* transgenic models, which we showed precedes cognitive impairment and synaptic deficits, respectively. Here, we studied BBB changes by dynamic contrast enhanced (DCE)-MRI in relation to brain structural volumetric changes with anatomical T2w sequence longitudinally in 5.5- and 9.5-month-old *APOE3* and *APOE4* knockin $(KI)^{flox/flox}$ floxed (F) mice, i.e., *E3F* (n=6) and *E4F* (n=6) alone and crossed with a mouse model of tauopathy (*P301S*), i.e., *E3F; P301S* (n=20) and *E4F; P301S* (n=15). Our data in 5.5-month-old mice show no significant changes in the lateral

ventricle and hippocampus between the four groups, whereas hippocampal BBB permeability ($K_{trans}$ $10^3$ $min^{-1}$) was increased in *E4F* mice compared to *E3F* mice by ~ 2-fold (i.e., 0.88±0.02 vs. 0.41±0.01, p<.0001), and was further increased by tauopathy in both genotypes by ~2-fold, but still in an *APOE* isoform-dependent manner (*APOE4 > APOE3*), i.e., *E4F;P301S* vs. *E3F; P301S* = 2.01±0.27 vs. 1.01±0.15, p=0.0021). In 9.5-month-old mice there was a significant, approximately 3-fold increase in the lateral ventricle size driven by tau-mediated neurodegeneration in both *E4F; P301S* vs. *E4F* mice and *E3F; P301S* vs. *E3F* mice associated with *APOE* isoform-dependent hippocampal atrophy, i.e., *E4F; P301S > E3F; P301S.* The BBB permeability in *E3F* vs, *E4F* mice remained comparable to that found in these mice at 5.5 months of age but was further exacerbated by tauopathy by 3-4-fold in *E3F; P301S* and *E4F; P301S* mice. Altogether, our data suggest that BBB breakdown precedes brain atrophy in *APOE3* and *APOE4* mice crossed *P301S* mice in an *APOE* isoform-dependent manner.

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**Title:** A common microvascular endophenotype in head injuries and Alzheimer's disease

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**Abstract:** Cerebrovascular injury is a common pathological feature of a spectrum of neurological disorders, including traumatic brain injury (TBI), stroke, Alzheimer’s disease (AD), and aging. Vascular manifestations among these conditions are similar indeed, including the breakdown of the blood-brain barrier (BBB). However, whether there is a unique molecular mechanism underlying the vascular changes among these conditions remains elusive. To explore how mild traumatic brain injury (mTBI) impacts the transcriptional and cellular profiles of vascular endothelial cells in adult mice across time, we performed single-cell RNA (scRNA-seq) sequencing and obtained 29,114 high-quality cells combined from ipsilateral hemispheres of WT C57BL/6J Sham mice and mTBI mice at day 1, 3 and 7. Joint clustering of all groups revealed 7 cell populations using their specific genetic markers, including vEC, capEC, aEC, microglia, oligodendrocytes, and mural cells. Next, we identified differential expression genes between Sham and day 1 or day 3 or day 7 post-mTBI within each endothelial cell cluster.Based on transcriptomic analysis on cerebrovascular scRNA-seq datasets, we identified a common molecular signature between mTBI and aging vasculature, involving a novel transmembrane protein gene *Tmem252*. *Tmem252* upregulation in brain endothelial cells may represent a shared endophenotype of microvascular injuries in different pathological conditions, and therapeutically targeted for treating BBB breakdown and vascular dysfunctions. Therefore, to further explore *Tmem252*-dependent microvascular endophenotype in mTBI and AD, we generated the *Tmem252* knock-in reporter and *Tmem252* knockout mice. To examine the function of Tmem252 *in vivo*, we induced a mild closed compact impact to the somatosensory cortex (S1) of *Tmem252*$^{-/-}$ and C57BL/6J 3-month adult mice and assessed its impact at day 1, 3 and 7 post-injuries. We

performed behavioral tests for sensorimotor functions and cognitive functions before and after mTBI. Interestingly, *Tmem252*$^{-/-}$ mice exhibited reduced astrogliosis and neuroinflammation after mTBI, as we found much less accumulation of astrocytes (GFAP**)** and microglia/macrophages (IBA1 and CD68) in brain cortex and hippocampus, while C57BL/6J mice showed significantly more activated astrocytes and microglia. These results indicating that *Tmem252* inhibition maybe beneficial for mTBI induced microvascular injury.

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**Title:** Effects of Gfap knock-down via anti-sense oligonucleotide on Aβ and pTau pathologies in Alzheimer’s disease mouse models

**Authors: *C. MUÑOZ-CASTRO**[1,2,3], M. A. HEALEY[1], M. JAISA-AAD[1,2], A. M. KHASNAVIS[1], L. A. WELIKOVITCH[1,2], B. T. HYMAN[1,2], A. SERRANO-POZO[1,2]; [1]Massachusetts Gen. Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Univ. de Sevilla, Sevilla, Spain

**Abstract:** Reactive astrocytes surround amyloid-β (Aβ) plaques and phospho-tau (pTau) neurofibrillary tangles in the Alzheimer’s disease (AD) brain yet their role on AD progression remains obscure. One of the best-known features of reactive astrocytes is the upregulation of the cytoskeletal intermediate filament glial fibrillary acidic protein (GFAP). GFAP has been attributed many key functions including vesicle intracellular trafficking, chaperone-mediated autophagy, and tripartite synapse integrity and function; however, the consequences of this GFAP upregulation with respect to AD neuropathology have not been fully elucidated. GFAP and vimentin (VIM) were implicated in the glial scar formation to restrict Aβ plaque growth, but two studies on APP/PS1×*Gfap*$^{-}$/*Vim*$^{-}$ mice are conflicting and may have been hampered by developmental compensations. Moreover, *Gfap* has never been knocked-down in tauopathy mice. We hypothesized that GFAP upregulation by reactive astrocytes is necessary to clear misfolded, aggregation-prone proteins such as Aβ and pTau. Specifically, we asked whether *Gfap* knock-down via anti-sense oligonucleotide (ASO) in astrocytes of adult AD transgenic mice worsens Aβ and pTau pathologies as well as downstream neurodegeneration. We injected a mouse-specific *Gfap* ASO (i.c.v.) into 4.5-mo-old wild-type, APP/PS1, THY-Tau22, and APP/PS1×THY-Tau22 double transgenic mice (n=8-15 per genotype and treatment, sex-balanced). Mice injected with saline and non-specific control ASO served as control groups. We euthanized all mice at 9 months of age (i.e., 4.5 months post-injection, prior to the plateau of the

pathology) and performed biochemical and immunohistochemical analyses. Mouse-specific *Gfap* ASO did not have obvious adverse effects (i.e., weight gain and survival) in any genotype. Relative to saline and control ASO, the mouse-specific *Gfap* ASO led to a 70-90% reduction of Gfap levels in hippocampus and cortex across the four genotypes. By contrast, Vim levels remained unaltered. There was no treatment effect on cortical or hippocampal Aβ plaque burden, or cortical Thioflavin-S+ plaque burden in either APP/PS1 or double transgenic mice, nor on hippocampal $pTau^{Ser202/Thr205}$ (AT8) immunoreactive area fraction in either THY-Tau22 or double transgenic mice. Lastly, there was no treatment effect on cortical or hippocampal areas across the four genotypes. In conclusion, blunting the Gfap upregulation characteristic of reactive astrocytes in adult AD transgenic mice with a specific ASO does not substantially impact the burden of Aβ deposits or pTau neurofibrillary tangles.

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**Presentation Number:** NANO55.08

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

**Support:** NIH Grant MH125903

**Title:** The role of astrocytic exocytosis of ATP in the progression of amyloid-β pathology

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**Abstract:** Alzheimer’s disease (AD) is a devastating neurodegenerative disease with no cure. It is characterized by amyloid-β (Aβ)-containing senile plaques and tau-containing neurofibrillary tangles in the brain. Among many mechanisms contributing to AD, excessive deposition of toxic Aβ peptides is believed to play a key role. Recent studies have shown that astrocytes and gliotransmitters released from astrocytes are involved in Aβ pathology.
Here, we aimed to investigate the potential role of astrocyte-derived purinergic signaling in Aβ pathology of AD. Aβ42 induced adenosine triphosphate (ATP) release in astrocytes. Further, 2-Me-SATP (an ATP analog) triggered a largely overlapping transcriptional response in astrocytes comparable to those treated with Aβ42, exemplified by the induction of inflammation, suppression of pathways involved in extracellular matrix, and regulation on phagocytosis. These data strongly suggest that in response to Aβ42 exposure, astrocytes may release ATP as a pro-inflammatory signal to trigger functional alterations in astrocytes and other surrounding cells. Astrocytes release ATP through multiple routes, including selective channels, connexin hemichannels, and exocytosis. To specifically investigate the role of exocytosis of ATP in astrocytes, we developed a unique mouse model to delete astrocytic vesicular nucleotide transporter (Vnut), which is an essential for loading cytosolic ATP into the secretory vesicles. Loss of Vnut significantly increased the uptake of HiLyte647-conjugated Aβ42 by primary astrocytes likely due to the increased activity of receptor-independent endocytosis/phagocytosis.

In agreement with the KO cell model, overexpression of Vnut inhibits Aβ42 uptake by astrocytes. To further examine the role of astrocytic Vnut in mouse model of Alzheimer's disease, we crossed Vnut-flox mice with astrocyte-specific *Aldh1l1*-CreERT2 and 5xFAD mice. Loss of Vnut in astrocytes of the female 5xFAD mice dramatically reduced Aβ plaques by ~50% at 6 months of age. Meanwhile, the expression of Gfap was also decreased. These alterations were most prominent in selective brain regions, including the prefrontal and motor cortex, lateral septum, and subiculum of the hippocampal formation. More importantly, loss of astrocytic Vnut greatly improved cognitive deficit in the female mice with 5xFAD background.
Our results suggest that Vnut-mediated vesicle storage and release of ATP is an important mechanism in astrocytes to regulate astrogliosis and Aβ pathology. Inhibiting astrocytic Vnut and astrocytic-derived purinergic signaling could represent a unique and novel glial-based therapeutic strategy for AD.

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**Title:** Iron-enriched microglia drive ferroptosis and white matter degeneration in aging brains

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**Abstract:** As the brain ages, recurrent cerebral white matter injury (WMI) causes disruptions in white matter integrity and myelination, contributing to the development of cognitive impairment seen in Alzheimer's disease (AD) and vascular dementia. We identified a significant population of microglia in aging human WMI that show degenerative changes linked to phagocytosis of myelin debris. Degenerative microglia (DM) are enriched in the iron-binding protein light chain ferritin and accumulate PLIN2-labeled lipid droplets. They also exhibit lipid peroxidation injury and increased expression of TOM20, a mitochondrial translocase, and oxidative stress sensor.

Analysis of the DNA fragmentation marker phospho-histone H2A.X suggests that iron-enriched DM are vulnerable to senescence-related degeneration through a mechanism consistent with ferroptosis. Relative to gray matter neurodegeneration, a distinct set of ferroptosis-related genes, which involve iron-mediated lipid dysmetabolism and oxidative stress, were found to be selectively expressed in WMI. Consequently, ferroptosis seems to be a significant mechanism underlying WMI in AD and vascular dementia. Failure of remyelination is linked to a large population of aging microglia that are sensitive to oxidative stress caused by phagocytosis and abnormal accumulation of myelin debris, thereby promoting microglial lipid peroxidation injury and ferroptosis-related degeneration.

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**Title:** The amyloid precursor protein (APP) and its role in oligodendrocytes and myelin

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**Abstract:** The myelin hypothesis of Alzheimer's disease is widely attributed to the work and writing of George Bartzokis who proposed that with aging “Cortical regions with the most protracted development are most vulnerable to AD pathology, and this protracted development is driven by oligodendrocytes.” He viewed the age-related breakdown of myelin as driving a feed-forward series of degenerative events that led to the features of the AD brain. Yet, while the correlation between myelin breakdown and loss of cognitive capacity is robust, a cell biological dimension has been missing. We present evidence that this cell biological connection may well be the amyloid precursor protein (APP). APP is widely known for its proteolytic breakdown product, the Aβ peptide, and for its genetic linkage to early onset forms of AD. Mining of public data bases reveals that the expression of APP in brain is higher in oligodendrocytes than in any other brain cell type. Cultured oligodendrocytes export APP to their processes. Using confocal microscopy, we validate earlier reports that APP is found in para-axonal myelin and that its levels there rise in AD and its mouse models. Co-immunoprecipitation will be done to test the association of APP with different myelin-related proteins. Finally, APP overexpression expands the node of Ranvier, a critical feature of all myelinated axons that represents a collaboration between the oligodendrocyte and the neuron. We propose that by acting as a cell adhesion molecule, APP helps mediate neuron-oligodendrocyte interactions and that these interactions are compromised during the development of AD.

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**Nanosymposium**

**NANO56: Basal Ganglia: Structure and Function**

**Location:** WCC 150

**Time:** Tuesday, November 14, 2023, 8:00 AM - 10:30 AM

**Presentation Number:** NANO56.01

**Topic:** E.03. Basal Ganglia

**Support:** Brain & Behavior Research Foundation,Young Investigator Grant
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**Title:** Neuron-type specific postsynaptic protein expression at striatal excitatory synapses

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**Abstract:** Combinatorial expression of postsynaptic proteins underlies synapse diversity within and between neuron types. Thus, characterization of neuron-type-specific postsynaptic proteomes is key to obtaining a deeper understanding of discrete synaptic properties and how selective dysfunction manifests in synaptopathies. To overcome the limitations associated with bulk measures of synaptic protein abundance, we developed a biotin proximity protein tagging probe to characterize neuron-type-specific postsynaptic proteomes *in vivo*. We found Shank3 protein isoforms are differentially expressed by direct and indirect pathway spiny projection neurons (dSPNs and iSPNs). Studies in mice lacking Shank3 gene exons 13-16 revealed a robust postsynaptic proteome alteration in iSPNs. We report unexpected cell-type specific synaptic protein isoform expression which could play a key causal role in specifying synapse diversity and selective synapse dysfunction in synaptopathies.

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**Topic:** E.03. Basal Ganglia

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**Title:** Basal ganglia gate transthalamic and cerebellar circuits through motor thalamus

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**Abstract:** The basal ganglia are traditionally described as part of an information loop, transmitting excitatory signals that originate in motor cortex to the motor thalamus, which then innervates motor cortex to close the loop. However, because the basal ganglia innervation of thalamus is GABAergic and inhibitory, we suggest instead that it serves to gate the relay of thalamocortical information to motor cortex. Accordingly, we investigated how GABAergic projections from the output nuclei of the basal ganglia, the internal segment of the globus pallidus (GPi) and substantia nigra (SNr), might interact with two excitatory, driving inputs to motor thalamus: that from layer 5 (L5) of primary motor cortex ($M1_{L5}$) that involves cortico-thalamo-cortical, or transthalamic, circuits, and that from the deep cerebellar nuclei (Cb). We did so using an optogenetic strategy in acute slices derived from L5-Cre mice of both sexes, recording from neurons in motor thalamus, and testing for convergence of these various input combinations. That is, we injected channelrhodopsin (ChR2) into one excitatory and one inhibitory area in each mouse according to the following scheme: cre-dependent ChR2 into M1 (L5 expression) or cre-independent ChR2 into Cb, paired with injections of an inhibitory neuron-specific ChR2 into GPi or SNr. Each input was identified by the character of the postsynaptic response and confirmed pharmacologically to be either glutamatergic ($M1_{L5}$ or Cb) or GABAergic (GPi or SNr). We discovered that GABAergic inputs from both the GPi and SNr converge with inputs from $M1_{L5}$, especially in the ventral motor thalamus. These findings demonstrate that both the GPi and SNr can gate signals traversing the transthalamic pathway originating in $M1_{L5}$, effectively controlling which cortical areas can communicate via both direct corticocortical and transthalamic circuits versus those only connected by direct (corticocortical) pathways. Somewhat surprisingly, GPi and SNr also converged with excitatory projections from Cb. That the basal ganglia also gates Cb inputs to motor thalamus suggests that an overall function for the basal ganglia is to control the flow of thalamocortical information through motor thalamus.

**Disclosures: K.P. Koster:** None. **S. Sherman:** None.

**Presentation Number:** NANO56.03

**Topic:** E.03. Basal Ganglia

**Support:** Barrow Neurological Foundation Grant

**Title:** Human basal ganglia recordings from fully internalized deep brain stimulation internal pulse generators reliably demonstrate movement-inhibition related increases in beta and theta power during the stop signal reaction time task.

**Authors: *B. ZAVALA**[1], S. FELSEN[1], M. OLSON[1], B. GREGER[2,1], Z. MIRZADEH[1], F. PONCE[1];
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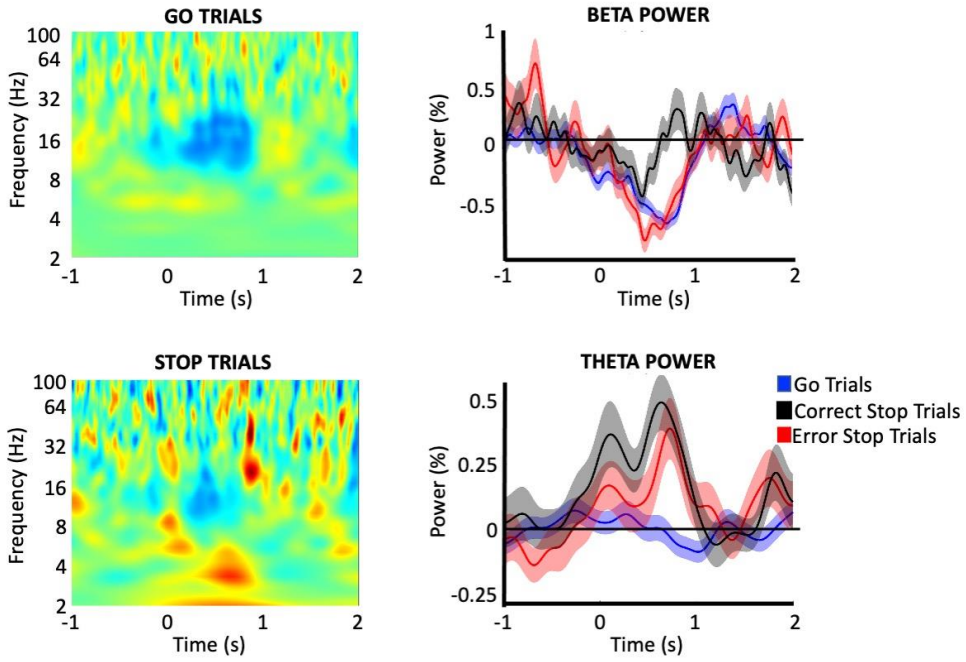
**Abstract:** Over the last 30 years, electrophysiological recordings from externalized deep brain stimulation (DBS) electrodes have greatly increase our understanding of neuronal oscillations in humans. Given that these recordings were previously limited to human subjects with electrodes protruding from their scalp, participation in these studies has generally been limited to the intraoperative setting or to inpatients admitted to large academic centers. The newest generation

of internal pulse generators (IPGs) have the potential to greatly expand access to electrophysiological recordings due to their ability to record data even when the entire system is fully internalized in a patient's body. If indeed these new tools are to reach their potential, however, they must first be shown to reliably reproduce known neurological biomarkers on and off stimulation. We recorded from 10 patients implanted with the Percept IPG (Medtronic) while they engaged in the well-established stop signal reaction time task. By sending 3 DBS pulses every 5 minutes and recording these pulses with EKG electrodes, we synchronized the electrophysiology from the brain to the behavioral task. We show that patients exhibited the classic movement-related decrease in beta power, as well as rapid increases in beta power during successful inhibition trials. We also demonstrate an increase in theta power during successful and unsuccessful response inhibition. Crucially, these signals remained present during DBS current delivery. Finally, the "On" stimulation recordings were more robust in patients implanted with the newest generation of DBS electrodes (SenSight, Medtronic). As the indications for DBS expand to include novel targets for new patient populations, it is as important as ever to study electrophysiological recordings from patients if we are to truly understand how therapeutic stimulation alters the brain. Here, we have demonstrated that commercially available fully implanted IPGs have the potential to dramatically increase access to these recordings for clinical and scientific purposes.

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**Topic:** E.03. Basal Ganglia

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**Title:** Neurometric determinants of gait variability: the influence of visuospatial processing and neurochemical signatures in basal ganglia

**Authors: *E. OFORI**[1], M. ORTEGA[2], S. SUN[1], M. HOOTEN[2], A. MOORE[2], A. SOLIS[3]; [1]Arizona State Univ., PHOENIX, AZ; [2]Arizona State Univ., Phoenix, AZ; [3]Univ. of Texas El Paso, El Paso, TX

**Abstract:** The complex relationship between cognitive processes and motor function remains underexplored. While gait variability has been associated with various neurological and geriatric conditions, the contributing cognitive elements and neurochemical factors are still largely unknown. We aimed to address this gap, focusing specifically on the role of visuospatial processing as assessed by NeuroTrax scores, and the associated neurochemical markers in the basal ganglia, as potential influencers of gait variability. We conducted a multimodal imaging study utilizing a cohort of 15 healthy older adults and 15 older adults with subjective memory complaints of diverse ages and both sexes. Data was collected through NeuroTrax, a standardized cognitive test battery, and gait was assessed under different conditions. Magnetic resonance spectroscopy (MRS) was employed to probe basal ganglia metabolites (Choline and Myo-inositol), and diffusion tensor imaging (DTI) was used to calculate free-water metrics. Our results demonstrated significant correlations between visuospatial processing scores and gait variability. More specifically, reduced visuospatial scores were associated with increased gait variability. Concurrently, alterations in Choline and Myo-inositol levels in the basal ganglia, as well as changes in free-water metrics, showed a strong association with both visuospatial processing scores and gait variability**.** Our study provides preliminary evidence linking cognitive processing, specifically visuospatial abilities, with gait variability, highlighting the potential underpinnings of various neurological disorders. Furthermore, it uncovers the essential role of neurochemical alterations in the basal ganglia, indicating a complex interplay between cognition, motor function, and underlying neurochemistry. This research might have far-reaching implications, opening new avenues for diagnosis and therapeutic intervention in neurodegenerative disorders affecting gait and cognitive function.

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**Title:** The coordinated dynamics of D1R and D2R neurons in striatum encode and modulate specific fine actions of the forelimb

**Authors: *V. R. ATHALYE**, I. RODRIGUES-VAZ, D. S. PETERKA, R. M. COSTA; Zuckerman Inst. for Mind, Brain, and Behavior, Columbia Univ., New York City, NY

**Abstract:** As animals interact with their environment, they must execute the appropriate action in order to achieve a desired outcome. While past work has identified that the striatum is critical for learning which action is appropriate based on reinforcement, the role that striatum plays in actually generating the appropriate action has been less studied. The concomitant activation of two subpopulations in the striatum (D1 receptor-expressing (D1R) striatonigral neurons and D2 receptor-expressing (D2R) striatopallidal neurons) encodes full-body movements, such as rearing, turning, and lever pressing. However, it is unclear if the striatum could encode and modulate specific forces and muscle patterns in the same body part, such as the forelimb. To test this, we designed a behavioral task in which head-fixed mice performed two forelimb actions consisting of a push or pull force on an immobile joystick without overt limb movement. We used 2-photon microscopy to image calcium activity (via GCaMP6f) of ~100 D1R and D2R neurons simultaneously in dorsolateral striatum through a GRIN lens. Notably, individual neurons divided into action-specific groups, with activity that was more correlated with a specific action's force on single trials. Population activity predicted which action was performed (through a linear Support Vector Machine classifier) as well as the action's force amplitude (through a linear regression model). Force amplitude was encoded in an action-specific manner, as a regression model trained to predict one action's force did not generalize to the other action, and nearly orthogonal dimensions of population activity encoded action-specific force. To analyze the coordination of the D1R and D2R subpopulations, we introduced a variant of Factor Analysis designed to analyze two subpopulations and which identified a low-dimensional latent state that captured how D1R and D2R neurons co-activated. A classifier that decoded the Factor Analysis latent state could predict action stably across days, revealing stable ensembles composed of both D1R and D2R neurons that encoded specific actions. To test their function, we developed a system to target 2-photon stimulation to action-specific ensembles through the GRIN lens, triggered in closed-loop when mice self-initiated an action that crossed a low force threshold. Our preliminary results (10 animal-sessions across 4 animals) showed that activating an ensemble specific to one action increased the force amplitude of that action but decreased the amplitude of the other action. Altogether, these results indicate that striatal ensembles encode and modulate action-specific forces during fine forelimb actions.

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**Title:** Direct striatopallidal pathways to thalamus and brainstem via the external globus pallidus differentially impact behavior

**Authors: *Z. GU**[1], J. TANG[1], A. MENDELSOHN[1], M. VICENTE[1], L. NIKOOBAKHT[1], S. ROSENBERG[1], J. LI[1], A. CHAKRAVARTHY[1], L. HAMMOND[1], E. THOMAS[2], C. RIMORIN[2], M. TIEU[2], D. BERTAGNOLLI[2], J. GOLDY[2], K. SMITH[2], B. TASIC[2], D. S. PETERKA[1], R. M. COSTA[2];

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**Abstract:** The external globus pallidus (GPe) is a key component of the basal ganglia system, yet its neuronal heterogeneity and contribution to action control have remained elusive. In the classical model of basal ganglia function, the GPe comprises a single, homogenous population of neurons that serves as a simple relay in the striatopallidal pathway to inhibit movements. Using a combination of anterograde and retrograde tracings, we first found that GPe neurons are highly heterogeneous: different subsets of GPe have distinct projection patterns, including previously uncharacterized subpopulations that project to the thalamus and brainstem directly. Second, using single-cell RNA sequencing, we characterized the neuronal diversity of GPe and found that parafascicular thalamic nucleus-projecting (GPe→Pf) and pedunculopontine nucleus-projecting (GPe→PPN) GPe neurons originate from different clusters of GPe neurons. Third, axon-specific calcium imaging demonstrated that the activity of $GPe^{Pf}$ and $GPe^{PPN}$ are differentially modulated, such that GPe→Pf is strongly modulated during the performance of a learned forelimb action (lever press) while GPe→PPN is more modulated during locomotion. Third, optogenetic activation of GPe-PPN projection impeded locomotion, while manipulation of GPe→Pf had a strong effect on the execution of lever press. Therefore, our study provides anatomical, physiological, and functional evidence to support that GPe is an output nucleus of the basal ganglia, questioning the classical prokinetic versus antikinetic dichotomy of direct and indirect pathways. Our data strongly suggest a new model of basal ganglia function, in which striatopallidal pathway can either promote (via the novel GPe projections to thalamus and midbrain) or inhibit (via the classical subthalamic nucleus/STN to the internal segment of the globus pallidus/GPi and the substantia nigra pars reticulata/SNr) movements depending upon the output channel(s). Supporting this notion, we further demonstrated that optogenetic activation of GPe-PPN projection blocks locomotion evoked by striatopallidal pathway stimulation in an open field exploratory task. Together, our data suggest that the striatopallidal pathway can function as direct output pathways via GPe projections to the brainstem and thalamus to facilitate locomotion and learned action respectively.

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**Topic:** E.03. Basal Ganglia

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**Title:** The therapeutic effect and underlying mechanism of oral splint for ameliorating tic symptoms in patients with Tourette syndrome

**Authors: *Y. TACHIBANA**[1], H. KUNO[1], N. TSUJI[1], A. YOSHIDA[2], T. TAKUMI[1]; [1]Kobe Univ. Grad. Sch. of Med., Kobe, Japan; [2]Dept. of Systematic Anat. and Neurobio., Osaka Univ. Grad. Sch. of Dent., Suita, Japan

**Abstract:** The therapeutic effect and underlying mechanism of oral splint for ameliorating tic symptoms in patients with Tourette syndrome
Yoshihisa Tachibana, Natsumi Tsuji, Fumihiko Sato, Toru Takumi, Atsushi Yoshida
Tourette syndrome (TS) is a neurodevelopmental disorder characterized by chronic multiple motor and vocal tics. Many TS patients also have comorbidities, such as attention-deficit/hyperactivity disorder and obsessive–compulsive disorder. Traditionally, behavioral, pharmacological, and surgical interventions are applied to TS patients. In contrast, we have recently reported that motor and vocal tics in TS patients are ameliorated by a removable oral splint, which has been commonly used in the dental treatment of temporomandibular joint disorder (Murakami et al., Mov Disord, 2019). Then, our question is why the oral splint is effective for improving tic symptoms. To answer this question, we first investigated the ascending pathway of masticatory muscle spindles, which are activated by the insertion of oral splint. We injected an anterograde tracer into the supratrigeminal nucleus, known to receive the orofacial muscle spindles, in rats. Many thalamic axon terminals were labeled mainly in a small area of the caudo-ventromedial edge of ventral posteromedial thalamic nucleus (VPMcvm). After the anterograde tracer injection into the VPMcvm, we further found that the orofacial proprioceptive inputs were finally transmitted to the dorsal part of granular insular cortex. The next question is why the modulation of orofacial proprioceptive inputs to the insula ameliorates tic symptoms. The abnormal activity in the striatum and insula has been reported in TS patients using imaging studies (Bohlhalter et al., Brain, 2006; Lerner et al., Neurology, 2007). On the other hand, the disinhibition of the striatum by local injection of GABA antagonist bicuculline induces tic-like symptoms in rodents (Bronfeld et al., Front Syst Neurosci, 2013) and primates (McCairn et al., Brain, 2009). We thus unilaterally injected the bicuculline into the motor region of mouse striatum, and successfully induced motor tic in the contralateral hand and face depending on injection sites. Then we investigated the activated brain regions by striatal disinhibition using c-Fos immunohistochemistry. We found that the insular cortex was activated although the structure does not receive direct inputs from the striatum. To summarize these data, we speculate that the neuromodulation of the insular activity triggered by inserting the oral splint may normalize the abnormal information processing in the motor (striatum)-limbic (insula) network observed in TS patients.

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**Title:** Using the novel genetically encoded biosensor iGABASnFR2.0 to investigate GABA dynamics in substantia nigra pars reticularis (SNr) upon modulation of direct pathway activity

**Authors: *S. NØRR**[1,2], B. J. HALL[2], U. GETHER[1], G. SØRENSEN[2], M. RICKHAG[1];
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**Abstract:** The coordinated activity of the direct pathway spiny projection neurons (dSPNs) and the indirect pathway SPNs (iSPNs) is a key system enabling orchestrated and efficient motor behavior selection in vertebrates. The action selection process takes place in the striatum (Str) and is governed by a complex interplay of different neurotransmitters, which is integrated by the SPNs into a binary firing rate. Here, we measured, *in vivo*, the resulting activity of dSPN related GABA dynamics in the output nucleus of the basal ganglia, the Substantia Nigra reticularis (SNr). For the measurements, we used a genetically encoded GABA sensor together with fiber photometry to show that perturbation of the dSPN/iSPN balance profoundly affect SNr GABA dynamics. 18 mice (9 DRD1-Cre and 9 WT littermates) were injected unilaterally with an adeno-associated virus (AAV) carrying the new ionotropic GABA biosensor iGABASnFR2.0 in SNr and a double-floxed Gq-DREADD in dorsal striatum (dStr) enabling selective chemogenetic activation of dSPNs in DR1D-cre mice. The mice were placed in an open field arena with video-based motor tracking and fiber-photometric assessment of SNr GABA dynamics for 2h45min. The mice were during this period sequentially pharmacologically challenged with a chemogenetic ligand (deschloroclozapine {DCZ}), a D1 agonist (SKF38393) and a D1 antagonist (SCH23390). Using a combination of canonical electrophysiology metrics and an unsupervised machine learning approach to describe SNr GABA dynamics, we obtained preliminary evidence that all treatment induced state transitions in SNr GABA dynamics, as well as abolishment or shifts in the temporal correlation between motor behavior and the SNr GABA signal. Summarized, the data show that SNr GABA dynamics, as measured with the novel GABA biosensor iGABASnFr2.0, is a viable and relevant metric when studying motor behavior in mice, that can offer unprecedented insight into the output dynamics of the basal ganglia and its correlation to motor behavior.

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**Presentation Number:** NANO56.09

**Topic:** E.03. Basal Ganglia

**Title:** Motor cortex responses and motor outcomes vary with beta phase during cortical phase-dependent stimulation in parkinson’s disease

**Authors: *Y. SALIMPOUR**[1], K. MILLS[2], W. ANDERSON[1];
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**Abstract:** Open-loop high-frequency stimulation is currently applied to subcortical structures for neuromodulation of the cortico-basal ganglia-thalamo-cortical network for Parkinson’s disease (PD). However, cortical neuromodulation of this network is limited by side effects and the inefficacy of high-frequency stimulation. Recently, the association between abnormal phase-amplitude coupling (PAC) in the motor cortex and Parkinson’s disease has been explored in PD patients as a biomarker of motor symptom severity. Current evidence suggests that stimulating the sensorimotor cortex with beta oscillations can induce short-term synaptic plasticity and the direction of the plasticity effect depends on the phase from which stimulation was triggered. Here, we measured the effects of cortical phase-dependent stimulation (PDS) on PD patients' network activities and motor functions during deep brain stimulation lead placement surgery.

During deep brain stimulation surgery for PD with 7 patients in the awake, unmedicated state, subdural high-density electrode strips were placed for ECoG recording and stimulation. We applied stimulation pulses triggered by specific phases of the beta oscillations of the motor cortex at rest and during a motor task with hand, position captured using two infrared light projectors and infrared cameras. Changes in hand position were analyzed as the velocity of movement. Each trial of the motor task has three temporal sequences including the rest phase, prepare, and go phases. We measured the effect of peak- or trough-targeted stimulation pulses on the temporal dynamics of the motor cortex, PAC modulation index in the motor cortex, and the dynamics of hand movements relative to the motor task temporal phases. Our results demonstrate that stimulation locked to the phase of the peak of beta increased beta power, and beta-gamma coupling, reduced the velocity of hand movement, and increase the severity of the motor symptoms during the execution phase. However, the opposite phase (trough) stimulation trended toward reducing the magnitude of the beta power, and beta-gamma coupling in the preparation phase, and increased the velocity of hand motion, reduction of the motor symptoms in the execution phase of the task. Our findings are in line with the idea that cortical beta oscillations provide a spatial and temporal substrate for short-term, activity-dependent synaptic plasticity. In conclusion, our results demonstrate the capacity of the motor cortex PDS to modulate oscillopathy signatures and alter the severity of the motor symptoms, allowing the targeting of cortical network nodes in the treatment of network-based brain disorders such as PD.

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**Presentation Number:** NANO56.10

**Topic:** E.03. Basal Ganglia

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**Title:** Triple-color fiber photometry recording of dopamine, glutamate and striatal neural activity

**Authors: *J. ZHOU**[1], G. CUI[2];
[1]NIEHS, Durham, NC; [2]NIH/NIEHS, NIH/NIEHS, Rtp, NC

**Abstract:** The striatum is the largest nucleus and the hub of the basal ganglia that integrates glutamatergic inputs from the cortex and the thalamus, and dopaminergic input from the substantia nigra pars compacta. It has been hypothesized that glutamate is the main driving force to excite striatal neurons and that dopamine differentially regulates the activities of striatonigral and striatopallidal spiny projection neurons (SPNs). To directly test this hypothesis, we used spectrally resolved fiber photometry and genetically encoded fluorescent indicators GRAB-DA2m (green), iGluSnFR3 (yellow) and jRGECO1a (red) to simultaneously measure the striatal dopamine and glutamate release and the neural activities of striatonigral or striatopallidal SPNs in D1-Cre or A2A-Cre mice. We show that the closely overlapped fluorescence emission spectra of GRAB-DA2m and iGluSnFR3 can be acutely extracted from the recorded spectra by linear spectral unmixing. These results show that spectral unmixing using spectrally resolved fiber photometry is a powerful tool to investigate the interactions between multiple elements in a brain circuit.

**Disclosures: J. Zhou:** None. **G. Cui:** None.

**Nanosymposium**

**NANO57: Circuit Mechanisms of Reward Processing and Feeding**

**Location:** WCC 147B

**Time:** Tuesday, November 14, 2023, 8:00 AM - 10:45 AM

**Presentation Number:** NANO57.01

**Topic:** G.02. Reward and Appetitive Learning and Memory

**Title:** Transformation of value signaling in a striatopallidal circuit

**Authors: *D. LEE**[1], L. LIU[2], C. M. ROOT[2];
[1]UCSD, San Diego, CA; [2]Univ. of California San Diego, La Jolla, CA

**Abstract:** The ways in which sensory stimuli acquire motivational valence through reward-association is one of the simplest forms of learning. Though we have identified many brain nuclei that play various roles in reward processing, a significant gap remains in understanding how valence encoding transforms through the layers of sensory processing. To address this gap, we carried out a comparative investigation of the olfactory tubercle (OT), and the ventral pallidum (VP)- 2 connected nuclei of the basal ganglia which have both been implicated in reward processing. First, using anterograde and retrograde tracing, we show that both D1 and D2 neurons of the OT project primarily to the VP and minimally elsewhere. Using 2-photon calcium imaging, we then investigated how the identity of the odor and the reward contingency of the odor are differently encoded by neurons in either structure during a classical conditioning paradigm. We find that VP neurons robustly encode value, but not identity, in low-dimensional space. In contrast, OT neurons primarily encode odor identity in high-dimensional space. Finally, using a novel conditioning paradigm that decouples reward contingency and licking, we show that both features are encoded by non-overlapping VP neurons. These results provide a novel framework for the striatopallidal circuit in which a high-dimensional encoding of stimulus identity is collapsed onto a low-dimensional encoding of motivational valence.

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**Title:** Genetically encoded GRAB sensors reveal the specific roles of serotonin and octopamine in olfactory associative learning

**Authors: *X. LI**[1,2], M. LV[1,2], J. ZENG[3], Z. ZHANGREN[1], Y. LI[1,3,2];
[1]Peking Univ., Beijing, China; [2]PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; [3]Inst. of Mol. Physiol., Shenzhen Bay Lab., Shenzhen, China

**Abstract:** Aminergic signals play critical roles in modulating learning and memory across species. In *Drosophila*, the mushroom body (MB), which is the olfactory learning center, receives heavy innervation from neuropils that release dopamine (DA), serotonin (5-HT), and octopamine (OA). Although the role of DA in encoding the unconditioned stimulus (US) has been well-established, the functions and dynamics of 5-HT and OA are still unclear. Despite recent connectomics studies of the related monoaminergic neurons, their functional input and output relationships in the complex neural circuitry remain elusive. To address these fundamental questions, we developed GPCR activation-based (GRAB) fluorescent sensors, namely, $GRAB_{5\text{-}HT1.0}$ and $GRAB_{OA1.0}$, and applied them to the MB to explore the physiological functions of 5-HT and OA. By performing *in vivo* two-photon imaging, we found that both odor (conditioned stimulus, CS) and electric shock (US) evoke time-locked and compartmental 5-HT release from the dorsal paired medial (DPM) neuron. This 5-HT signal is elicited by acetylcholine (ACh) from Kenyon cells (KCs), and the 5-HT, in turn, feeds back to KCs to inhibit the tone of ACh. Interestingly, we found that this 5-HT signal bidirectionally regulates the coincidence time window of odor-shock pairing for inducing synaptic depression in the MB and achieving associative learning. Unlike the spatially heterogeneous pattern of the 5-HT signal, the odor- or shock-evoked OA signal is relatively homogeneous in the MB. This OA signal is also activated by ACh from KCs, and it mainly targets the dopaminergic (DANs) to amplify the US-induced DA release. We found that this octopaminergic regulation facilitates changes in synaptic plasticity and learning behavior. In summary, this study employed new technologies to elucidate the dynamics of 5-HT and OA in the MB with high spatiotemporal resolution and revealed their critical functions during olfactory learning.

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**Title:** Serotonin predictively encodes value

**Authors: *E. F. HARKIN**, J.-C. BÉÏQUE, R. NAUD;
Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

**Abstract:** The *in vivo* responses of dorsal raphe nucleus (DRN) serotonin neurons to emotionally-salient stimuli are a puzzle. They are phasically activated by both rewards and punishments, they prefer rewards that are surprising over those that are predicted but have no such preference for punishments, and their tonic activity is modulated by reward and punishment context (Cohen et al., Elife, 2015; Matias et al., Elife, 2017; Paquelet et al., Neuron, 2022). Several competing reinforcement-learning- and predictive-coding-inspired qualitative theories have been proposed to explain different aspects of the activity patterns of serotonin neurons, but

none account for all three of the tuning features listed above. By combining reinforcement learning and predictive coding in a single quantitative model, we find a consistent explanation in the amount of reward associated with the current state, a quantity called value in reinforcement learning theory, transformed by spike frequency adaptation. Through simulations of trace conditioning experiments common in the serotonin literature, we show that adapting value accounts for phasic activation of serotonin neurons by rewards while also explaining activation by punishments. Similarly, the model accounts for the preference of serotonin neurons for surprising rewards, while explaining why this preference does not apply to punishments. Finally, as the model applies to both phasic and tonic activity, it also explains slow modulation of tonic activity by reward and punishment context. An adapting value code explains and generalizes the observed responses of serotonin neurons to emotionally-salient stimuli, offering specific predictions that can be tested through new experiments and/or analysis. To test two of the main predictions of our theory, we re-analyzed a recently-published dataset of *in vivo* tetrode recordings of genetically-identified serotonin neurons collected from mice learning to associate an odour with a water reward (Cohen et al., Dryad, 2021). We found that individual serotonin neurons and serotonergic populations exhibited both within-trial cue- and reward-associated firing activity dynamics and between-trial reward-history-associated activity modulation consistent with our theory. Explicitly comparing against quantitative formulations of previous qualitative theories shows that adapting value provides the best description of both aspects of activity by a large margin. Thus, our adapting value theory resolves apparent conflicts in the interpretation of serotonin neuron responses to emotionally-salient stimuli, a key step in understanding the role of serotonergic signaling in the brain.

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**Title:** Cerebellar spatiotemporal coding for orchestrating dopamine signals in the midbrain reward system

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**Abstract:** Reward-based learning is a fundamental mechanism for optimizing behavioral responses to the outside world. Mounting evidence has linked the cerebellar contribution to the midbrain ventral tegmental area (VTA) dopamine system, which directly regulates rewarding behaviors. However, the precise neuronal coding of cerebellum-to-VTA activity and its regulation of reward-based behaviors remains unclear. Here, we report that neurons in the deep cerebellar nuclei (DCN) represent reward-related information with a specific spatial-temporal organization and causally modulate VTA reward coding and reward-driven behaviors. Using single-photon miniature microscopy to image calcium activity and *in vivo* electrophysiology recording in a two-choice reward task in mice, we observed a higher spatial coherence of DCN neuronal ensembles during reward encoding, but not an increase in DCN single-unit firing rate. Dendritic calcium activity of cerebellar Purkinje cells also exhibited regional phasic synchronization within the two-photon imaging space. To further investigate the specific coding mechanism in the DCN-to-VTA pathway, we analyzed time-frequency features in local field potentials (LFPs) and performed fiber photometry recordings to monitor VTA-projecting DCN axonal calcium activity. We found that the low-frequency LFPs in the DCN and VTA strongly oscillated specifically during the reward encoding period, and reward information was carried by DCN axonal calcium activity. Using optogenetic approaches and direct DC current injection to manipulate the DCN-to-VTA projection, we found that reward-driven behaviors can be modulated by increasing the spatial coherence of DCN inputs, which perturbs VTA dopamine activity. Our results provide evidence that reward information is organized in the cerebellum through a spatial-temporal coding mechanism, supporting the view that cerebellar circuits encode dynamic reward-based cognitive processes in addition to traditional motion perspectives.

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**Presentation Number:** NANO57.05

**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** NIH Grant DK121883

**Title:** Motivational state-dependent control of reward seeking by an extended amygdala to lateral hypothalamic area pathway

**Authors: *M. A. ROSSI**, K. SHRIVASTAVA, V. ATHREYA;
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**Abstract:** The lateral hypothalamic area (LHA) is a critical mediator of motivated behavior, including feeding and reward-seeking. The LHA exerts control over behavior through functionally distinct cell types and diverse synaptic connectivity. Glutamatergic LHA neurons suppress food intake, and their activity is modified by fasting, feeding hormones, and diet-induced obesity. However, the circuit mechanisms by which changing motivational state guides LHA glutamate neuron activity are unknown. A major source of synaptic input to the LHA arises from GABAergic neurons of the bed nucleus of the stria terminalis (BNST), which preferentially target LHA glutamatergic neurons. Manipulations of this pathway influence feeding and reward behavior. We therefore hypothesized that input from the BNST contributes to changes in LHA

glutamate neuron activity that promote reward seeking according to current energy demands. Using optogenetic perturbations, we show that the function of the BNST-LHA pathway is sensitive to changes in motivational state (fasting and over-feeding). Ex vivo electrophysiology confirms that this is caused by tuning synaptic strength. By combining in vivo multiphoton imaging with optogenetic perturbation, we demonstrate that LHA glutamate neurons exhibit prolonged inhibition in response to BNST input activation. The extent of inhibition depends on motivational state and predicts reward seeking. Together, these results suggest one way by which the activity of lateral hypothalamic glutamate neurons is modified by presynaptic neurons to orchestrate situationally appropriate feeding behavior.

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**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** NRF Grant 2021R1A6A3A13042965

**Title:** Behavioral and Molecular Profile of Lateral Hypothalamic Leptin Recepter Expressing Neurons

**Authors: *Y. LEE**, K. PARK, Y.-B. KIM, K. KIM, M. JEON, J. JANG, G. RYU, S. LEE, J.-I. KIM, H. CHOI;
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**Abstract:** For survival, it is crucial for eating behaviours to be sequenced through two distinct seeking and consummatory phases. Heterogeneous lateral hypothalamus (LH) neurons are known to regulate motivated behaviours, yet which subpopulation drives food seeking and consummatory behaviours have not been fully addressed. Micro-endoscope recording of the LH LepR neurons demonstrated that one subpopulation is time-locked to seeking behaviours and the other subpopulation time-locked to consummatory behaviours. Behavioural dependent neuronal labeling elucidated neural circuit mechanisms of LH LepR food-seeking and consummatory subpopulations. Further, molecular profiles of LH LepR subpopulations are analyzed by single nucleus RNA sequencing and spatial transcriptomics. This result provides crucial scientific foundation for the development of novel pharmaceuticals tailored to specific eating behaviors for appetite and obesity.

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**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** NIH Grant R01MH112739 (NIMH)

**Title:** Parallel genetic strategies to access transcriptionally distinct melanin-concentrating hormone neuron subpopulations in the lateral hypothalamus

**Authors: *M. ANTONY**, A. FUJITA, A. C. JACKSON;
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**Abstract:** The lateral hypothalamic area (LHA) coordinates crucial innate behaviors through diverse, yet poorly understood neuronal populations. Melanin-concentrating hormone (MCH; encoded by *Pmch*)-expressing neurons, comprise a distinct cell population in the LHA and are key contributors to this complex physiological control. In rodents, excitation of MCH neurons lead to increases in both REM sleep and increases in wake-related behaviors, such as feeding, learning, and reward. Given its contradictory role in promoting wake and sleep related processes, it is hypothesized that MCH neurons are functionally and molecularly heterogeneous. Building upon foundational anatomical and developmental studies, our single cell transcriptomic analysis of the LHA described a large set of discriminatory markers that differentiate MCH neuron subpopulations. One of these is the neurokinin-3 receptor of the tachykinin family (NK3R; encoded by *Tacr3*), which is expressed robustly in one MCH subpopulation but not the other ($MCH^{Tacr3+}$ and $MCH^{Tacr3-}$ neurons). However, further exploration of MCH subpopulations has been limited by available genetic tools, as these subpopulations are spatially intermixed within the LHA. We hypothesize that MCH subpopulations operate through functionally distinct, parallel subcircuits that exhibit unique neuroanatomical projections, and that *Tacr3* expression may be leveraged to dissociate these subcircuits. Our project has defined a multi-pronged approach to gain genetic access to MCH subpopulations and their neuroanatomical projection targets through use of novel mouse lines, cell ablation strategies, and recombinase-specific viruses. We first demonstrate the cell-type specificity in the proposed mouse lines and ablation reagents through fluorescence *in situ* hybridization and immunohistochemical techniques, quantified through an unbiased cell counting pipelines. After validation, we use ablation and viral tools to specifically ablate $MCH^{Tacr3+}$ neurons and target $MCH^{Tacr3-}$ axonal projections. In parallel, we have utilized an intersectional genetic strategy to access the $MCH^{Tacr3+}$ subpopulation. Using these approaches, we have identified unique regions of the brain that are enriched in axons from specific MCH subpopulations. Ultimately, we aim to characterize the unique anatomical properties of MCH subcircuits to further our understanding of circuit-level synaptic and behavioral mechanisms mediated by transcriptionally distinct MCH subpopulations.

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**Presentation Number:** NANO57.08

**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** CF-2023-G-518

**Title:** The Neuroscience of Hedonic Feeding: Role of the Lateral Hypothalamus

**Authors: *R. GUTIERREZ**;
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**Abstract:** Overconsumption of palatable food is one major factor in developing obesity. In this talk, you will learn about the lateral hypothalamus's critical role in controlling hedonic feeding. We propose that GABAergic and glutamatergic neurons play opposite and bidirectional roles in reward and feeding behaviors. In particular, GABAergic neurons are highly responsive to the nearest and most palatable food available, especially sugars and fats. After exposure to a high-fat

diet during childhood and adolescence, a subset of GABAergic neurons is hypersensitized to sugars in mice. These neuronal adaptations may contribute to the overconsumption of palatable foods observed in obesity.

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**Topic:** G.02. Reward and Appetitive Learning and Memory

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**Title:** Glp-1 signaling in the hypothalamic-brain stem descending circuit regulates energy homeostasis

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**Abstract:** Central nervous system (CNS) control of metabolism plays a pivotal role in maintaining energy homeostasis. Glucagon-like peptide 1 (GLP-1, encoded by *Gcg*), secreted by a distinct population of neurons located within the Nucleus Tractus solitarius, suppresses feeding. Central and peripheral GLP-1 work independently to suppress feeding. However, *the cellular and circuit mechanisms mediating endogenous GLP-1 action in the CNS are still poorly understood, mainly due to* diverse neuronal subtypes and complex central neuronal connectivity. We previously found that NTS GLP-1 projection to the paraventricular hypothalamic nucleus (PVN) enhances glutamatergic synaptic transmission, which is sufficient to suppress food intake, and ablation of PVN GLP-1R causes overeating and obesity. Here we investigate the impact of PVN GLP-1R neuronal descending neuronal ensemble, particularly the brain stem dorsal vagal complex (DVC), in energy homeostasis. Using multiple anterograde pathways tracing, we found PVN GLP-1R neurons form synapses with DVC neurons release glutamate and are regulated by GLP-1 presynaptically. Using retrograde Patch-Seq, we revealed the complex neuronal identity of PVN GLP-1R neurons, including oxytocinergic and non-oxytocinergic vGlut2/GLP-1R neurons. Chemogenetic activation of the PVN-to-DVC GLP-1R circuit is sufficient to suppress food intake in different motivational states Loss-of-function studies by blocking the PVN GLP-1R-to-DVC synaptic release or ablation of GLP-1R in the PVN-to-DVC projecting neurons increases food intake and cause type 2 diabetes, including obesity, elevated blood glucose, and deficits in glucose metabolism. These findings suggest that GLP-1 signaling regulates the PVN-to-brain stem descending circuit and regulates energy homeostasis.

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**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** NHLBI Fellowship F31HL165896
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**Title:** Characterization of cell type-specific synaptic protein expression and function in hypothalamic arousal neurons

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[1]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Biol., Williams Col., Williamstown, MA; [3]Neurosci., UConn Hlth., Farmington, CT

**Abstract:** Hypocretin/orexin (H/OX) is an excitatory neuropeptide expressed exclusively in a subpopulation of neurons within the lateral hypothalamic area (LHA), which are critical regulators of the wake state. Across vertebrate phylogeny, H/OX neuron dysfunction results in defects in sleep-wake architecture. In humans, loss of H/OX signaling results in the sleep disorder narcolepsy, which is characterized by excessive daytime sleepiness, fragmented sleep and, in the most severe cases, cataplexy. Despite a large body of work describing the circuitry and behavioral role of H/OX neurons, little is known about the molecular building blocks that comprise their wake-promoting excitatory synapses. Through single-cell RNA sequencing, we identified a suite of molecular markers that, within the LHA, are uniquely and robustly expressed in H/OX neurons. One of these markers is *C1ql3*, a transcript encoding complement component 1q-like protein 3 (C1QL3), which in other regions of the brain is known to be an important synaptic organizing protein. We hypothesize that C1QL3 may also be crucial in the integrity of H/OX excitatory synapses and contribute to their role in the regulation of sleep-wake states. To address this hypothesis, we first confirmed *C1ql3* mRNA expression in virtually all H/OX neurons using fluorescence *in situ* hybridization (FISH) in both male and female adult mice. We likewise demonstrated C1QL3 protein localization in H/OX soma and axons through immunohistochemistry. Next, we conditionally knocked out (cKO) *C1ql3* from H/OX neurons by virally expressing Cre recombinase in the LHA of *C1ql3*$^{flox/flox}$-mVenus mice. We found that, compared to uninjected littermate controls, *C1ql3* H/OX-cKO mice had significantly diminished H/OX-IR puncta in the locus coeruleus (LC), an important wake-promoting target of H/OX neurons. This is consistent with previous work in which *C1ql3* cKO in other regions of the brain resulted in diminished excitatory synapses formed onto targets. To determine if this diminished connectivity resulted in behavioral deficits, we carried out EEG/EMG recordings of *C1ql3* H/OX-cKO mice and, following blinded scoring, found several signatures of H/OX dysfunction in their sleep-wake architecture as compared with controls. These results support our hypothesis that C1QL3 may be an important regulator of both H/OX synaptic function and sleep-wake behavior.

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**Title:** Source and percentage of high-fat diet differentially impacts food-choice related motivational drives

**Authors: *A. SUTTON HICKEY**[1], E. KAROLCZAK[2], J. J. BECKER[2], S. C. DUANE[2], C. LI[2], M. J. KRASHES[2];
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**Abstract:** Industrial food manufacturing, while originally designed to increase the shelf life of foods, has increased worldwide in the past century and coincides with increases in a variety of dysregulated feeding diseases (e.g., Binge-Eating Disorder (BED)). While still poorly understood, studies suggest that varying ingredients of processed foods are a contributing factor to this increase in dysregulated feeding. In particular, the elevated fat content prevalent in processed foods has been identified as a potential cause of the food's addictive characteristics. Indeed, recent studies from our research group and others demonstrate that exposure of high-fat diet (HFD) promotes devaluation of nutritionally balanced standard chow in mice, and that this process is mediated, at least in part, by hunger-promoting agouti-related peptide (AgRP) neurons. Yet, it is unknown if this devaluation is based on a particular percentage of fat in the diet, and/or how varying types of fat impact standard diet (SD) devaluation. To test this, we performed a combination of acute and longitudinal feeding and behavioral assays with exposure to varying diet compositions. We paired these experiments with *in vivo* fiber photometry to measure if AgRP population activity is impacted by varying levels of HFD access. Our results indicate that SD devaluation, and AgRP activity, is contingent on both fat content and composition. Future studies intend to identify how artificial activation of AgRP neurons alters HFD intake in first vs. repeated exposure. Our studies indicate that HFD content and composition alter SD intake and hypothalamic hunger neurons, and thus suggest that this could underlie some of the food addictive behaviors observed in individuals experiencing dysregulated feeding diseases.

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**Nanosymposium**

**NANO58: Computational Models for Decision-Making**

**Location:** WCC 144

**Time:** Tuesday, November 14, 2023, 8:00 AM - 11:15 AM

**Presentation Number:** NANO58.01

**Topic:** H.03. Decision Making

**Title:** Neurocomputational investigation of human schema-based learning, decision making and their modulators in ecological settings

**Authors:** J. CHEN[1], A. PADANYI[2], Y. SHANG[3], L. HAMERSZTEIN[5], H. ZHU[6], X. SHAO[3], H. LI[7], S. LIANG[5], J. ZHENG[3], J. SHEN[8], Z. HAN[9], C. DU[10], Z. YU[4], L. NGUYEN[5], R. HILL[5], D. TSE[11], ***G. LUKSYS**[5];
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**Abstract:** Schemas are known to facilitate learning and memory consolidation in humans and rodents. However, their role in decision making is not so well known. Besides, the effects of task factors like risk, novelty, prior knowledge, arousal and attention on this process are under-explored. A new computer-based experiment was designed and performed where participants could firstly explore a list of different author's paintings or quotes and then select items with the same authorship. To investigate the effect of risk, although a large bonus is delivered if they can find all items from the same author, only "low risk" participants receive a small bonus if they can identify three out of four. Items from new authors are introduced half-way through the experiment to study the effect of novelty. Questionnaires of background information and participants' eye movements, pupillometry and heart rates were also collected to discover other modulators of this process. Most participants showed improvement in performance, yet factors, including previous progress, risk and novelty conditions, pupil diameter and heart rate, prior knowledge, English proficiency and task type affected different aspects of their performance. Previous progress in the task encouraged the participants switch from choosing low payoff items to high payoff items, leading to further improvement. Such improvement and high task difficulty also led to decreasing attention and arousal levels, suggesting a possible interaction between these modulatory factors. Eye tracking analysis revealed that participants firstly screened all items to choose a schema, and then selected the matching items, shown by the difference in certain metrics, like response times or numbers of observed items. Inspired by those results, a computational model based on reinforcement learning and drift diffusion processes was designed. In our model, choice options are integrated and selected based on schema confidence (which is affected by exploration and performance feedback of that schema), payoffs for different schemas, and attentional factors. The model simulation replicated the observed patterns in choice behaviour and various cognitive measures. Using model fitting, we investigate which cognitive components are influenced by those modulators. We show that schemas assist learning and decision making with the modulation of individual differences, biomarker levels and environmental factors, which interact in a complex manner.

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**Presentation Number:** NANO58.02

**Topic:** H.03. Decision Making

**Title:** Different sources of noise under divisive normalization explain opposing contextual modulation effects in value-based decision-making

**Authors: *B. SHEN**[1], D. NGUYEN[1], J. WILSON[3], K. LOUIE[2], P. W. GLIMCHER[1];
[1]Neurosci. Institute, Sch. of Med., [2]Ctr. for Neural Sci., New York Univ., New York, NY;
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**Abstract:** Objective: Cognitive noise is ubiquitous in decision-making. It remains unknown whether different sources of noise have different effects on value coding and choice behavior. Here we study two types of noise: noise in value representation and noise in option selection. We examine the impact of noise on context-dependent choice behavior; the impact of a third option on the choice between the other two options. Methods: We employed behavioral testing, theoretical simulations, and neural imaging (fMRI) to study how noise impacts context-dependent behavior. In each trial of the behavioral study ($N$ = 57), the participants chose between three different consumer goods. The third item was either familiar to the subject (low *representation* noise) or relatively unfamiliar (high *representation* noise). Low and high time pressure was set over blocks to induce low and high *selection* noise. In the simulations, we assumed representation noise added to option-value before normalization. Selection noise was added after normalization. Normalization was implemented in a divisive manner. Option value was normalized by the summed value in the choice set. In the fMRI study, we scanned 10 subjects on a similar trinary choice task (an additional 40 subjects are planned). Results: Behavioral data shows that higher representation noise produces a positive contextual modulation: choice accuracy between two target items significantly increases with the third item's value. In contrast, higher selection noise produces a negative contextual modulation. Simulation explains both behavioral findings: increasing the third item value under high representation noise enhances Signal-to-noise-ratio (SNR) thus facilitating choice accuracy; whereas increasing the third item value under high selection noise reduces SNR thus impairing choice accuracy. fMRI data shows that the BOLD signal in the ventral striatum negatively codes the interaction term between the third item's value and the targets' values, suggesting a contextual inhibition regardless of representation or selection noise. Interestingly, the BOLD signal exhibits larger noise when the representation noise of the items is higher, consistent with divisive normalization. Conclusion: We show that a contextual item can facilitate or impair choice accuracy depending on the source of cognitive noise, reconciling controversies in the literature about the direction of contextual effects. These findings support divisively normalized value coding with two types of noises as a novel computational mechanism of contextual value coding.

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**Presentation Number:** NANO58.03

**Topic:** H.03. Decision Making

**Title:** Allocating attention for information gain during decision making

**Authors: *J. GOTTLIEB**, J. D. DOMINGUEZ-ZAMORA, G. P. HORGA;
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**Abstract:** To make adaptive decisions in complex natural settings, we must not only commit to an action but also determine which information to gather for guiding that action. Eye movements and selective attention sample decision-relevant information and, according to Bayesian theory, should be controlled to maximize expected information gains (EIG). In turn, EIG depends on the integration of diagnosticity (e.g., the predictive validity of a visual feature or location) and prior uncertainty (e.g., the decision maker's uncertainty about the optimal choice). Monkey parietal neurons were shown to encode diagnosticity and prior uncertainty, but a key question is how these quantities are integrated. To examine this question, we used a task in which humans inferred the identity of a hidden state (a jar containing mostly upright or mostly inverted letter T's) after viewing two evidence samples (two letters randomly drawn from the jar). We measured the participants' d' for discriminating the 2nd letter orientation to infer how they allocated attention to it based on diagnosticity and decision uncertainty. Consistent with Bayesian EIG, d' was biased toward the expected location of a high versus low-diagnosticity 2nd letter (hiD vs loD) and, consistent with the role of the fronto parietal network in proactive spatial attention control, multivariate analyses produced above-chance decoding of the hiD location (right vs left hemifield) from areas V3, IPS and FEF. Crucially, the d' bias produced by diagnosticity increased under higher uncertainty, providing direct evidence for the integration of these quantities. This integration was specifically encoded by areas V3 and IPS in the left hemisphere, which showed more accurate decoding of the hiD location under high versus low decision uncertainty, with the strength of the interactions corresponding with individual d' sensitivity. Psychophysiological interaction analyses revealed that functional connectivity between an uncertainty-encoding region of the ACC and the left V3 was significantly modulated by uncertainty. The findings suggest that uncertainty information reaches the FP network from the ACC and is integrated with diagnosticity in the left V3 and IPS for orienting attention.

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**Topic:** H.03. Decision Making

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**Title:** Distractor effects in decision making depends on individual's style of integrating choice attributes

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**Abstract:** The presence of irrelevant distractor options can lead people to make irrational decisions. However, there has not been a consensus on whether the distractor effect operates at the level of component attributes of distractor options or at the level of their overall value, as well as on whether the presence of a highly rewarding distractor facilitates or impairs decision

making. Here, we argue that, first, the precise effect of distractors varies across individuals. Second, to understand this variability, it is important to consider the individual differences in how people make decisions in view of a recent debate about whether choice attributes are combined additively or multiplicatively. To address these debates, we analysed a multi-laboratory study dataset that employed the same decision-making paradigm to demonstrate that people engaged in a mix of both approaches to varying extents in their decision making process. Specifically, we found this variability correlated with the effect of distractor on decision making whereby a positive distractor effect was associated with individuals using the multiplicative approach to choice attribute combination, but a negative distractor effect (divisive normalisation) was evident in those using the additive approach. Our findings coincide with recent findings in behavioural and neuroscience research reporting multiple distractor effects and that these effects can co-exist.

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**Topic:** H.03. Decision Making

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**Title:** Distinct roles of reward and information gains in prioritizing decision-relevant stimuli

**Authors: *Y. LI**[1], J. P. GOTTLIEB[2];
[2]Columbia Univ., [1]Columbia Univ., New York, NY

**Abstract:** As we navigate complex visual environments, we decide which stimuli to prioritize to guide our actions. In normative theories, decisions for rapid eye movements (saccades) depend on information gains and rewards, but the neural mechanisms are not understood. To fill this gap, we designed a two-step task in which monkeys first made a saccadic decision to obtain information from one of two sources (visual masks) followed by a final decision to obtain a reward based on the information. We orthogonally manipulated (1) mask diagnosticity (the probability of delivering accurate information), (2) prior uncertainty (the probability of a correct final choice without information), and (3) reward magnitude for a correct final choice. We show that monkeys preferentially sample the higher-diagnosticity mask and this bias increases with prior uncertainty but shows minimal effects of rewards, suggesting a stochastic comparison process that becomes more reliable under higher uncertainty. Distinct subsets of neurons in the anterior cingulate cortex (ACC) encode uncertainty and reward magnitude, showing positive and negative modulations (enhancement vs suppression by each variable). These ACC responses are qualitatively distinct from those we previously described in the lateral intraparietal area, consistent with reinforcement meta-learning model predictions that the two areas encode, respectively, cost-benefit tradeoffs and implementation of attentional policies.

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**Topic:** H.03. Decision Making

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**Title:** Decoding deliberation during decision making

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**Abstract:** Decision-making is a complex cognitive phenomenon that requires coordinated activity of multiple brain areas over a variable length of time. However, the extent of the contribution across brain regions and how these brain regions contribute to deliberation during decision making is unclear, in part due to the constraints imposed by non-invasive neural recording methods. Non-invasive neural recording methods (e.g. electroencephalogram, EE) have relatively low spatial resolution and a loss of information compared to intracranial EEG (iEEG), limiting the efficacy of cutting edge nonlinear computational models. To address this methodological impediment, we conducted a study on a cohort of fifteen epilepsy patients undergoing intracranial electrophysiological monitoring. In this experiment, we recorded neural activity from brain regions selected for clinical relevance related to the subjects' epilepsy symptoms. Brain regions included the prefrontal and parietal cortices, as well as deep temporal lobe structures. While being monitored, the participants participated in a risky decision-making task, which required them to choose between a safe bet and a risky gamble for potentially a higher monetary reward. Here, we modeled our data on a patient-by-patient basis using non-linear methods that maintain interpretability while obtaining high classification accuracy of whether or not the participant would gamble. Specifically, we employed 1-D Convolutional Neural Networks analyzed with Class Activation Maps and Random Forests analyzed with Gini Importance. These models both achieved a significantly higher leave-one-out classification accuracy than previous linear models and allowed greater insight into accumulation of information prior to choice. We found that information about choice is highly distributed across all brain regions tested, suggesting high inter-region communication. Additionally, over the time prior to choice, information about choice alternates between the two options until ~200ms before the choice is made. This alternating occurs at roughly 2-5hz, consistently across trials, within subject. This alternating pattern of information encoding may be linked to an individual's delta waves, which have also been shown to be linked to alternating attentional patterns. Future studies will examine the possibility of real-time choice decoding and neuromodulation to alter subject's risk preference.

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**Topic:** H.03. Decision Making

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**Title:** Predictive representation captures dynamics of structure learning during context-dependent decision-making

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**Abstract:** Flexible human behavior requires rapidly updating responses and expectations in the face of changing contingencies or goals. One approach is to identify a latent state, or "context", that governs the relationships between stimuli, responses, and outcomes. While context-dependent decision-making is widely studied, the process by which the human brain learns and represents latent structure remains an open question. In particular, is context represented as an explicit variable within a hierarchical framework? Or is it sufficient to represent only the observable states, but in a way that encodes their shared features, such that context is implied by the relationship between states? To address this question, we developed a model of structure learning based on the successor representation (SR), a predictive model of temporal abstraction previously applied to planning and navigation. The SR—in which context is implied, not explicit—can be read out directly to infer context-dependent states, or used downstream to compute an explicit representation of context for hierarchical inference. We applied the SR to a reversal learning task in which the correct responses and outcomes for a set of stimuli depended on a latent context. Specifically, human subjects learned two sets of stimulus-response-outcome (SRO) mappings, which switched back-and-forth, without cue, in alternating blocks of trials, thereby defining two temporal contexts. We found that subjects learned and exploited this hidden structure to guide choices: after an un-cued change in the SRO mapping for one stimulus, choice accuracy was above chance on the first encounter with the remaining stimuli. Given the 8 unique SRO combinations, or states, the SR model learned the long-run occupancy of state *s*' given prior state $s_t$, which the model then used to infer the current state and thereby select the correct response. This process reproduced the observed learning dynamics at multiple timescales, including the rapid updating at context switches and the gradual structure learning across blocks. Moreover, the information encoded by the SR afforded a compact, trial-by-trial summary of each subject's beliefs about task structure that explained individual differences in learning. Finally, subjects transferred meta-knowledge of the task structure to new instances of the task, each with novel stimuli, requiring an extension of the standard SR model to generalize abstract structure to novel problems. In summary, our results offer a parsimonious framework for learning and utilizing latent, context-dependent structure that obviates the need for an explicit representation of latent context.

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**Topic:** H.03. Decision Making

**Title:** A decision-theoretic model of perceptual multistability: perceptual switches as internal actions

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**Abstract:** Perceptual multistability (when two or more percepts alternate in response to a single ambiguous sensory input) has been studied for centuries using myriad approaches, and has illuminated diverse cognitive functions (e.g., perceptual inference, attention, visual awareness). Traditionally, multistability has been viewed in Helmholtzian terms, i.e., treating perception as a passive [Bayesian] inference about the contents of the world. However, this view neglects the crucial role played by value: e.g., percepts paired with reward tend to dominate for longer periods than unpaired ones. We reformulate visual multistability in terms of a decision process, employing the formalism of a partially observable Markov decision process (POMDP). Each percept is potentially associated with different sources of rewards or punishments (including aesthetic value), and switching between percepts is a form of (costly) internal action - the attentional equivalent of the external action of moving eye gaze between objects. Selecting one percept is accompanied by reduced observation noise, and ultimately stronger beliefs about the perceived state (dominant percept). The solution of the POMDP is the (approximately) optimal perceptual policy; this replicates and explains several classic and elusive aspects of rivalry. It reproduces apparently spontaneous random switches, with roughly gamma-distributed dominance periods. It captures the modulation by reward. It explains the rich temporal dynamics of perceptual switching rates, i.e. the increase in switching rate initially observed in naive participants, then decreases within single observation periods in subsequent sessions, and finally slowly increasing switching rates across days. To our knowledge, this model is unique in explaining the last two observations. Overall, our value-based decision-making account of perceptual multistability synergizes with previous accounts and also offers a more comprehensive treatment of computational and algorithmic facets of multistability. Furthermore, the dynamic nature of value in our framework might help explain the differences reported between psychiatric and healthy populations that concern the temporal dynamics of perception (e.g., the rate of perceptual switching).

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**Title:** Cognitive control in the face of drowsiness and exertion: a study on non-pharmacological altered arousal states

**Authors: *C. ALAMEDA JIMÉNEZ**[1], C. AVANCINI[1], D. SANABRIA[1], T. BEKINSCHTEIN[2], A. CANALES-JOHNSON[2], L. CIRIA[1];

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**Abstract:** Throughout a day, humans naturally enter different states of physical arousal and alertness, which can have an impact on cognition and task performance. However, these daily fluctuations of the arousal level we experience are rarely taken into account in cognitive neuroscience. In the present study, we aimed at characterizing cognitive performance at both sides of the arousal spectrum. In two different experiments, participants performed an auditory conflict task either in a state of drowsiness (N = 33) or while exercising (indoor cycling) at high intensity (N = 39). In the drowsiness experiment, drowsy trials were determined by applying an algorithm developed by Jagannathan et al. (2018) to the EEG data, whereas in the exercise experiment, participants cycled at 80% of their maximum VO2 consumption. In line with our pre-registered hypothesis, linear mixed-effects model analysis indicated that conflict and conflict adaptation effects were preserved during both altered arousal states. While overall task performance was markedly poorer at the lower side of the spectrum, this impairment was not observed at the upper side, consistent with the 'task-difficulty' assumption within the Yerkes-Dodson's (1908) framework. Drift-diffusion modeling analyses revealed that deterioration in performance during drowsiness could be explained by a temporary loss of efficiency in some decision making processes, such as slower rate of evidence accumulation, wider separation of decision boundaries and longer non-decision time, in line with recent accounts (Xu et al., 2023). At the neural level, the reconfiguration of the brain networks putatively responsible for information processing and conflict resolution shown with EEG data from our same database (e.g., the disappearance of the classic conflict-induced theta-band power neural marker; Canales-Johnson et al., 2020) could be associated with some of the reported changes in decision-making mechanisms. Notably, although increased levels of arousal showed minimal performance changes, they were associated with a decrease in the amplitude (interference) of task-irrelevant information processing, which may again be linked to changes in brain activity during states of physiological disturbance. Altogether, these results provide evidence that high and low arousal differentially impact overall performance and decision-making processes during automated tasks, and show the resilience of cognitive control mechanisms in face of naturally induced physiological fluctuations.

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**Title:** Influence of working memory limitations and dopamine on evidence accumulation

**Authors: *C. AGHAMOHAMMADI**[1], J. VAN KEMPEN[2], M. STAPLETON[2], A. GIESELMANN[2], C. LANGDON[3], T. ENGEL[3], A. THIELE[2];

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**Abstract:** Complex decisions involve evidence accumulation over long periods, which requires maintaining and updating the representation of the cumulative evidence in the working memory. Both working memory and decision-making depend on the prefrontal and parietal cortical areas and are sensitive to dopamine. Despite the shared behavioral demands and neural circuitry, the contribution of working memory to decision-making is unappreciated. Tasks used to study decision-making usually pose low working-memory demands due to short trials. As a result, whether and how the limited duration and capacity of working memory constrain decision-making performance is unknown.

We trained two monkeys to perform a task that required integrating sequential pieces of evidence with varying reliability over long periods. The monkeys made decisions based on a sequence of shapes, each providing probabilistic evidence for two choice alternatives. While the cumulative evidence increased with the number of observed shapes, the monkeys' collected reward saturated, indicating that the animals did not use all available evidence to guide their choices. To identify processes leading to the evidence loss, we modeled the choice behavior considering several hypotheses for working memory limitations: finite capacity, temporal decay, primacy, recency, and priming. Memory decay was necessary to explain the choice behavior and account for differences between the animals.

We constructed a neural circuit model to show that memory decay observed in monkeys' decision-making behavior can arise from a biophysically plausible mechanism based on competition between two excitatory populations. The rate of memory decay in the circuit model is controlled by the strength of recurrent self-excitation.

We further tested the role of dopamine in regulating the working memory constraints on decision-making using systemic drug applications. The behavioral model revealed that dopamine modulated both the gain of sensory evidence and the rate of memory decay.

During the task, we recorded spiking activity with multielectrode arrays from dorsolateral prefrontal (dlPFC) and lateral intraparietal (LIP) cortical areas. Neural responses in dlPFC and LIP were heterogeneous, with single neurons encoding both the weights of individual shapes and the cumulative evidence. Our work identifies mechanisms by which working memory limitations affect decision-making and indicates that dopamine regulates these limitations.

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**Topic:** H.03. Decision Making

**Support:** NYU Research Catalyst Prize

**Title:** Ambiguity aversion arises from nonlinear forward sampling of future reward states

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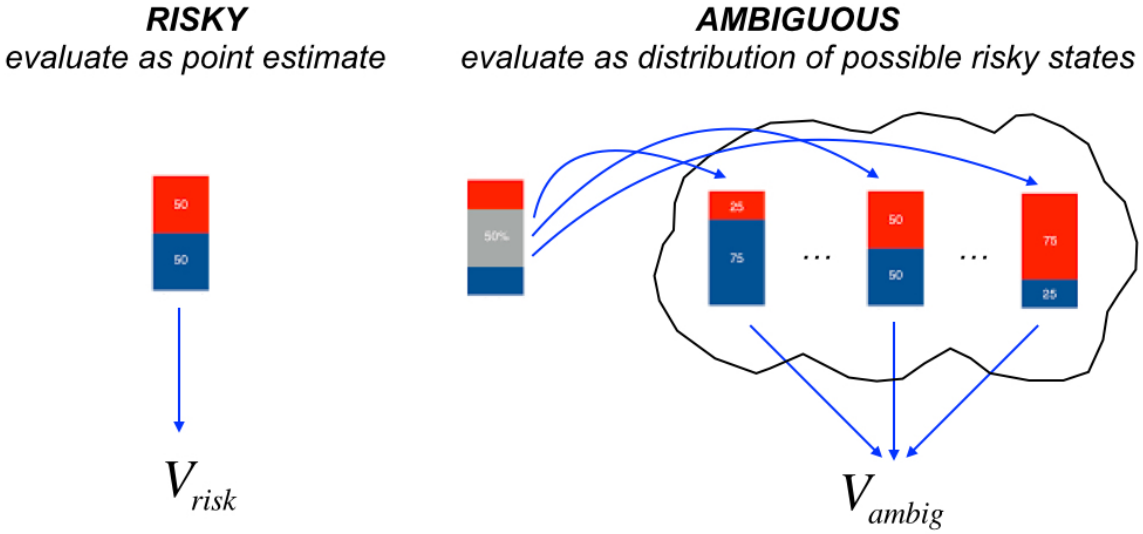
**Abstract:** Empirical decision-making in biological choosers depends markedly on outcome uncertainty. Such uncertainty can differ in the degree of knowledge held by a chooser: in

decisions under risk, outcomes are probabilistic but those probabilities are known; in decisions under ambiguity, the probabilities themselves are unknown or uncertain. While human choosers generally exhibit aversion to both risk and ambiguity, these two types of uncertainty preferences differ in fundamental ways. Risk aversion can be rationally derived as a normative tradeoff between magnitude and probability according to individual chooser utility functions. In contrast, ambiguity aversion is normatively irrational and cannot be explained by existing models of valuation and choice. Here, we show that ambiguity aversion arises naturally in agents employing (1) a normalized value representation and (2) a distributional sampling of possible outcome states under ambiguity. This nonlinear forward sampling model replicates known characteristics of empirical ambiguity aversion, including: preference for risky over ambiguous options, a quasi-linear relationship between valuation and ambiguity, and a dissociation between risk and ambiguity preferences. At the behavioral level, this model makes the counterintuitive (and testable) prediction that the degree of ambiguity aversion varies inversely with the density of forward sampling. At the neural level, the state sampling inherent in the model suggests a mechanism for ambiguity preference via distributional reinforcement learning, a process recently linked to normalized value representations. Together, these results offer a simple explanation for ambiguity preferences based on normalized value coding and forward inference, and argue for an incorporation of biologically valid value functions in computational models of decision-making.




**Ambiguity aversion as distributional sampling of risky states.** While risky options (left) are evaluated as a point estimate, ambiguous options (right) are evaluated using an average of sampled possible future states, each of which is a risky lottery.

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**Title:** Domain specificity and generality of adaptive coding

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**Abstract:** Adaptive coding is a fundamental mechanism for efficient information processing because it allows the brain to prioritize the most likely inputs within the current context. Adaptive coding occurs across many different domains, from basic sensory processing to higher-level perception and processing of reward magnitude. However, it remained unclear whether the capacity to adapt is domain-specific or domain-general. We investigated this question in healthy human volunteers (N=93) who performed an orientation-processing task (basic sensory processing), a face-house discrimination task (higher-level perception) and a monetary incentive delay task (reward processing).
To operationalize and quantify adaptive coding in each of these tasks at the neural level, we determined the extent to which the context affected the processing of current stimuli. Specifically, for orientation processing, we extracted activity from visual cortex and determined the extent to which surround-gratings suppressed activity elicited by center gratings. For face-house discrimination, we extracted activity from the fusiform face area (FFA) and the parahippocampal place area (PPA) and determined the extent to which the slope of activity increased from contexts with a wide range of face vs. house morph proportions to contexts with a narrow range of face vs. house morph proportions. For reward processing, we extracted activity from the ventral striatum and determined how strongly the slope of activity increased from contexts with a wide range of reward magnitudes to contexts with a narrow range.
Within the domain of higher-level perception, we found a substantial correlation between adaptive coding in FFA and adaptive coding in PPA (r=0.53; Pearson correlation). Across domains, adaptive coding in higher-level perception correlated with adaptive reward processing (FFA: r=0.33; PPA: r=0.23) but not with basic sensory processing (FFA: r=-0.03; PPA: r=-0.18). Moreover, we also found no relation between adaptive reward and basic sensory processing (r=-0.05). These findings suggest domain-generality in adaption to the range of inputs for higher-level visual perception and reward processing. By contrast, for basic sensory and higher level processing, adaptive coding appears to be domain-specific.

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**Topic:** H.03. Decision Making

**Support:** Australian Research Council grant DP 19010048

**Title:** Inefficient Divisive Normalization: Human Choosers Employ Divisive Normalization Even When They Should Not

**Authors: *V. KURTZ DAVID**[1], V. ALLADI[5], S. SINHA[2], S. BUCHER[6], A. BRANDENBURGER[3], K. LOUIE[4], A. DEWAN[2], P. W. GLIMCHER[2], A. TYMULA[5]; [2]Sch. of Med., [3]Stern Sch. of Business, NYU Tandon, and NYU Shanghai, [4]Ctr. for Neural Sci., [1]New York Univ., New York, NY; [5]Univ. of Sydney, Sydney, Australia; [6]Tübingen AI Ctr. and Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany

**Abstract:** The Divisive Normalization (DN) function is often viewed as a canonical neural encoding mechanism. However, DN maximizes mutual information, and thus is efficient, only for input stimuli coming from a very specific class of inputs: heavy-tailed multivariate Pareto distributions. Given the same infomax criterion, DN is not efficient for stimuli or rewards coming from other distributions. Using a behavioral paradigm and computational modeling of our results, we tested whether the brain uses DN both in environments in which it is and in which it is not efficient. That is, we ask whether choices are well described as arising from a DN representation even when that mechanism is inefficient - evidence of a physiological constraint that requires DN. We perform our experiment in a biphasic risky-choice experiment, where subjects had to choose amongst lotteries drawn from four different types of input distributions. In Phase 1 of our experiment, subjects (N=78) reported the most they would pay to purchase a given lottery, using a Becker-DeGroot-Marschak (1964) auction with a large set of lotteries. The subject-specific estimates from these valuations were used to generate two classes of continuous distributions of subjective valuations (SVs): (1) Uniform distributions, for which the DN encoding function is inefficient; and (2) multivariate Pareto type-III distributions, for which the DN function is efficient. These distributions were then used in Phase 2, where subjects made 1,280 choices in a 2*2 design (320 trials per treatment) that manipulated the number of choice options (2 vs. 6), as well as the class of SVs distributions (uniform vs Pareto). We used pooled and hierarchical maximum likelihood estimations to recover the DN functional parameters. The pattern of stochasticity in the choices of our subjects clearly indicated that subjects employed a divisive representation under both classes of input distributions. This was true regardless of the computational method used to estimate the underlying normalization, in both the pooled and hierarchical estimations. The magnitude of the curvature of the DN function, however, did vary across treatments, suggesting that subjects adapted to the different choice environments. Our results suggest that people are obligate DN-choosers, and that at least for adaptation periods of this duration, a certain degree of embedded inefficiency exists in choice induced by DN. These findings correspond to previous empirical results, which showed that many real-world naturalistic stimuli have long-tail asymmetric distributions, and perhaps imply an evolutionary origin of the neural encoding mechanism that constrains it to employ DN.

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**Nanosymposium**

**NANO59: Integrative Networks of Language Processing and Production**

**Location:** WCC 140

**Time:** Tuesday, November 14, 2023, 8:00 AM - 10:15 AM

**Presentation Number:** NANO59.01

**Topic:** H.11. Language

**Support:** NIH NINDS U01 NS128921

**Title:** Convergent hierarchical dynamics within the language network for speech listening and silent reading

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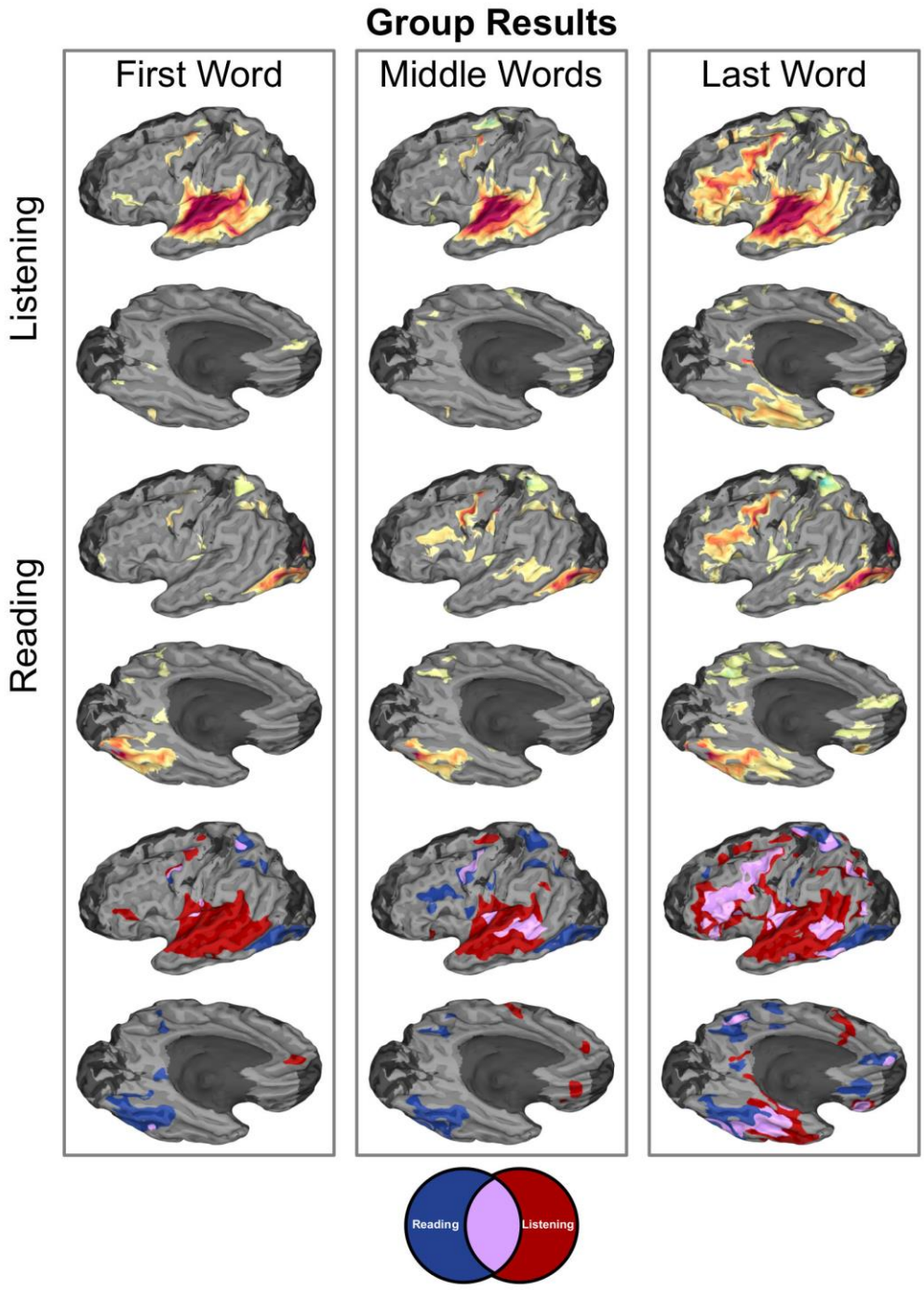
**Abstract:** Spoken and written language are crucial to communication and likely recruit shared brain networks to map phonology and orthography to meaning. Models of speech perception for both domains suggest overlapping processes. The cortical instantiation of these models is critical to our understanding of comprehension, but the convergent neural mechanisms are unclear. We investigate the dynamics of shared networks for listening and silent reading using ECoG.
62 patients underwent ECoG with subdural grids or depths during cued naming to matched spoken and written descriptions. The last word in each prompt was crucial to bind semantic concepts. We analyzed broadband gamma activity (BGA; 70-150Hz) and integrated group responses with mixed effects multilevel analyses.
For each spoken word, activity was first seen in posterior superior temporal gyrus (pSTG; 128% BGA) and was sustained throughout the prompt. Activity was then seen in posterior middle temporal gyrus (pMTG; 41% BGA). For each written word, visual cortex activity was followed by activation of lateral occipitotemporal cortex and intraparietal sulcus (IPS). Activity then converged with listening in pSTG (20% BGA) and pMTG (29% BGA). For both modalities, the last word triggered an identical lexical processing network (pMTG, fusiform gyrus, IPS, pars triangularis).
Activity from depths along STG (n=37) revealed a consistent anteroposterior gradient. Electrodes in Heschl's gyrus showed sustained activation during listening consistent with acoustic processing and were quiescent during reading. Electrodes in secondary auditory cortex (planum temporale; PT) showed transient activity at speech onset and recurrent activity for written words. Our prior work implicates PT in predictive encoding during production - this work suggests the same occurs in silent reading.
Using large-scale ECoG, we derived new insights into the neural basis of spoken and written language. Ultimately, we believe that this work would provide important insights to optimize the design of neural prosthetics for the treatment of reading-related disorders.

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**Presentation Number:** NANO59.02

**Topic:** H.11. Language

**Support:** NIH Grant NINDS 1R01NS109367
NIH Grant NINDS 1R01NS115929
NIH Grant NIDCD R01DC018805

**Title:** A Language-Specific Left-Lateralized Network for Auditory Naming

**Authors:** ***L. YU**[1,2], N. M. CHAPOCHNIKOV[3], W. K. DOYLE[4], O. DEVINSKY[3], A. FLINKER[3];
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**Abstract:** Naming is a fundamental cognitive function and an integral component of language assessment in clinical settings. Recent clinical stimulation mapping and neuroimaging evidence indicate that auditory naming, within the context of everyday linguistic discourse, recruits distinct prefrontal cortex regions compared to picture naming. However, the underlying neural dynamics of these processes remain poorly understood. In this study, we aimed to investigate whether and how auditory naming engages language-specific prefrontal regions to process increasing semantic load. We conducted a battery of language production tasks, including picture naming, auditory naming ("what a king wears on his head"), with visual word reading and auditory word repetition serving as control tasks, on 50 neurosurgical patients undergoing Electrocorticographic (ECoG) monitoring. Region of interest analysis of high gamma broadband activity (70-150 Hz) revealed sustained enhancement in left inferior frontal gyrus (IFG) and left middle frontal gyrus (MFG) specifically during auditory naming preceding articulation. We confirmed the effect with multifactor linear regression model (interaction for auditory and semantic features, *p*<0.01). Employing an unsupervised clustering approach, we discovered a novel network in the frontal cortex, centered on the border of IFG and MFG. This network exhibited task-selectivity for auditory naming peaking around 450 ms before articulation, displaying significantly greater activity across all electrodes. This network was distinct from another cluster exhibiting pre-articulatory responses irrespective of task within IFG and portions of precentral gyrus, which peaks around 250 ms before articulation. To investigate the nature of semantic load within the naming network, we applied three encoding models (acoustic, semantic integration, and task-based attention) across all active electrodes. Our results provided evidence that the naming network primarily encodes increasing semantic load, rather than acoustic or task-based attention ($r^2 = 0.006$ for the semantic integration model after variance partitioning, above permutation baseline, p<0.01). Lastly, we examined the laterality of this network in both hemispheres to assess language specificity. Our findings demonstrated a strong left lateralization within the naming network, while networks associated with sensory perception and motor execution exhibited minor differences in laterality. In conclusion, our study uncovers a novel left-lateralized naming network centered around the border of IFG and MFG, specifically involved in processing increased semantic load.

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**Topic:** H.11. Language

**Support:** National Natural Science Foundation of China (62293550, 62293551, 61977008)

**Title:** Neural synchrony underlies the positive effect of shared book reading on children's language ability

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**Abstract:** Although it is well recognized that parent-child shared book reading produces positive effects on children's language ability, the underlying neurocognitive mechanisms are not well understood. Here, we addressed this issue by measuring brain activities from mother-child dyads simultaneously during a shared book reading task using functional near infrared spectroscopy (fNIRS) hyperscanning. The behavioral results showed that the long-term experience of shared reading significantly predicted children's language ability. Interestingly, the prediction was modulated by the age of children: for older children over 30 months, the more the shared reading experience, the better the language performance; for younger children below 30 months, however, no significant relationship was observed. The brain results showed significant interpersonal neural synchronization (INS) between mothers and children at the superior temporal cortex (STC), which mediated the positive impact of long-term experience of shared reading on older children's language. Finally, the results showed that the instantaneous quality of shared reading contributes to children's language ability through enhancing INS and increasing long-term experiences. Based on these findings, we tentatively proposed a theoretical model for the relationship among INS, shared reading, and children's language ability. These findings will facilitate our understanding of the role of shared reading in children's language development.
**Keywords:** Social interaction, Shared reading, Language ability, Interpersonal neural synchronization, fNIRS

**Disclosures: Y. Zhai:** None. **H. Xie:** None. **H. Zhao:** None. **W. Wang:** None. **C. Lu:** None.

**Presentation Number:** NANO59.04

**Topic:** H.11. Language

**Support:** R01AT010627

**Title:** Verbal movie recall reveals heightened self reference in individuals with heroin use disorder

**Authors: *S. G. KING**[1], G. KRONBERG[2], N. MCCLAIN[3], A. O. CECELI[5], J. GRAY[3], N. ALIA-KLEIN[6], R. Z. GOLDSTEIN[4];
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**Abstract:** Drug addiction is marked by enhanced salience attribution to an individual's substance of choice, including to substance-related cues in the natural environment, which potentiates further drug use. Unstructured, spontaneously generated speech can provide unique insights into a person's current psychological state, such as following exposure to salient drug stimuli, potentially enhancing the arsenal of clinically meaningful measures for predicting future behaviors. In this study, 55 participants with heroin use disorder (HUD) in inpatient medication-

assisted treatment and 29 control participants (CTL) viewed 17 minutes from the movie *Trainspotting*, heavily featuring heroin use and addiction, then verbally recalled the events and their subjective experience of the movie. Participants completed the same task at a second session 8 weeks later while still in treatment. Word frequency was analyzed for the use of first, second, and third person pronouns, and drug-related terms, as proportions of the total word count. A 2 (Group) x 2 (Session) x 3 (Pronoun) ANOVA revealed a significant main effect of Pronoun ($F=50.66$, $p<.001$) quantified by a significant Group x Session x Pronoun interaction effect ($F=3.47$, $p=.033$). Post hoc testing at Session 1 [2 (Group) x 3 (Pronoun) ANOVA] showed a significant Group x Pronoun interaction effect ($F=4.64$, $p=.012$), with group differences in all pronouns (first and second person: HUD>CTL; third person: CTL>HUD), suggesting more self-directed speech in HUD. In contrast, there was no significant Group x Pronoun interaction effect at Session 2 ($p>.05$). Post hoc testing in HUD showed significant decreases in first (paired $t=3.79$, $p=.001$) and second (paired $t=3.94$, $p<.001$), and an increase in third (paired $t=2.56$, $p=.019$), person pronoun usage at Session 2, while the CTL group showed no changes with time (all $p>.05$). A 2 (Group) x 2 (Session) ANOVA for drug word frequency revealed a significant effect of Session ($F=24.13$, $p<.001$, Session 1 > Session 2), with no significant effects of Group or the Group x Session interaction ($p>.05$). Despite the absence of a group effect in drug words, the pronoun use measure demonstrates a quantifiable and malleable linguistic marker of the subjective experience/attribution of salient drug-related stimuli to the self in HUD. Further analyses will leverage the observed changes over time in HUD to investigate the contribution of individual differences in clinical severity, outcome measures, and underlying neurobiology assessed with functional MRI during movie viewing. Linguistic markers of latent contextual variability measured using natural language processing techniques will also be explored.

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**Presentation Number:** NANO59.05

**Topic:** H.11. Language

**Title:** Pre-articulatory recruitment of frontal cortex during imagined speech

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**Abstract:** How does the brain represent imagined speech? The majority of us have internal dialogues, but the cortical networks that support imagined speech are not well understood. While it is well established that the Inferior Frontal Gyrus (IFG) plays a critical role in speech preparation, it is not clear which prefrontal regions support imagined speech and if their cortical temporal dynamics align with overt speech. The aim of this study is to identify cortical candidates that support imagined speech and establish pre-articulatory processes regardless if speech is imagined or overt in nature. We obtained neurosurgical electrocorticography (ECoG) data when patients (N = 4) were performing a series of speech production tasks requiring either imagined or overt speech responses, including: picture naming, auditory repetition, visual reading, and a control task of passive listening. All tasks utilized the same set of 50 words, but

with different retrieval routes. T-tests were conducted to determine the statistical significance of differences in neural activity within high gamma broadband (70-150 Hz) during overt and imagined speech locked to articulation (the onset of articulation in imagined condition was estimated with RT in overt condition and then cross correlated with sensory responses). We find that electrodes within IFG show significant activation 250 ms prior to articulation during imagined speech in contrast to neighboring motor cortex sites which are significantly active only for overt speech. The same set of electrodes also showed the same level of activity during overt speech, providing evidence for both imagined and overt pre-articulatory processing in IFG. Further, a subset of IFG electrodes showed stronger activity for overt compared with imagined speech, showing the modulation by task conditions. This study helps establish the role of IFG in imagined speech and provides important insights for speech decoding applications in patients who are unable to speak.

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**Title:** Language Acquisition in Brains and Algorithms: towards a systematic tracking of the evolution of language representations using stereoelectroencephalography recordings in children and deep learning.

**Authors: *L. EVANSON**[1,2], C. BULTEAU[2], M. CHIPPAUX[2], G. DORFMÜLER[2], S. FERRAND-SORBETS[2], E. RAFFO[2], S. ROSENBERG[2], P. BOURDILLON[2], J.-R. KING[1]; [1]Ecole normale supérieure, Paris, France; [2]Rothschild Fndn. Hosp., Paris, France

**Abstract:** The human brain is unique in its ability to acquire language: in a few years, it learns to combine a limited set of known elements (words) into a representation that is both novel and meaningful. However, the neuronal computations allowing this feat remain largely unknown. Here, we study the evolution of language representations in the brain throughout development. To this end, we collected stereoelectroencephalography (sEEG) signals in 30 French-speaking children, aged between 3 and 19 years, while they listened to an audiobook, Le Petit Prince by Antoine de Saint-Exupéry. We then use encoding and decoding analyses of the brain signal to track the formation of phonetic and lexical representations during speech listening. Our analyses reveal three main findings. First, phonetic as well as lexical representations can be significantly decoded in the posterior superior temporal sulcus in most participants, including in the 3-year old children. Second, we observe a systematic speed up of the neural response latency with age: the lexical representations built in the brain of older children rise more quickly and are maintained for a longer amount of time than younger children. Third, in young children, response times are similar across brain areas, however, in older children there is a delay that increases with distance from the primary auditory cortex indicating that there is integration of multiple levels of

information. Overall, these results provide a stepping stone to understanding the neural bases of language during brain maturation, and provide important constraints to the neuroscientific theories of language acquisition.

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**Presentation Number:** NANO59.07

**Topic:** D.06. Vision

**Title:** Different neural codes representing animate,inanimate and abstract concepts in a low-dimensional semantic space

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**Abstract:** Generating a picture for "apples" is much less challenging than for "rules" for both humans and AI. What cause this difference? According to the theory of dual-coding model, "apples" is a concrete concept that can be understood through either a sensor code (i.e. visual image) or verbal code, in contrast to "rules" which is an abstract concept that is primarily processed using verbal code. However, the neural basis of sensor code and verbal code is not well understood, though imaging studies suggest that concrete and abstract concepts have a partially distinct neural basis. Recent research utilizing representational similarity analysis has revealed that both the human brain and deep neural networks employ distinct sensory codes to represent animate and inanimate images. Here, inspired by the image representation research, motivated by image represent study, we used a lexical decision task combined with MEG recordings to explore how the brain represents animate, inanimate and abstract concepts. In the behavior experiment (subjects n=19), participants were instructed to distinguish words (animate, inanimate and abstract word) and pseudo-words, while in the MEG experiment (subjects n=20), participants were instructed to detect a specific word "mind", while viewing a serious of animate, inanimate and abstract word list. The behavioral experiment revealed that compared to pseudo-words, people respond faster to animate words than to abstract words, while their response to inanimate words was slower than to abstract words. During the MEG experiment, classification-based multi-voxel pattern analysis (MVPA) revealed two main results. First,between 300-400 ms after a word is presented, the brain exhibits a distinct response to animate, inanimate and abstract words. Second, animate and inanimate concepts involve the utilization of both hemispheres, while abstract concepts demonstrate a left-lateralized representation. Furthermore , we employed representational similarity analysis (RSA) combined with Multidimensional scaling analysis (MDS) to explore the neural geometry of the concept representation. Our analysis reveals that in the low-dimensional semantic space, one dimension is devoted to word frequency(high vs. low), with the other dimensions distinguishing animate, inanimate and abstract concepts. Together, our study has provided neural evidence that the brain uses different codes to signify animate, inanimate and abstract concepts.

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**Title:** Analysis of electrophysiological markers and correlated components of neural responses to discourse coherence

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**Abstract:** Constructing meaning from spoken language is invaluable for learning, social interaction, and communication. In clinical populations with developmental disorders of speech comprehension, the severity of disruption can persist and vary from limiting occupational opportunities to lower performance outcomes. Previous research has reported an event-related potential (ERP) neural positivity over right hemisphere lateral anterior sites in response to semantic and discourse processing. Although useful as a marker for clinical populations of autism spectrum disorder (ASD) and developmental language disorder (DLD), little is understood about the dynamics and neural sources of this biological marker. In addition to traditional methods of ERP analysis, this investigation utilizes methods for analyzing correlated components to determine meaningful sources of neural activity shared across this population of healthy adults. Based on previously published findings by Neumann et al. (2014), it was hypothesized in the current study that a positivity index of discourse processing would be detected at right lateral anterior sites starting 600 ms after sentence onset and persisting for 300 ms. The current study replicated previous findings and confirmed that a persistent positivity is detectable for 300 ms over right lateral anterior sites during late-stage semantic processing. The results of this analysis also revealed a significant positivity at parietal sites when listening to discourse, which started 1000 ms following sentence onset and persisted for 200 ms. In addition, a significant negativity when listening to discourse over right lateral anterior sites started at 1000 ms following sentence onset and persisted for 200 ms. The results from this study reveal a more complex, biphasic dynamic of potentiation not observed in previous findings which had an epoch limit less than 1000 ms following sentence onset. The correlated components of evoked responses were determined using inter-subject correlation (ISC) and found to be consistent with the pattern observed for ERPs. The current study confirmed that correlation is stronger for coherent, ordered sentences when compared with randomly-ordered sentences, or with nonsense syllabic speech. These findings suggest a novel understanding of the processes involved in the neural construction of coherent discourse comprehension.

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**Topic:** H.11. Language

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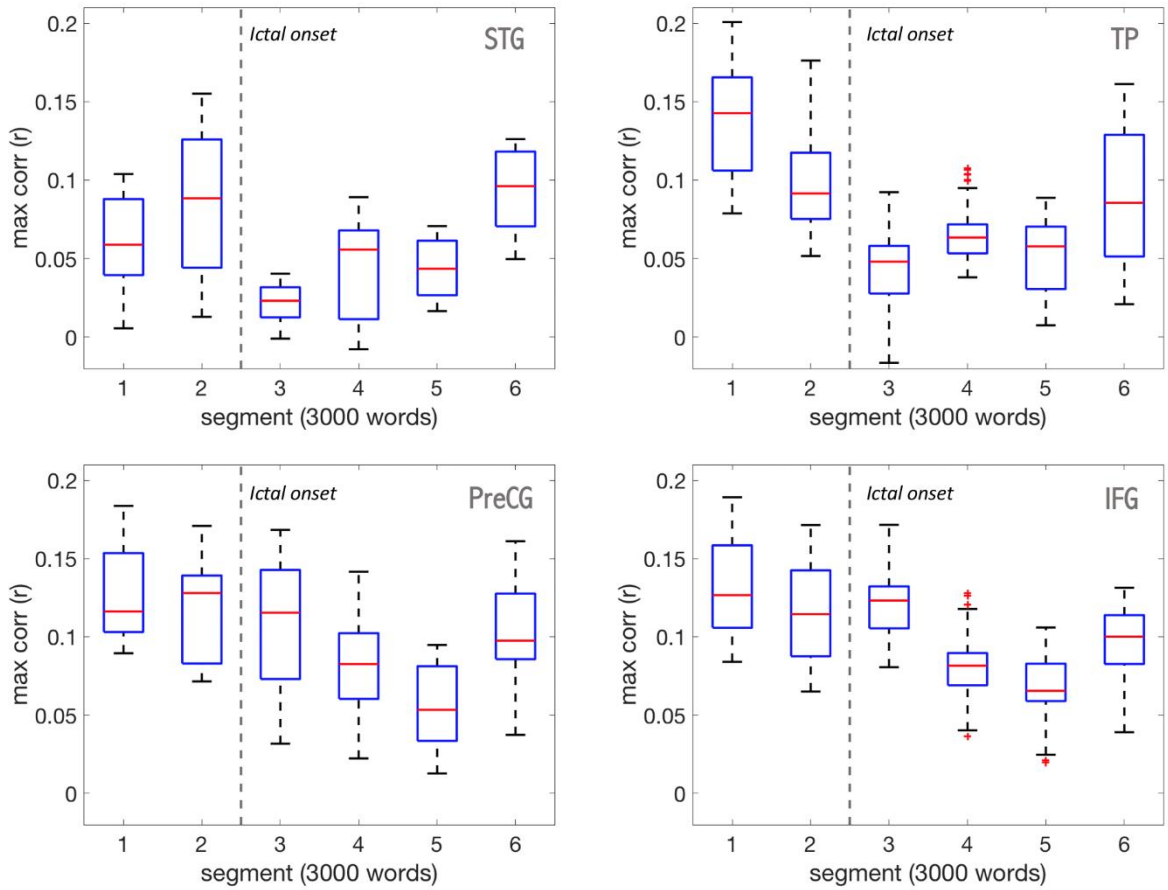
**Title:** Applying language models to study the neural basis of language production in the real world before, during, and after ictal aphasia

**Authors: *B. AUBREY**[1], A. GOLDSTEIN[1], Z. ZADA[2], L. HASENFRATZ[3], W. DOYLE[4], D. FRIEDMAN[6], P. DUGAN[7], L. MELLONI[4], S. DEVORE[5], O. DEVINSKY[6], A. FLINKER[6], U. HASSON[1];
[2]Psychology, [3]Neurosci. Inst., [1]Princeton Univ., Princeton, NJ; [4]New York Univ., New York, NY; [5]New York Univ., Brooklyn, NY; [6]New York Univ. Langone Med. Ctr., New York, NY; [7]Dept. of Neurol., New York Univ. Sch. of Med., New York City, NY

**Abstract:** General models of linguistic impairments provide an incomplete picture when applied to the individual brain. Additionally, we rarely have the opportunity to record and analyze the recovery process from the onset of impairment to the recovery of typical communication abilities. This study investigates language processing in a single epilepsy patient (53, F) undergoing electrocorticography (ECoG) monitoring over a one-week period in which we recorded and transcribed their day-to-day conversations. Midway through their stay, they spontaneously experienced ictal aphasia, categorized by a difficulty or inability to retrieve words when speaking and an inability to repeat phrases, which lasted approximately one day. To assess these production deficits in the brain over time, we split all words produced (32,012 words) into chronological segments of 3,000 words. We used the first two segments to train an encoding model to predict the neural activity of each word using embeddings derived from language models (e.g. GloVe, GPT-2). We then evaluated the trained model's performance on the following six segments by predicting the brain signal and correlating the prediction with the actual neural activity. Prior to ictal onset, several brain regions achieved strong encoding performance. However, performance rapidly degraded after the ictal episode in the superior temporal gyrus (STG) and temporal pole (TP). Interestingly, the inferior frontal gyrus (IFG) and precentral gyrus (PreCG) did not show a decrease in model performance in the first segment following the seizure but did decline in the following segments. Finally, encoding performance in the final chronological segment, after the patient's speech fully recovered, was comparable to performance in the segments prior to seizure activity in all four language regions. These findings point to differing contributions from certain language areas to the observed ictal language deficit and suggest that language models may be useful in monitoring the neural signatures of language recovery in the real world.

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**Nanosymposium**

**NANO60: Biochemical and Molecular Techniques**

**Location:** WCC 152A

**Time:** Tuesday, November 14, 2023, 8:00 AM - 10:30 AM

**Presentation Number:** NANO60.01

**Topic:** I.04. Physiological Methods

**Support:** NSF GRFP DGE 1841052

**Title:** Development of integrating reporters for detection of protein-protein interactions with enzymatic readout

**Authors: *J. SESCIL**;
Dept. of Chem., Univ. of Michigan, Ann Arbor, Ann Arbor, MI

**Abstract:** Temporally gated integration reporter for detecting PPIs with an immediate enzyme activation readout Protein-protein interactions (PPIs) play crucial roles in numerous biological processes, and their dysregulation is implicated in numerous diseases. Therefore, numerous methods have been developed to monitor PPIs, such as real-time sensors like Fluorescence Resonance Energy Transfer. This technique produces a transient signal that can elucidate dynamic information about the event, but the temporary signal limits further study of involved cells. Another class of reporters, integrating reporters, permanently mark cells involved in PPIs, allowing further investigation of labeled cells, such as RNA sequencing or proteomic analysis. For example, a PPI can reconstitute a split fluorophore, producing a direct fluorescent readout that leaves a fluorescent mark on involved cells. However, many of these existing split protein reporters lack temporal gating, and therefore have a larger time window to accumulate false positive signal from irreversible split protein reconstitution. Furthermore, the direct fluorescent readout is not amplified, leading to a decreased readout in response to low signal input and worsening detection limit. Transcription-based integrator sensors with temporal gating have also been reported, but these can only detect PPIs that occur in the cytosol and require over eight hours for signal readout. The Wang lab has developed a new reporter motif, which partially fulfills the need for a temporally gated PPI detector with a bright, amplified signal. This motif, CLAPon, or Cleavage of Leucine zipper- caged APEX for Protease detectiON, relies on the activation of the enhanced ascorbate peroxidase (APEX) enzyme for the minutes-long readout. In this poster, I will show further design and optimization of this reporter motif, which will have increased functionality for broader applications.

**Disclosures: J. Sescil:** None.

**Presentation Number:** NANO60.02

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Title:** Microfluidic system coupled with homogeneous proximity ligation assay to measure low-abundance protein biomarkers

**Authors: *J. WAN**, H. NGUYEN, M. SHANNON;
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**Abstract:** Alzheimer's disease (AD) is a progressive neurodegenerative condition marked by pathologic amyloid Aβ and tau protein aggregation that eventually leads to cognitive impairment. With increasing cases of AD and a high disease burden, there has been a growing interest in developing tools to detect low-abundance biomarkers in readily accessible serum or plasma. In this work, we present a microfluidic-based platform coupled with proximity ligation assay (PLA) technology for measuring the AD protein biomarker, tau phosphorylated at serine-181 ($pTau_{181}$) with high accuracy and sensitivity. In these studies, we measure and compare the limit of detection (LoD) of $pTau_{181}$ protein with the traditional qPCR-based PLA platform and a novel microfluidic-based digital PLA (dPLA) performed on the Applied Biosystems™ Absolute Q™ digital PCR System*. The LoD for both PLA and dPLA were comparable at 0.1 pg/mL, which is more sensitive to many ELISA-based assays. Further, we tested serum samples of donors with and without AD for the presence of $pTau_{181}$. Both PLA and dPLA were able to identify samples containing elevated levels of $pTau_{181}$, thus demonstrating both approaches as methods to test donor samples for AD biomarkers. AD positive samples had significantly higher

$pTau_{181}$ than the no protein control (NPC) and AD negative sample (Tukey's multiple comparisons test, **p<0.01, ***p<0.001, ****p<0.0001). While both PLA and dPLA have comparable LoD and can differentiate $pTau_{181}$ levels of AD positive and negative samples, we further investigated whether both techniques can quantify more subtle differences in protein levels. The dPCR-based PLA was able to distinguish statistically significant small differences between samples in the range of 30% (Tukey's multiple comparisons test, *p<0.05); however, qPCR-based PLA was not able to statistically separate protein concentrations in the same range. While measuring elevated levels of $pTau_{181}$ is key for early detection of AD, it is also important to measure subtle differences in signal. These small changes in the levels of $pTau_{181}$ are likely more informative, and dPLA is a potential tool to help appreciate these small changes with high accuracy. This technique is likely extendable beyond AD for other protein-based biomarkers where quantifying amounts and changes in protein content are essential.
*For Research Use Only. Not for use in diagnostic procedures

**Disclosures: J. Wan:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **H. Nguyen:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific. **M. Shannon:** A. Employment/Salary (full or part-time):; Thermo Fisher Scientific.

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**Title:** Halo GluSnFR: A hybrid chemigenetic fluorescent biosensor for neuronal glutamate release

**Authors: *N. TJAHJONO**[1], A. ANDREONI[2], J. A. CHOUINARD[2], E. WRIGHT[2], L. PARRA[4], Y. JIN[3], J. SUN[1], B. J. GARCIA[6], J. SUTEDJO[6], C. DEO[7], J. GRIMM[7], S. ROY[5], E. R. SCHREITER[8], L. D. LAVIS[9], L. TIAN[1];
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**Abstract:** Glutamate is the predominant excitatory neurotransmitter in the brain, underlying regulation of cell excitability, synaptic plasticity, learning and memory. The diversity of glutamatergic neurons and their interplay with neurons that release neuromodulators--some of which corelease or cotransmit glutamate-- necessitates tools for specific, multiplexed detection of neurochemicals. Genetically encoded biosensors allow for sensitive, cell-type-specific, and non-invasive glutamate measurements. However, the most used indicators for detecting glutamate require excitation with blue light, which induces autofluorescence, produces high cytotoxicity, and limits imaging to shallower depths compared to red-shifted excitation. Furthermore, most optimized biosensors for other neuromodulators utilize GFP as a fluorescent reporter, enforcing spectral limitations on multiplexed imaging. In this study, we engineered,

validated, and demonstrated the utility of a modular fluorescent indicator, “Halo GluSnFR” as a new class of glutamate biosensors. Halo GluSnFR consists of a genetically encoded protein scaffold based on the self-labeling protein HaloTag that binds to exogenously applied fluorescent dye ligands. Upon glutamate binding to the sensor-dye conjugate, the fluorophore increases its fluorescence emission. Multiple rounds of engineering and optimization were performed on Halo GluSnFR through rational design and site-saturated mutagenesis, resulting in sensors with high sensitivity, speed, and dynamic range. We exploited the modularity of the sensor by characterizing different dye-sensor combinations with a palette of red-shifted fluorogenic molecules. By fusing Halo GluSnFR to different transmembrane domains, we expanded its potential applications for dissecting the functional roles of glutamate, targeting the sensor to pre-synaptic or post-synaptic sites. We demonstrated that Halo GluSnFR can detect neuronal glutamate release evoked from single action potentials and it is suitable for multiplexed recording with other fluorescent biosensors and optogenetic tools. With Halo GluSnFR, neuroscientists have a new tool for multiplexed, non-invasive glutamate imaging in deeper brain regions, to answer previously intractable questions in behavior and disease.

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**Title:** Dual-color GRAB sensors for monitoring spatiotemporal serotonin release in vivo

**Authors: *F. DENG**[1], J. WAN[1], G. LI[1], H. DONG[1], X. XIA[1], Y. WANG[1], X. LI[1], C. ZHUANG[2], Y. ZHENG[1], L. LIU[1], Y. YAN[1], J. FENG[1], Y. ZHAO[1], H. XIE[2], Y. LI[1];
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**Abstract:** The serotonergic system plays important roles in both physiological and pathological processes, and is a widely used therapeutic target for many psychiatric disorders. Although several genetically encoded GFP-based serotonin (5-HT) sensors were recently developed and advanced our knowledge of serotonergic neurotransmission, their sensitivities and spectral profiles are limited, which hinders further dissecting 5-HT signals from complex conditions. To overcome these limitations, we optimized green fluorescent G-protein-coupled receptor (GPCR)-activation-based 5-HT ($GRAB_{5\text{-}HT}$) sensors and developed a new red fluorescent $GRAB_{5\text{-}HT}$ sensor. These sensors exhibit excellent cell surface trafficking, high specificity, sensitivity, and spatiotemporal resolution, making them suitable for monitoring 5-HT dynamics *in vivo*. With these new 5-HT sensors, the subcortical 5-HT release was recorded in freely moving mice using fiber photometry. Interestingly, we observed both uniform and gradient 5-HT release in the mouse dorsal cortex with mesoscopic imaging under different circumstances. Using dual-color imaging, seizure-induced waves of 5-HT release throughout the cortex following calcium and endocannabinoid waves were observed. What's more, by expressing the most sensitive green $GRAB_{5\text{-}HT}$ sensor in endothelial cells with a novel transgenic mouse line, we monitored the spatiotemporal dynamics of 5-HT in blood vessels using 2-photon imaging. In summary, these new 5-HT sensors offer unprecedented opportunities to study serotonergic neurotransmission in both physiological and pathological states.

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**Presentation Number:** NANO60.05

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH Grant 008914

**Title:** Development of a new version of SPOTIT for a permanent fluorescent read out of dopamine release with cellular-resolution

**Authors: *S. M. HAVENS**, W. WANG;
Univ. of Michigan, Ann Arbor, Ann Arbor, MI

**Abstract:** Dopamine is a neuromodulator which is essential for motor control, reward processing, and motivation through G-protein coupled receptor (GPCR) signaling. Recent GPCR-based real-time sensors allow for optical monitoring of dopamine release in behaving animals. However, there is still a need for high resolution mapping of dopamine release throughout the brain. To fill this need, we have developed a new GPCR-based sensor to detect dopamine with a permanent fluorescent mark through a combination of computational modeling and rational design. Our new sensor detects dopamine with high specificity and generates a permanent green fluorescent signal with up to a 9-fold signal-to-background ratio while maintaining high brightness and cellular-resolution. This paves the way for further studies into dopamine release throughout the brain and enables the possibility of whole-brain dopamine mapping.

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**Presentation Number:** NANO60.06

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** U01NS120820

**Title:** Toward the next generation of dopamine sensors with the dLight3.0 series

**Authors: *J. A. CHOUINARD**[1,2], R. DALANGIN[1], S. TAKAHASHI[2], E. C. SCOTT[1], N. TJAHJONO[1], P. T. FREITAS[1], D. NOSAKA[2], N. KITAMURA[2], K. KURIMA[2], J. R. WICKENS[2], L. TIAN[1];
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**Abstract:** Recent advances in biosensor technology have paved the way for an unprecedented level of inquiry into the spatiotemporal dynamics of neurochemical release in the brain. These sensors directly and specifically report synaptic signals as they are received by their receptors. dLight is a family of dopamine sensors with broadly tuned apparent affinity and dynamic range suitable to measure dopamine release between pM and µM range. They exhibit high affinity, millisecond response times, high signal-to-noise ratio (SNR), along with expression and targeting suitable for use *in vitro* and *in vivo* to track neuromodulation. We created Cre-dependent viruses optimized to express these genetically encoded sensors pre- or post-synaptically in transgenic animals. Taking into consideration the heterogeneity of brain regions dopaminergic levels and to improve upon the brightness of previously published dopamine indicators, we engineered new optimized dopamine sensors: dLight3.0 series. Members of the dLight3.0 series have improved SNR whereby their performances were compared to dLight1.3b in both prefrontal cortex (PFC) and dorsolateral striatum (DLS) with single and 2-photon imaging. For example, dLight3.6 was engineered with a Kd of 25 nM and a higher basal fluorescence for low dopamine innervated regions. dLight3.8 was optimized with a Kd of 250 nM, a dim baseline fluorescence, and a high dynamic range, and is best suited for regions with high dopamine innervation or pharmacological testing. We also performed dopamine imaging on acute brain slices with and without the dopamine reuptake inhibitor methylphenidate at the pre- and post-synaptic level which provided novel insights compared to fast scan cyclic voltammetry. A comparison between sensors revealed differences in dopamine levels and spatiotemporal dynamics that are dependent on expression level and pattern. Our data show that the dLight sensor family can pave the way towards a more complete understanding of neurotransmitter dynamics in the basal ganglia circuitry and beyond in less studied regions where previous methods have not yet been successful. Combined with improved imaging and analysis methods, these biosensors could be valuable tools to decipher neural activity into its composite molecular signaling events.

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**Topic:** I.04. Physiological Methods

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**Title:** A toolkit of highly selective and sensitive genetically encoded neuropeptide sensors

**Authors: *H. WANG**[1], T. QIAN[1], Y. ZHAO[1], Y. ZHUO[1], T. OSAKADA[2], P. CHEN[3], Z. CHEN[4], L. GENG[1], S. FU[1], Y. YAN[1], H. REN[1], Y. ZHU[4], D. LIN[2], J.-N. ZHOU[3], Y. LI[1];
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**Abstract:** Neuropeptides are key signaling molecules in the endocrine and nervous systems that regulate many critical physiological processes, including energy balance, sleep and circadian rhythms, stress, and social behaviors. Understanding the functions of neuropeptides in vivo requires the ability to monitor their dynamics with high specificity, sensitivity, and spatiotemporal resolution; however, this has been hindered by the lack of direct, sensitive and non-invasive tools. Here, we developed a series of GRAB (G protein-coupled receptor activation–based) sensors for detecting somatostatin (SST), corticotropin-releasing factor (CRF), cholecystokinin (CCK), neuropeptide Y (NPY), neurotensin (NTS), and vasoactive intestinal peptide (VIP). These fluorescent sensors utilize the corresponding GPCRs as the neuropeptide-sensing module with the insertion of a circular-permutated GFP as the optical reporter, enabling detection of specific neuropeptide binding at nanomolar concentration with a robust increase in fluorescence. We used the SST sensor to measure the spatiotemporal dynamics of endogenous SST release in isolated pancreatic islets and monitored SST changes in basal lateral amygdala of mouse brain during Pavlovian conditioning. The CRF sensor reported the release of CRF in acute brain slices. Moreover, we detect endogenous CRF release induced by stressful experiences in vivo using fiber photometry and 2-photon imaging in mice. Together, these new sensors establish a robust toolkit for studying the release, function, and regulation of neuropeptides under both physiological and pathophysiological conditions.

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**Presentation Number:** NANO60.08

**Topic:** I.04. Physiological Methods

**Support:** Howard Hughes Medical Institute (HHMI)

**Title:** In vivo functional imaging in the green to near-infrared with chemigenetic fluorescent indicators

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Campus, Ashburn, VA; [3]Dept. of Diagnos. Radiology and Nuclear Med., [4]Univ. of Maryland Sch. of Med., Univ. of Maryland Sch. of Med., Baltimore, MD; [5]Janelia Res. Campus, Janelia Res. Campus, Ashburn, VA; [6]Howard Hughes Med. Institute, Janelia Farm Res. Campus, Howard Hughes Med. Institute, Janelia Farm Res. Campus, Ashburn, VA

**Abstract:** Functional fluorescence imaging has been vital for understanding the dynamics of cellular activities in behaving animals. Genetically encoded indicators built from fluorescent proteins have been especially useful, but they have a fixed emission color, and we currently lack bright indicators in the near infrared. Chemigenetic indicators, built from genetically encoded proteins and bright small-molecule dye-ligands, allow flexibility in the emission color. Here, we present a chemigentic calcium ion indicator: WHaloCaMP, with emission from green to near-infrared that can be delivered to the central nervous system in animals. We have used WHaloCaMP with near-infrared emission to image neuronal activity in the brains of zebrafish, fruit flies and mice, and we have performed simultaneous three-color functional imaging in zebrafish larvae together with green and red indicators. As WHaloCaMP has >2 ns change in fluorescence lifetime on calcium addition, we also performed quantitative fluorescence lifetime imaging microscopy (FLIM) of calcium ion transients in cells and in zebrafish larvae. We believe this chemigenetic approach to be a general way to leverage the favorable optical properties of synthetic dyes in building indicators to report on dynamic cellular activities during animal behavior.

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**Presentation Number:** NANO60.09

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Title:** Temporally-gated activity integrators in Drosophila

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**Abstract:** Genetic access to the neuronal ensembles activated during specific behavioral paradigm is important for studying neuronal pathways underlying cognitive processes and behaviors. Temporally-gated transcriptional integrators, which convert transient neuronal activities into a gene expression signal, can label neurons activated during a user-specified time window with any transgenes. However, such tools have not been demonstrated in *Drosophila* yet. To address this gap, I have engineered a new generation of light- and calcium-gated transcriptional integrator, termed cytoFLARE. cytoFLARE can detect 1-minute calcium rise with a signal-to-noise ratio of 44-fold in HEK293T cells. Compared to the benchmark integrator, cytoFLARE showed a higher activation efficiency during a short time window. We also demonstrated that cytoFLARE can report activated second-order neurons upon noxious stimulation in *Drosophila* larvae. We will further compare cytoFLARE to existing integrators in neuronal culture and apply cytoFLARE to study the somatosensory system of behaving *Drosophila* larvae. Overall, cytoFLARE will be the first temporally-gated transcriptional

integrator for reporting neuronal activity in *Drosophila* and it can also be potentially adapted for applications in other animal models.

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**Presentation Number:** NANO60.10

**Topic:** I.04. Physiological Methods

**Title:** Development of a multimodal approach to follow the transit of adeno associated viruses inside cells

**Authors: *L. PAQUET**[1,2], J. ECHANOVE[1], J. MERONE[2], A. BARBEAU[1], R. DALANGIN[1], A. GODIN[1,2], M.-E. PAQUET[1,2];
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**Abstract:** Viral vectors are indispensable tools in neuroscience and bio-imaging as they are used for modulating the expression of specific genes in cells and enabling direct observation of targeted proteins. Recombinant adeno associated viruses (rAAVs) are the vehicles of choice for the delivery of optogenetic tools inside cells, or more specifically neurons. These viral vectors, made of 25 nm capsids, establish a serotype specific and long-term expression. They were shown to be non-pathogenic and can infect non-dividing cells such as neurons. They can also be used for central nervous system gene therapy with the help of focused ultrasounds (FUS). However, while rAAVs enable *in vivo* observations of a panoply of biological mechanisms, the transduction of the viral particle itself remains very intricate. Thus, the goal of this study is to develop a multimodal method to follow the journey of rAAV throughout neurons in real time. First, AAV capsids will be labeled through a novel and innovative tagging strategy to monitor its progress during the infection process. Second, subcellular regions like the cell membrane, the nuclear envelope and other organelles will be tagged to create checkpoints during the AAV infection. Third, the monitoring of the AAV infection will be made using real-time videorate super-resolution fluorescence microscopy. Super-resolution microscopy will help unravel events occurring on the nanometer scale. Altogether, the new capsid labeling method will allow a very specific and versatile labeling with minimal impacts on its integrity and its transduction properties. Fluorescently labelled capsids will be easily detectable using fluorescent microscopy and will provide a unique opportunity to track viral particles with live imaging. Furthermore, the labelling of subcellular components will not only allow to reveal precise AAV intracellular localization, but it will also allow quantification of the transduction efficiency of different rAAVs serotypes at specific steps. This project will allow the development of a multimodal method for direct *in vivo* observation of AAV transduction in neurons. Furthermore, once the observation strategy is developed, a vast selection of parameters can be modified and compared (AAV serotypes, cellular models) which will open the door to new studies to further understand the AAV transduction mechanisms. The development of this multimodal method to observe the journey of AAVs throughout neurons will bring significant data on AAV infection and contribute to the use of viral vector in neuroscience.

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**Nanosymposium**

**NANO61: Gene Targeting Approaches for Brain Function and Disease**

**Location:** WCC 146C

**Time:** Tuesday, November 14, 2023, 8:00 AM - 9:45 AM

**Presentation Number:** NANO61.01

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Support:** NIH Grant NS109532
CJD Foundation Research Grant

**Title:** Development of an effective gene therapy for sporadic CJD

**Authors:** J. ZHANG[1], M. CAMACHO[1], C. CHOI[1], V. WARADY[1], T. F. SHAY[2], V. GRADINARU[3], ***Q. KONG**[1];
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**Abstract:** Creutzfeldt-Jakob disease (CJD) is an invariably fatal human prion disease that progresses rapidly and has no treatments. An effective and lasting treatment for CJD is urgently needed. The cellular prion protein (PrP) is essential for both prion replication and prion pathogenesis but expendable for life, making it an attractive target for prion therapeutics development. Knocking down the PrP gene expression through RNA interference, such as with antisense oligonucleotides (ASOs), has been shown to be safe and effective against rodent prions in mouse models. But the RNA interference strategy targeting the PrP gene with ASO or shRNA/siRNA has not been tested against human prions, and there has been no report of rAAV-based shRNA gene therapy for any prion disease. We designed four shRNAs (shRNA 1-4) for the human PrP gene that showed ~90% knockdown of human PrP protein level in a human neuroblastoma cell line. We created rAAVs carrying the shRNA2 using either the AAV-PHP.eB (or PHP.eB) capsid or the AAV.CAP-B10 (or CAP-B10) capsid, then tested CAP-B10-shRNA2 in a humanized CJD mouse model (Tg40h) that overexpresses human PrP-129M at 3-4 times the normal level and was inoculated with the most common sporadic CJD prion subtype (sCJDMM1) before the rAAV treatment. When administered retro-orbitally at near clinical onset, a single dose of CAP-B10-shRNA2 ($1.6 \times 10^{12}$ vg/mouse) knocked down human PrP level by ~26% in the brain and led to a 9.6% extension of survival of the sCJDMM1-inoculated mice ($p=0.0003$). As we found that CAP-B10 brain transduction potency is significantly reduced in the Tg40h mice compared to C57BL/6, we also tested PHP.eB, whose CNS potency is retained in our model. Retro-orbital delivery of PHP.eB-shRNA2 at a much lower dose ($2.4 \times 10^{11}$ vg/mouse) reduced human PrP level by 52.7% in the brain of the Tg40h mice, suggesting that a much more extensive extension of survival with the systematic rAAV-shRNA delivery approach is within reach if the PHP.eB capsid is utilized to package the rAAV-shRNA genome, even in human PrP overexpressing mice. Our data strongly suggest that a single systematic administration of an optimized rAAV-shRNA targeting PrP is a highly promising and realistic approach for effective CJD treatment when given at clinical onset.

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**Presentation Number:** NANO61.02

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Title:** In silico prediction and in vivo testing of promoters targeting GABAergic inhibitory neurons

**Authors: *J. B. SMITH**[1], Y. NIIBORI[2], R. DUBA-KISS[2], J. T. BRUDER[1], D. R. HAMPSON[2]; [1]R&ED, REGENXBIO Inc., Rockville, MD; [2]Univ. of Toronto, Toronto, ON, Canada

**Abstract:** Impairment of GABAergic inhibitory neuronal function is linked to epilepsy and other neurological and psychiatric disorders. One therapeutic strategy for treating disorders where GABAergic inhibition is impeded, is to enhance GABA neuronal activity in the brain. Recombinant adeno-associated virus (rAAV)-based gene therapy targeting GABAergic neurons is a promising treatment for GABA-associated disorders. However, there is a need to develop rAAV-compatible gene regulatory elements capable of selectively driving expression in GABAergic neurons throughout the brain. Here, we designed several novel GABAergic gene promoters. *In silico* analyses, including evolutionarily conserved DNA sequence alignments and transcription factor binding site searches among GABAergic neuronal genes, were carried out to reveal novel sequences for use as rAAV-compatible promoters. rAAV (serotype 9) vectors were injected into the brain of neonatal and adult mice to assess promoter specificity. In mice injected neonatally, transgene expression was detected in multiple brain regions with very high neuronal specificity, and moderate to high GABAergic neuronal selectivity. The GABA promoters differed greatly in their levels of expression, and in some brain regions, showed strikingly different patterns of GABAergic neuron transduction. For example, one promoter expressed in medium spiny neurons in the striatum, whereas another was restricted to parvalbumin (PV) interneurons in the striatum. This study is the first report of rAAV vectors that are functional in multiple brain regions using promoters designed by *in silico* analyses from multiple GABAergic genes. These novel GABA targeting vectors may be useful tools to advance gene therapy for GABA-associated disorders.

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**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

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**Title:** CRISPR-Cas13d-mediated reduction of ataxin-2 extends survival and improves motor function in a mouse model of a TDP-43 proteinopathy

**Authors:** M. ZEBALLOS C., H. MOORE, T. SMITH, J. POWELL, ***N. AHSAN**, S. ZHANG, T. GAJ;
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**Abstract:** The mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43) is a pathological feature in TDP-43 proteinopathies, which include amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Given its apparent central role in ALS and FTD, TDP-43 has emerged as an important and potentially broadly applicable therapeutic target for both disorders. However, TDP-43 performs essential regulatory functions, which likely preclude its targeting. As such, numerous studies have been conducted to identify novel gene targets whose perturbation can modify TDP-43-mediated toxicity without affecting its underlying expression. One such modifier is ataxin-2, a polyQ-containing RNA-binding protein that, when modulated, can modify TDP-43-associated toxicity. Thus, strategies for targeting ataxin-2 (ATXN2) may hold potential for ALS-FTD. Gene silencing modalities such as antisense oligonucleotides (ASOs) have been used to target ATXN2 and demonstrated promise in preclinical models. However, ASOs have a transient lifecycle that necessitates redosing, a limitation that could impose a physical burden on patients. For this reason, we explored the utility of a CRISPR technology to target ATXN2, as these platforms can be delivered by a viral vector to mediate their continuous expression. Among the CRISPR-based platforms that can achieve this goal are RNA-targeting CRISPR effector proteins, such as Cas13 and Cas7-11, which, along with a CRISPR RNA, can facilitate the degradation of target RNAs. Here, we demonstrate that RNA-targeting CRISPR effector proteins can target ATXN2 to ameliorate a TDP-43 proteinopathy. We show that reducing ATXN2 with CRISPR-Cas13 can reduce the size and number of TDP-43 protein inclusions, and its localization to stress granules, transient structures that can form in response to stress, in several cell culture models. We further validate our approach by delivering an ATXN2-targeting CRISPR-Cas13 system to a mouse model of TDP-43 proteinopathy. We find that CRISPR-Cas13 rescued motor impairment, enhanced motor coordination and grip strength, dramatically extended survival (~56% median $P < 0.01$), and decreased the severity of several neuropathological hallmarks (neuronal survival, TDP-43 phosphorylation, and neuroinflammation). Finally, we probe the targeting specificity of high-fidelity effector proteins, finding that second-generation effectors possess improved capabilities to the original Cas13 protein when targeting ATXN2. Our results thus demonstrate that RNA-targeting CRISPR technologies can be programmed to silence disease-modifying proteins and hold potential for ALS and FTD.

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**Presentation Number:** NANO61.04

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Support:** NIH Grant 5UG3MH120102

**Title:** Engineering viral vectors for acoustically targeted gene delivery to the brain across species

**Authors: *H. LI**[1], J. HEATH[1], M. HARB[3], J. TRIPPETT[4], M. SHAPIRO[2], J. SZABLOWSKI[4]; [1]Biol. and Biol. Engin., [2]Chem. and Chem. Engin., Caltech, Pasadena, CA; [3]Bioengineering, Rice Univ., Pasadena, CA; [4]Bioengineering, Rice Univ., Houston, TX

**Abstract:** Spatially targeted gene delivery to the brain has the potential to treat prevalent neurological and psychiatric diseases. However, the site-specific delivery of vectors such as adeno-associated viruses (AAVs) is typically performed via invasive injections, limiting scope of clinical applications. Over recent decades, focused ultrasound blood-brain-barrier opening (FUS-BBBO) has emerged as a noninvasive procedure enabling the site-specific entry of small viral vectors into the brain from systemic circulation. However, when used in conjunction with natural AAV serotypes, this approach has limited transduction efficiency, and results in undesirable transduction of peripheral organs.

In this project we employ *in vivo* evolution to develop new AAV serotypes with enhanced transduction at sites of FUS-BBBO and decreased transduction peripherally in rodents and nonhuman primates (NHPs). In our screening strategy, a library of AAVs with mutated capsids based on AAV9 is injected intravenously into hSyn1-Cre mouse and delivered via FUS-BBBO to one hemisphere. When a particular AAV variant transduces Cre-expressing neurons, its viral genome is modified, becoming detectable by a Cre-dependent PCR and next-generation sequencing (NGS). Repeated rounds of selection for vectors that uniquely appear in the targeted hemisphere led to desired novel AAV vectors (AAV.FUS) with 12.1-fold improvement in overall tissue specificity and above 56% improved neuronal tropism in brain. AAV.FUS's capability of improved FUS-mediated delivery to brain is validated across multiple murine strains. As a bonus, AAV.FUS has 2.5-fold improved transduction efficiency over AAV9 when delivered via intracranial injection.

To develop improved viral vectors for FUS-BBBO in nonhuman primates (NHPs), we performed viral screening in macaques with a strategy similar to that of rodent model but introduced neuronal Cre expression through AAV9-hSyn1-Cre transduction due to lack of transgenic NHP. After two rounds of evolutions, 145 viral variants are selected for further screening based on their unique enrichment in acoustically targeted brain regions of NHP.

Overall, this study shows that the molecular engineering of AAV capsids can lead to improved noninvasive, site-specific ultrasound-mediated gene delivery to the brain across different species, paving the way for large-scale disease model studies and potential future human clinical translation.

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**Presentation Number:** NANO61.05

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Support:** R01NS121073

**Title:** Hemogenetic tools for brain-wide functional imaging of cells and circuits

**Authors: *Y. KE**, D. TIRUKOTI, Y. JIANG, A. JASANOFF;
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**Abstract:** Numerous cellular and circuit-level processes distributed throughout the brain interact to orchestrate behavior and plasticity. Understanding mechanisms of brain function will depend on tools for monitoring these processes noninvasively, throughout intact living animals. Our recent work introduces a family of genetic probes, called NOSTICs, that allow cellular-level activity to be sensitively monitored using functional magnetic resonance imaging (fMRI). NOSTICs are engineered enzymes that transduce the cytosolic calcium dynamics of the probe-expressing cells into localized hemodynamic responses. We show how first generation probes have been used to study information flow in neural circuitry during rewarding or sensory stimulation, in an approach we refer to as hemogenetic imaging. We next describe three ongoing directions aimed at improving the current NOSTICs and broadening their applications. The first direction involves a high-throughput engineering method to optimize the NOSTIC reporters and better differentiate them from endogenous background signals. In the second direction, we are establishing a diverse set of viral vectors suitable for delivering and controlling NOSTIC genes. The vectors include Cre-dependent NOSTIC constructs, vectors that express NOSTICs in combination with optical reporters, and compact adeno-associated vectors (AAVs) that exploit advances in viral technology for delivery and circuit labeling. The third direction entails the creation and validation of novel drug-induced activatable hemogenetic probes, termed diaNOSTICs. These probes can be switched on by delivery of small molecules, revealing expression profiles or facilitating probe multiplexing. Together, the strategies we describe will facilitate hemogenetic imaging-based assessments of a variety of complex neural phenomena in health and disease.

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**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

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**Title:** Human Blood-Brain Barrier Receptor-Guided Evolution of AAVs for Brain Gene Therapy

**Authors: *C. LIN**, X. CHEN, E. SULLIVAN, X. DING, Y. FAN, A. CHUNG, T. GAWDA, S. JANG, T. F. SHAY, V. GRADINARU;
the California Inst. of Technol., Pasadena, CA

**Abstract:** Crossing the blood-brain barrier (BBB) poses challenges for treating brain disorders. Adeno-associated viral vectors (AAVs) offer an attractive solution due to their safety and specificity (Challis et al. 2022). Yet AAVs engineered in rodents often underperform in primates due to species-specific differences. Until now, most efforts to bypass this problem have focused

on directed evolution of AAVs to target conserved transcytosis pathways by screening in multiple species. However, this approach is costly in time and resources, particularly primate subjects, and has met with limited success due to differences in receptors between humans and other species.
In response, we have devised a new strategy for animal-free, in vitro evolution of AAVs for specific binding to human BBB receptors, such as one we recently identified: carbonic anhydrase IV (CA4) (Shay et al. 2023). CA4 is highly conserved from rodents to primates, including humans. The mouse homolog (msCar4) mediates BBB transcytosis of the engineered AAVs 9P31 and 9P36, leading to broad CNS transduction (Nonnenmacher et al. 2021). However, sequence differences in the AAV binding pocket prevent human CA4 (huCA4) from binding 9P31 and 9P36.
We therefore set out to engineer huCA4-targeting AAVs. We developed an in vitro resin-based pulldown assay to identify AAVs that efficiently bind to huCA4. We validated our approach using LY6A, which yielded the known binders PHP.B and PHP.eB. Similarly, msCar4 yielded 9P31 and 9P36. After two rounds of resin-based selection for huCA4, we identified 2,005 potentially-interacting AAVs. A third round, combining resin-based and in vitro cell-based selections, led us to a final set of candidate AAVs: huCA4-AAVs. We characterized the enhanced huCA4 binding of these huCA4-AAVs by pulldown assays and surface plasmon resonance (SPR). The huCA4-AAVs displayed a huCA4-dependent infectivity boost in an in-vitro cell-culture assay. Some also demonstrated huCA4-dependent tissue transduction in transgenic rodents expressing huCA4 (delivered by AAV1-X1 to endothelial cells (Chen et al. 2023)). Using the same strategy, we also identified AAV variants that recognize rhesus macaque CA4, which we are now validating in vivo.
Overall, we developed a pipeline to engineer human receptor-targeting AAVs in an animal-free system, which offers the potential to find better gene delivery vehicles compatible with existing mechanisms in humans. The AAVs identified by this mechanism-driven approach could enable efficient gene delivery to the CNS for next-generation neurotherapeutic interventions.

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**Presentation Number:** NANO61.07

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Title:** Identification of a novel OHC-specific enhancer fragment for precision gene therapy in the cochlea

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**Abstract:** Cochlear hair cells, comprising inner hair cells (IHCs) and outer hair cells (OHCs), play a vital role in auditory perception. Among these, OHCs exhibit a remarkable capability to elongate and contract in response to sound, a process essential for sound sensing mediated by the motor protein Prestin, which is exclusively expressed in OHCs. Adeno-associated virus (AAV) has demonstrated efficacy in delivering gene therapy to enhance auditory function in murine models of hereditary hearing loss. However, the diverse forms of AAVs exhibit variable infectivity towards different cell types within the cochlea, and a highly specific AAV capable of targeting OHCs is yet to be available. Consequently, the development of an OHC-specific enhancer fragment is imperative for effective gene therapeutic interventions in cases of OHC damage, a prevalent cause of deafness. Our hypothesis centered on the notion that deletion of a specific cis-regulatory DNA fragment within the Slc26a5 gene would have an impact on its expression. To investigate this, we employed CRISPR/Cas9-mediated large fragment deletions in combination with immunohistochemistry, in situ hybridization, and auditory brainstem response (ABR) testing. Initially, a 13 kbp fragment deletion within Slc26a5 intron 2 was found to induce a developmental delay in Prestin expression and hearing function. Subsequently, through a stepwise approach, we progressively narrowed down the critical enhancer fragment to 1.4 kbp, and its knockout successfully replicated the phenotype observed in the 13 kbp deletion. Furthermore, when combined with the Hsp68 promoter, the 1.4 kbp enhancer fragment facilitated the specific expression of green fluorescent protein (EGFP) solely in OHCs, affirming its sufficiency and necessity for precise Prestin expression in a timely manner. Notably, the homologous 398 bp fragment within the human Slc26a5 gene also drove specific EGFP expression, indicating the potential evolutionary conservation of the enhancer across different species. Leveraging the high infectivity of AAV2.7m8 towards both IHCs and OHCs, we employed this capsid to infect hair cells (HCs) and utilized the newly identified 398 bp CRE of Prestin in combination with the Hsp68 promoter to selectively drive EGFP expression in OHCs. The distinct EGFP signals observed solely in OHCs highlighted the efficiency and specificity of this newly developed tool. This novel finding bears significant clinical significance as it paves the way for developing AAVs specifically targeting OHCs for the treatment of deafness.

**Disclosures: D. Zhang:** None. **Y. Sun:** None. **G. Wang:** None. **Z. Liu:** None.

**Nanosymposium**

**NANO62: Modulation of Calcium Channels in Health and Disease**

**Location:** WCC 207A

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO62.01

**Topic:** B.03. Ion Channels

**Support:** NIH R01 DK123463-01
NIH R01 DK131118-01

**Title:** Primary cilia regulation of hippocampal excitability

**Authors:** T. VIEN, M. C. TA, L. F. KIMURA, ***P. DECAEN**;
Northwestern Univ., Chicago, IL

**Abstract:** Primary cilia are antenna-like organelles that represent a frontier of knowledge in neuroscience research. They were first reported by Duncan and Dahl more than sixty years ago, and although they are implicated in neurodevelopmental diseases, our understanding of their function in neurons is limited. The authors presentation will demonstrate that the primary cilia is an excitable organelle richly populated with PKD2L1 TRP channels which . Using microelectrode electrophysiology and mouse genetics, the authors find PKD2L1 channels in the cilia contributes to high frequency action potential firing and their loss of function primarily impacts interneuron excitability. Loss of channel expression impairs cilia maturation in mice, which behaviorally exhibit autism-like features and seizure susceptibility that may have implications to human neuronal ciliopathy conditions. In total, the findings identify PKD2L1 channels as regulators of hippocampal excitability and the neuronal primary cilia as organelle mediators of brain electrical signaling. These results relate to the recently (May 22, 2023) published article in *PNAS* (doi: 10.1073/pnas.2219686120. PMID:37216541)

**Disclosures: T. Vien:** None. **M.C. Ta:** None. **L.F. Kimura:** None. **P. Decaen:** None.

**Presentation Number:** NANO62.02

**Topic:** B.03. Ion Channels

**Support:** 1RF1AG077103
1RF1AG065628

**Title:** A cascade of intracellular organelle defects drives cellular pathophysiology in Alzheimer's disease and Down syndrome neurons

**Authors: *G. E. STUTZMANN**[1], S. MUSTALY[2], W. GALLEGOS[3], R. A. MARR[2], M. ALLDRED[4], S. GINSBERG[5];
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**Abstract:** Alzheimer's disease (AD) is a neurodegenerative disorder characterized by abnormal protein aggregates, synaptic deficits, metabolic defects, and memory loss. This series of neuronal pathophysiological features reflects dysfunctional coordination among key intracellular organelles, namely the endoplasmic reticulum (ER) through aberrant protein trafficking and $Ca^{2+}$ release, lysosomes through defective proteolysis, and mitochondria through altered bioenergetics and free radical production. Notably, AD and Down syndrome (DS) share many pathological

components, and the majority of DS individuals will convert to AD as they age. While the cellular phenotypes and differentially expressed gene gene pathways are fairly well described in these conditions, the underlying pathophysiological mechanisms are still poorly understood. To address this gap and attempt to identify shared mechanisms in AD and DS, we used a series of fluorescent biosensors and optical imaging approaches in model cells, mouse models, and human induced neurons (HiN) derived from AD and DS patients, to explore functions of neuronal organelles implicated in AD and DS. We identified a novel cellular signaling cascade mediated by intracellular calcium dysregulation which underlies protein mishandling and mitochondrial dysfunction in AD neurons. Increased $Ca^{2+}$ release via the endoplasmic reticulum (ER) resident ryanodine receptor (RyR) was associated with reduced expression of the lysosome proton pump vATPase subunits, resulting in lysosome deacidification and disrupted proteolytic activity in AD mouse models and human induced neurons (HiN). As a result of impaired lysosome proteolytic capacity, mature autophagosomes accumulated containing increased hyperphoshorylated tau levels, exacerbating proteinopathy. Furthermore, calcium dyshomeostasis contributes to a steady-state increase in oxidative free radicals, depolarized membrane potential, and increased calcium within the inner mitochondrial matrix in the AD and DS HiN. Normalizing aberrant RyR-$Ca^{2+}$ signaling with dantrolene (Ryanodex) restored physiological functioning of these critical organelles in both conditions. These results highlight that aberrant ER $Ca^{2+}$ signaling disrupts a series of key organelle interactions that collectively drive a neuronal AD phenotype, and point to shared pathogenic mechanisms between AD and DS. Furthermore, pharmacological suppression of RyR-$Ca^{2+}$ release rescues organelle function, revealing a target for therapeutic intervention that has demonstrated effects in clinically-relevant assays.

**Disclosures: G.E. Stutzmann:** None. **S. Mustaly:** None. **W. Gallegos:** None. **R.A. Marr:** None. **M. Alldred:** None. **S. Ginsberg:** None.

**Presentation Number:** NANO62.03

**Topic:** B.03. Ion Channels

**Support:** CIHR Grant PJT-152914
Krembil Foundation

**Title:** Limiting RyR2 open time protects neurons from transient ischemic attack (TIA) induced hyperactivity and dysfunction

**Authors: *J. YAO**, A. LYER, M. MILLER, Z. SONG, J. P. ESTILLORE, W. S. R. CHEN; Hotchkiss Brain Inst., Calgary, AB, Canada

**Abstract:** Transient ischemic attack (TIA) is often known as a “ministroke” resulting from temporary focal cerebral ischemia. Although TIA has been associated with an increased risk of cognitive decline and dementia, its underlying mechanisms remain incompletely understood, partly due to the challenges of modeling TIA in small animal models. To address this gap, we employed the "stroke induced with magnetic particles" (SIMPLE) approach, functional ultrasound (fUS)imaging system, and multi-photon imaging technology to investigate the effects of TIA on the central nervous system at the levels of single neurons and brain network. Two-photon calcium imaging studies showed that transient cerebral hypoperfusion induced by oxygen-glucose deprivation (OGD) increased the excitability of hippocampal CA1 pyramidal

neurons in brain slices. Notably, this finding aligns with neuronal hyperactivity observed in Alzheimer's disease (AD) patients and AD animal models. Furthermore, using fUS neuroimaging, we observed that TIA induced by magnetic nanobeads reduced neuronal activity in the barrel cortex in response to whisker stimulation. This observation is consistent with the reduced response to whisker stimulation in the 5xFAD mice, a well-established AD animal model. To investigate the role of ryanodine receptor 2 (RyR2) in TIA, we employed a RyR2 mutant (E4872Q) mouse model that displays reduced duration of channel opening. Excitingly, we found that genetically reducing the RyR2 open time (the E4872Q mutation) protected neurons against OGD-induced hyperactivity and TIA-induced impaired response to whisker stimulation. This is consistent with our previous finding that limiting RyR2 open time protects neurons from AD-induced hyperactivity and cognitive decline. These preliminary findings suggest that TIA may increase neuronal activity in the affected area, potentially leading to disruption of connectivity in the brain network and eventual cognitive decline and dementia. Limiting RyR2 open time may be an effective strategy to shield neurons from TIA-induced damage. Clearly, further research is necessary to fully understand the mechanisms underlying TIA-induced neuronal hyperactivity and to develop effective interventions (Supported by Krembil Foundation and CIHR).

**Disclosures: J. Yao:** None. **A. Lyer:** None. **M. Miller:** None. **Z. Song:** None. **J.P. Estillore:** None. **W.S.R. Chen:** None.

**Presentation Number:** NANO62.04

**Topic:** B.03. Ion Channels

**Support:** NIH grant NS101880
NIH grant NS132398

**Title:** Disrupting chloride homeostasis preferentially augments NMDA receptor activity in excitatory spinal dorsal horn neurons via α2δ-1

**Authors: *Y. HUANG**, H. CHEN, S.-R. CHEN, H.-L. PAN;
Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

**Abstract:** The potassium-chloride cotransporter-2 (KCC2) controls chloride homeostasis and maintains normal synaptic inhibition in the spinal dorsal horn. Nerve injury increases NMDA receptor (NMDAR) activity in spinal dorsal horn neurons, which leads to KCC2 cleavage and impairment of synaptic inhibition by GABA and glycine. However, it is unclear whether and how KCC2 activity controls NMDAR activity in spinal dorsal horn neurons. In the present study, we showed that treatment with VU0463271, a potent and selective KCC2 inhibitor, significantly increased puff NMDA currents in vesicular glutamate transporter 2 (VGluT2)-expressing excitatory neurons in the spinal cord. In VGluT2 dorsal horn neurons, VU0463271 also significantly increased the frequency of miniature EPSC (mEPSC), which was readily reversed by the NMDAR antagonist AP5. By contrast, treatment with VU0463271 had no effect on puff NMDA currents or mEPSCs in vesicular GABA transporter (VGAT)-expressing inhibitory neurons in the spinal dorsal horn. In α2δ-1 KO mice, VU0463271 failed to alter puff NMDA currents or the mEPSC frequency in spinal dorsal horn neurons. Furthermore, intrathecal injection of 5 μg VU0463271 rapidly induced tactile, pressure, and heat hypersensitivity in wild-

type mice, but it had no such effect in α2δ-1 KO mice. This VU0463271-induced pain hypersensitivity in wild-type mice was completely blocked by prior intrathecal application of 5 μg AP5 or 5 μg pregabalin, a clinically used α2δ-1 inhibitory ligand. In addition, intrathecal administration of 1 μg α2δ-1 C terminus-interfering peptide, which inhibits the α2δ-1-NMDAR interaction, abolished the pain hypersensitivity induced by intrathecal injection of VU0463271. Together, our findings suggest that intrinsic KCC2 activity constitutively controls presynaptic and postsynaptic NMDAR activity in excitatory spinal dorsal horn neurons. Disrupting KCC2-mediated chloride homeostasis potentiates excitatory nociceptive transmission in the spinal dorsal horn via α2δ-1-mediated NMDAR hyperactivity.

**Disclosures: Y. Huang:** None. **H. Chen:** None. **S. Chen:** None. **H. Pan:** None.

**Presentation Number:** NANO62.05

**Topic:** B.03. Ion Channels

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University of Iowa distinguished scholarship
R03TR004161-01
AHA Predoctoral fellowship 23PRE1019152

**Title:** Cav2.1 alpha1 subunit motifs that control Cav2.1 subtype abundance are distinct from preference

**Authors: *J. LI**[1], S. M. YOUNG, Jr[2], P. VEERARAGHAVAN[1];
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**Abstract: $Ca_V2.1$ $\alpha_1$ subunit motifs that control presynaptic $Ca_V2.1$ subtype abundance are distinct from $Ca_V2.1$ preference**Jianing Li[1,2], Priyadharishini Veeraraghavan[1], Samuel M. Young, Jr[1,3^ 1]Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA, USA. [2]Cell Developmental Biology Graduate Program, University of Iowa, Iowa City, IA 52242, USA[3]Department of Otolaryngology, Iowa Neuroscience Institute, University of Iowa, Iowa City, IA, USA.Presynaptic voltage-gated $Ca^{2+}$ channels ($Ca_V$) subtype abundance at mammalian synapses regulates synaptic transmission in health and disease. In the mammalian central nervous system, most presynaptic terminals are $Ca_V2.1$ dominant with a developmental reduction in $Ca_V2.2$ and $Ca_V2.3$ levels, and $Ca_V2$ subtype levels are altered in various diseases. However, the molecular mechanisms controlling presynaptic $Ca_V2$ subtype levels are largely unsolved. Since the $Ca_V2$ $\alpha_1$ subunit cytoplasmic regions contain varying levels of sequence conservation, these regions are proposed to control presynaptic $Ca_V2$ subtype preference and abundance. To investigate the potential role of these regions, we expressed chimeric $Ca_V2$ $\alpha_1$ subunits containing swapped motifs with the $Ca_V2.2$ and $Ca_V2.3$ $\alpha_1$ subunit on a $Ca_V2.1/Ca_V2.2$ null background at the calyx of Held presynaptic terminal. We found that expression of $Ca_V2.1$ $\alpha_1$ subunit chimeras containing the $Ca_V2.3$ loop II-III region or cytoplasmic C-terminus (CT) resulted in a large reduction of presynaptic $Ca^{2+}$ currents compared to the $Ca_V2$ $\alpha_1$ subunit. However, the $Ca^{2+}$ current sensitivity to the $Ca_V2.1$ blocker Agatoxin-IVA, was the same between the chimeras and the $Ca_V2.1$ $\alpha_1$ subunit. Additionally, we found no reduction in presynaptic $Ca^{2+}$ currents with $Ca_V2.1/2.2$ cytoplasmic CT chimeras. We conclude that the

motifs in the Cav2.1 loop II-III and CT do not individually regulate Cav2.1 preference, but these motifs control Cav2.1 levels and the Cav2.3 CT contains motifs that negatively regulate presynaptic Cav2.3 levels. We propose that the motifs controlling presynaptic Cav2.1 preference are distinct from those regulating Cav2.1 levels and may act synergistically to impact pathways regulating Cav2.1 preference and abundance.

**Disclosures: J. Li:** A. Employment/Salary (full or part-time):; University of Iowa. 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.; American heart association. **S.M. Young:** A. Employment/Salary (full or part-time):; University of Iowa. 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. **P. Veeraraghavan:** None.

**Presentation Number:** NANO62.06

**Topic:** B.03. Ion Channels

**Support:** NINDS 1R01NS125271
NINDS T32NS086750

**Title:** Novel technique for optically controlling L-type calcium channel function shows local signaling from dendritic spine to nucleus in conjunction with NMDA receptors.

**Authors: *N. J. MANDELBERG**[1], H. KHALED[2], R. W. TSIEN[3];
[1]New York Univ. Sch. of Med., New York, NY; [2]New York Univ., New York, NY; [3]Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY

**Abstract:** Neurons perform a remarkable range of tasks by changing their physiology in response to external stimuli. L-type voltage gated $Ca^{2+}$ (Cav1) channels are critical for plasticity because of their privileged role in regulating transcription in response to depolarization. However, it is not generally clear how activation of synapses up to hundreds of microns away from the cell body controls nuclear transcription. The coupling mechanism between synaptic activity and activation of gene expression predetermines the types of synaptic or electrical activity that can affect neurons' genetic programs. We developed a technique to optically isolate dendritic or somatic Cav1 activity using a photolabile Cav1 channel antagonist. We show that Cav1 channels act from dendritic spines to drive CaMKII-dependent activation of the powerful transcription factor CREB, synergizing with N-methyl-*D*-aspartate receptors (NMDARs), even in the absence of spikes. These same synaptic molecules enable both mEPSPs and action potentials to signal to the nucleus. We find that Cav1 channels cooperate with NMDARs to drive signaling to the nucleus from dendritic spines, and that activity from even a handful of spines can have impact on a neuron's transcriptional program.

**Disclosures: N.J. Mandelberg:** None. **H. Khaled:** None. **R.W. Tsien:** None.

**Presentation Number:** NANO62.07

**Topic:** B.03. Ion Channels

**Support:** NIH award R01 NS114210

**Title:** Defining Junctophilin-3 ER-PM junctions in the brain

**Authors: *L. RIBEIRO SILVA**[1], K. D. MURRAY[1,2], N. C. VIERRA[1], J. S. TRIMMER[1]; [1]Physiol. and Membrane Biol., [2]Psychiatry and Behavioral Sci., Univ. of California, Davis, Davis, CA

**Abstract:** ER-plasma membrane (PM) junctions are specialized membrane contact sites found in all eukaryotic cells. These domains serve as signaling hubs as the close apposition of the ER and PM membranes promotes the enrichment of specific molecules, increasing their local concentration and thus augmenting the subsequent signaling cascades. Junctophilins (JPH) are a family of ER-resident proteins that interact with PM lipids forming ER-PM junctions. JPH-3 and 4, the isoforms expressed in the brain, have not been as extensively characterized as their skeletal muscle and heart counterparts, JPH-1 and JPH-2. JPH-3 has been implicated in the pathophysiology of Huntington's Disease-like 2, and JPH-3 knockout mice display behavioral abnormalities, which are more severe in JPH3/JPH4 double knockout mice, which also exhibit aberrant neuronal calcium signaling and excitability. To better understand the role of JPH-3 in the brain, we developed a panel of novel anti-JPH-3 monoclonal antibodies. When employed in immunohistochemistry of rat brain, we found, consistent with *in situ* hybridization analyses, that at the cellular level JPH-3 immunolabeling was widespread with higher levels in neocortex, hippocampus, and cerebellum. Throughout, cellular labeling appeared to be somatic, mostly cytoplasmic in nature but with a punctate appearance at higher magnifications as expected from an ER-PM junction forming protein. A prominent population of ER-PM junctions in brain neurons is formed by the interaction of PM Kv2.1 potassium channels with ER VAP proteins. While there is substantial overlap in JPH-3 and Kv2.1 immunolabeling at the cellular level, there exist populations of neurons highly enriched in one and not the other. This is also true at the subcellular level in that neurons co-expressing both proteins exhibit distinct populations of JPH-3 and Kv2.1. We have previously immunopurified Kv2.1-containing protein complexes from crosslinked mouse brain homogenates and further analyzed their protein constituents by mass spectrometry to identify the molecular components of neuronal ER-PM junctions. This detected JPH-3 as one of the proteins specifically copurifying with Kv2.1 from crosslinked mouse brain samples. Using this same approach, we used mass spectrometry to analyze proteins immunopurifying with JPH-3. This revealed a set of proteins involved in calcium signaling selectively enriched in immunopurified JPH-3-containing protein complexes. These studies support that JPH-3 is widely expressed in brain neurons where it participates in calcium signaling at ER-PM junctions.

**Disclosures: L. Ribeiro Silva:** None. **K.D. Murray:** None. **N.C. Vierra:** None. **J.S. Trimmer:** None.

**Presentation Number:** NANO62.08

**Topic:** B.03. Ion Channels

**Title:** Dystonia in Timothy Syndrome mouse model with a gain-of-function mutation in Cav1.2

**Authors: *P. MITRANO-TOWERS**, M. MATSUI, G. PITT;
Cardiovasc. Res. Institute, Med., Cornell Univ. / Weill Cornell Medic Neurosci. Program, New York, NY

**Abstract:** Dystonia, characterized by involuntary movement, is the third most common movement disorder. There are many monogenic causes of dystonia that widely range in cellular mechanisms with no clear convergent molecular pathway. Ultimately imbalanced signaling between the basal ganglia, thalamus, and cerebellum have been implicated in the onset of motor dysfunction. A subset of mutations associated with dystonia affect cellular metabolism, including mutations that lead to pyruvate dehydrogenase (PDH) deficiency. Separately, increasing neuronal $Ca^{2+}$ influx through voltage-gated calcium channels can cause motor dysfunction, including dystonia in mice. Our lab has generated a genetic model of increased neuronal $Ca^{2+}$ influx by mimicking Timothy syndrome (TS), which is caused by gain-of-function mutations in *CACNA1C* and resulting reduced $Ca_V1.2$ channel inactivation. This mouse exhibits muscle contractions and postures consistent with dystonia, and mice are more sensitive (compared to WT) to exacerbation of dystonia with a dihydropyridine $Ca^{2+}$ channel agonist. Further, we find that dystonia is exacerbated in TS mice by acute supplementation of pyruvate, and exacerbation is prevented by pretreating with calcium channel blockers (CCBs) that are blood brain barrier penetrant but not by CCBs that are excluded from the CNS. To understand the cell types and circuits driving this $Ca^{2+}$ influx dependent and pyruvate exacerbated dystonia, we generated mice in which the gain-of-function mutant $Ca_V1.2$ was specifically expressed in inhibitory neurons or cortical excitatory neurons, neither of which cause the dystonia or sensitivity to metabolic substrates. To identify pathways that may contribute to dystonia, targeted polar metabolite profiling of TS mice was performed. This revealed minimal changes in overall metabolism and only a small number of metabolites with differential abundance. We are currently investigating the roles of additional cell types in the brain as well cellular mechanisms related to metabolism and altered calcium flux in driving dystonia in TS mice.

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**Topic:** B.03. Ion Channels

**Support:** NINDS Grant T32 NS086750
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**Title:** NMDAR $ca^{2+}$ flux and LTCC voltage-dependent conformational change cooperate locally and sequentially to signal to the nucleus

**Authors: *H. G. KHALED**[1], N. MANDELBERG[2], X. WANG[2], R. W. TSIEN[2];
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**Abstract:** Enduring plasticity depends on neurons' ability to couple electrical signals at the surface membrane to gene expression in the nucleus, a process called excitation-transcription (E-T) coupling. L-type voltage gated $Ca^{2+}$ channels (LTCCs) are critical for E-T coupling. Switching on LTCC initiates a signaling cascade relying upon calcium/calmodulin-dependent

kinase II (CaMKII) and leading to activation of transcription factors such as cAMP response element binding protein (CREB), critical for neural plasticity and long-term memory. Previous studies of LTCC have often made the implicit assumption that they act as $Ca^{2+}$ sources, parallel to the N-methyl-d-aspartate receptor (NMDAR), and generate synaptic plasticity that is best studied with NMDAR blocked. On the contrary, we have recently demonstrated that excitatory neurons use two distinct signals to synergistically mediate CaMKII mobilization to dendritic spines and activity-dependent gene transcription: not just a local rise in $Ca^{2+}$, but also a voltage-dependent conformational change (VΔC) of LTCC. However, much remains unknown about how LTCC VΔC might cooperate with other postsynaptic $Ca^{2+}$ sources such as NMDAR to drive excitation-transcription coupling. We find that NMDAR activation is sufficient to drive synergy between Ca2+ elevation and LTCC VΔC in cultured cortical neurons when LTCC $Ca^{2+}$ flux is pharmacologically blocked. LTCC VΔC is required for NMDA-induced CaMKII mobilization and pCREB increases, which is blocked by the addition of LTCC-blocker nimodipine to prevent the conformational change. We investigated the temporal scale of LTCC VΔC-NMDAR $Ca^{2+}$ synergy by measuring the effects of blocking LTCC VΔC at intermediate time points after NMDAR activation. Our results show that CaMKII mobilization increases independently of LTCC VΔC at early time points (15s) but not later ones (30-60s). These results suggest that NMDAR $Ca^{2+}$-LTCC VΔC synergy functions in series rather than in parallel, whereby NMDAR plays a dominant role initially while LTCC is more influential later on. The transition appears to involve phosphorylation of Ser1303 of NMDAR subunit GluN2B, a substrate of CaMKII previously shown to decrease CaMKII binding affinity. This partnership may extend to distinct roles for each channel in synaptic plasticity: NMDAR in driving CaMKII mobilization, and LTCC in sustaining it. Studies are currently under way to investigate the temporal dynamics of protein-protein interactions between CaMKII and either LTCC or NMDAR using FRET imaging. Together, our findings provide a molecular basis for how NMDAR and LTCC may cooperate to drive long-term synaptic changes.

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**Presentation Number:** NANO62.10

**Topic:** B.03. Ion Channels

**Support:** NIH grant DC004450

**Title:** $Ca^{2+}$-dependent $Ca^{2+}$-channel inactivation may underlie burst firing of auditory efferent neurons

**Authors: *H. HONG**, L. O. TRUSSELL;
Oregon Hearing Res. Ctr., Oregon Hlth. and Sci. Univ., Portland, OR

**Abstract:** Lateral olivocochlear (LOC) efferent neurons are believed to play an important role in protection of hearing sensitivity. We have examined the electrophysiological properties of these neurons in brain slices from juvenile mice. Previously we showed that LOC neurons exhibit an infra-slow (~0.1 Hz) burst firing pattern, the genesis of which is dependent on L-type $Ca^{2+}$ channels. However, it has not been clear what triggers the termination of burst firing. Here we characterized the properties of $Ca^{2+}$ current in LOC neurons using the whole-cell patch-clamp technique, and show that termination of $Ca^{2+}$ channel activity by $Ca^{2+}$-dependent $Ca^{2+}$ channel

inactivation (CDI) may contribute to the termination of bursts. The voltage sensitivity and pharmacology of LOC $Ca^{2+}$ current indicated that LOC neurons express multiple $Ca^{2+}$ channel subtypes, including T- and L-type channels, but mainly L-type are active during long depolarizations that accompany the burst. With 0.1 mM EGTA in the intracellular solution and 1.2 mM $Ca^{2+}$ in the bath, the decay phase of $Ca^{2+}$ current could be fitted with a double exponential, with a tau-1 of ~50 ms and a tau-2 of ~600 ms. When 10 mM BAPTA was used to chelate intracellular $Ca^{2+}$, tau-2 was significantly prolonged, thus reducing the degree of inactivation. A more drastic effect was observed by switching the external $Ca^{2+}$ to 1.2 mM $Ba^{2+}$. $Ba^{2+}$ prolonged both time constants, and reduced the fractional contribution of the fast tau. The use of BAPTA and $Ba^{2+}$ together reduced the degree of inactivation (measured at the end of a 2-s pulse) from 95% to 67%. A dual-pulse protocol indicated that $Ca^{2+}$ flux may inhibit the $Ca^{2+}$ current, and this effect on current amplitude was attenuated with BAPTA or $Ba^{2+}$. We also explored outward currents that might also contribute to repolarization, but found that block of $Ca^{2+}$-activated potassium channels, Kv2 or inwardly-rectifying potassium channels failed to abolish the patterned firing in LOC neurons. However, an antagonist of Kv7 abolished bursts, but only in a subset of LOC neurons, suggesting heterogeneity of LOC neurons and that other $K^+$ channels must contribute to repolarization. Altogether, we propose that CDI of $Ca^{2+}$ channels, working synergistically with Kv7 and/or potassium leak conductance, contribute to the termination of bursts. This self-regulating mechanism might be fundamental to ensure constant efferent control of the cochlea.

**Disclosures: H. Hong:** None. **L.O. Trussell:** None.

**Nanosymposium**

**NANO63: Therapeutic Strategies: Animal Models**

**Location:** WCC 150

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:45 PM

**Presentation Number:** NANO63.01

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

**Support:** NIH R01 NS124084
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NIH R01 AG080104
P30AG066507 JHU-ADRC

**Title:** Senolytic therapy mitigates microglia senescence and alleviates cerebral hypometabolism and BBB dysfunction in the PS19 mouse model

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**Abstract:** Cellular senescence has been observed in both Alzheimer's disease (AD) patients and animal models. In this study, we investigated the effects of senolytic therapy in the PS19 mouse model, which expresses the P301S mutant form of human microtubule-associated protein tau (MAPT) under the direction of the mouse prion protein promoter. This model represents a tauopathy model, characterized by neuronal loss and brain atrophy, principally in the hippocampus but spreading to other brain regions, at approximately 8-9 months of age, and widespread neurofibrillary tangle-like inclusions. We aimed to examine both functional and structural changes in the brain of PS19 mice using human translatable MRI measures and explore the mechanisms involved in senolytic therapy with this model. 9-month-old PS19 mice displayed significantly reduced cerebral metabolic rate of oxygen (CMRO2) detected by noninvasive T2 relaxation under spin tagging (TRUST) and phase contrast (PC) MRI, and increased blood-brain barrier (BBB) permeability to water by using water-extraction-with-phase-contrast-arterial-spin-tagging (WEPCAST) MRI. Meanwhile, brain microstructural changes were determined by the diffusion tensor imaging (DTI), and cognitive function was evaluated using the tracing fear conditioning test. Our results indicate that treating 3-month-old mice with dasatinib plus quercetin (D+Q) senolytic therapy for 6 months mitigated cerebral hypometabolism (indicated by improved CMRO2), maintained BBB integrity (indicated by reduced permeability surface area product and water extraction fraction), attenuated hippocampal atrophy, and reduced tauopathy. Furthermore, the D+Q senolytic therapy reduced a senescence-like phenotype of microglia characterized by upregulation of the senescence marker p21/CDKN1A. Moreover, the D+Q treatment led to a shift of microglia from a disease-associated subtype to a homeostatic subtype. Notably, the D+Q-treated mice exhibited improved performance in the tracing fear conditioning test. These findings provide evidence for the potential therapeutic benefits of senolytic therapy and highlight the involvement of microglia in the underlying mechanisms. The use of human translatable biomarkers in the PS19 mouse model contributes to our understanding of the brain functional changes associated with tauopathy and the effects of senolytic therapy.
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**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Title:** Synergistic effect of Apigenin and Safranal on oxidative and neurological deficits in Alzheimer's disease rats

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**Abstract: ABSTRACT:BACKGROUND:** Alzheimer's disease (AD) is a prevalent neurodegenerative condition that is characterized by a reduction in cognitive ability as well as

disrupted neural connections. The causative damage occurs due to oxidative stress and neuroinflammation, the two most important contributors for the onset of AD. Natural compounds possessing anti-inflammatory and antioxidant characteristics have emerged as potential therapy possibilities. **AIMS AND OBJECTIVES:** The objective of the present study was to investigate the potential synergistic effects of Apigenin and Safranal on the oxidative damage and neurological dysfunction in rats having AD-like dementia. **METHODOLOGY:** In this study, 36 albino Wistar rats were divided into six groups. The healthy control group received normal saline while the disease control group received scopolamine (2.5 mg/kg, i.p). Donepezil, Apigenin, Safranal, and API+SAF groups received scopolamine (2.5 mg/kg, i.p) followed by oral administration of donepezil (1.5 mg/kg), Apigenin (50mg/kg), and Safranal (0.2ml/kg) and API+SAF (Safranal 0.2 ml/kg; and Apigenin 50mg/kg) respectively. The study lasted four weeks, after which cognitive function and behavioral changes were assessed to monitor cognitive function, depression, and anxiety. After analysis, rats were euthanized and specific brain regions were collected for neurochemical assessment. Various biomarkers related to oxidative stress and brain function were measured, such as MDA, SOD, catalase, GPx, GSH, total protein content, and acetylcholine levels. **RESULTS:** The results showed that the combination of Apigenin and Safranal exerted a significant neuroprotective effect compared with the disease control group and the treatment group alone i.e., Apigenin and Safranal as observed in the behavioral and neurochemical results. Furthermore, the synergistic group showed a significant increase in acetylcholine levels as well as also showed a reduction in oxidative stress, as indicated by decreased MDA levels and increased SOD, catalase, GPx and GSH activities. **CONCLUSION:** These findings suggest that the combined treatment of Safranal and Apigenin, at the given doses, has a synergistic impact in improving cognitive deficits, reducing oxidative stress, and alleviating anxiety in the Scopolamine-induced rat model of Alzheimer's disease. Hence, proves that these natural compounds hold potential as therapeutic agents for Alzheimer's disease but need further investigation to understand their underlying mechanisms of action. **Keywords:** Alzheimer's disease, Oxidative stress, Antioxidant, Natural compounds.

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

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**Title:** Folliculin manipulation in the dorsal hippocampus sex-specifically affects learning and autophagic-lysosomal functions in C57BL/6 and 5xFAD mice

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**Abstract:** Folliculin is a lysosomal protein that functions as a GTPase-activating protein to RagC/D, capable of modulating lysosomal activity based on nutrient availability via displacement of mTORC1 from the lysosomal surface. Although its expression has been confirmed throughout the brain, a functional role has yet to be evaluated. We first evaluated

whether folliculin manipulation within the dorsal CA1 affected learning behavior in female and male C57BL/6J mice, and characterized candidate molecular changes. Folliculin shRNA produced a sex-specific cognitive change, with females demonstrating impaired watermaze learning and probe retention. Increasing folliculin had a more modest effect in improving probe measures in males only. Molecularly, folliculin shRNA reduced female transcript levels of FNIP1, FNIP2, TFEB, TFE3, GABARAP, PPARGC1α, SQSTM1, and NPC1. Conversely, increased folliculin resulted in higher TFEB and NPC1 transcripts in males only, with reduced pAMPK and pULK S757 signaling. As lysosomal dysfunction is heavily implicated in Alzheimer's Disease etiology, we also investigated whether augmenting CA1 folliculin would improve behavioral and/or molecular outcomes in five-month old female and male 5xFAD mice. Folliculin enhancement did not significantly affect watermaze learning, likely due to the extreme phenotype present in even young 5xFAD mice. However, increasing folliculin did continue to affect pAMPK and pULK signaling, primarily in male mice, reinforcing AMPK and ULK as hippocampal targets of folliculin. We are continuing with qPCR analysis of the same molecular targets from Experiment 1. Thus far, folliculin has a sex-dependent role in the dorsal hippocampus, it interacts with lysosomal signaling and appears capable of controlling the CLEAR network of genes via enhancement or suppression of TFEB and TFE3. Due to its ability to increase TFEB, NPC1, and autophagic activity by inhibiting pULK1 at S757, increasing folliculin in the hippocampus should continue to be investigated for therapeutic intervention, albeit in a less severe disease model than 5xFAD mice.

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

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**Title:** Hypusinated eIF5A drives TDP43 pathology via regulation of brain glucose hemostasis and mitochondrial energetics in ADRD

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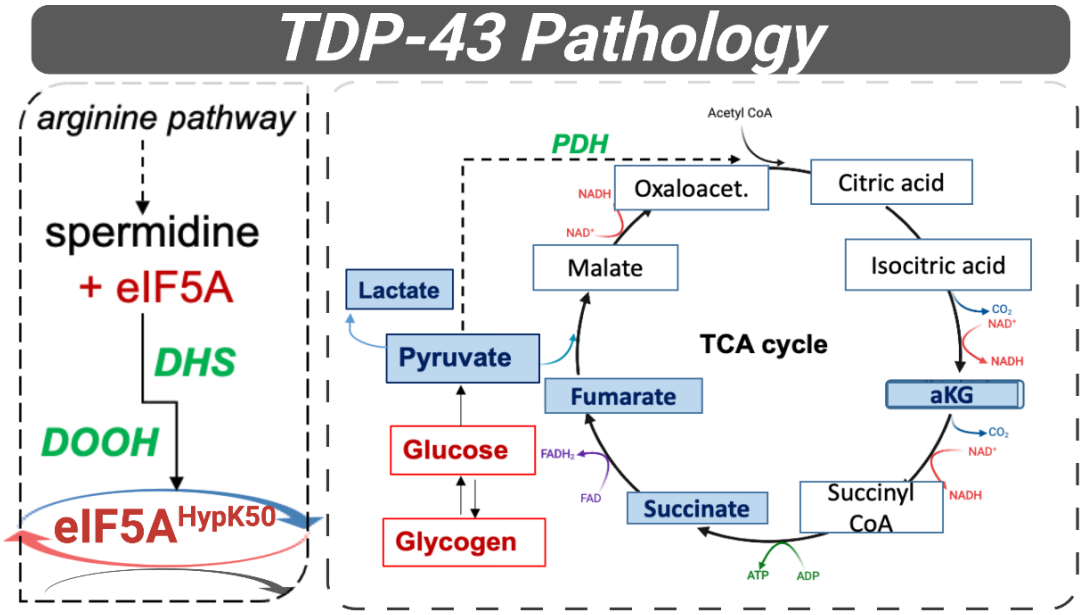
**Abstract:** TAR DNA-binding protein 43 (TDP-43) pathology is associated with clinical dementia in Alzheimer's disease (AD) patients and limbic-predominant TDP-43 encephalopathy (LATE). Regional decline in glucose metabolic rate linked to tau and amyloid-beta pathologies suggest that impaired brain metabolism is one of the earliest and most consistent features of AD. Despite decade-long efforts, the impact of TDP-43 pathology on neurometabolic dysregulation observed in the TDP-43 ADRD disease spectrum remain poorly understood. We have recently shown mechanistic evidence on the role of eukaryotic translation initiation factor 5A (**eIF5A**) in TDP-43 pathology during cellular stress. eIF5A is the only protein undergoing **hypusination** (eIF5A$^{Hyp}$) via deoxyhypusine synthase (**DHS**) and deoxyhypusine hydroxylase (**DOHH**) activity converting a single lysine to a hypusine moiety. Further, we found that TDP-43 pathology induced DHS expression and hypusine levels in the brain of AD and TDP-43 mouse

models. Comprehensive cortical tissue metabolome coverage in a heterozygous TDP-43 mouse model (TAR$^{het}$, mild pathology) showed increased brain glycolytic rate and TCA cycle metabolite levels, supporting a heightened bioenergetic state. *These findings mimic the brain metabolic state reported in ALS patients with early TDP-43 pathology*. Surprisingly, albeit induced neuronal eIF5A$^{Hyp}$ levels via rAAV-DHS/DOHH expression in TAR$^{het}$ mice further increased glucose uptake, aberrant increase in hypusination led to uncoupling of the downstream pathways and significant reduction in pyruvate/lactate levels. Considering that pyruvate is the primary safeguard against oxidative stress, we suggest that induced eIF5A$^{Hyp}$ is a metabolic switch in regulating pyruvate supply to TCA cycle and ATP production, and responsible for the cerebral metabolic dysregulation observed in rAAV-DHS/DOHH mice *in vivo*. In agreement, our RNAseq and the NanoString nSolver$^{TM}$ analyses identified upregulation of disease-associated gene expression involved in UPR, mitochondrial OXPHOS pathway, carbohydrate metabolism, and oxidative stress uniquely pertinent to DHS/DOHH- but not eIF5A-expressing mice. Our findings provide pioneering evidence for eIF5A$^{Hyp}$ regulation of brain glucose hemostasis and mitochondrial impairment under energy-demanding TDP-43 proteinopathy state in disease.

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**Title:** Targeting the MuSK-BMP pathway with antisense oligonucleotides to promote adult hippocampal neurogenesis in neurological and psychiatric disease

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**Abstract:** New neurons are created in the adult brain throughout life and play a key role in learning and memory, resilience to stress, rejuvenation, and the replenishment of lost neurons. Adult hippocampal neurogenesis (AHN) is abundant in healthy aged humans but is reduced from the earliest stages of Alzheimer's, other neurodegenerative diseases, and major depressive disorder. Work in animal models has underscored the role of AHN in improving cognition in the face of AD pathology as well as in improving stress induced depressive-like symptoms. The accumulation of negative signals degrades the neurogenic niche and contributes to the reduction in newborn neurons in AD, stress, and aging. Bone morphogenetic proteins (BMPs) are niche components that restrain neurogenesis. Importantly, their levels are increased in AD patients and mouse models. Decreasing BMP signaling in neural stem cells (NSCs) promotes the formation of new neurons and their integration into the mature circuitry. We recently showed that the transmembrane protein MuSK is a BMP co-receptor that augments and shapes BMP signaling. We term this novel mechanism the 'MuSK-BMP pathway' (Yilmaz et al., 2016; Fish and Fallon, 2020). The MuSK Ig3 domain is necessary for promoting BMP binding and signaling, but is dispensable for MuSK's role in neuromuscular junction formation. To perturb the MuSK-BMP pathway we created mice lacking the MuSK Ig3 domain ('ΔIg3-MuSK' Jaime et al., bioRxiv, 2022). Here we show that freshly isolated adult WT NSCs express MuSK transcript and protein, and that cultured ΔIg3-MuSK NSCs show reduced BMP responsiveness. Immunohistochemistry revealed that MuSK is localized in both the hippocampal and the subventricular zone neurogenic niches in the adult brain. EdU injection studies show that AHN is increased >2 fold in ΔIg3-MuSK mice as judged by the scoring EdU+/doublecortin+ cells in the dentate gyrus. Importantly, ΔIg3-MuSK mice show improved hippocampal-dependent learning as assessed by a novel object location task. As a first step towards targeting the MuSK-BMP pathway pharmacologically we have identified splice-modifying antisense oligonucleotides that induce the skipping of the exons encoding the MuSK Ig3 domain. Targeting the MuSK-BMP pathway by ASO-mediated exon skipping of the Ig3 domain offers the potential to selectively manipulate this signaling pathway in the CNS to promote AHN in a range of neurodegenerative and psychiatric diseases. We are currently testing these exon-skipping ASO in both wild type and disease models to support clinical trials in one or more of these disorders.

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

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**Title:** Alzped: an open science tool raising the standards for preclinical testing of candidate therapeutics in alzheimer's disease animal models

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**Abstract: Background:** Positive findings from testing therapeutics in Alzheimer's disease (AD) animal models are often not translated to effective treatments due to the poor methodological rigor and inadequate reporting practices of therapeutic efficacy studies. The Alzheimer's Disease Preclinical Efficacy Database (AlzPED), developed by the NIA, is a searchable and publicly available knowledgebase that prioritizes and promotes the use of rigorous methodology to ameliorate this translation gap in AD therapy development. Through a checklist of experimental design elements - the Rigor Report Card - AlzPED highlights reporting recommendations and standards while providing a practical tool that enables the planning of rigorous therapeutic studies in animals. AlzPED also serves as a platform for reporting negative findings to mitigate the publication bias favoring positive reports. **Methods:** Key word-driven literature searches are used to acquire and curate published studies. Two expert curators extract bibliographic details, funding source, study goals and principal findings, data on relevant translational criteria like therapy type, therapeutic agent, therapeutic target, animal models, and AD-related outcome measures, prior to publication in AlzPED. Rigor in study design and methodology is evaluated with the Rigor Report Card. All analytics including reports from negative findings are shared on AlzPED under the principles of open science. **Results:** AlzPED hosts curated summaries from 1400 published preclinical therapeutic studies in AD animal models, data related to 274 therapeutic targets, 1201 therapeutic agents, 226 animal models, more than 3000 AD-related outcome measures, and thousands of principal findings. Evaluation of Rigor Report Cards demonstrates significant under-reporting of critical elements of methodology such as power/sample size calculation, blinding, randomization, balancing for sex, inclusion/exclusion criteria, these being reported by fewer than 35% of the 1400 curated studies. These deficiencies in reporting critical elements of methodology diminish the scientific rigor, reproducibility, and translational value of preclinical studies. **Conclusion:** Rigorous experimental design and transparent reporting are essential to inform future research, science policies, and successful clinical trials. Adopting a standardized set of best practices like those proposed by AlzPED can improve the predictive power of preclinical studies in AD animal models and promote the effective translation of drug testing data to the clinic.

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**Title:** Discovery of a Dual-Action of G9a Inhibitors for the Treatment of Alzheimer's Disease

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**Abstract:** Alzheimer's disease (AD) is the most common cause of dementia, making the disease a global health crisis that must be addressed. Until now, none of the approved AD treatments turned out to be a success. AD is unknown but involves a combination of genetic, biochemical, and environmental factors, being one of the reasons why single-target-directed drugs have failed to reach clinical trials. As a new strategy in drug discovery for AD, multifunctional molecules avoid drug-drug interactions, off-target adverse effects, poor patient compliance, and high development costs compared to combination therapies. Multiple lines of evidence suggest that epigenetic alterations and tau pathology are two of the crucial causes of AD. Strikingly, overexpression of G9a and the other protein serve as drivers of the cognitive impairment, leading to synaptic plasticity reduction, autophagy dysfunction, increasing Tau pathology, OS and neuroinflammation. Here, we synthesized the compound AMC-1, a new chemical scaffold with high potency micromolar (μM) to inhibit both targets. Moreover, other interesting characteristics are that AMC-1 is selective to G9a respect GLP (another histone/lysine methyltransferase), exhibits a high PAMPA-BBB permeability, no presented hERG toxicity and a good drug metabolism and pharmacokinetics. Besides, treatment with AMC-1 in SAMP8 mice rescued

cognitive decline measured via NORT. G9a is responsible for methylating Histone 3, being capable to repress the expression of genes related to learning and memory formation, we evaluated several repressive histone marks in the SAMP8 mice model. Furthermore, we evaluated the Tau phosphorylation, observing that AMC-1 was able to reduce its levels in SAMP8. In addition, the density of dendritic spines and the length of dendritic branches were evaluated, showing an increase in the treated group. Therefore, our dual approach is an innovative and promising multifaceted therapeutic strategy for AD treatment.

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

**Support:** NIH Grant 1R01 AG079859-01

**Title:** Antisense-oliognucleotides based targeting of Tau-tubulin kinase 1 reduces phosphorylated tau level in tauopathy mouse model

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**Abstract:** Tau -tubulin kinase 1(TTBK1), a neuron-specific tau kinase, is highly expressed in the entorhinal cortex and hippocampal regions where early tau pathology evolves in Alzheimer's disease (AD) brains. We attempted to knockdown *Ttbk1* in PS19 tauopathy mouse model by antisense oligonucleotide-based approach to understand the therapeutic efficacy of targeting TTBK1 in AD animal model. 700ug of ASOs targeting murine *Ttbk1* or control ASO were intracerebroventricularly administered in PS19 mice at 6 months old age. Mice were euthanized for biochemical and pathological analysis at 8 weeks post injection. The soluble and insoluble form of tau were isolated from the mouse hippocampal tissues to examine the effect of knocking down *Ttbk1* on phosphorylated tau level. Immunofluorescence against pS422, pre-tangle marker and PHF1, paired helical filaments, were performed using mouse hippocampal tissues. We further performed bulk RNA sequencing of the temporal cortex brain tissue to examine the global effect of ASO-*Ttbk1* treatment. Administration of ASO-*Ttbk1* showed specific down regulation in mRNA level of *Ttbk1* in the temporal cortex without affecting the level of *Ttbk2*. The ASO-*Ttbk1* treatment significantly reduced the level of several phosphor epitopes relevant to AD pathology, including pT231, pT181, and pS396 tau in sarkosyl soluble and insoluble fractions isolated from the hippocampal tissue as determined by ELISA. The level of pS422 tau in soluble fraction was significantly reduced by ASO-*Ttbk1* as measured by western blot analysis. Immunofluorescence against pS422 tau antibodies showed that ASO-*Ttbk1* significantly reduced phosphorylated tau intensity measurement in Mossy fibers of the dentate

gyrus. The Bulk RNA sequence analysis of the temporal cortex tissue revealed enriched signaling pathways for interferon-gamma and complement and increased expression in MHC class II genes involved in antigen presentation (Cd 86, Cd74, and H2-Aa) by ASO-*Ttbk1* treatment, suggesting its potential off-target effect on microglial phenotype. Taken together, the bolus intracerebroventricular injection of ASO-*Ttbk1* significantly reduced tau pathology development in PS19 mice at 8 weeks post injection. Further investigation is warranted for understanding the potency of the therapeutic application of ASO- *Ttbk1* in preclinical models of AD.

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

**Title:** Compound intervention affects pathology in a novel humanized APP knock-in model ($APP^{SAA}$), strengthening its relevance as a tool in preclinical research

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**Abstract:** To improve clinical translatability of non-clinical in-vivo Alzheimer's disease (AD) models, a humanized APP knock-in mouse model (APPSAA) was recently created (Xia, D. et al., 2022). This homozygous APP knock-in model carries a humanized Aβ1-42 sequence and 3 disease causing mutations (Swedish, Arctic, and Austrian). These modifications lead to increased Aβ42/40 ratios in AD relevant tissues, resulting in an age-dependent amyloid deposition in the brain. The highest plaque density is found in cortical and hippocampal regions. As one of the few available models, plaques of these mice contain dystrophic neurites positive for phosphorylated Tau. In addition, APPSAA mice display clear neuroinflammation and an increase in fluid biomarkers of AD (NF-L and total Tau in CSF). Here we assess the value of this model as a tool for non-clinical efficacy studies of experimental drugs with diverse mechanisms of action to facilitate the development of novel AD therapeutics. APPSAA mice and WT controls were aged and sacrificed at various time points. In addition, APPSAA mice were treated with MCC950 (an NLRP3-inhibitor), an in-house compound (Calcium homeostasis) or vehicle, on a daily basis for 3 months from the age of 3 months onward. The major pathological hallmarks of this model and the treatment effect were investigated with biochemical and immunohistological assays. Aβ40, Aβ42 and pyroglutamate modified Aβ42 (N3pE-42) levels were shown to be affected with age while compound intervention was able to counteract certain types of Aβ. Cortical amyloid plaques, surrounded with activated microglia, were shown to be present already at an age of 3 months, albeit at a low level. With age, the number of cortical plaques infiltrated with activated microglia, increased. The effect of compound intervention and whether other brain regions show similar pathology is currently being investigated. Interestingly we were able to confirm the presence of endogenous phospho-Tau positive dystrophic neurites within these plaques, clearly demonstrating the translational relevance of this model. Since APPSAA mice lack an obvious

behavioral phenotype, synaptic plasticity was investigated to serve as a functional readout. However, with the current stimulation protocol, at an age of 4 and 6 months no defects in synaptic plasticity were observed. These data lead us to conclude that this knock-in model is a broadly applicable tool to investigate efficiency of disease-modifying drugs with diverse mechanisms of action. Treatments targeting the Abeta/Tau pathway specifically would likely show larger effects on the abovementioned pathology.

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**Title:** Treatments with a tart cherry extract and omega-3 fatty acid compound decrease amyloid beta protein in the entorhinal cortex in the 3xTg mouse model of Alzheimer's disease

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**Abstract:** Alzheimer's disease (AD) is a progressive neurodegenerative disease marked by memory loss, accumulation of amyloid beta plaques, and formation of phosphorylated tau in the various brain regions, as well as increased oxidative stress and gliosis, leading to dementia. In our previous studies, we have shown that the oral administration of a proprietary nutraceutical, Total Body Rhythm (TBR), which is a mixture of tart cherry extract, omega-3 fatty acids from Nordic fish oil, and Emu oil, which exert antioxidant effects that diminish the cognitive impairment in mu-p75 saporin (SAP)-induced mouse model of AD and in 6- and 12-month-old 5xFAD mice. In the present study, we have administered the TBR orally in 22-month-old triple-transgenic (3xTg-AD) mice, every other day for 1 month. The mice were divided into 5 different groups: (1) 3xTG mice receiving 30 mg/kg of TBR; (2) 3xTg mice receiving 60 mg/kg of TBR; 3xTg mice receiving 90 mg/kg of TBR; 3xTg mice receiving vehicle (methylcellulose); and the wild-type mice receiving vehicle. After one month, all mice were euthanized, and the dentate gyrus (DG), CA1, CA3, prefrontal cortex (PFC), retrosplenial agranular cortex (RSA), and entorhinal cortex (EC) regions of the brain were extracted separately. Western blot assays were performed on all these brain regions with different antibodies, including: GAPDH

(glyceraldehyde-3-phosphate dehydrogenase), GFAP (glial fibrillary acidic protein), APP (amyloid β (Aβ) precursor protein), normal tau, and Phospho-Tau. Our results indicated that TBR has the potential to reduce amyloid beta protein (Aβ) in the EC of 22-month-old 3xTg mice, but not in the other sampled brain regions. When combined with our previous findings, the present data suggests that TBR has the potential to mitigate some of the behavioral and neuropathological deficits associated with AD.

**Disclosures: N. Mojarad:** None. **R. Schalau:** None. **A. Poudel:** None. **B. Srinageshwar:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

**Presentation Number:** NANO63.11

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

**Support:** AMED Grant JP23ym0126113

**Title:** Development of Therapeutics for Tauopathies through Antisense Modulation of Tau Isoforms

**Authors: *S. ISHIGAKI**[1], K. ENDO[2], K. KAWAI[2], Y. FUJIOKA[1], K. SAHASHI[2], G. SOBUE[3];
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**Abstract:** Tau, a microtubule-binding protein, is associated with Alzheimer's disease and tauopathies, such as frontotemporal lobar degeneration (FTLD). It is categorized into 3-repeat (3R) and 4-repeat (4R) isoforms based on repeat sequences. The accumulation of 4R-tau is implicated in FTLD, progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD). Our previous study has demonstrated the efficacy of normalizing increased 4R-tau levels by introducing an adeno-associated virus (AAV) expressing shRNA against 4R-tau, leading to the recovery of FTLD-like phenotypes in FUS KD mice in which 4R/ 3R tau ratio was increased. In this study, we developed 2'-O, 4'-C-ethylene-bridged nucleic acid (ENA)-modified antisense oligonucleotides (ENA-ASOs) capable of skipping MAPT exon to restore the 4R/3R-tau ratio. Specifically, we identified an efficient ENA-ASO named NK-18, which effectively skips MAPT exon 10. Intracerebroventricular administration of NK-18 normalized the imbalanced 4R/3R-tau ratio in FUS-silenced humanized tau mice. Notably, NK-18 also ameliorated disease phenotypes, including aberrant behaviors and neurodegeneration, in FUS-silenced humanized tau mice. Moreover, NK-18 remained in the brain, retaining its splicing correction ability for up to 24 months post-injection with minimal inflammatory reactions. These findings highlight the therapeutic potential of ENA-ASO targeting MAPT exon 10 for treating 4R-tau-associated tauopathies, such as FTLD, PSP, and CBD.

**Disclosures: S. Ishigaki:** None. **K. Endo:** None. **K. Kawai:** None. **Y. Fujioka:** None. **K. Sahashi:** None. **G. Sobue:** None.

**Nanosymposium**

**NANO64: Spinal Cord Injury, Neural Regeneration, and Repair**

**Location:** WCC 140

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO64.01

**Topic:** A.04. Transplantation and Regeneration

**Support:** NIH award R56NS128413
Morton Cure Paralysis Fund

**Title:** Creb primarily drives the preconditioning signal that enhances neuroregeneration and modulates transcriptional levels in c. elegans

**Authors: *N. W. F. GROOMS**, T. T. NGUYEN HOANG, S. H. CHUNG;
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**Abstract:** In lesion conditioning, a peripheral axon cut enhances central axon regeneration following injury. The peripheral, but not central, lesion drives broad, transcriptional changes in “regeneration-associated” genes (RAGs) that confer regenerative capacity. While these changes are well described, the upstream (i.e., “preconditioning”) signal that initiates them remains unknown. Moreover, some regeneration mechanisms are cell-type specific, and independent regeneration pathways (conditioned/two-cut vs. “conventional"/one-cut) exist, but the distinctions are poorly understood. To address these issues, we leverage our lesion conditioning model in the roundworm *C. elegans*, a powerful *in vivo* model animal for genetic analysis. We assess neuron regeneration in mutants of dual-leucine zipper kinase (DLK) and cAMP response element binding protein (CREB), $n \geq 20$ animals each. DLK is a primary driver of conventional regeneration in several *C. elegans* neurons and in other animals. CREB potentially transduces signals from multiple conditioning genes in our model. We also assess changes in expression of thioredoxin TXN, a RAG that limits neuron degeneration and promotes neuroprotection in mammals. We demonstrate that multiple neurons functionally depend on DLK for conventional regeneration and CREB for conditioned regeneration. Loss of CREB, but not loss of DLK, largely eliminates the conditioning effect indicating that CREB drives conditioned regeneration. Conditioning strongly upregulates TXN in our model. We demonstrate that TXN upregulation in CREB, but not DLK, is strongly diminished, even after peripheral lesion. CREB is integral for driving the transcriptional changes required for conditioning. Our work in multiple neurons establishes CREB as a principal driver of conditioned regeneration and RAG upregulation. Several genes involved in regeneration are cell-specific while others are shared. Our working hypothesis is that cell-specific and shared pathways converge to phosphorylate CREB at specific residues to modulate transcription for conditioned regeneration. In our presentation, we will describe our current model that explains how peripheral lesion alters cAMP, cGMP, and activity levels to change the phosphorylation state of CREB. Future plans are to establish a mechanistic understanding of how peripheral lesion alters secondary messenger pathways and to directly assess CREB activation and transcriptional changes that permit a growth-associated state.

**Disclosures: N.W.F. Grooms:** None. **T.T. Nguyen Hoang:** None. **S.H. Chung:** None.

**Presentation Number:** NANO64.02

**Topic:** A.04. Transplantation and Regeneration

**Support:** NIH grant R01NS126821
Whitehall Foundation
Martina Stern Memorial Fund
Dan and Diane Riccio Fund for Neuroscience

**Title:** Gliotransmission and adenosine signaling promote axon regeneration

**Authors: *Y. XIANG**[1], F. WANG[1], K. RUPPELL[1], S. ZHOU[2], Y. QU[2], J. GONG[1], Y. SHANG[1], J. WU[2], X. LIU[2], W. DIAO[2], Y. LI[2];
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**Abstract:** How glia control axon regeneration remains incompletely understood. We investigate how glia-neuron interactions could regulate different regenerative abilities of closely related Drosophila larval sensory neuron subtypes. Axotomy elicits Ca2+ signals in ensheathing glia, which release the gliotransmitter adenosine. Adenosine acts on the regenerative neuron subtype to mount the axon regenerative programs including neuron burst firing and Ras activity. In contrast, the non-regenerative neuron subtype does not respond to glial stimulation or adenosine. Such neuronal subtype-specific responses to gliotransmission result from specific expressions of adenosine receptors in the regenerative neuron subtype. Disrupting gliotransmission impedes axon regeneration of the regenerative neuron subtype, and ectopic adenosine receptor expression in non-regenerative neuron subtype is sufficient to activate the regenerative programs and induce axon regeneration. Furthermore, stimulating gliotransmission or activating the mammalian ortholog of Drosophila adenosine receptors in retinal ganglion cells (RGCs) promotes axon regrowth after optic nerve crush in adult mice. Altogether, our findings demonstrate that gliotransmission orchestrates neuronal subtype-specific axon regeneration in Drosophila and suggest that targeting gliotransmission or adenosine signaling is a new strategy for mammalian central nervous system repair.

**Disclosures: Y. Xiang:** None. **F. Wang:** None. **K. Ruppell:** None. **S. Zhou:** None. **Y. Qu:** None. **J. Gong:** None. **Y. Shang:** None. **J. Wu:** None. **X. Liu:** None. **W. Diao:** None. **Y. Li:** None.

**Presentation Number:** NANO64.03

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** Indiana Spinal Cord & Brain Injury Research Fund from the Indiana State Department of Health
NIH (5R01NS117701)

**Title:** A zebrafish drug screen identifies HDAC inhibitors as regeneration enhancing compounds after spinal cord injury

**Authors: *G. L. ANDREWS**[1,2], G. M. G. ANDREWS[3,7], J. R. MANSELL[3,8], A. M. FRIEDMAN[1], Y. F. LEUNG[1,2], D. M. SUTER[1,2,4,5,6];
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**Abstract:** Traumatic spinal cord injuries (SCIs) affect an estimated 250,000 patients worldwide annually, with a particularly heavy concentration occurring in lower-income countries. Despite their prevalence and the debilitating affect they have on longevity and quality of life, treatment options that allow preservation or restoration of central nervous system function for SCI patients are still extremely limited. Motivated by the critical need for new therapies, a small molecule FDA-approved drug library containing 2747 unique compounds was screened to test for potential regenerative effects on spinal cord injuries using a larval zebrafish SCI model system designed specifically for this study. The study was split into three phases—a toxicity screen, a primary screen, and a secondary screen. For all study phases, a 10 μM drug concentration with a 2 day exposure period that began at 5 days post fertilization (dpf) larvae was used. After drugs toxic to healthy zebrafish larvae were eliminated (toxicity screen, 639 in total), SCI was performed and drug treatment administered, followed by assessment of functional recovery compared to matched controls (primary screen). Functional recovery was evaluated with a visual motor response assay; a tool that utilizes a video monitoring system to track larval swimming distance in response to a startling light stimulus. Drug compounds that promoted statistically significant improvement in swimming abilities post-injury were re-tested for confirmation (secondary screen). Those compounds that were determined to improve functional recovery from the first quarter of the library screened are being used to seed a data driven approach for identifying and investigating other potential regeneration-enhancing drugs in the library with similar chemical structures and cellular targets. In the early phase of the drug screen, histone deacetylase (HDAC) inhibitors were identified as drugs of interest. Of the 12 drugs HDAC inhibitors tested, 5 showed significant promise at promoting functional recovery. A key feature of this group is their inhibition of class I and class IIa HDACs. Our findings suggest that several already FDA-approved drugs including HDAC inhibitors enhance functional nerve regeneration in *danio rerio* and may have similar effects in other species.

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**Presentation Number:** NANO64.04

**Topic:** A.04. Transplantation and Regeneration

**Title:** Regeneration of neurons by reprogramming glial cells in adult mice

**Authors: *T. HOANG**[1], N. LE[2], S. BLACKSHAW[3];
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**Abstract:** One of the major challenges in regenerative medicine is to effectively regenerate desired cell types that are lost due to diseases. Neurons in the retina, like elsewhere in the central

nervous system (CNS), are highly susceptible to diseases, and their loss often result in blindness. Some non-mammalian vertebrates, such as zebrafish, *Xenopus* and chick, are able to regenerate a diverse set of retinal neurons via reprogramming of Müller glia (MG) cells. In contrast, in mammals such as mice and humans, MG do not spontaneously regenerate lost retinal neurons following injury. Therefore, the identification of the cellular and molecular mechanisms underlying MG-mediated regeneration will facilitate the development of cell-based therapies for human retinal degeneration. We found that suppressionof Notch signaling by deletion of *Rbpj* deletion resulted in MG dedifferentiation and activation of genes involved in neurogenic competence in MG such as *Ascl1* and *Neurog2*. A subset of *Rbpj*-deficient MG cells gives rise to multiple neuronal cell types, including neurons expressing markers of bipolar and amacrine cells, in adult mice. Furthermore, a combinational deletion of *NFIa/b/x* factors with *Rbpj* results in >80% MG reprogramming into retinal neurons. Taken together, our study highlights the importance of repressing negative regulators for efficient glial cell reprogramming.

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**Presentation Number:** NANO64.05

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** NIH Grant NS105038
NIH Grant EY030138
UCSF Program for Breakthrough Biomedical Research: New Frontier Research Award
Howard Hughes Medical Institute Hanna Gray Fellowship
Croucher Fellowship for Postdoctoral Research
Damon Runyon Cancer Research Foundation Fellowship

**Title:** Endogenous opioid signaling regulates proliferation of spinal cord ependymal cells

**Authors: *W. YUE**[1], K. K. TOUHARA[1], K. TOMA[2], X. DUAN[2], D. JULIUS[1];
[1]Physiol., [2]Ophthalmology, Univ. of California, San Francisco, San Francisco, CA

**Abstract:** Mammalian spinal cords respond to injury by developing scar tissues, which can be beneficial in sealing off the damaged area and preventing further injury. However, excessive scarring can also inhibit neural regeneration and functional recovery. Therefore, an effective therapeutic approach may require dynamic control of the extent of scar formation. While previous research has focused mainly on the role of glial cells in scar formation, recent evidence indicates that ependymal cells also contribute to this process. Ependymal cells normally constitute the epithelial layer surrounding the central canal but following injury they undergo significant proliferation and differentiation into astroglial cells, becoming a critical component of scar tissue. However, the precise mechanisms governing ependymal proliferation *in vivo*, both in healthy and injured conditions, are still not fully understood.
In this study, we uncovered an intercellular kappa (κ) opioid signaling pathway that regulates endogenous ependymal proliferation. Specifically, we identified the expression of the κ opioid receptor, OPRK1, in an intriguing group of cells known as the cerebrospinal fluid-contacting neurons (CSF-cNs), which are intercalated among the ependymal cells. Additionally, we have discovered a neighboring cell population that produces the cognate ligand, prodynorphin

(PDYN). While opioid receptors typically couple to inhibitory signaling pathways, we demonstrated that CSF-cNs represent a rare exception in which activation of the κ opioid receptor is excitatory. Using genetic and pharmacological tools, we showed that constitutive activation of this endogenous κ opioid signaling pathway suppresses ependymal proliferation under normal, healthy conditions. Moreover, we found that spinal cord injury disrupts this tonic opioid signaling cascade, thereby relieving CSF-cN-mediated suppression of ependymal cell proliferation to facilitate scar formation. Collectively, our findings provide a mechanistic basis for potentially utilizing κ opioids to modulate scar formation and promote recovery in individuals with spinal cord injuries.

**Disclosures: W. Yue:** None. **K.K. Touhara:** None. **K. Toma:** None. **X. Duan:** None. **D. Julius:** None.

**Presentation Number:** NANO64.06

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Title:** Sun11602 exerted bfgf-like neuroprotective activity supporting tissue regeneration and modulating neuroinflammation in a subacutein vivomodel of spinal cord injury

**Authors: *A. ARDIZZONE**[1], V. BOVA[1], A. FILIPPONE[1], I. PATERNITI[1], E. ESPOSITO[2], S. CUZZOCREA[1];
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**Abstract:** Spinal cord injury (SCI) is defined as a debilitating traumatic event to the spinal cord that usually triggers permanent changes in motor, sensory and autonomic functions. Injured tissue becomes susceptible to secondary mechanisms caused by spinal cord injury, including ischemia, edema, and release of pro-inflammatory cytokines, which activate microglia and astrocytes, which are the first cells to respond to tissue damage, increasing neuronal sensibility and producing factors that contrast the Central Nervous System (CNS) regeneration. In this regard, the knowledge of important functions exerted on the CNS, such as modulation of cell proliferation, neurotrophic activity, promotion of neurites survival and tissue repair, and basic fibroblast growth factor (bFGF) results in a good therapeutic approach for long-term consequences of CNS traumatic event. Its therapeutic use is limited due to the undesirable effects developed following its administration. Therefore, the synthetic compound, mimetic of bFGF, SUN11602 (with chemical name 4-[[4-[[2-[(4-Amino-2,3,5,6-tetramethylphenyl)amino]acetyl]methylamino]-1-piperidinyl]methyl]benzamide) has been reported to show neuroprotective activities similar to those of bFGF, but with greater safety. Here, we aimed to investigate the neuroprotective effects and the ability of SUN11602 to restore motor function and neurodegeneration in a subacute mouse model of SCI.

**Disclosures: A. Ardizzone:** None. **V. Bova:** None. **A. Filippone:** None. **I. Paterniti:** None. **E. Esposito:** None. **S. Cuzzocrea:** None.

**Presentation Number:** NANO64.07

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** CIHR
PVA Research Foundation

**Title:** Pharmacological Treatments to Improve Remyelination & Recovery in Aged Mice with Spinal Cord Injury

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**Abstract:** Spinal cord injury (SCI) causes devastating deficits in sensory, motor, and autonomic function. The SCI-induced loss of myelin following SCI contributes to dysfunction, particularly when injury is sustained at an older age. Worse outcomes for the elderly after SCI present a growing concern given the aging population of North America. Repurposing approved pharmaceuticals to promote recovery by improving remyelination is an active field of research in SCI and other demyelinating conditions. Two drugs of interest, metformin and clemastine, may promote remyelination and myelin plasticity. Research shows both can promote differentiation of oligodendrocyte precursor cells (OPCs) to oligodendrocytes, the myelin producing cells of the central nervous system. A clinical trial is exploring the combinatorial use of metformin and clemastine in multiple sclerosis. Rejuvenation of OPCs is particularly promising as a therapeutic in the elderly, as age-related OPC decline decreases responsiveness to differentiation cues. To assess if metformin or metformin in combination with clemastine improves recovery in aged SCI, male and female 18-month-old or 3-month-old C57BL6J mice sustained a 70 kDyne thoracic level 9 (T9) contusion injury using the Infinite Horizons impactor. Animals were given water with metformin (300mg/kg), metformin + clemastine (300mg/kg + 10mg/kg), or their standard water for 8 weeks after injury. Neither treatment had an effect on motor recovery in either age group as compared to injured animals drinking standard water as assessed by the Basso Mouse Scale or Catwalk gait analysis. There were no memory or anxiety effects, as measured by the spontaneous alternation Y-maze or elevated plus maze, respectively. Histological analyses will show if OPC or oligodendrocyte numbers and turnover differ at the lesion and surrounding cord or if inflammatory responses are influenced by treatment. Future research will include more detailed analyses of gait to ascertain if there are minor benefits with treatment. Ongoing work is assessing the effects of these drugs in chronic SCI. The present results are in contrast to studies which describe behavioral improvements with metformin after compression or weight drop injury. The injury models as well as the pleiotropic and dosage dependent mechanisms of action for these drugs may explain these discrepancies. Given these findings, the translation of metformin for SCI requires more research to determine if, and under what conditions, metformin alone or in combination with clemastine represents a viable therapeutic candidate for SCI.

**Disclosures: B.R. Kondiles:** None. **S. Wheeler:** None. **S. Duenwald:** None. **H. Briggs:** None. **M. Lu:** None. **J. Fan:** None. **O. Seira:** None. **C. Wellington:** None. **W. Tetzlaff:** None.

**Presentation Number:** NANO64.08

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** NIH Grant R01NS111761

**Title:** Investigating the impact of TNFR1 activation on spinal cord interneurons after spinal cord injury

**Authors: *T. MARTYNYUK**, V. BRACCHI-RICARD, J. J. RICARD, J. R. BETHEA; Drexel Univ., Philadelphia, PA

**Abstract:** Spinal cord injury (SCI) is a devastating condition with 250,000 to 500,000 new cases globally each year. Respiratory infections, e.g., pneumonia and influenza are the leading cause of death after SCI. An individual with SCI is 37 times more likely to become infected and die from the flu. Although progress in the field has been made, there is still a poor understanding of how altered neuro-immune communication impacts an individual's outcome to infection. In humans and rodents, SCI leads to maladaptive changes in the spinal-sympathetic reflex (SSR) circuit which is crucial to sympathetic function. The cause of the impaired immune function may be related to harmful neuroinflammation which is detrimental to homeostatic neuronal function, aberrant plasticity, and hyperexcitable circuits. Soluble tumor necrosis factor (sTNF) is a pro-inflammatory cytokine that is elevated in the CNS after SCI and remains elevated for several months after injury. We recently demonstrated that pharmacologically blocking TNFR1 activation in the CNS decreased the maladaptive excitability of Spinal interneurons (SpINs) in the SSR circuit leading to improved immune response to infection after SCI. We hypothesize that persistent TNFR1 activation leads to hyper-excitability of glutamatergic SpINs after SCI that contributes to immune dysfunction. To test this, we utilized viral mediated targeted knock-down of TNFR1 on excitatory SpINs prior to mid-thoracic (T9) contusion and demonstrated that inhibiting TNFR1 on excitatory SpINs contributes to improved immune function, including an increase in virus-specific CD8+ T cells in the spleen and decrease in viral load remaining in the lung after X31 H3N2 influenza infection. Ongoing mechanistic studies are aimed at investigating how persistent TNFR1 activation on SpINs negatively affects the SSR circuit and the spleen, which is a major contributor of CD8+ T cells. These studies include investigating spleen architecture and immune cell exhaustion in the spleen after SCI. In addition, we are investigating how NF-kB, a downstream mediator of TNFR1 signaling, contributes to immune dysfunction. Our goal is to uncover mechanisms by which SCI negatively impacts immune function for future therapeutic approaches to increase the quality of life by those affected with SCI.

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**Presentation Number:** NANO64.09

**Topic:** C.11. Spinal Cord Injury and Plasticity

**Support:** NSF Grant RES515698//DBI-2015317
CHN Foundation Grant 649297

**Title:** Unmasking the culprit: Muscle spasms after spinal cord injury stem from prominently altered motoneuron properties, not synaptic excitation

**Authors: *A. MAHROUS**[1], D. BIRCH[2], V. M. TYSSELING[2,1], C. J. HECKMAN[1,2];
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**Abstract:** Muscle spasms are a common occurrence in the chronic phase of spinal cord injury (SCI), posing challenges to rehabilitation and daily activities. Even though motoneuron excitability is known to increase after SCI, current pharmacological management of spasms relies on suppression of excitatory inputs, an approach known to hinder motor recovery. Here, we investigated whether changes in excitatory inputs or motoneuron excitability contribute to muscle spasms. In adult mice (3 mo old), we induced either a complete (transection) or incomplete (impact) SCI at the lower thoracic segments and let animals recover for 6 months (chronic SCI). Animals with incomplete injury were further divided into either low-function or high-function groups based on their motor recovery (BMS score). The whole-tissue sacrocaudal spinal cord along with its ventral and dorsal roots was then extracted and used *ex vivo* to study plasticity below injury level. Tissue from age-matched animals with no prior spinal injuries was used as a control. Electrical stimulation of the dorsal roots elicited prolonged activity in multiple spinal segments (spasm-like activity) in all injury models as compared to very brief responses in the control preparations. We developed a scoring system for this *in vitro* spasm activity based on the intensity/frequency of dorsal root stimulation needed to evoke it. The data showed that both complete and incomplete chronic SCI caused a higher spasm score. To study whether these spasms were due to increased synaptic excitation, we first blocked synaptic inhibition pharmacologically to avoid confounding effects of mixed synaptic inputs. We then measured excitatory postsynaptic currents (EPSCs) evoked by dorsal root stimulation into motoneurons. The data showed no difference in the amplitude of EPSCs measured from spinal cords with chronic injuries as compared to the control. Nonetheless, we found that motoneuron persistent inward currents (PICs) activated by the EPSCs were increased to varied degrees in different models of chronic SCI. Therefore, we studied the intrinsic properties and firing styles of motoneurons in response to current injection. The data showed that motoneurons switched to more excitable phenotypes after chronic SCI, accompanied by the emergence of highly excitable firing styles that were not observed in the control. These findings suggest that major changes in motoneuron excitability, rather than synaptic excitation, contribute to spasms after SCI and could be targeted for more effective therapeutic interventions.

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**Presentation Number:** NANO64.10

**Topic:** A.04. Transplantation and Regeneration

**Support:** R37NS-071785-07

**Title:** Host brain environmental influences on transplanted medial ganglionic eminence progenitors

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**Abstract:** Interneuron progenitor transplantation ameliorates disease symptoms in preclinical models for a variety of neurological disorders. This strategy could have significant therapeutic impact and is based on transplantation of embryonic progenitors from medial ganglionic eminence (MGE). Elucidating host brain environment influences on these interneuron progenitors as they integrate is critical to optimizing this transplantation strategy across different disease states. Here we systematically evaluated how age and brain region influence survival, migration and differentiation of transplant-derived cells. We find that early postnatal MGE transplantation, between postnatal days 2 and 4, yields superior survival and more extensive migratory capabilities compared to juvenile or adult ages. Transplant-derived interneurons migrate more widely and extensively in cortex compared to hippocampus at all ages. At adult and juvenile ages, transplant-derived interneuron expressing parvalbumin was reduced compared to early postnatal ages. In addition, MGE progenitors transplanted into a sub-region of the early postnatal hippocampus (dentate gyrus) can locally differentiate into astrocytes. Our results suggest that host brain environment critically regulates survival, spatial distribution and maturation of MGE-derived interneuron subtypes following transplantation. These findings inform and enable optimal conditions for interneuron transplant therapies.

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**Nanosymposium**

**NANO65: Auditory, Visual, and Audiovisual Processing**

**Location:** WCC 152B

**Time:** Tuesday, November 14, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO65.01

**Topic:** D.08. Multisensory Integration

**Support:** NIH Grant R01DC016915
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NIH Grant R01DC017991
NIH Grant P41EB015896
NIH Grant K99MH120054

**Title:** Non-invasive spatiotemporal characterization of feedforward and feedback influences in human auditory cortex

**Authors: *K. LANKINEN**[1], S. P. AHLFORS[1], F. MAMASHLI[1], M. JAS[1], I. ULUC[1], T. TURPIN[2], A. BLAZEJEWSKA[1], T. RAIJ[1], J. R. POLIMENI[1], J. AHVENINEN[1];
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**Abstract:** Animal studies have demonstrated that intracortical laminar patterns can provide information about the type of inputs into a brain region. Feedforward (FF) input typically first arrives to the middle cortical layer, whereas feedback (FB) arrives to superficial and deep layers. However, it has been difficult to non-invasively test whether these principles apply to humans.

We investigated the spatiotemporal characteristics of FF and FB inputs using ultra-high resolution 7-tesla functional MRI (fMRI), magnetoencephalography (MEG), and computational modeling. We studied the responses to simple auditory and visual stimuli (noise bursts and checkerboard).In the fMRI study, 1-mm isotropic resolution 3D echo-planar imaging at 7T was used to investigate the intracortical depth profiles of blood oxygenation level dependent (BOLD) signals. BOLD percent-signal-changes were estimated at 11 equally spaced intracortical depths in regions-of-interest in auditory (Heschl's gyrus and sulcus, planum temporale, posterior superior temporal gyrus) and polymodal (middle and posterior superior temporal sulcus) areas. The BOLD depth profiles were significantly different for auditory vs. visual stimuli in auditory cortices, but not in polymodal areas. Thus, the depth profiles could reflect FF vs. FB influences, previously shown in laminar recordings in nonhuman primates. In the MEG study, the estimated source waveforms in auditory cortex showed peaks at 37 and 90 ms in response to auditory stimuli and at 125 ms in response to visual stimuli. These auditory cortex waveforms were modeled through FF- and FB-type connections targeting different cortical layers using the Human Neocortical Neurosolver (HNN), which consists of a neocortical circuit model linking the cellular- and circuit-level mechanisms to MEG. The HNN models suggested that the auditory response could be explained by an FF input centered at 35 ms and an FB input at 75 ms, and the cross-sensory visual response by an FB input at 125 ms. The fMRI results suggest that intracortical BOLD depth profiles could help distinguish between FF and FB influences in the human brain. The combined MEG and computational modeling results support the hypothesis that cross-sensory visual input in the auditory cortex is of FB type, whereas sensory-specific auditory input includes an initial FF input followed by an FB. Taken together, these studies illustrate how the patterns of the estimated layer-specific activity have potential to characterize inputs into cortical areas in terms of hierarchical organization.

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**Presentation Number:** NANO65.02

**Topic:** D.08. Multisensory Integration

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**Title:** Effect of audiovisual speech training on word recognition in adult cochlear implant users

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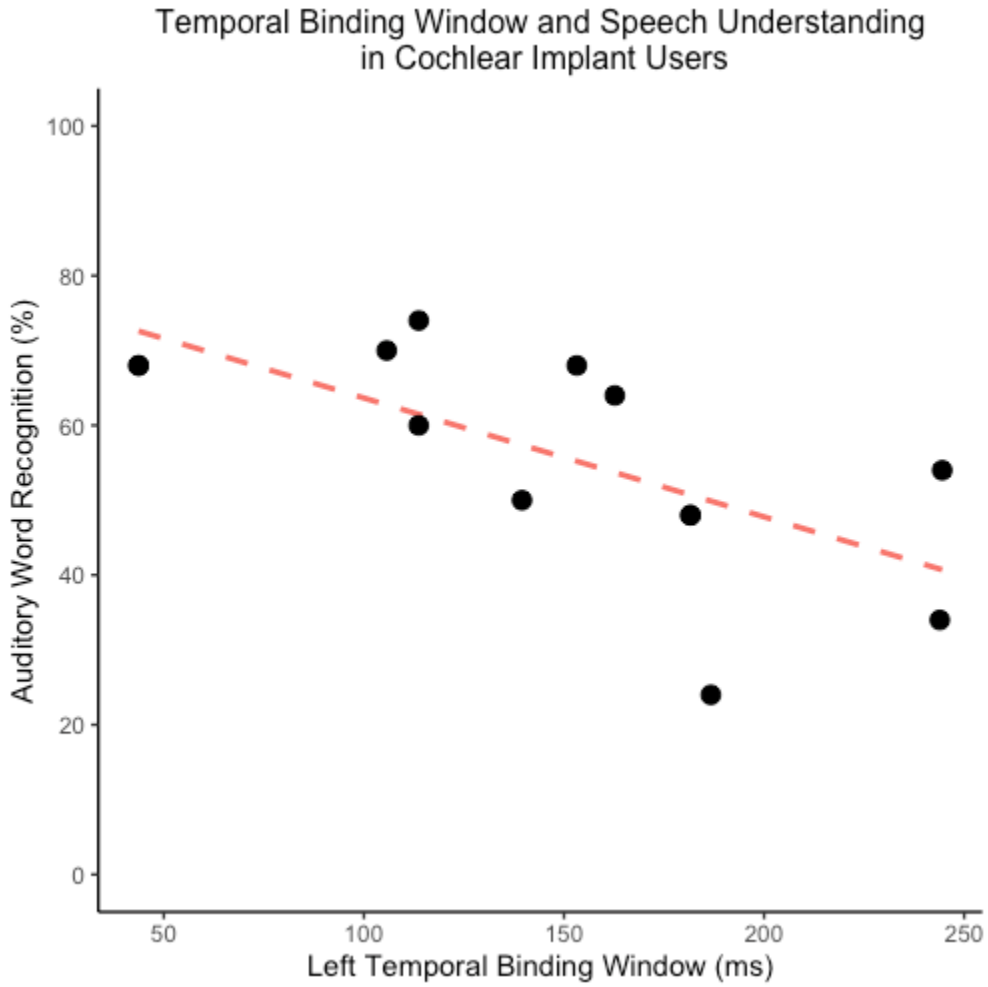
**Abstract:** Cochlear implants (CIs) can restore hearing in many deaf adults, but speech recognition outcomes are highly variable. Speech understanding is a complex phenomenon that involves the integration of auditory and visual speech cues. The temporal binding window (TBW) is a measure of audiovisual temporal acuity, and our lab has previously shown that the

size of the TBW can be narrowed through training using a simultaneity judgment (SJ) task. We predict that SJ training will have a comparable effect in CI users, and that this improvement in audiovisual temporal acuity will result in improvements in speech understanding. Prior to training, TBW size was measured in CI users using an SJ task (N=17 ears). The relationship between TBW size and Consonant-Nucleus-Consonant (CNC) scores was analyzed using a multiple linear regression model, controlling for age at implantation. Then a cohort of CI users participated in a computerized SJ training program over 3-4 days (N=4 ears). In this group, TBW size, auditory-only, visual-only, and audiovisual word recognition in noise were evaluated before and after SJ training. Before training, a significant negative correlation was found between left TBW size and binaural auditory-only word recognition (p=0.0179). In other words, CI users with better audiovisual temporal acuity also had better auditory word recognition. Following training, all participants demonstrated improvements in the width of their total TBWs (589.8 ms to 482.1 ms) and improvements in auditory-only word recognition in noise (16.7% to 22.5%). There were no significant group-level associations between TBW size and word recognition scores. We found that smaller TBW size, indicating better audiovisual temporal acuity, is associated with better auditory-only speech understanding in CI users. We also demonstrate for the first time that the audiovisual temporal acuity of CI users can be narrowed through SJ training. In summary, audiovisual integration plays a key role in speech understanding, and audiovisual temporal acuity can be improved through training in CI users.



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**Topic:** D.06. Vision

**Support:** F31 EY034016
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**Title:** Visual snow is alleviated by contrast adaptation

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**Abstract:** Visual Snow Syndrome (VSS)—in which a veil of small flickering dots covers the visual field—affects about 2% of the population and its symptoms can interfere with daily tasks. However, little is known about the mechanisms underlying VSS, and effective treatments are lacking. Here, we present a method to temporarily but reliably reduce the visibility of the snow symptom via adaptation to visual dynamic noise. Prolonged exposure to a high contrast stimulus reduces sensitivity to subsequent stimuli with similar spatial and temporal properties. Whether such visual adaptation could affect the appearance of the visual snow symptom was unknown. Participants with visual snow viewed high-contrast dynamic noise, resembling television static, shown on one side of the screen, then judged the strength of the visual snow compared to the other side of the screen and pressed a button when the two sides matched. For most observers, the visual snow was temporarily reduced in strength to the point that it disappeared, especially at longer exposure durations. For participants in our first experiment (n = 5), the visual snow disappeared for a mean of 14.1 sec and took a mean of 40.0 sec to regain full strength following 135.0 sec exposure to the adapter. The effect followed typical trends of adaptation for physical stimuli in normally sighted observers. Effect duration increased monotonically with duration of exposure to the adapter (ANOVA with linear effect of adapter duration: $F_{1,4} = 351.7$, $p = 5.1 \times 10^{-40}$) . Additionally, the effects on visual snow were specific to dynamic noise; adapting to a high contrast striped pattern had little effect on visual snow (ANOVA: interaction between adapter and test: $F_{1,4} = 553.2$, $p = 1.5 \times 10^{-42}$, Cohen's $d = 5.3$ for adapt noise test snow vs. adapt striped pattern test snow). In a larger sample (n = 25), 60% of participants reported their visual snow fully disappeared or weakened after adapting to dynamic noise and all but one participant experienced increased effect duration with longer exposure to the adapter. Adaptation provides reliable experimental control over the visual snow symptom, and represents a promising tool for understanding its neural origins, developing diagnostic tests, and providing a basis for treatment.

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Helen Blank: Funded by the German Research Foundation (Grant No DFG BL 1736/1-1)

**Title:** Stimulus-evoked EEG response patterns more strongly encode expected than unexpected image components

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**Abstract:** Perception is the result of integrating incoming sensory information with our prior knowledge and current expectations about our environment. How the human brain accomplishes this remains an outstanding neuroscientific challenge. In particular, it is unclear whether expectations give rise to enhanced encoding of expected stimuli (Bayesian integration), enhance the representations of deviant stimuli (prediction error coding), or if both occur at separate stages of stimulus-evoked responses (Press et al., 2020).
To investigate this, we conducted an EEG experiment aimed at measuring the relative encoding strength of expected and unexpected image components over time using multivariate analysis. During the experiment, we presented repetitive predictable sequences of natural images with interspersed image overlays that were composed of the currently expected and an unexpected image (see figure below). We recorded EEG data during six sessions for each participant using all six possible stimulus sequences to prevent any confounding effects of stimulus sequence. EEG response patterns evoked by the four natural images revealed robust information about stimulus identity. Most importantly, EEG response patterns also revealed increased stimulus identity encoding for the expected image components during the early (100-150ms) and late (300-450ms, 500-600ms) stages of the evoked EEG response.
Our findings are consistent with the Bayesian hypothesis that perceptual representations are biased toward our prior expectations. However, we did not observe any relative increase in image information encoding for unexpected image components, which would have been in line with prediction error coding. One possible explanation for this could be that unexpected components did not induce an update of subjects' beliefs because they were inconsequential for the ongoing repetitive sequence.
Press, C., Kok, P., & Yon, D. (2020). The Perceptual Prediction Paradox. Trends in Cognitive Sciences, 24(1), 13-24.


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**Topic:** H.03. Decision Making

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**Title:** Perceptual matching operates on efficient, holistic sensory representations

**Authors: *A. STOCKER**, J. MAO;
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**Abstract:** Sensory perception is widely considered to be an inference process where the percept reflects the best guess of a stimulus feature based on uncertain sensory information. We challenge this reductionist view. We instead propose that perception is a holistic inference process that operates not only at the feature but jointly at all levels of the representational hierarchy. We test this hypothesis in the context of a commonly used psychophysical matching task in which subjects are asked to report their perceived visual orientation of a test stimulus by adjusting a probe stimulus (method-of-adjustment). We introduce a holistic matching model that assumes that subjects' reports reflect an optimal match between the test and probe stimulus, both in terms of their inferred feature (orientation) but also their higher-level representation (orientation category). Validation against five existing datasets demonstrates that the model accurately and comprehensively predicts subjects' response behavior, and outperforms previous models both quantitatively and qualitatively. Moreover, the model generalizes to other feature domains and offers an alternative account for categorical color perception. Our results suggest that categorical effects in perceptual decision tasks are ubiquitous and can be parsimoniously explained as optimal behavior based on efficiently encoded, holistic sensory representations. These findings have substantial implications for our understanding of the neural information pathways and mechanisms underlying perceptual decision-making.

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**Presentation Number:** NANO65.06

**Topic:** D.08. Multisensory Integration

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**Title:** Attentional prioritization scales with audiovisual semantic relatedness

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**Abstract:** Knowledge about a visual scene and the objects in it guides attentional prioritization. However, the role of semantic information in guiding attention in multisensory, rather than unisensory, contexts is not as well understood. Previous studies show that a task-irrelevant sound improves search performance for a matched visual target (hearing a bark speeds up search for a dog image compared to an unmatched sound). Sounds can be semantically related to an image without being a matched target (e.g., a cat is more semantically related to a dog than a cow). Whether this audiovisual search benefit extends to more distant semantic relationships is unclear. It is possible that the attention facilitation between matched sounds and images is supported by neural mechansims, such as multisensory integration, that are sensitive to matches specifically and the same audiovisual attentional prioritization may be observed for sounds and images with more distant semantic relationships. To elucidate the role of semantic information in guiding audiovisual attention, we created a database of crossmodal semantic relatedness values and directly examined whether semantic relatedness modulates search speeds. Participants searched for images accompanied by a sound. Search efficiency scaled with semantic relatedness, such that target images were found more quickly when the sound was more closely related to the image. This result suggests that semantic information guides attention in a continuous manner, rather than in a manner specific to matched sounds and images. Ongoing neuroimaging work is further investigating what neural mechanisms underlie the semantic guidance of attention in audioisual contexts.

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**Topic:** D.08. Multisensory Integration

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SSHRC Doctoral Fellowship
University of Western Ontario Faculty Development Research Fund

**Title:** Neural Underpinnings of Audiovisual Multisensory Integration in adults with ADHD: An EEG Investigation

**Authors: *C. HARE**[1], M. LUSZAWSKI[1], C. ATTA[1], G. ZHAI[2], Y. LI[3], R. A. STEVENSON[1];
[1]Psychology, [2]Neurosci., [3]Med. Sci., Univ. of Western Ontario, London, ON, Canada

**Abstract:** Increasing evidence suggests that sensory processing may be impacted in attention-deficit/hyperactivity disorder (ADHD), specifically hyper- and hypo-sensitivities to sensory

information in different domains. Whether the specific sensory process of multisensory integration is affected in ADHD has shown mixed results in behavioural studies. However, multiple imaging studies have now shown that even when little to no behavioural differences in multisensory integration are observed, differences in the neural mechanisms underlying integration are still seen. In this study, we examined whether audiovisual multisensory integration is affected in adults with ADHD (n= 25) compared to Neurotypical adults (n= 27) using two speeded response tasks paired with electroencephalography (EEG) measures. Participants were presented with auditory pure tones, visual Gabor patches, or a combination thereof, all embedded in audiovisual white noise. Participants responded as quickly as possible when they detected any stimulus. Participants completed two versions of the task - one with stimuli presented at the participants' unisensory detection threshold, determined via a psychophysical staircase procedure (perceptually matched), and a second, stimulus-matched detection task. No group differences in accuracy gain were found in either task. There was no difference in the magnitude or number of violations of Miller's race model, a measure of multisensory gain, between the groups. However, when using the additive criteria preliminary analysis suggests there are neural differences in key sensory areas (e.g., parietal, and occipital regions) between the two groups. Taken together, these results suggest that neural differences for multisensory integration may exist in individuals with ADHD compared to neurotypical adults, despite a lack of behavioural differences. Additionally, having a perceptually matched and stimulus-matched detection task allows us to account for the sensory sensitivities that may exist in ADHD.

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**Nanosymposium**

**NANO66: Advances in Neuroprosthetics**

**Location:** WCC 146C

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:45 PM

**Presentation Number:** NANO66.01

**Topic:** E.05. Brain-Machine Interface

**Support:** UH3 NS107714
R35 NS122333

**Title:** Expanding the repertoire of artificial touch: illusory edges and motion via patterned microstimulation of human somatosensory cortex

**Authors: *G. VALLE**[1], R. LIENKAMPER[2], C. M. GREENSPON[1], T. HOBBS[2], C. VERBAARSCHOT[2], J. DOWNEY[1], R. GAUNT[2,3], S. BENSMAIA[1,4];
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**Abstract:** Intracortical microstimulation (ICMS) of somatosensory cortex (S1) evokes vivid touch sensations, the properties of which can by systematically manipulated by varying the parameters of stimulation. This phenomenon can be leveraged to convey feedback via a brain-controlled bionic hand about object interactions, the locations on the hand that contact the object and the forces it exerts on it. However, natural touch conveys much richer information about objects and our interactions with them, which supports dexterous manipulation. We seek to expand the repertoire of ICMS-based artificial touch to include features beyond location and force to confer greater dexterity to brain-controlled bionic hands. To this end, we designed stimulation paradigms to evoke touch sensations imbued with specific features and tested these in human participants implanted with electrode arrays in S1. Specifically, we sought to convey information about the local geometrical features of objects and about their motion, leveraging our understanding of how these sensory features are encoded in primate S1. First, we simultaneously delivered ICMS through multiple electrodes whose projected fields (PFs) - the patch of skin over which the sensation is experienced - were arranged in a line. Unprompted, the participants reported the sensation of an edge. By varying the stimulated electrodes, and the alignment of their respective PFs, we could give rise to edges at different orientations. We investigate the ability of this approach to evoke sensations with arbitrary shapes. Second, we sequentially delivered ICMS through electrodes with spatially displaced PFs. The participants reported the sensation of an object moving across their skin. By selecting sets of electrodes with different configurations of PFs, we could systematically manipulate the direction of perceived motion across the skin. We conclude that, by judiciously designing spatiotemporal patterns of ICMS inspired by our understanding of tactile coding in S1, we can expand the repertoire of artificial touch.

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**Presentation Number:** NANO66.02

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** Ministry of Trade, Industry and Energy (No. 1415181023), and the name of the project is Alchemist Brain to X (B2X) (No. 20012355).

**Title:** Evoking Experiential Phenomena Using Single and Multi-site Direct Cortical Stimulation (DCS)

**Authors: *Y. HONG**[1], J. KIM[2], C. CHUNG[3];
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**Abstract:** The encoding of complex perceptions is a multi-modal process, involving not only the primary auditory cortex and the temporal lobe but other areas of the brain. Thus, targeting multiple nodes of brain networks simultaneously may play a key role in the encoding of various information through electrical stimulation. However, despite the growing popularity of BCIs and brain stimulation, the induction of higher cognitive perceptions with single and Multi-Site Stimulation(MSS) remains largely unexplored, and its potential has yet to be determined. In this study, three drug-resistant epilepsy patients underwent invasive video-EEG monitoring with

intracranial electrodes at Seoul National University Hospital. To target auditory and semantic areas of the brain, the patients were instructed to listen to audio files of words and non-words. We used brain recordings generated during the listening task and clinical mapping results to find electrode channels that showed high activity across all frequencies, specifically gamma activity. Then, we stimulated pairs of electrodes to evoke Experiential Phenomena(EP), mainly auditory and visual hallucinations. Both single-site and multi-site stimulation and various parameters were used to evoke consistent perceptions. *Results:* Three epilepsy patients reported experiencing EP during and after stimulation. Two patients in our study described witnessing vivid mental scenes and auditory hallucinations. The third patient reported seeing complex visual hallucinations only. Interestingly, MSS in all three patients evoked different phenomena than the ones evoked using single-site stimulation. We elicited multiple types of EP in patient 1 using MSS, while single-site stimulation evoked one type of EP at one electrode site. Patient 2 reported experiencing more vivid and detailed EPs during MSS compared to single-site stimulation. Finally, in patient 3, the same object (clouds) with vivid rainbow-like colors were observed in MSS whereas in single-site stimulation, the object was only one color. Our findings provide unique reports of complex auditory hallucinations and mental visual imagery evoked during single and multi-site stimulation of the temporal, frontal, and occipital lobes. New perceptions were evoked when stimulation of two electrode pairs rather than one, which may explain the dynamic nature of complex perceptions. Higher cognitive processes, such as speech and memory, require the activation of multiple brain regions; these perceptions cannot be localized to a distinct area like visual or motor perceptions. Thus, MSS may be a promising technique for evoking complex perceptions.

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**Presentation Number:** NANO66.03

**Topic:** E.05. Brain-Machine Interface

**Support:** T&C Chen Brain-machine Interface Center
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NIH/NINDS Grant U01NS123127
Neilsen Postdoctoral Fellowship Research Grant 731621

**Title:** Charge delivered via multi-channel intra-cortical micro-stimulation (ICMS) modulates intensity and reaction times across in human primary sensory cortex in two participants

**Authors: *D. A. BJA°NES**[1], K. PEJSA[1], B. LEE[2], C. Y. LIU[3], R. A. ANDERSEN[4];
[1]Caltech, Pasadena, CA; [2]Keck Sch. of Med. of USC, Los Angeles, CA; [3]Neurosurg., USC, Los Angeles, CA; [4]BBE, Calif Inst. of Technol., Pasadena, CA

**Abstract:** Somatosensory brain-machine interfaces (BMIs) can create naturalistic sensations by modulating activity of neural populations in the brain. By utilizing different spatial or temporal patterns of intra-cortical micro-stimulation (ICMS) in primary somatosensory cortex (S1), human patients suffering somatosensory loss can experience both cutaneous and proprioceptive sensation. As evidenced by motor deficits in deafferented patients, rapid somatosensory feedback is critical for dexterous motor ability, in part because visual feedback is much slower than natural occurring somatosensory input. Our previous work demonstrated evoked sensations via multi-

channel patterns of ICMS could be cognitively processed significantly faster than natural stimuli (visual or vibrotacile) in a single human participant; however, it was unclear whether this was due to an increase in delivered charge or an increase in perceived intensity of the evoked sensation.
Two human tetraplegic participants were implanted with NeuroPort microelectrode arrays in somatosensory cortex. Single- and multi-channel electrical stimulation patterns elicited naturalistic somatosensory percepts in the arm and hand. Reaction times (RTs) to sensations evoked via ICMS were quantitatively compared to RTs from naturally occurring visual and tactile stimuli. Single- and multi-channel ICMS patterns were chosen to produce stable, reproducible cutaneous somatosensory percepts. A vibrotactor was used to generate naturally occurring tactile sensations in sensate locations near locations of evoked ICMS percepts.
In both participants, sensations evoked via multi-channel ICMS were cognitively processed with significantly faster latencies than visual stimuli, as measured via the reaction time task. We delivered several different spatial patterns of multi-channel ICMS, to modulate perceived intensity, while constraining the total amount of delivered charge. We identified patterns of multi-channel ICMS yielding equally fast reaction times, with differing levels of perceived intensity; suggesting intensity alone is not solely responsible for observed decreases in reaction times from single- to multi-channel ICMS.
This work builds on our comprehensive investigation of multi-channel ICMS evoked sensations showing several improvements (stability, reliability, “naturalness” of descriptions, and decreased detection thresholds) over comparable single-channel stimulation patterns. These findings are significant advances toward development of state-of-the-art sensory BMIs and may improve control accuracy and increase embodiment for human users of motor BMI devices.

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**Presentation Number:** NANO66.04

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** NWO 453-15-008

**Title:** Frequency- and state-specific hippocampal-parietal connectivity enhancement by 5Hz transcranial electric stimulation: a simultaneous tACS-fMRI study

**Authors:** M. KAISER[1], F. DÜCKER[1], S. TEN OEVER[1], A. T. SACK[1], Y. WANG[2], ***V. VAN DE VEN**[1];
[1]Dept. of Cognitive Neurosci., Fac. of Psychology and Neuroscience, Maastricht Univ., Maastricht, Netherlands; [2]Univ. of Maastricht, Maastricht, Netherlands

**Abstract:** There is much interest to experimentally stimulate hippocampal function to better understand its role in cognition, mental health and disease. Direct stimulation is constrained to invasive procedures in animals and brain surgery patients, but recent developments have showcased indirect hippocampal stimulation through non-invasive brain stimulation (NIBS) over cortical areas that are strongly connected to the hippocampus. Trancranial alternating current stimulation (tACS) is an appealing NIBS method that allows oscillatory stimulation at a frequency of interest, is safe to use and easily administered with little side effects. However, it is

unknown if tACS can modulate hippocampal function. Here, we aimed to modulate hippocampal-cortical connectivity in a frequency- and mental state-dependent manner by administering tACS over hippocampal-connected parietal cortex in healthy human participants (N=18). METHODS: We applied tACS at 2 mA (peak-to-peak) over P4 (EEG 10-20 system) in blocks of 120 s at various frequencies (5, 10, 20 or 40 Hz, or sham) during 3T fMRI to measure brain function. We used two concentric electrodes comprising an inner (3 cm diameter) and an outer circular electrode (11 cm diameter) for focal stimulation (verified with SimNIBS). During each tACS condition, two task blocks of 30 s of attentional orienting trials were alternated with 30 s of continued resting state with eyes open. TACS condition order was pseudo-randomized across participants. RESULTS: All tACS conditions, compared to sham, significantly enhanced right (but not left) hippocampal-parietal connectivity during resting states, with the strongest enhancement for 5 Hz tACS (frequency-specific effect). The parietal hotspot of strongest modulation was situated in-between the two electrodes. During the task, none of the tACS conditions modulated hippocampal-cortical connectivity or task performance (state-dependent effect). A whole-brain psychophysiological interaction (PPI) with right hippocampus as "physiological" factor and the 2 x 2 conditions of tACS (sham, 5 Hz) and state (task, rest) as "psychological" factor again revealed a significant right parietal cluster in-between the electrodes, and a left inferior frontal cluster. Our findings of a frequency-, state- and regionally-specific effect of 5 Hz tACS on hippocampal-cortical connectivity during resting state aligns well with reported strong hippocampal-cortical default mode network connectivity during rest, and the presence of hippocampal theta oscillations. Our study could lay the groundwork for research invested in studying hippocampal function in cognition, in health and disease.

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**Topic:** I.08. Methods to Modulate Neural Activity

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**Title:** Investigate the neuroprotective effects of electrical stimulation following acute ischemic stroke in nonhuman primates

**Authors: *J. ZHOU**[1], K. KHATEEB[1], A. S. GALA[3,1], M. RAHIMI[1], A. YAZDAN-SHAHMORAD[2];
[2]Bioengineering and Electrical Engin., [1]Univ. of Washington, Seattle, WA; [3]Stanford Univ., Palo Alto, CA

**Abstract:** Brain stimulation has emerged as a novel therapy for ischemic stroke, a major cause of brain injury that often results in lifelong disability. Although previous rodent studies have demonstrated neuroprotection using electrical stimulation acutely after stroke, it remains challenging to translate these to humans due to significant anatomical differences and a limited

understanding of the physiological response to post-stroke stimulation. To bridge this gap, we combined electrophysiology and histology to study the effects of electrical stimulation following cortical ischemic stroke in nonhuman primates (NHPs). We used the photothrombotic method to induce controlled focal ischemic lesions in the sensorimotor cortex of five macaques while collecting electrocorticography (ECoG) signals bilaterally. In two of the macaques, we applied continuous theta-burst electrical stimulation with 1 kHz pulses at one hour after stroke through an ECoG electrode adjacent to the lesion. We used ECoG signal power as an electrophysiological marker to investigate the protective effects of stimulation on neural activity. We also performed histological analysis including Nissl and immunohistochemistry staining to evaluate the tissue response to ischemic injury. In comparison to controls, ECoG signals showed decreased gamma power across the sensorimotor cortex in stimulated animals. Meanwhile, histology revealed smaller lesion volumes for the stimulated group, as well as reduced neuronal activation and inflammation as measured by c-Fos immunoreactivity and Iba1-positive microglial morphology respectively. These results suggest that electrical stimulation may exert neuroprotection by suppressing post-ischemic neural activity, thereby conserving energy, decreasing excitotoxicity, and reducing injury. Since the size of irreversible ischemic damage is a major determining factor of patient mortality and outcome, our results suggest that cortical electrical stimulation can be used safely and effectively as an acute stroke intervention. Furthermore, this acute stimulation paradigm can be tested in combination with different brain stimulation devices available in the clinic. Together, these studies will provide valuable insights into the mechanisms of stimulation-induced neuroprotection and its effect on patient recovery, thus facilitating the clinical translation of acute neuromodulation therapies to alleviate the growing burden of stroke.

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**Presentation Number:** NANO66.06

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH Grant UH3 NS095557

**Title:** Reducing the fading of phosphenes generated by the Intracortical Visual Prosthesis in a blind human by cycling among nonoverlapping electrode groups within an electrode array

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**Abstract:** Neural interfaces can stimulate a blind human's visual system to create crude artificial vision. Such visual prostheses are often hampered by rapid fading of percepts (phosphenes) after a few seconds of stimulation. We investigated whether cycling among nearby electrode groups could extend the time stimulation is perceived. The first human participant (P1) of the

Intracortical Visual Prosthesis study received 25 wireless floating microelectrode arrays (WFMAs) in dorsolateral visual cortex in February 2022. P1 had only bare light perception before implantation, but previously had decades of usable vision. Each subdural WFMA has 16 stimulating electrodes. Camera-driven stimulation to WFMAs allowed P1 to perform many visual tasks, including localizing people, or a pill, and discriminating grating orientations at the level of 20/800 acuity. To measure fading of percepts used in such tasks, P1 used a controller to sustain stimulation until phosphenes disappeared. Duration of stimulation was recorded automatically. One WFMA was stimulated per trial, using two paired electrodes at a time. Stimulation parameters included: current at 2x threshold, 200 Hz, 200 µs cathodic phases in cathodic-first pulses, and 60 µs delays between successive electrode onsets. Off-durations of 50-100 ms separated trains of 500 ms to avoid phosphene persistence after stimulation offset. Three runs of 24 trials measured fading with two nonoverlapping pairs of electrodes in 8 WFMAs. For a trial, a WFMA was stimulated using the first (A) or second (B) pair only, or cycling between the same 2 pairs (C). Cycles stimulated on 1 pair for 500 ms, waited 50-100 ms, then stimulated with the other pair for 500 ms; these cycles were repeated throughout the trial. Trial order was randomized, balanced, and unknown to P1. Mean differences were calculated between times until fading for each cycle (C) and the cycle's constituent pairs (A, B) in the same run. Measurements were randomly permuted $10^6$ times within WFMA and trial run for analysis. Across all trials, times until fading without cycling averaged 13.3 s (SD: 7.3 s), while times until fading for cycles averaged 20.8 s ± 8.5 s. Within WFMA and trial run, cycling often more than doubled the time phosphenes were perceived: times until fading for cycles were 7.5 s ± 6.4 s and 112% ± 69% greater on average than those of their constituent pairs ($n = 24$, $p < 0.002$, permutation resampling). By increasing the time until phosphenes fade, this technique, and future enhancements, can increase the time visual prosthesis users can view a scene and increase the utility of artificial vision. Further experiments will investigate the effects of more than 2 electrode groups per cycle.

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**Presentation Number:** NANO66.07

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** UK Dementia Research Institute
Alzheimer Association

**Title:** Non-invasive temporal interference electrical stimulation of the human hippocampus

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**Abstract:** Electrical brain stimulation is a key technique in research and clinical neuroscience studies and also is in increasingly widespread use from a therapeutic standpoint. However, to date, all methods of electrical stimulation of the brain either require surgery to implant an electrode at a defined site or involve the application of non-focal electric fields to large fractions of the brain. We recently discovered a strategy for sculpting electrical fields to enable focused yet noninvasive neural stimulation at depth using temporal interference (TI) of kHz electric fields with a difference frequency within the range of neural activity (Grossman et al., Cell 2017; Grossman, Science 2018). We validated the TI stimulation concept in rodents, demonstrating noninvasive stimulation of hippocampus neurons without recruiting overlying cortex neurons and steerable stimulation of cortical region without moving the electrodes.Here we report the translation of the non-invasive DBS concept to humans. Earlier human studies tested TI stimulation of cortical structures, but the crucial non-invasive DBS capability has not been validated. We first used electric field modelling and measurements in a human cadaver to verify that the locus of transcranial TI stimulation can be steerably localised to the human hippocampus with minimal exposure of the overlying cortex. We then performed simultaneous TI and functional magnetic resonance imaging (fMRI) experiments designed to explore physiological changes in brain activity in response to stimulation and provide evidence for target engagement. Finally, we tested the behavioural impact of delivering TI stimulation to the hippocampus in healthy participants. We demonstrate the safety and tolerability of TI stimulation in humans, the ability to focally target the stimulation locus to the hippocampus, and the capacity to modulate hippocampal activity and associated memory performance.TI stimulation may represent a new method of brain stimulation using familiar and well-tested electric fields, but able to achieve focal deep stimulation without neurosurgery. These results provide crucial proof-of-concept validation of the first non-invasive DBS in humans. The hippocampus is important in many brain functions, including learning, memory, and emotional behaviour. It also plays a central role in many of the most common brain disorders. By modulating hippocampal neural activity noninvasively, TI stimulation offers new opportunities to probe and treat its functions.

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**Presentation Number:** NANO66.08

**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** T32MH125786

**Title:** An active learning framework for personalized deep brain stimulation

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**Abstract:** Background: To personalize deep brain stimulation (DBS), it is crucial to establish a connection between DBS parameters and an individual's neural response. The current approach, which relies on random sampling (RS), is not only time-consuming and costly but also impractical in a clinical setting. Consequently, it hinders the exploration of novel settings when faced with challenging situations. To tackle this issue, we have developed a novel algorithmic framework centered around active learning (AL). This framework enables us to acquire the optimal model for the relationship between DBS parameters and brain response, while simultaneously reducing the number of experiments required.
Methods: We utilized a computational model of Parkinson's disease to generate synthetic data. By sweeping through various parameters such as subthalamic nucleus DBS amplitude, frequency, and pulse width, we estimated the power of globus pallidus internus (GPi) beta (13-30 Hz) for each DBS parameter. This process yielded a total of 200 distinct samples. Subsequently, we randomly allocated 80% of this data for pool training, reserving the remaining 20% as unseen test data. For establishing the link between DBS parameters and GPi beta power, we employed linear regression and non-linear regression models. To train these models, we initially used three training samples, employing both the RS and AL approaches. Following an iterative process, we added one training sample at a time to both models based on the AL and RS approaches until we had a total of 20 training samples. At each iteration, we assessed the performance of both models on the unseen test data and calculated the root mean squared error (RMSE). We repeated this entire process 1000 times.
Results: The mean RMSE for the AL and RS approaches was 0.043 and 0.039, respectively. The results demonstrated that the AL-based model exhibited superior performance compared to the RS-based model, as evidenced by significantly fewer errors on the unseen dataset. This difference was statistically significant, as determined by a two-sample t-test ($p = 2.33e-07$, $N = 1000$). Conclusion: Our study confirms the superiority of our AL approach over the existing method in establishing a personalized connection between DBS parameters and brain response, all while reducing the duration of the experimental procedure.

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**Title:** ConnectToBrain: Toward non-invasive wireless connection to the networks of the human brain

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**Abstract:** An everlasting pursuit in neuroscience is to understand how multiple brain regions work together to express behavior, cognition, and emotion. Transcranial magnetic stimulation (TMS) offers a non-invasive, wireless connection to the brain to evoke targeted neuronal activation. However, conventional TMS technology does not allow fast or concurrent stimulation of adjacent targets and relies on the slow physical movement of a coil placed over the scalp, limiting the delivery of stimulation to only one brain region at a time. This method is indifferent to the network properties of brain function and makes its applications slow, operator dependent, and limited in accuracy and efficacy. We aim to develop a fully automated, non-invasive brain stimulation platform that enables fast and accurate targeting of multiple cortical regions, informed by anatomical and functional measures.
The first prototype of our system comprises a custom five-coil multi-locus TMS (mTMS) device, assisted by a collaborative robotic arm (Elfin 5; Han's Robot, China). The five-coil set allows electronically moving and rotating the peak electric field within a 30-mm diameter area on the cortical surface. In turn, the robot keeps the coil set in place and handles the large-scale physical movement. The robot and the mTMS device are controlled via custom neuronavigation software (InVesalius 3), connected to a motion-capture setup (Flex13; OptiTrack, USA). An automated algorithm guides the stimulation based on electroencephalography (EEG) or -myography (EMG) data recorded with a NeurOne device (Bittium, Finland). We tested our robot-guided mTMS with an automated motor mapping algorithm in two healthy men (aged 25-29). The optimal cortical site evoking maximal muscular responses in three intrinsic hand muscles could be found within 45 trials (about 3 min) and with a 3.7-mm mean error without any operator interference.

This pilot study demonstrates the feasibility of full automation in robot-guided mTMS. Our system offers hands-free and fast electronic control of the location and orientation of the electric field induced in the brain within milliseconds without the need for the operator to move the TMS coils physically. The modular hardware and software architectures offer flexible input and output for incorporating custom algorithms. We anticipate that wireless and automated access to networked brain areas will enable the development of highly efficient diagnostic and treatment protocols for neurological disorders such as tumors, epilepsy, depression, Alzheimer's disease, and Parkinson's disease.

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**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** HFSP Fellowship
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**Title:** A living biohybrid neural interface for synaptic modulation of neural activity in the visual thalamus

**Authors:** L. SIFRINGER[1], A. FRATZL[3], S. J. IHLE[1], P. CHANSORIA[1], J. DURU[1], B. CLEMENT[1], S. CONOLLY[1], B. MAURER[1], C. M. TRINGIDES[1], L. MÖNKEMÖLLER[1], G. AMOS[1], S. WEAVER[1], S. GIRARDIN[1], K. VULIC[1], S. STEFFENS[1], A. BELTRAMINELLI[1], E. CEYLAN[1], S. MADDURI[4], M. ZENOBY-WONG[1], B. ROSKA[3], J. VÖRÖS[1], ***T. RUFF**[2]; [2]Information Technol. and Electrical Engin., [1]ETH Zürich, Zürich, Switzerland; [3]IOB Basel, Basel, Switzerland; [4]Univ. Geneva, Geneva, Switzerland

**Abstract:** Restoring functional vision in blind patients lacking a healthy optic nerve requires bypassing retinal circuits. This can be achieved by high resolution stimulation of the visual thalamus, which is located deep inside the brain and serves as the main input to cortical circuits underlying vision. However, available deep brain stimulation electrodes suffer from low stimulation resolution, limited biocompatibility and limited coverage of the targeted tissue. To overcome those limitations, we propose a novel living biohybrid neural interface with the goal of restoring functional vision in the blind without a healthy optic nerve. The interface uses living on-chip grown retinal neurons as relays to convert electrical signals from a stretchable microelectrode array into synaptic stimulation of a neural target tissue. The interface is based on

a stretchable micropatterned microelectrode[1,2] array onto which we align axon guiding microfluidic structures that enable unidirectional guidance and merging of axons to form an artificial optic nerve. We increase the biocompatibility of the device by replacing the Polydimethylsiloxane (PDMS) based nerve forming channel with a collagen or gelatin methacryloyl (GelMA)[3] tube that is only 300 µm in diameter and directly integrated onto the PDMS device. We demonstrate the seeding of retinal spheroids into our biohybrid devices using a modified fluid force microscope. The retinal ganglion cells form an artificial optic nerve up to 3mm long that can transit from the device into the PDMS-bound hydrogel tube to reinnervate a matrigel-based target structure in vitro. We show that individual retinal spheroids can be stimulated using our stretchable microelectrode array. We also present in vitro data on how spikes propagate within the biohybrid implant to modulate thalamic target activity using glass and CMOS multielectrode arrays. Finally, we present first progress towards in vivo implantation.

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**Topic:** I.08. Methods to Modulate Neural Activity

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**Title:** Multifunctional soft hydrogel probes for neural modulation and recording

**Authors: *S. RAO**[1], S. HUANG[2], Q. WANG[2], R. XIAO[1];
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**Abstract:** Neural probe technologies link the neural activity at cellular and circuit levels with the behavioral outcome and consequently assist to reveal the neural mechanisms. By utilizing soft materials, such as hydrogels, we can equip these probes with tissue-like mechanical properties that offer adaptive advantages for neural modulation and recording during various behaviors. In

this study, we introduce a suite of multifunctional neural probes derived from soft materials, which incorporate optical waveguides, microelectrodes, and microfluidic channels within compact devices. Through in-situ crosslinking and control over the growth of polymer nano-crystalline, we can manage the volumetric shrinkage during hydrogel probe fabrication and enable the integration of various components. Furthermore, we have demonstrated the capabilities of these integrated soft devices in the context of optogenetic stimulation, fiber photometry recording, electrical recording, and pharmacological interventions in the brains of behaving mice.

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**Nanosymposium**

**NANO67: Sleep Regulation and Entrainment**

**Location:** WCC 201

**Time:** Tuesday, November 14, 2023, 1:00 PM - 4:00 PM

**Presentation Number:** NANO67.01

**Topic:** F.07. Biological Rhythms and Sleep

**Support:** Cora May Poncin grant to Elizabeth Medina
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**Title:** The effect of mutations in Shank3 on the ontogenesis of the transcriptional response to sleep loss in the mouse cortex

**Authors: *E. MEDINA**, C. MUHEIM, K. FORD, K. SINGLETARY, M. FRANK, L. PEIXOTO;
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**Abstract:** Sleep deprivation (SD) is known to produce large changes in gene expression in the brain, and the identification of affected genes has been helpful to understand the molecular mechanisms mediating the detrimental effects of sleep loss. However, much less is known about the molecular response to sleep loss during development. Sleep problems, such as problems falling asleep and reduced sleep time, occur at a higher rate in the neurodevelopmental disorder Autism Spectrum Disorders (ASD) affecting up to 93% of individuals. We have previously shown that adult male mice carrying a c-terminal deletion in high-confidence ASD gene *Shank3* (Shank3$^{\Delta C}$) sleep less and have an increase in the number of genes affected by SD in the prefrontal cortex (PFC), an area particularly affected by sleep loss. This suggests an interaction between the Shank3$^{\Delta C}$ mutation and SD on the regulation of gene expression. In addition, our recent study in wildtype (WT) mice reported that around postnatal day 24 (P24), the age where hallmarks of sleep are still developing, the PFC transcriptome seems to be especially vulnerable to prolonged wakefulness. The goal of this study was to investigate, for the first time, the interaction between age and the Shank3$^{\Delta C}$ mutation on the cortical transcriptional response to SD. To do so both Shank3$^{\Delta C}$ and WT male littermates were either SD or allowed to sleep at two different ages: P24 and P30. RNA was extracted from the PFC and sequenced using Illumina

technology. RNA-seq from young mice was integrated with our previously published adult (P70-90) data. Transcript quantification was then performed using Salmon. Normalization and differential gene expression were performed using R/Bioconductor. Reproducibility of our results was evaluated by comparing the number of differentially expressed genes (DEG) after SD to a list of 663 positive control genes established from previous studies. Our positive control recovery rate was over 80% in adult WT animals. Functional enrichment analysis of genes differentially expressed was done using DAVID. Our results show that the transcriptional response to SD differs across development in a genotype specific manner.

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**Presentation Number:** NANO67.02

**Topic:** F.07. Biological Rhythms and Sleep

**Support:** NIH R01 AG064231
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**Title:** Sleeping Less, Learning Less: The Lasting Effects of Restricted Sleep on Cognitive Performance and Neuronal Health in Young Mice

**Authors: *N. NAIDOO**;
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**Abstract:** Chronic inadequate sleep is widespread in modern societies. Epidemiological studies indicate that chronic short sleep and/or disrupted sleep are all associated with metabolic dysfunction, cardiovascular risk, cognitive impairments, and increased risk for Alzheimer's disease. We have shown that sleep deprivation disrupts proteostasis, leading to the activation of an adaptive endoplasmic reticulum (ER) stress response known as the unfolded protein response (UPR). However, prolonged ER stress triggers the integrated stress response, which has been implicated in memory impairments. In this study, we investigated the effects of chronic short sleep (CSS) exposure in young adult wild-type (WT) mice on learning, proteostasis, and cellular senescence over the course of one year. 2-month-old mice (n=40) subjected to 8 weeks of CSS for 3 days/week and tested at 4-week intervals for hippocampal dependent learning across a year performed worse than rested mice. The difference in performance was evident at 28 weeks of age until 52 weeks of age when both CSS and undisturbed mice showed no difference in cognitive performance. A drop in the ER chaperone BiP and BDNF preceded the decline in memory in the CSS mice while p-CREB was reduced in CSS mice hippocampi at 28 weeks when compared to that in undisturbed mice. The senescence marker B-gal was increased in the cortices of CSS mice compared to undisturbed mice at 52 weeks. Results from this study suggest that perturbed proteostasis precedes cognitive decline and that chronic sleep loss early in life accelerates cognitive decline and aging.

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**Presentation Number:** NANO67.03

**Topic:** F.07. Biological Rhythms and Sleep

**Support:** NIH Grant 1R03AG073906-01A1

**Title:** Transcriptomics-wide association study highlights upregulation of excitatory neuronal genes associated with sleep duration

**Authors: *I. PIRAS**, M. NAYMIK, M. J. HUENTELMAN;
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**Abstract:** Abnormal sleep durations have been increasingly recognized as a public health concern due to their potential association with a range of health disorders. We conducted Transcriptome-Wide Association (TWAS) referencing to 11 brain regions. We leveraged four large GWAS from UK Biobank to investigate sleep duration phenotypes (SD): self-reported SD (SR-SD), short sleep (SR-SS), long sleep (SR-LS), and accelerometer derived SD (AD-SD). Following joint/conditional and permutation testing, and accounting for multiple comparisons at the brain region level through Bonferroni method, we uncovered 345 significant signals, linked to 165 distinct genes. The most significant genes included *FANCL* (SR-SD; Z = 8.885; $p = 8.4^{-19}$), *PAM* (SR-SD; Z = 6.684; $p = 6.7^{-12}$), and *GIN1* (SR-SD; Z = 6.81; $p = 9.69^{-12}$). Of the 165 genes, 125 were unobserved in the original GWAS, according to our annotation criteria that assign a SNP to a gene when it falls within -2,000 bp and +500 bp. Among the novel most significant associated genes there were *MAPK8IP1P2* (z = -6.777; $p = 2.4^{-11}$; SR-LS and SR-SD), and *GNAZ* (Z = 6.088; $p = 1.1^{-09}$; SR-SD). *GNAZ* gene (G Protein Subunit Alpha Z) is known to play a critical role in connecting the circadian clockwork to G protein-mediated signaling in the retina (PMC5663513). Additionally, *GNAZ* cell mutants exhibit increased ERK 1/2 activation (PMID: 34913528), a signaling pathway that links waking experience-induced neuronal gene expression to both sleep duration and quality (PMID: 28119463). Our Cell-Set Enrichment analysis suggested a significant enrichment of excitatory neuronal genes in upregulated genes associated with SR-SS (adj-p = 0.039), and SD, when integrating self-reported and accelerometer-derived TWAS results (adj-p = 0.026). It is worth noting that excitatory neurons' role in regulating sleep and wakefulness has recently been linked to the signaling cascade LKB1-SIK3-HDAC4 (PMID: 36477539). Finally, Gene Ontology analysis highlighted an overrepresentation of ion homeostasis among upregulated genes associated with SR-LS (adj-p < 0.01; *GRN*, *ATP2C1*, and *GRIA1*), a biological process involved in the control of sleep-wake cycle (PMC5441687).
To conclude, our findings highlight the key influence of excitatory neurons and ion homeostasis in SD. We have identified several sleep-associated genes not identified in the original GWAS. Through TWAS, we have been able to prioritize candidate causal genes, providing a clearer picture of the molecular mechanisms underlying sleep disturbances, and pointing toward novel potential therapeutic targets. It is important, however, to validate these potential causal relationships further using in vivo models.

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**Topic:** F.07. Biological Rhythms and Sleep

**Support:** NS078410

**Title:** Hydrogen-rich water reduces sleep latency and enhances forebrain neuronal activity in mice

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**Abstract:** Poor sleep is a hallmark of modern society. Nearly 30% of American adults average ≤ 6 hours of daily sleep, and more than 10% of the global population has experienced some form of insomnia. Sleep loss contributes to various health issues and impairs neurological function. Behavioral interventions for improving sleep can be effective but are often inadequate to resolve common sleep disturbances. If behavioral interventions fall short, pharmaceutical hypnotics are often prescribed because they are fast-acting. However, undesirable side effects often accompany these drugs, and long-term use can lead to drug dependence. Therefore, the value of an intervention that improves sleep quality or reduces the consequences of sleep loss without deleterious side effects cannot be easily overstated.Molecular hydrogen has recently gained popularity as a non-toxic ergogenic and health promoter. Recent work in humans demonstrates that hydrogen-rich water (HRW) may increase alertness and cognitive function and in the face of pharmacological or chemical challenges, it appears to act as a neuroprotectant in the hippocampus. While numerous reports have demonstrated that HRW can favorably modulate various neurobiological processes and behavior, its effects on sleep and sleep-related neural systems remain unexplored.In this study, we tested the ability of 7 days of *ad libitum* access to HRW to alter baseline sleep-wake architecture and the response to acute sleep deprivation in wildtype C57BL/6J mice. We used polysomnography to assess several electrophysiological and behavioral markers of circadian activity and sleep pressure in freely moving mice. Separately, we performed a between-subjects assessment of neuronal activity in known sleep- and wake-related brain regions following the same HRW treatment regimen using cFos immunohistochemical staining.Our findings suggest that HRW decreases sleep latency, increases sleep consolidation in undisturbed mice, and increases NREM and REM sleep amount in sleep-deprived mice following sleep deprivation. Additionally, mapping of cFos protein immunoreactivity shows that neuronal activity in the lateral septum, medial septum, ventrolateral preoptic area, and median preoptic area of mice treated with HRW was significantly altered.Together, these results suggest that HRW can improve sleep consolidation and can act as a neuroprotectant in known sleep regulatory regions during a chemical challenge. As the most abundant chemical substance on the planet, hydrogen enriched in water may serve as a simple, effective treatment to improve recovery after acute sleep loss.

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**Topic:** F.07. Biological Rhythms and Sleep

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**Title:** Chemogenetic deletion of the NOP receptor disrupts both spontaneous and agonist-induced sleep

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**Abstract:** We have previously shown that NOPR agonism potently induced sleep and increased EEG delta power in rats, mice and non-human primates[1], suggesting that the N/OFQ-NOPR system may have a previously unrecognized role in sleep/wake regulation. In the present study, we tested the hypothesis that the N/OFQ-NOPR system is a component of the endogenous sleep/wake regulatory system. We bred mice homozygous *NOPR*$^{loxlox}$ mice with B6.Cg-*Ndor1*$^{Tg(UBCcre/ERT2)1Ejb}$/1J mice to produce a strain (iNOPR mice) in which deletion of NOPRs could be induced throughout the brain by tamoxifen (TMX) administration. Since homozygous Cre- offspring are TMX-insensitive, comparisons of post-TMX iNOPR mice could be made to the same mice in pre-TMX (NOPR intact) condition as well as to post-TMX Cre- mice. At ~16 weeks of age, both Cre+ iNOPR and Cre- control male mice were implanted with telemetry devices to measure EEG, EMG, activity and $T_b$ (DSI, Inc.). TMX administration (150 mg/kg, i.p.) for 5 days to homozygous Cre+ iNOPR mice resulted in either global deletion of the NOPR gene or a massive knockdown of NOPR expression as determined by Western blotting and RNAscope hybridization. Whereas administration of the NOPR agonist Ro64-6198 (3 mg/kg, i.p.) suppressed activity, decreased $T_b$ and increased NREM sleep in the Cre+ iNOPR mice before TMX administration and in Cre- mice both before and after TMX administration, these effects were abolished following TMX treatment in the Cre+ iNOPR mice, providing pharmacological confirmation of successful NOPR deletion. Within-animal comparisons of pre vs. post TMX-treated Cre+ iNOPR mice revealed that NOPR deletion produced partial insomnia under baseline conditions with 11.5±1.4% more Wake time during the light phase ($p < 0.001$), the major sleep period in mice, and 5.3±1.4% over 24-h ($p = 0.028$). Conversely, NREM sleep decreased by 10.4±1.4% ($p < 0.001$) during the light phase and 4.7±1.4% ($p = 0.04$) over 24-h. NOPR deletion also reduced baseline $T_b$ and activity during the dark phase, particularly during the first 4 h after the light-to-dark transition. These results are consistent with our previous observations that NOPR agonism is NREM sleep-promoting and wake-suppressing and supports the hypothesis that loss of endogenous NOPR tone increases wake and reduces sleep amounts.
[1] Morairty SR, Sun Y, Toll L, Bruchas MR, Kilduff TS. Activation of the nociceptin/orphanin-FQ receptor promotes NREM sleep and EEG slow wave activity. Proc Natl Acad Sci USA 2023;120:e2214171120.

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**Topic:** F.07. Biological Rhythms and Sleep

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**Title:** A preoptic neuronal population encodes and controls sleep homeostasis

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**Abstract:** Sleep is a necessary process for the survival and living of all animals. Yet, our understanding of the key neuronal types and their brain-wide interactions underlying sleep regulation is far from complete. While previous studies identified the hypothalamic preoptic area (POA) as a key brain region for sleep control, the sleep-active neurons and wake-active neurons are spatially and molecular identically intermingled, including GABAergic neurons and Gal neurons, impeding our further understanding and treatment for sleep disorders. To uncover specific molecular marker and mechanism for sleep modulation in the POA, we applied single-cell RNA-sequencing to screen neuronal subtypes activated in different sleep states, and identified a rebound sleep-active transcriptomic cell type in the POA. Single-cell level *in vivo* calcium imaging of the group of neurons revealed that their activity faithfully encoded both the brain states and sleep pressure in single sleep episode level. The group of neurons bidirectionally regulate sleep homeostasis, and their excitability reflects homeostatic strength. Moreover, administration of its top marker agonists can elevate their neuronal excitability and restore the insomnia in animal model. Thus, we identified a preoptic neuronal population plays a key function in sleep homeostasis, and agonists of its marker are potential drugs for treating sleep homeostasis-related sleep disorders.

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**Topic:** F.07. Biological Rhythms and Sleep

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**Title:** Circadian rhythms of intracellular cAMP are controlled by VIP-associated network in the suprachiasmatic nucleus

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**Abstract:** Various biological functions are influenced by second messengers like cAMP and $Ca^{2+}$. Signaling transmits signals from cell surface receptors to target molecules within the cell, amplifying the signaling process. Eventually, the signal modifies gene expression, leading to changes in cellular functions. In the mammalian central clock, known as the suprachiasmatic nucleus (SCN), intracellular cAMP and $Ca^{2+}$ are believed to be involved in the input and/or output of the molecular circadian clock. However, the precise functional roles of cAMP and $Ca^{2+}$ and their dynamics in the SCN neuronal network remain largely unknown. To investigate the functional roles of cAMP, we visualized the spatiotemporal patterns of circadian rhythms in the SCN using our developed bioluminescent cAMP probes (Okiluc-aCT). For comparison, we also measured the circadian rhythms of $Ca^{2+}$ using a fluorescent $Ca^{2+}$ probe (GCaMP6s). We found

that when we inhibited the function of the neural network, the rhythm of cAMP disappeared, while the rhythm of Ca2+ persisted. This suggests that in the SCN, the circadian rhythm of cAMP is controlled by the neural network, while the rhythm of $Ca^{2+}$ is regulated by intracellular mechanisms. Next, our focus turned to a signaling molecule called vasoactive intestinal peptide (VIP), which is present extracellularly. Its receptor is known to modulate cAMP in the SCN. To analyze the impact of VIP on the cAMP rhythm, we blocked VIP signaling. The results showed a high dose of VIP application or its antagonist loss of the cAMP rhythm, indicating that intracellular cAMP rhythms are regulated by VIP in the SCN. If this is correct, there should also be a circadian rhythm in the release of VIP. To verify this, we employed a green fluorescent protein-based G-protein-coupled receptor-activation-based (GRAB) VIP sensor. Time-lapse imaging of VIP release in the SCN revealed a distinct circadian rhythm. Furthermore, blocking the function of the neural network abolished this VIP release rhythm. These findings indicate that VIP is released rhythmically based on neuronal activity and that the VIP release rhythm regulates the intracellular cAMP rhythm. In conclusion, our study demonstrates that intracellular cAMP is a crucial molecule in the composition of the circadian neuronal network in the SCN.

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**Topic:** F.07. Biological Rhythms and Sleep

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**Title:** The functional outputs from the suprachiasmatic nucleus in mice

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**Abstract:** In mammals, the generation of behavioral/physiological rhythms and the entrainment of these rhythms to the light/dark cycle are mediated by the suprachiasmatic nucleus (SCN) of the hypothalamus. Although there are a lot of studies on the mechanism of SCN outputs to regulate rhythmic events, it still remains unclear what kind of outputs is critical to circadian rhythms. The circadian locomotor rhythms disappeared in experimental animals with “the isolation of the SCN (iSCN)” which severs afferent/efferent neural connections of the SCN, indicating that neural outputs from the SCN are essential for the circadian locomotor rhythms (Stephan & Nunez, Behav Biol 1977). On the other hand, a transplant of a fetal SCN coated by semipermeable membrane restored circadian locomotor rhythms in the SCN-lesioned animal, indicating that diffusible outputs from the SCN are essential for the circadian locomotor rhythms (Silver et al., Nature 1996). In the present study, we challenged to clear the discrepancy in functional outputs from the SCN to circadian behavioral/physiological rhythms. We investigated the effects of iSCN on circadian behavioral/physiological rhythms with mice. We designed a micro-knife known as “Halász-knife” and made a physical cut of all neural fibers from the SCN except the retinal input by rotating the knife above the SCN. And then, we observed the wheel-running activity, multiple unit neural activity (MUA) rhythms *in vivo* and estrous cycle with

iSCN mice. iSCN mice showed decreased activity levels and fluctuated activity onsets in wheel-running activity after the surgery, however, they maintained a normal circadian period. *In vivo* MUA recordings, iSCN mice showed low amplitudes in MUA rhythms in the striatum while maintaining a normal circadian period. In contrast, 4- or 5-day estrous cycles disappeared in female iSCN mice as measured by vaginal smear cytology. These results suggest that neural outputs from the SCN determine the circadian robustness, such as amplitudes of circadian rhythms and adjustments of timing signals. In addition, it is suggested that some sort of output independent of physical connections drives the circadian rhythm itself. We describe the functional connectome of the SCN and an entity of diffusible factors to drive circadian rhythms in this presentation.

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**Title:** Gastrin releasing peptide-producing neurons in the hypothalamic suprachiasmatic nucleus mediate photic entrainment

**Authors: *R. LI**[1,2], P. VERGARA[2], Y. CHERASSE[2], R. INOUE[3], H. MORI[3], A. HIRANO[1,2], T. SAKURAI[1,2];
[1]Univ. of Tsukuba, Tsukuba, Japan; [2]Intl. Inst. for Integrative Sleep Med., Tsukuba, Japan; [3]Univ. of Toyama, Toyama, Japan

**Abstract:** Most organisms display 24-hour fluctuations of various physiological phenomena (circadian rhythms). In mammals, the master clock that regulates the circadian rhythms is the hypothalamic suprachiasmatic nucleus (SCN). The SCN consists of many different subtypes of neurons that produce distinct neurotransmitters. Among these, the gastrin releasing peptide-producing neurons (GRPNs) are scarce, and their role in regulating circadian rhythm is unclear. Therefore, we aimed to clarify the functions of these neurons. In order to specifically manipulate the SCN GRPNs, we used *Grp-iCre* knock-in mice and virus vector that Cre-dependently expresses target genes. First, we expressed the fusion protein channelrhodopsin2-enhanced yellow fluorescent protein in SCN GRPNs to visualize their projections and found that they mostly project to the thalamus and hypothalamus. We then used glycoprotein-deleted rabies to retrogradely visualize the neurons that project to the GRPNs and discovered that they mostly receive projections from within the SCN. We also examined the neural activity of the GRPNs using calcium indicator protein GCaMP6s in free-moving mice. We found that the GRPNs

display 24-hour firing rhythms that peak during the first half of the subjective night. These neurons also show a rapid increase in neural activity when the mice were exposed to bright white light, suggesting that they may convey photic information from the retina. Next, we determined whether the SCN GRPNs play a crucial role in generating circadian rhythm. We specifically expressed Caspase3, an enzyme that induces cell death, in the GRPNs to ablate these neurons (GRPNx). Spontaneous activity of the mice was recorded to determine their activity rhythms, but the GRPNx mice did not show attenuation in the robustness of the behavioral rhythm. As GRPNs reacted to photic stimulation through the retina, we hypothesized that they may be involved in photic entrainment. To examine this, we subjected the GRPNx mice to jetlag, and we discovered that the GRPNx mice entrained significantly slower to the new light-dark cycle after jetlag. We then expressed hM3Dq (excitatory DREADD) in the SCN GRPNs and intraperitoneally injected the ligand CNO to stimulate these neurons at the onset of jetlag. We discovered that mice entrained significantly faster to the new light-dark cycle when the GRPNs are stimulated. From these results, we conclude that the SCN GRPNs may not be necessary for the generation of circadian rhythms but are essential for photic entrainment.

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**Presentation Number:** NANO67.10

**Topic:** F.07. Biological Rhythms and Sleep

**Title:** Neural signatures of Recovery of Consciousness from External and Intrinsic processes

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**Abstract:** Understanding the mechanisms underlying the transition from unconsciousness to consciousness is a fundamental pursuit in neuroscience. This study investigates two novel neural markers of Recovery-Of-Consciousness (ROC) in humans, one based on externally evoked responses and the other on intrinsic brain processes. Namely, we first examine the differential response of the human brain to external electrical stimulation during anaesthesia and after the recovery of consciousness. And secondly, we explore whether band-limited complexity, represented by permutation entropy, in various frequency bands of intrinsic brain activity can serve as an indicator for the sharp step transition from coma to consciousness. Electrocorticography (ECoG) was employed to record brain activity in 11 patients who underwent awake brain tumor surgery. We utilized interleaved blocks of steady-state somatosensory electric stimulation and resting-state measurements, spanning from anesthesia-induced unconsciousness to full wakefulness. Regarding the external somatosensory stimulation, we found a significant reduction in magnitude of the corresponding event-related potentials (ERPs) after the recovery of consciousness. This diminished response is a novel finding and it is likely attributable to increased top-down processing in the wakeful state. Regarding the intrinsic activity, permutation entropy identified a notable sharp, steplike increase in the peak frequency of ongoing alpha oscillations, right at the moment of the patients' initial behavioral response after

coma. Further spectral analysis revealed that this peak-frequency increase in the alpha band was due to a "seesaw" shift of the spectral power around a pivot frequency point. This pivot frequency was found in the alpha band, and its power was not affected by ROC. The "seesaw" shift was instantiated by the power of the adjacent higher frequencies increasing and/or the power of adjacent lower frequencies decreasing after ROC. These findings offer two significant novel contributions. 1) Our results extend the range of possible functional roles of alpha oscillations, beyond facilitatory and inhibitory. Here we find that in many areas of the human brain there seems to be a reserved stationary "pivot" alpha frequency, which is not affected by the state-of-consciousness. 2) The use of band-limited permutation entropy identifies the sharp step transition from unconsciousness to consciousness, with obvious implications for clinical application. Overall, our study presents significant insights into the understanding of the brain mechanisms underlying human recovery-of-consciousness.

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**Topic:** F.07. Biological Rhythms and Sleep

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**Title:** Exogenous electric fields induce phase precession of single units in awake non-human primate

**Authors: *H. TRAN**[1], M. WISCHNEWSKI[1], Z. ZHAO[1], I. ALEKSEICHUK[1], S. SHIRINPOUR[1], J. ZIMMERMANN[2], A. OPITZ[1];
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**Abstract:** Neural activity can be modulated by exogeneous electric fields such as transcranial alternating current stimulation (tACS). Animal studies have shown that spike timing can occur at a specific phase of an induced oscillation. The gradual shifting of preferred neural spiking relative to local field potentials (LFPs), known as phase precession, plays a prominent role in neural coding. However, no studies have investigated the shift in phase preference during a continuous stimulation. To investigate whether a phase preference of single units shifts during tACS, we performed in-vivo recordings of single-unit activity in awake non-human primate (8-yo male) while applying tACS in the anterior-posterior direction with an intensity of 1mA. The animal was implanted with 128 microdrives covering a large area of the left brain hemisphere. The stimulation conditions consisted of 8 blocks of 6 min each - 4 blocks at 10Hz (alpha rhythm) and 4 blocks at 20Hz (beta rhythm). We were able to detect and classify 81 single units and we quantified neural entrainment and phase shifting - 46 (56.8%) and 48 (59.3%) were significantly entrained during AC stimulation at alpha and beta frequencies respectively. For alpha stimulation, 8 neurons showed a clockwise (negative) phase shift (mean: -38.26°), meaning that spikes occurred progressively earlier in the oscillatory cycle. We observed a counter-clockwise (positive) phase shift in 7 neurons (mean: 31.94°) suggesting that neural spiking preference moved to later in the oscillatory cycle. For beta stimulation, 4 neurons showed a clockwise (negative) phase shift (mean: -38.51°) while 9 neurons showed a counter-clockwise (positive) phase shift (mean: 27.23°). In conclusion, we show for the first time that

spiking neurons' preferred phase shift during extended periods of tACS. Moreover, it highlights neural directional sensitivity which may be crucial for understanding (human) brain networks interactions in normally and abnormally functioning brains.

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**Title:** Macroscopic phase precession induced by exogenous electric fields in humans

**Authors: *M. WISCHNEWSKI**[1], H. TRAN[2], Z. ZHAO[2], S. SHIRINPOUR[2], Z. J. HAIGH[2], J. ROTTEVEEL[2], I. ALEKSEICHUK[2], J. ZIMMERMANN[2], A. OPITZ[2];
[1]Univ. of Minnesota, Saint Paul, MN; [2]Univ. of Minnesota, Univ. of Minnesota, Minneapolis, MN

**Abstract:** Spike-field coupling refers to the observation that neural spiking occurs preferentially at specific phases of endogenous local field potentials. Preferred phases gradually shift during learning, suggesting that phase precession is a marker for neural coding and synaptic plasticity. Currently, evidence of phase precession in animals and humans is observational, while causal evidence is lacking. The aim of the present study was to show that neuromodulation of ongoing local field potentials can induce phase precession. We applied non-invasive exogenous electric fields, known as transcranial altering current stimulation (tACS), to 20 healthy volunteers in a double-blind cross-over study. This method has been shown to bias spike timing in rodents, non-human primates, and humans. To probe phase precession, transcranial magnetic stimulation (TMS) was used to induce group-level neural spiking in the motor cortex. Resulting motor-evoked potentials (MEP) serve as a measure of corticospinal excitability. TMS was applied at different phases of the ongoing exogenous alternating current, enabling the calculation of preferred excitability phase, as well as shifts in phase over time. TACS at an intensity of 2 mA (peak-to-peak) was applied in two sessions to modulate individual alpha (9.81 ± 0.22 Hz) or beta (20.24 ± 0.89 Hz) oscillations. During four tACS blocks (~6.5 min each), 600 single TMS pulses were applied at the peak, trough, rising phase and falling phase of each oscillation. Results showed an overall main effect of phase ($F = 8.62$, $p < 0.001$), which was similar for alpha and beta (interaction $p = 0.765$). Specifically, MEPs were larger at the trough/falling phase, compared to peak/rising phase. Averaging MEPs using a sliding window showed phase precession through the block (circular-linear correlation, alpha: $r = 0.655$, $p = 0.014$, beta: $r =$

0.825, p = 0.001). For alpha oscillations, phase maximal MEPs started at the falling phase (92.7°) and drifted to the trough (163.6°) at the end of block. Similarly, for beta tACS, the preferred phase transitioned from falling/trough (131.8°) to trough/rising (189.2°). These results complement single-unit data in non-human primates, which show that subsets of neurons shift in preferred phase when tACS is applied. Overall, the results provide evidence phase precession can be induced by the modulation of ongoing local field potentials by the application of exogenous alternating currents. Furthermore, the ability to modulate endogenous brain activity with tACS demonstrates its therapeutic potential for the treatment of disorders associated with abnormal neural oscillation patterns.

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**Nanosymposium**

**NANO68: Reward, Motivation, and Neuronal Circuits**

**Location:** WCC 152A

**Time:** Tuesday, November 14, 2023, 1:00 PM - 4:15 PM

**Presentation Number:** NANO68.01

**Topic:** G.03. Motivation

**Support:** R01 MH108643

**Title:** Therapeutic doses of ketamine acutely attenuate the aversive effect of losses during decision-making

**Authors: *H. SEO**[1,3], M. OEMISCH[2];
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**Abstract:** The discovery of rapid-acting antidepressant, ketamine has opened a pathway to a new generation of treatments for depression, and inspired neuroscientific investigation based on a new perspective that non-adaptive changes in the intrinsic excitatory and inhibitory circuitry might underlie the pathophysiology of depression. Nevertheless, it still remains largely unknown how the hypothesized molecular and synaptic levels of changes in the circuitry might mediate behavioral and neuropsychological changes underlying depression, and how ketamine might restore adaptive behavior. Here, we used computational models to analyze behavioral changes induced by therapeutic doses of ketamine, while rhesus macaques were iteratively making decisions based on gains and losses of tokens. When administered intramuscularly or intranasally, ketamine reduced the aversiveness of undesirable outcomes such as losses of tokens without significantly affecting the evaluation of gains, behavioral perseveration, motivation and other cognitive aspects of learning such as temporal credit assignment and time scales of choice and outcome memory. Ketamine's potentially-antidepressant effect was separable from other side effects such as fixation errors, which unlike outcome evaluation, was readily countered with

strong motivation to avoid errors. We discuss how the acute effect of ketamine to reduce the initial impact of negative events could potentially mediate longer-term antidepressant effects through mitigating the cumulative effect of those events produced by slowly decaying memory, and how the disruption-resistant affective memory might pose challenges in treating depression. Our study also invites future investigations on ketamine's antidepressant action over diverse mood states and with affective events exerting their impacts at diverse time scales.

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**Presentation Number:** NANO68.02

**Topic:** G.03. Motivation

**Support:** ZIA MH002928 (BA)

**Title:** A state-based value model predicts motivation during a reinforcement learning task with token reinforcement

**Authors: *D. BURK**, C. TASWELL, H. TANG, B. AVERBECK;
NIH, Bethesda, MD

**Abstract:** Reinforcement learning (RL) is a theoretical framework that describes how agents learn to select options that maximize rewards and minimize punishments over time. In many RL tasks, reward size and/or probability are varied, and animals learn to make choices to maximize the total reward received. We often make choices, however, to obtain symbolic reinforcers (e.g. money, points) that can later be exchanged for primary reinforcers (e.g. food, drink). Although symbolic reinforcers are highly motivating, little is understood about the computational and behavioral mechanisms that shape motivation to earn them. In the present study, we examined how monkeys learn to make choices that maximize fluid rewards through reinforcement with tokens. The monkeys learned through trial and error which visual images were associated with gaining tokens and which images were associated with losing tokens. Every 4-6 trials, tokens were exchanged for fluid rewards. The question addressed here is how state value, which is a complex function of task features (e.g. current number of accumulated tokens, cue pair, task epoch, trials since last delivery of primary reinforcer, etc.), affects motivation. We assessed motivation using three behavioral readouts: (1) reaction times to acquire fixation, (2) choice reaction times, and the (3) probability of aborting a trial. We constructed a Markov decision process model (MDP) that computes state values given task features in order to capture the motivational state of the animal and make predictions about behavior and neural activity. The model calculates the state value of each moment within a trial, based on the combined factors present in the tokens task. Fixation times, choice reaction times, and abort frequency were all significantly related to the state values and changes in state value during the tokens task (n=5 monkeys). Furthermore, the model makes predictions for how neural responses can change, on a moment-by-moment basis, relative to changes in motivational state that are driven by changes in state value. Together, this task and model allow us to capture learning and behavior related to symbolic reinforcers in a way that stateless RL models cannot.

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**Presentation Number:** NANO68.03

**Topic:** G.03. Motivation

**Title:** The Motivational Role of the Ventral Striatum and Amygdala in Learning from Gains and Losses

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**Abstract:** Adaptive behavior requires animals to be sensitive to the environment and to modulate their effort in response accordingly. The ventral striatum (VS) and amygdala are two structures often implicated as essential structures for learning. The literature addressing the contribution of these areas to learning, however, is not entirely consistent. We propose that these inconsistencies are due to learning environments and the effect they have on motivation. To differentiate aspects of learning from environmental factors that affect motivation, we conditioned tokens as reinforcers in tasks where animals could both gain and lose tokens. We ran three experiments; two used deterministic reinforcement and one used stochastic reinforcement. We compared rhesus monkeys with VS lesions, amygdala lesions, and unoperated controls across the three tasks. We completed within- and between-group analyses of performance across the three experiments and found that for all three groups, performance varied by experiment. All three groups modulated their behavior in the same directions, to varying degrees, across the three experiments. This level of behavior modulation is why we find deficits in some experiments but not others, meaning the amount of effort animals were willing to give differed depending on the learning environment. Importantly, all three of these tasks had the same information load, which suggest that the performance differences we found across tasks is due to motivation and not learning ability. Our results suggest that the VS is important for the amount of effort animals will give in rich deterministic, and relatively leaner stochastic learning environments. We also showed that monkeys with amygdala lesions had no learning deficits in learning environments with loss and conditioned reinforcers. Further, we discussed how this finding supports the idea that the amygdala is critical for motivation in concurrent appetitive learning environments. These results show that learning environments shape motivation and that the VS and amygdala are essential for distinct aspects of motivated behavior.

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**Title:** Explore-exploit tradeoffs regulated through amygdala inputs to ventral striatum

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**Abstract:** Decision-makers will choose to explore options with unknown consequences rather than exploit options whose consequences are known, a tradeoff known as the explore-exploit

dilemma. In nonhuman primates, explore-exploit decision making is typically studied in the context of maximizing gains, but is understudied in the context of minimizing losses because of ethics surrounding use of aversive reinforcement. We developed a three-arm-bandit task that utilizes novel choice cues and token rewards that were cashed out for juice when the number of tokens earned reached a preset threshold. Using tokens as a secondary reinforcer allowed us to include aversive conditions where animals can lose tokens. Animals must learn which option provides them with the best outcome, which could mean maximizing token gains or minimizing token losses. Explore-exploit tradeoffs were induced by introducing novel options associated with either token gains or losses. We fit a reinforcement learning (RL) model to the behavior of two rhesus macaques and found that both learned faster from cues associated with token losses than gains. However, the prospect of losses did not reduce monkeys' bias to explore novel options. Instead, novelty-seeking was highest when monkeys already learned that choosing a familiar option would result in the token loss. Their novelty-seeking did decrease as the likelihood of cashing out the tokens they had already earned increased. Thus, the monkeys could flexibly decide when to value exploration to maximize long-term gain versus valuing exploitation to increase the immediacy of their token cash out. Using pathway-specific chemogenetics, we evaluated the effects of inhibiting excitatory amygdala inputs to the ventral striatum on exploratory decision-making in this task, given that neurons in each region code the value of exploring novelty (Costa et al., 2019). We found that chemogenetic inhibition of amygdala inputs to ventral striatum reduces exploration of novelty and increases exploration of less sampled but familiar options. Chemogenetic inhibition of this pathway also has a valence dependent effect on learning, which only impedes learning about the value of options associated with gains. This is the first demonstration that nonhuman primates manage explore-exploit tradeoffs similarly whether choices are associated with primary or secondary reinforcers, and that the prospect of losses has considerable effects on exploratory decision-making. It is also the first demonstration that chemogenetics can be used to reliably manipulate a specific amygdalar circuit to modulate multiple aspects of reinforcement learning and decision making.

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**Title:** Differential roles of anterior insular and lateral prefrontal cortex in decisions under risk

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**Abstract:** Human risk attitude is highly context-dependent and is influenced by the current wealth level and on whether the decision is made in the face of possible gains or losses (Tversky and Kahneman, 1979). To study the neuronal mechanisms responsible for the changes in risk attitude across contexts, we trained monkeys in a token-based gambling task. In this task, the monkey must collect six tokens (secondary reinforcer) for the exchange to a fluid reward. By varying the decision context (gain or lose) and the composition (the amount and probability of

gaining/losing tokens) of each gamble option across trials, this task allows us to examine the effect of decision context, as well as the token asset (the 'wealth level' of monkeys), on the monkeys' preferences for the same gamble option.
We found macaques, like humans, change their risk attitude across wealth levels and gain/loss contexts. Neurons in the monkey anterior insular cortex (AIC) encode the current wealth level (tokens), which serves as a reference point, and gain/loss-specific value signals (Yang et al., Nat. Comm. 2022). AIC neurons also reflect 'loss aversion' (i.e., option value signals are more sensitive to change in the loss than in the gain context). However, AIC neurons do not strongly predict the choices of the monkeys on individual trials.
We therefore recorded activity in the Lateral Prefrontal Cortex (LPFC), a cortical area closer related to action selection. We found that LPFC, just as AIC, encode the reference point and gain/loss-specific value signals in independent neuronal populations. This suggests that representing value in a reference-dependent framework might be universal in the brain. However, we also found a number of differences between AIC and LPFC: (1) AIC neurons more commonly encode the gain/loss context of the choice, while LPFC more frequently encode comparative value signals. (2) While AIC more commonly represent loss-value signals, LPFC neurons represent gain- and loss-value with equal frequency. (3) LPFC neurons are equally sensitive to loss and gain and do not reflect loss-aversion. (4) The monkey's choices (saccade direction toward chosen option) in each individual trial can be decoded from the activity of LPFC neurons, but not AIC neurons. (5) LPFC mostly represent secondary rewards (token changes), while AIC mostly represent primary reward (fluid delivery).
These findings suggest that AIC plays a more significant role in representing the decision context. In contrast, PFC is more involved in comparing the value of the options and facilitating the choice.

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**Support:** College of Medicine, University of Tennessee Health Science Center (UTHSC)

**Title:** Neuromodulation of aversion resistant substance seeking in C. elegans

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**Abstract:** Aversion-resistant seeking (ARS) has been a valuable model of compulsive-like alcohol and drug use in humans. ARS is characterized by an imbalance between the strong drive to substance and disruption in control of substance use, stemming from disturbances in the intricate electrical activity of the brain. To gain a comprehensive understanding of ARS, it is crucial to investigate the neural signals and regulatory processes associated with reward-seeking behavior, including communication within and across brain regions, integration of sensory information, and behavioral regulation. We exploit the strengths of the ARS model in *C. elegans*, analysis of complex behavioral paradigms within a simplified system, which offers the advantage of a rapid genetic workflow and a transparent nervous system, as its neural network

has been fully mapped down to the level of individual neurons. The ARS model of the worm, which encompasses an imbalance between the superior drive to consume alcohol and a loss of control over alcohol intake, is a relevant tool for modeling compulsive-like alcohol use in humans. We utilized two conflicting behavioral programs in the worms: seeking ethanol and avoiding aversive stimuli that block ethanol-seeking. Following chronic ethanol exposure, worms that are dependent on ethanol repeatedly attempt to cross an aversion barrier to reach the ethanol area beyond the barrier, ultimately succeeding in crossing it. Indeed, the chemotaxis to ethanol of these worms exhibits a highly proactive goal-directed behavior, where they traverse a distance approximately 100 times their own body size (<1mm) to navigate towards the ethanol spot. We hypothesized that chronic ethanol exposure alters the adaptation threshold to aversion/nociception in dependent animals, which in turn affects decision-making during multisensory integration under conflicting conditions, eventually contributing to the development of ARS. Our quantitative analysis of the optogenetic induction of aversive response revealed a notable enhancement in the adaptation to repeated optogenetic stimulation of primary sensory neurons for aversion in animals exposed to ethanol treatment. Currently, we are investigating the following questions: 1) the specificity of adaptation alteration, 2) the involvement of peripheral or central modulation, and 3) the pivotal neural substrates responsible for modulating this behavioral tolerance.

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**Title:** Revealing cell-type specific striatum-wide representation of stimuli value and location during value-based approach behavior with multi-fiber arrays

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**Abstract:** Value-based behavior requires animals to perceive, evaluate, and localize stimuli to direct actions accurately. The striatum is a crucial brain structure regulating these processes, as it can integrate and filter converging external sensory and internal value inputs to regulate appropriate action strategies. Striatum-dependent action control is facilitated by the concurrent activation of the direct and indirect pathway spiny projection neurons. Past studies have suggested that different striatal subregions make distinct, simultaneous contributions to value learning and action. However, due to technical limitations in simultaneously recording cell-type specific neural activity across the striatum, it remains unclear how dSPN and iSPN signals in various striatal regions support different aspects of value-based approach behavior.
We developed a novel multi-optical-fiber array photometry approach to record neural activity at over 70 locations simultaneously across the entire 3-D volume of the striatum. We applied this technology to measure striatum-wide calcium signals in head-fixed mice from dSPNs and iSPNs during tasks requiring evaluation, localization, and movements toward visual cues. To isolate representations of cue value and spatial location, mice were initially presented with either a Cs+

cue or a Cs- cue presented at different locations in their visual field. To establish dynamics related to the execution of a value-based approach action, mice were then trained to execute appropriate approach responses directed towards the CS+ cue.
Preliminary results indicate that representations of stimulus location, relative value, and actions vary across cell types and striatum space. Notably, the anterior ventral striatum (aVS) and anterior dorsal medial striatum (aDMS) are more sensitive in learning positive relative values, whereas the posterior dorsomedial striatum (pDMS) is more sensitive in learning to neglect neutral values. At the learned stage, the representations of relative stimulus value were similar between dSPNs and iSPNs in the aVMS and aDMS but were divergent in the pDMS. Moreover, stimulus location was represented in the aDMS and pDMS but not the aVMS, suggesting that the DMS may integrate stimulus value with location to generate learned, cell-type specific activity patterns that influence proper orienting during value-based action selection. We hypothesized that the differential direction-sensitive representation of relative value by dSPNs and iSPNs in aDMS and pDMS indicates that these two regions might be differentially involved in influencing the orienting behavior during value-based approach or avoidance.

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**Title:** Acetylcholine signaling in the dorsomedial striatum is associated with behavioral flexibility in mice learning a virtual reality response task

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**Abstract:** A striking and defining feature of the brain is its ability to adapt flexibly. Making decisions adaptively is essential in changing environments. Findings in humans and experimental animals have shown that the dorsomedial striatum (DMS) is a node in the network of brain systems for behavioral flexibility. In rodents, lesioning of DMS cholinergic interneurons, and pharmacological manipulation of acetylcholine (ACh) release impair flexible behaviors. However, the activity of ACh during behavioral switching, when conditions demand a shift in response, is not yet known. To address this, we designed a virtual reality response learning (Y-maze) task for head-fixed mice (ChAT-Cre; 3-8 months, n=7, 2F) using the JetBall system (PhenoSys). We imaged ACh dynamics using 2-photon microscopy via a GRIN lens implanted above the DMS and a genetically encoded biosensor that permits ultrafast cellular resolution imaging of ACh, iAChSnFR. We measured ACh concentration while mice learned a Y-maze requiring choice of the left or right arm for reward. The behavioral task comprised acquisition and reversal phases lasting 10-12 days. Mice were trained daily in sessions of about 40 trials. When they reached an acquisition criterion of 80% correct performance the rewarded arm was switched, and ACh measurements were continued during reversal. Trained mice adapted their behavior to reversal in several ways. Selection of the rewarded arm of the maze increased over sessions, during which the rate of correct choices scaled with reward probability. In motivational

aspects, we found significant differences in both velocity, anticipatory licking, and latency (time taken to complete a trial) with which mice approached the rewarded arm. Preliminary measures of ACh dynamics following the reversal revealed a delayed, progressive increase in the spatially averaged concentration of ACh during the outcome period when mice chose the previously-reinforced arm (error trials). In later trials after reversal, the magnitude of ACh concentration appeared to decrease in response to error. The context-dependent increase in ACh release in the initial trials after reversal might serve to inhibit the expression of the initial response patterns until the new response pattern is reliably executed (lose-switch). We propose that this behaviorally-induced ACh release and spatiotemporal distribution in response to error trials may provide a teaching signal to extinguish invalid responses. These findings suggest a model in which striatal ACh facilitate disengagement from irrelevant responses and the engagement of new, valid responses based on reinforcement contingencies.

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**Title:** Behavioral and neural alterations of the ventral tegmental area by exposure to junk food in rats

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**Abstract:** The brain reward system plays a crucial role in regulating appetitive and consummatory behaviors in response to different incentive stimuli. Among these stimuli, junk food is particularly known for its high palatability and the potential for excessive consumption. Previous research has indicated that excessive junk food consumption can affect the reward circuitry, but the underlying mechanisms remains unclear. Furthermore, it is unknown whether the functionality of this brain system is also altered when responding to other types of natural rewards. Our study aimed to investigate whether excessive consumption of junk food, in the form of a cafeteria diet (CAF), could alter the neural activity patterns of the ventral tegmental area (VTA) in response to food and sexual rewards. To this end, sexually experienced female rats were unilaterally infused with 750 nl AAV5-hsyn-GCaMP6s in the VTA (coordinates: AP -5.0, ML ±0.9, DV -8.5 from skull) with an optic fiber implanted above the injection site. Following surgery, the rats were divided into three groups and were exposed to a control diet (standard chow), High-fat high-sugar diet (HFHS) and CAF diet for 6 weeks. After diet exposure, the rats underwent tests to assess their responses to food and sexual rewards. A food reward test was conducted five times, while a sexual reward test was conducted three times. Additionally, a dissatisfaction test involving the standard chow reward was performed. Each test comprised three phases: baseline, pre-reward, and reward. Throughout these phases, various appetitive and consummatory behaviors were monitored, while neural activity in the VTA was

recorded. The findings of our study reveal that rats on a long-term CAF diet exhibited reduced interest in the junk food reward and consumed smaller amounts of it compared to rats on a control diet. These behavioral changes were accompanied by diminished neural activity responses in the VTA when the rats were sniffing or eating the food reward. Similarly, although to a lesser extent, reductions in VTA activity responses were observed in relation to a sexual partner. However, no significant differences were observed between the control and CAF rats in terms of appetitive or consummatory behaviors during sexual interactions. Overall, these results suggest that prolonged exposure to junk food leads to a desensitization of VTA neurons, resulting in behavioral changes specifically in response to food rewards but not sexual rewards.

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**Title:** Functional neuroimaging of apathy in traumatic brain injury.

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**Abstract:** Apathy, which refers to a significant loss of motivation, is a common and impairing clinical symptom in patients with mild-to-moderate traumatic brain injury (mmTBI). Previous research has suggested that damage to specific motivational neural circuits anchored in the ventromedial prefrontal cortex (vmPFC) can lead to increased apathy in mmTBI. However, few studies have examined the precise brain functions underlying this symptom in humans using task-based neuroimaging. To address this gap, we conducted a study where both mmTBI patients and non-brain-injured control participants completed two motivated decision-making tasks while undergoing functional magnetic resonance imaging (fMRI). The tasks included a reinforcement learning task and a physical effort-based decision-making task. We found that mmTBI patients with higher apathy showed impaired behavioral performance on both tasks. Specifically, they exhibited impaired estimation of the relative future value of choice options, alongside a reduced willingness to exert physical effort for rewards. Importantly, our computational model-based fMRI analyses revealed that these behavioral differences were driven by BOTH blunted encoding of expected future value and effort-based blunting of value signals in brain networks involved in motivation, including the vmPFC. These findings not only highlight specific neural targets for future brain-based treatments for apathy in mmTBI, but also provide further causal

evidence for the role of these functional brain networks in driving normative motivated behavior in humans.

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**Topic:** G.03. Motivation

**Support:** ROC salary support from FPWR: Targeting the orexin system to treat Prader-Willi syndrome associated hyperphagia

**Title:** Habenular circuits link threat level to food value and drive obesity-associated compulsive eating in mice

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**Abstract:** Hunger disinhibits high-risk foraging strategies that are usually suppressed to avoid predation and other negative outcomes. Little is known about the neural mechanisms that link energy balance to risk tolerance or their involvement in obesity-related behaviors. Here, we investigated the role of hypothalamic to habenular interactions in guiding foraging behaviors and their relevance to obesity. Using in vivo calcium imaging in mice we show that lateral habenula (LHb) neurons signal the detection of food in a manner that depends on palatability, current energy status, and environmental threat level. Furthermore, weight gain reconfigures LHb responses such that palatable food comes to elicit starvation-like “value signals” even in threatening environments. Single cell sequencing of RNA transcripts from mouse revealed excitatory neurons in the lateral hypothalamus (LH) show marked obesity-associated alterations in gene expression. Specifically, transcripts from genes associated with glutamatergic neurotransmission were significantly lower in tissue harvested from obese mice. Using electrophysiology and whole brain clearing with automated quantification of synaptic contacts, we found this transcriptional plasticity accompanied the emergence of LH glutamatergic hypofunction and a profound restructuring of hypothalamic output to a range of brain loci including the LHb and dorsal raphe nucleus (DRN). To assess the brain wide impact of such LH glutamatergic hypofunction we used viral tagging of synaptic output combined with light-sheet

microscopy and Multiplexed Analysis of Projections by Sequencing (MAP-seq). We found these same LH glutamatergic neurons broadcast food value signals concurrently to the LHb and other brain regions that regulate approach/avoidance behaviors. Furthermore, weight gain functionally remodels the habenula-regulated networks that converges on the DRN. Using viral manipulations of neuronal function we found these habenular centered networks precipitates high-risk foraging for palatable food. Thus, obesity induced remodeling of LH activity leads to a wide-ranging functional reorganization of a broad network of brain sites controlled by glutamatergic neurons. As such, our research posits rescuing excitatory hypothalamic output as a novel therapeutic strategy for reversing the hyperphagia and motivational shifts associated with obesity and thus correcting caloric intake to match energy expenditure.

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**Title:** Insulo-frontal projection conveys prior outcome to guide set-shifting

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**Abstract:** Cognitive flexibility is the ability to adapt behavioral choices to complex environmental changes. Individuals need to integrate diverse cognitive functions to accomplish performance adaptation. Among them, outcome updates, especially error-related conflict updates, are of great significance for guiding the configuration of new rules. However, it is unclear which brain regions are responsible for updating outcomes in cognitive flexibility tasks. The anterior insular cortex (aIC) is a key node of the salience network, encoding information about bodily states, and mediating interoceptive attention. Here we found, the aIC plays a vital role in flexible decision-making by conveying prior outcomes to the medial prefrontal cortex (mPFC), a region indispensable for cognitive flexibility. We use attentional set-shifting task (AST), a widely used behavior test to study cognitive flexibility. In AST, the animal is presented with a series of compound sensory stimuli (e.g., digging medium and odor), in which the cue-reward contingency is switched; how quickly the animal adapts to new contingency rules reflects its cognitive flexibility. We found that the population calcium (Ca) activity of mPFC-projecting aIC (aIC→mPFC) neurons is significantly higher after trial outcomes in incorrect choices than in correct ones. And such differences persist into the next trial. In contrast, similar differences are seen in mPFC after the outcome but disappear around the trial ends. Indeed, both inhibition of aIC activity by DREADD and increase of aIC→mPFC projection activity by optogenetics impair the shifting process. We next performed the Minisocpe Ca imaging at mPFC, to study how aIC inputs modulate neuronal coding during AST. The results revealed that mPFC neuronal ensembles carry behavior-relevant information and gradually stabilize over trials. Optogenetic

activation of aIC→mPFC projection not only disrupts the ensemble stabilization but also impairs the decoding of prior trial outcomes from mPFC neurons. We further found that activation of aIC→mPFC projection exerts a predominantly inhibitory effect on mPFC. Pharmacogenetic inhibition of parvalbumin-expressing inhibitory interneurons (PV+ INs) in mPFC prevents the behavioral impairment caused by optogenetic activation of aIC→mPFC projection. Together, our study suggests that aIC plays a key role in flexible decision-making by conveying prior outcomes to mPFC through feedforward inhibition mediated by PV+ INs.

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**Title:** Dopamine and glutamate receptor heteromers as a common molecular substrate for substance use disorder and comorbid depression

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**Abstract:** Drug addiction is a psychiatric disorder defined as a compulsive pattern of drug-seeking/taking behavior despite negative consequences with recurrent episodes of abstinence and relapse. Addictive drugs increase dopamine (DA) in the striatum, especially in its ventral part (i.e. the nucleus accumbens (NAc)), where it persistently shapes excitatory glutamate transmission within the reward circuit, thereby hijacking natural reward processing. The NAc is a key target structure of addictive drugs that integrates convergent glutamate inputs from limbic, thalamic and cortical regions, encoding components of drug-associated stimuli, and DA signals that mediate incentive values. DA-glutamate signal integration is achieved by the two mostly segregated subpopulations of GABAergic medium-sized spiny neurons (MSN) expressing either DA receptors (DAR) type 1 (D1R) or type 2 (D2R). Recently, we provide evidence, from mice to humans, that psychostimulants and opiates alter DA-glutamate signal integration in the NAc through a drug-evoked heteromerization (i.e direct physical interaction) of glutamate NMDA receptors (NMDAR) with D1R or D2R. Using a temporally-controlled inhibition of D1R-NMDAR heteromerization, we show their selective implication in early phases of cocaine-evoked adaptations, whereas preventing D2R-NMDAR heteromerization blocked the persistence

of these adaptations. Interfering with these heteromers spared natural reward processing. Because the high prevalence of comorbidities between addiction and mood disorders suggests that brain dysfunctions underlying these disorders may rely on partly shared mechanisms, we asked whether DAR-NMDAR heteromerization in the NAc could constitute a common molecular switch in addiction and depression. Using the chronic defeat stress paradigm (CSDS) as a preclinical model of depression, we found that mice susceptible to stress that developed depressive-like behavior, but not resilient mice, exhibit an increased D1R-NMDAR heteromerization, which disruption fully prevents the onset and persistence of depressive-like symptoms. These findings contribute to a better understanding of common molecular mechanisms underlying addiction and depression and uncover DAR-NMDAR heteromers as targets with potential therapeutic value for multiple psychiatric diseases associated with alterations in dopamine and glutamate-dependent transmissions.

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**Nanosymposium**

**NANO69: Memory Consolidation, Molecular Mechanisms**

**Location:** WCC 144

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO69.01

**Topic:** H.07. Long-Term Memory

**Support:** NIH Grant NS122316

**Title:** Neurons repurpose the Integrated Stress Response effector GADD34 to enhance protein synthesis in response to neuronal activity

**Authors: *M. OLIVEIRA**[1], M. MOHAMED[2], M. K. ELDER[3], K. BANEGAS-MORALES[4], M. DONOHUE[4], E. LU[4], E. GOLHAN[4], N. NAVRANGE[4], S. CHATTERJEE[5], T. ABEL[6], E. KLANN[3];
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**Abstract:** Neurons are highly plastic cells, rapidly changing their proteome as an adaptive response to environmental cues. These changes are generally governed by both changes in the pace at which neurons can produce new proteins and the nature of the mRNAs that are being translated. Although it is well known that de novo protein synthesis is a core process that is required to consolidate long-term memory, many details remain obscure, including: (1) the molecular mechanisms that trigger the increase in protein synthesis and (2) how can neurons drive prolonged increases in protein synthesis as a consequence of learning. Here, we sought to

determine the identity of mRNAs that are translated very early during the memory consolidation process, aiming to identify molecules that may mediate prolonged increases in protein synthesis that drive the conversion of short-term to long-term memory. We used a combinatorial approach of contextual threat conditioning and TRAP-sequencing to identify mRNAs that were differentially loaded onto neuronal ribosomes 15 min following learning. Among mRNAs that were enriched when learning was induced, we identified *PPP1R15A*, which encodes GADD34, a potent modulator of translation initiation, as a candidate to mediate learning-induced increases in protein synthesis. Indeed, knocking out GADD34 in excitatory neurons impaired long-term contextual memory. Using *in vitro* models, we further found that GADD34 is rapidly synthesized following neuronal activity, controls eIF2α-dependent increases in neuronal translation, and that this affects the translation of numerous mRNAs that are crucial for promoting synaptic plasticity. Overall, our results identify GADD34 as an important player in the activity-driven increases in mRNA translation and offer important insights regarding the role of translation initiation for synaptic plasticity and memory. Supported by NIH grant NS122316 (E.K.).

**Disclosures: M. Oliveira:** None. **M. Mohamed:** None. **M.K. Elder:** None. **K. Banegas-Morales:** None. **M. Donohue:** None. **E. Lu:** None. **E. Golhan:** None. **N. Navrange:** None. **S. Chatterjee:** None. **T. Abel:** None. **E. Klann:** None.

**Presentation Number:** NANO69.02

**Topic:** H.07. Long-Term Memory

**Support:** NIH grant NS121786
Alzheimer's Association Research Fellowship
Leon Levy Postdoctoral Fellowship
Rainwater Foundation Research Leadership Fellowship

**Title:** Visualization of long-term memory-induced mRNA translation in mice

**Authors: *H. T. EVANS**, D. ADLER, S. VENKATESAN KALAVAI, J. ALAPIN, A. YU, M. M. OLIVEIRA, E. A. GOLHAN, E. KLANN;
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**Abstract:** The formation of new long-term memories is dependent upon multiple windows of tightly controlled mRNA translation, with these windows thought to occur both in different brain regions and at different times depending on the type of memory being formed. Previously described methods for labelling the *de novo* proteome of awake and behaving mice have been unable to distinguish between these various windows due to the long labelling periods necessary to tag nascent proteins. To overcome these limitations, here we describe a novel technique which, through the retro-orbital injection of the methionine surrogate azidohomoalanine (AHA), allows for the labelling and visualization of the *de novo* proteome through-out the rodent brain in time periods as short as one hour. Using this technique, we identified both brain region and cell-type specific changes in mRNA translation in mice immediately following training via various behavioral paradigms, including auditory threat conditioning and contextual threat conditioning. We also demonstrated that hippocampal long-term memory-induced protein synthesis is impaired in the PS19 mouse model of tauopathy, hinting at a potential mechanism by which memory is impaired in these mice. Together these results highlight the versatility of our method

for examining rapid, behavioral-induced changes in mRNA translation. This work was funded by the Alzheimer's Association (H.T.E), the Leon Levy foundation (H.T.E.), the Rainwater foundation (H.T.E.) and NIH grant NS121786 (E.K.).

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**Presentation Number:** NANO69.03

**Topic:** H.08. Learning and Memory

**Support:** DA056288
DA13137
DA059310
NS100624
GM109091

**Title:** Mediator kinase inhibition improves spatial cognition in aged mice

**Authors: *H. LI**[1], K. MCLAURIN[1], C. F. MACTUTUS[2], A. C. SHARKO[3], E. BROUDE[1], I. RONINSON[1], R. BOOZE[4];
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**Abstract:** Age-associated cognitive decline involves both neuroinflammation and hippocampal neuronal senescence. We explored whether a new class of drugs, acting via selective inhibition of CDK8/19 Mediator kinase, would improve age-related cognitive decline. To investigate how the inhibition of mediator kinase influences cognitive decline, 26 male and female aged mice (21 months old) were treated with either a medicated diet (brain-permeable CDK8/19 inhibitor - Compound 25, $n$=12) or rodent chow ($n$=14) for two months. Subsequently, the cognitive domains of spatial learning and memory were evaluated using the Barnes Maze, a task that challenges animals to use spatial cues to find a hidden tunnel. Animals were assessed on two trials per day for six consecutive days. During each trial, animals had four minutes to enter the tunnel, whereby the dependent measures of interest include the latency to enter the tunnel, the number of errors, and the position of the mice on the maze. Mediator kinase inhibition, induced via treatment with Compound 25 chow, significantly improved spatial learning and memory in aged mice. Specifically, the aged mice fed Compound 25 chow made significantly fewer errors localizing the hidden tunnel [$F(3,176)=3.68$, $p<0.013$] and did so with a significantly lower latency relative to their control counterparts [$F(3,176)=4.51$, $p<0.005$]. Postmortem evaluation of galactosidase activity in the CA1 region of the hippocampus, revealed significantly decreased galactosidase levels in mice treated with Compound 25; supporting treatment-induced improvements in age-related hippocampal senescence by the brain-permeable Compound 25. Pharmacological treatments able to effectively slow or prevent the onset and development of age-related cognitive and neuronal decline remains a major challenge. Overall, our data support Mediator kinase inhibition as a potential therapeutic approach to enhance cognitive function during aging.

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**Presentation Number:** NANO69.04

**Topic:** G.01. Fear and Aversive Learning and Memory

**Support:** CIMVHR Master's Scholarship
CIHR CGS-M
NSERC CGS-D
CIHR Project Grant 2019

**Title:** Identifying unique cell types and molecules involved in fear memory.

**Authors: *K. SULLIVAN**[1], A. I. KINMAN[1], S. C. WOOD[2], L. WANG[3], A. LEMIRE[3], J. CLEMENTS[3], M. S. CEMBROWSKI[1];
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**Abstract:** Following a traumatic event, some individuals experience adverse and persistent psychological symptoms, resulting in post-traumatic stress disorder (PTSD). To better understand this disordered type of memory, we must first understand the cells and molecules involved in experimentally well-controlled models of fear memory, so that effective interventional strategies for PTSD can be developed.
Our research has shown that classic "textbook" cell types of many brain regions can be divided into distinct subtypes based on the genes they express. Here, we sought to identify the unique subtypes of cells participating in the creation and recollection of fear memories in the mouse subiculum, a primary output region of the hippocampal formation.
We assayed cell-type transcriptomic changes following fear memory using a mouse line allowing for the tagging of cells active during a given time window. Using a combination of single cell RNA sequencing and multiplexed in situ hybridization, we investigated the cell-type-specific participation and gene expression patterns arising from exposure to a fear-associated environment, relative to both a novel environment and a home cage environment.
We revealed unique cell types in the ventral subiculum that preferentially participate in fear memory. Furthermore, we uncovered unique transcriptional signatures of memory, thus elucidating cellular and molecular targets related to fear memory. These findings provide a vital framework for future experiments investigating the therapeutic relevance of these targets in interventions for PTSD.

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**Presentation Number:** NANO69.05

**Topic:** H.07. Long-Term Memory

**Support:** HFSP RGY0063/2017
NWO/NWA.1228.191.208
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Ubbo Emmius foundation

**Title:** Restoring access to hippocampal memories thought-to-be lost in the sleep-deprived brain

**Authors:** Y. G. BOLSIUS[1], P. HECKMAN[2], C. PARACIANI[1], S. WILHELM[1], F. RAVEN[3], R. L. MEIJER[1], M. KAS[1], S. RAMIREZ[4], P. MEERLO[1], ***R. HAVEKES**[1];
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**Abstract:** Sleep loss is a common problem in our modern 24/7 society due to social and economic demands. Sleep deprivation negatively impacts brain function and particularly affects cognitive processes that require the hippocampus. It is unclear, however, whether sleep deprivation-induced amnesia is due to a failed storage of information or rather a result of attenuated retrievability of the stored information in the sleep-deprived brain. In our recent studies, we used optogenetic engram technologies as well as FDA-approved drugs to show that sleep deprivation does in fact not lead to the loss of information, but rather results in memories that are 'hidden' in the brain and difficult to retrieve. Moreover, by combining these optogenetic and pharmacological strategies, we could successfully make these 'dormant' memories permanently accessible again. These findings underscore the exciting possibility that more information is stored in the brain under sleep deprivation conditions than previously considered and may support the development of novel therapeutic strategies to combat cognitive deficits associated with sleep deprivation.

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**Presentation Number:** NANO69.06

**Topic:** H.07. Long-Term Memory

**Support:** NIH Grant AG062398

**Title:** Mtor-mediated translation after acute and chronic sleep loss

**Authors: J. M. MCCARTHY**, A. A. COLMAN, A. A. LEONE, L. C. GIVVINES, O. SKWIERAWSKI, S. P. FEENEY, E. N. WASH, J. G. GRANA, C. R. PETRUCONIS, M. COSTIN, M. E. DECARLO, N. A. BURKERT, I. K. SUCCI, L. NARAYANAM, *J. TUDOR;
St. Joseph's Univ., Philadelphia, PA

**Abstract:** Memory formation requires protein synthesis, which is dependent on several signal transduction pathways, including mammalian target of rapamycin (mTOR) signaling. Previous research has shown that five hours of acute sleep deprivation attenuates mTOR-mediated protein synthesis in the hippocampus of male mice. But it was not known whether this effect was limited to males or the hippocampus. Interestingly, we found that mTOR activity was also significantly reduced in the cerebellum of acutely sleep deprived male mice. A chronic sleep restriction of 20 hours of REM sleep loss per day for seven days also significantly reduced mTOR activity in the hippocampus and cerebellum of male mice. In female mice, five hours of acute sleep deprivation only significantly reduced mTOR activity in the hippocampus, and not the cerebellum, and chronic sleep restriction did not impact mTOR activity in either brain region, which differs from findings in males. Further, we determined that fluctuating levels of hormones during the female

estrous cycle did not affect mTOR activity in control conditions, or after acute sleep deprivation. In both sexes we also found that 5 hours of acute sleep deprivation significantly reduced hippocampal protein synthesis and impaired spatial memory, as measured in the object place recognition task. To rescue these deficits, we injected an adeno-associated virus containing a mutant form of eukaryotic initiation factor 4E-binding protein 2 (4EBP2) under a CaMKII alpha promoter into the hippocampus of male mice. This virus contained four alanine point mutations at phosphorylation sites, rendering this "phospho-mimetic" 4EBP2 (M4EBP2) constitutively inactive and preventing 4EBP2-mediated inhibition of protein synthesis. We found that expression of M4EBP2 in excitatory hippocampal neurons of male mice rescued the spatial memory impairments observed after sleep deprivation. Work is ongoing to determine whether this result is sex-specific. Our results show that while sleep loss affects mTOR activity in the hippocampus of male and female mice similarly, other brain regions may be differentially affected based on sex.

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**Presentation Number:** NANO69.07

**Topic:** H.07. Long-Term Memory

**Support:** National Science Foundation Graduate Research Fellowship
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**Title:** Replay as context-driven memory reactivation

**Authors: *Z. ZHOU**, M. J. KAHANA, A. C. SCHAPIRO;
Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Replay in the brain is not a simple recapitulation of recent experience, with awake replay often unrolling in reverse temporal order upon receipt of reward, in a manner dependent on reward magnitude. These findings have led to the proposal that replay is optimized for learning value-based predictions in accordance with reinforcement learning theories. However, other characteristics of replay are in tension with this proposal, leaving it unclear whether one set of principles governs all replay. We offer a parsimonious memory-focused account, suggesting that the brain associates experiences with the contexts in which they are encoded, at rates modulated by the salience of each experience. During periods of quiescence, replay emerges when contextual cues trigger a cascade of reactivations driven by the reinstatement of each memory's encoding context, which in turn facilitates memory consolidation. We show that a computational model instantiating this account unifies numerous replay phenomena, including findings that existing models fail to account for. First, in our model, the content and structure of replay sequences vary according to task and behavioral contexts as observed in rodent studies. Second, the model captures prominent effects of valence on properties of replay despite not maintaining nor updating value representations. Third, in line with empirical studies, replay is not restricted to direct recent experience: The model reactivates non-local and never-experienced

novel trajectories. Fourth, our model captures counterintuitive effects of repeated task exposure on replay. Finally, offline replay benefits memory consolidation in the model, in ways that align with prior observations and theories. As a whole, we outline a general, mechanistic framework that unifies a wide range of the empirically observed characteristics of replay across rest and sleep.

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**Presentation Number:** NANO69.08

**Topic:** H.07. Long-Term Memory

**Support:** European Research Council
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**Title:** Forget the Engram: Experience shapes memory

**Authors: *J. O'LEARY**[1], R. BRUCKNER[3,4,5], L. AUTORE[6,4], T. RYAN[2,4,7,8];
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**Abstract:** Memories are stored as ensembles of engram neurons and their successful recall involves the reactivation of these cellular networks. While progress has been made in understanding the biology of engrams, significant gaps remain in connecting these cell ensembles with the process of forgetting. Here, we examine whether forgetting is governed by changes in engram plasticity and suggest that it helps animals prioritize relevant memory representations for adaptive behavior. We utilized a mouse model of object memory and investigated the conditions in which a memory could be preserved, retrieved, or forgotten. We found that engram activity correlated with the rate of forgetting. Direct modulation of engram activity via optogenetic stimulation or inhibition either facilitated or prevented the recall of an object memory. In addition, the modulation of engram activity was able to prevent forgetting itself. Moreover, through pharmacological and behavioral interventions, we successfully prevented or accelerated forgetting of an object memory. Finally, we show that these results can be explained by a computational model in which engrams that are subjectively less relevant for adaptive behavior are more likely to be forgotten. Based on these findings we suggest that natural forgetting maybe considered as a form of adaptive learning and that miscalibrated learning rates governing memory accessibility could give rise to pathological forgetting.

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**Nanosymposium**

**NANO70: Spatial Representations Across Species**

**Location:** WCC 147A

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO70.01

**Topic:** H.09. Spatial Navigation

**Support:** NSERC (PDF-516867-2018)
FRQNT(B3X-258512-2018)
5R01MH129046
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McGovern Institute of Brain Research

**Title:** Generalizable relational inference with cognitive maps in a hippocampal model and primates

**Authors: *S. NEUPANE**, J. HWANG, I. R. FIETE, M. JAZAYERI;
MIT, Cambridge, MA

**Abstract:** Humans and animals exhibit a remarkable capacity to form memories and use those memories for flexible cognitive behavior, such as rapidly learning the layout of a new city or planning a novel route within a familiar city. To investigate how artificial and biological neural networks give rise to such generalization behavior, we employed a newly developed mental navigation task (Neupane et al. 2022) in humans, monkeys, and machines. In brief, agents travel virtually between two points on a 1-dimensional path containing a sequence of equidistant landmarks. On each trial, agents must deflect the joystick in the correct direction and for the appropriate duration to move from a start to a target landmark along the path. Crucially, the task had to be solved mentally, i.e., without sensory feedback about the intervening landmarks. Both humans and monkeys showed generalization to unseen landmark pairs. Humans, in addition, showed rapid generalization to novel environments and exhibited resistance to catastrophic forgetting. Conventional recurrent networks succeeded in learning the task but failed at generalizations. We then built a multi-modular network model with a structured neocortical-entorhinal-hippocampal circuit, the Memory Scaffold with Heteroassociation (MESH) adapted from Sharma et al. (2022), concatenated with a continuous-time recurrent policy neural network (CTRNN). The network learned the mental navigation task, generalized to unseen pairs of start and target landmarks, and showed memory capacity resilient to catastrophic forgetting. The internal states of CTRNN showed a representation of distance and direction inferred from start and target landmark inputs prior to navigation onset. We recorded neural activity from the hippocampus (HC), entorhinal cortex (EC), and posterior parietal cortex (PPC) of monkeys performing mental navigation and found the representation of distance and direction in HC and PPC during the inference epoch prior to movement onset, consistent with the CTRNN's internal states. In HC, we also found a high dimensional representation of landmark images temporally prior to the emergence of distance and direction encoding. Together, our modeling work and

neural data offer an understanding of how the architecture of cortico-hippocampal circuits supports rapid learning and flexible generalization.

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**Presentation Number:** NANO70.02

**Topic:** H.09. Spatial Navigation

**Support:** NIH NRSA Grant F32NS116023
NIH Grant R01MH101297

**Title:** Entorhinal-hippocampal spatial representations during multisensory navigation

**Authors: *H. DAVOUDI**, J. B. ISSA, J. R. CLIMER, D. A. DOMBECK;
Northwestern Univ., Evanston, IL

**Abstract:** As rodents navigate their habitat, they use multiple spatially-tuned sensory cues, such as visual and olfactory signals, to form a neural representation of space. The entorhinal-hippocampal network is a candidate brain region for constructing a multisensory cognitive map of the environment. Yet, in spatial navigation studies, the spatial information of different sensory inputs (e.g., visual and olfactory) can be difficult to independently and precisely control or measure. To achieve this aim, our lab has built a multisensory virtual reality environment with independently controlled visual and olfactory inputs. Using this apparatus, we studied the multisensory cognitive map and determined how visual-spatial and olfactory-spatial information are represented in the entorhinal-hippocampal network. We have previously shown that hippocampal CA1 neurons respond to both visual-spatial and olfactory-spatial virtual coordinates in a task-dependent manner (Radvansky et al., 2021), opening the question of where in the brain spatial representations of different sensory modalities first emerge. CA1 receives cortical input from the functionally distinct medial and lateral entorhinal cortices (MEC and LEC), but it is unknown whether MEC and LEC both contribute to visual-spatial and olfactory-spatial processing or each of them preferentially encodes one sensory-spatial modality. To answer this question, we imaged neural populations in MEC and LEC in separate mice receiving rewards at visual or olfactory targets in a multi-sensory spatial environment. We classified the neurons by the space they best coded, and we found that both MEC and LEC represent visual-spatial and olfactory-spatial information, yet in significantly different proportions. While MEC primarily encodes visual-spatial information, LEC primarily encodes olfactory-spatial information. Moreover, the level of encoding of visual and olfactory spaces depends on the context of different tasks that mice perform. These findings may shed further light on the neural mechanisms of multisensory spatial representations in the entorhinal-hippocampal network.

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**Presentation Number:** NANO70.03

**Topic:** H.09. Spatial Navigation

**Support:** FG20621

**Title:** Two distinct pathways within retrosplenial cortex granular layer facilitate mice action during navigation.

**Authors: *X. LIN**[1], A. GHAFURI[1], X. CHEN[1], M. KAZMI[1], D. A. NITZ[2], X. XU[3];
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**Abstract:** The retrosplenial cortex (RSC) contributes to complex cognitive functions in primates and rodents, including spatial navigation, mnemonic processing, and planning. RSC has reciprocal connections with many cortical and subcortical brain regions. It has been suggested that the corticocortical connections between the RSC and secondary motor cortex (M2), as well as corticothalamic connections between the RSC and anterodorsal thalamus (AD), function as semi-independent, but parallel pathways that regulate spatial information in distinct ways during navigation. To examine distinctions in connectivity and function among different projection-specific populations of RSC neurons, we used retrograde and anterograde viral tracers alongside a monosynaptic retrograde rabies virus to quantitively characterize and compare the afferent and efferent distributions of projection-defined RSC neuron sub-groups. We find that M2-projecting RSC neurons obtain more extensive afferent input from the dorsal subiculum, lateral dorsal and lateral posterior thalamus, and sensory cortices compared to AD-projecting neurons. AD-projecting RSC neurons obtain greater afferent input from the anterior cingulate cortex and the medial septum. While AD-projecting and M2-projecting RSC neurons overlap in their projections to other brain regions, they do not project to M2 and AD, respectively. To test the functional role of these projection-specific RSC populations, we performed chemogenetic inhibition of M2- and AD-projecting RSC neurons and examined its impact on object-location memory, object-recognition, open-field exploration, and place-action association. Our findings indicate that inhibition of M2-projecting RSC neurons impairs object location memory as well as place-action association, while the RSC to AD pathway impacts only object-location memory. Our study demonstrates that RSC connectivity and function is organized as a set of semi-independent circuits that integrate information from distinct sets of RSC afferents. The findings highlight the importance of investigating the roles of association cortices such as RSC in cognitive processes through the characterization and manipulation of its specific input/output circuitries.

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**Presentation Number:** NANO70.04

**Topic:** H.09. Spatial Navigation

**Support:** BBSRC BB/M009513/1

**Title:** Evaluating hippocampal replay without a ground truth

**Authors: M. TAKIGAWA**[1], M. HUELIN GORRIZ[1], M. TIROLE[2], *D. BENDOR[1];
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**Abstract:** During rest and sleep, memory traces replay in the brain. The dialogue between brain regions during replay is thought to stabilize labile memory traces for long-term storage.

However, because replay is an internally-driven, spontaneous phenomenon, it does not have a ground truth - an external reference that can validate whether a memory has truly been replayed. Instead, replay detection is based on the similarity between the sequential neural activity comprising the replay event and the corresponding template of neural activity generated during active locomotion. If the statistical likelihood of observing such a match by chance is sufficiently low, the candidate replay event is inferred to be replaying that specific memory. However, without the ability to evaluate whether replay detection methods are successfully detecting true events and correctly rejecting non-events, the evaluation and comparison of different replay methods is challenging. To circumvent this problem, we present a new framework for evaluating replay, tested using hippocampal neural recordings from rats exploring two novel linear tracks. Using this two-track paradigm, our framework selects replay events based on their temporal fidelity (sequence-based detection), and applies a validation step using each event's trajectory discriminability, where sequenceless decoding across both tracks is used to quantify whether the track replaying is also the most likely track being reactivated.

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**Topic:** H.09. Spatial Navigation

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**Title:** Impairment of Remapping in Spatiotemporal Object and Trace Coding in Lateral Entorhinal Cortex in Alzheimer's Disease

**Authors: *R. RAGHURAMAN**, M. HERMAN, A. AOUN, O. SHETLER, S. ABID HUSSAINI;
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**Abstract:** LEC neurons are among the first to be affected in Alzheimer's Disease (AD). However, there are no studies that have assessed their neuronal function in the context of AD. It is noteworthy that early AD symptoms entail misplacing items, olfactory dysfunction and distorted perception of time which may be due to pathology and the associated impairments in the computations of this region. Our work attempted to assess the spatial firing properties and the remapping fields in AD using behavioral and *in-vivo* electrophysiology techniques that followed with the monitoring of large population of LEC neurons which pointed to impairments in object and odor memory. Our data points to a distinct decline in the Skagg's information content and selectivity for the AD group, which falls in line with the increase in sparsity and coherence of the spatial firing fields, as compared to the control. The increase in firing rate observed in AD group indicates a trend in hyperactivity of neurons in LEC region. Furthermore, there is an impairment in the remapping metric in the AD group, also demonstrated by the poor decoding accuracy of object and trace cells. Our results thus far show behavioral dysfunction seen from Object-Context Recognition task, Odor-discrimination task and in T-maze and an impairment in object and trace field maps in the 18 months old EC-APP/Tau mice.

**Disclosures: R. Raghuraman:** None. **M. Herman:** None. **A. Aoun:** None. **O. Shetler:** None. **S. Abid Hussaini:** None.

**Presentation Number:** NANO70.06

**Topic:** H.09. Spatial Navigation

**Support:** The Research Foundation – Flanders (FWO) grant G0D7516N
The Research Foundation – Flanders (FWO) grant G077321N

**Title:** Theta cycle dynamics of spatial representations in the lateral septum

**Authors:** K. BZYMEK, ***F. KLOOSTERMAN**;
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**Abstract:** The ability to navigate the environment in search for food is crucial for the survival of mammals. An internal representation of the environment - or map - allows animals to evaluate multiple routes and adapt their navigation strategy to current needs and future goals. The hippocampal formation plays a crucial role in learning a spatial map and using the map for goal-directed navigation. The lateral septum forms a major node for connections between the hippocampus and subcortical brain regions, including reward processing centers such as the ventral tegmental area (VTA). However, it remains understudied how the lateral septum contributes to processing of spatial information and route planning.
In this study we investigated the temporal dynamics of spatial representations in the lateral septum. Neuropixels probes were used to record cellular activity along the dorsal-ventral extent of the lateral septum while rats performed one of two spatial navigation tasks in a Y-maze. The activity of a large fraction of cells was theta rhythmic and a subset of cells showed evidence of being active on alternate theta cycles (theta cycle skipping). Both theta rhythmicity and cycle skipping were strongest in the dorsal lateral septum. Similarly, spatially selective firing was most prominent in the dorsal later septum. Using neural decoding, we show that the lateral septum cell population encodes both the current location and alternatingly the possible future paths within single theta cycles when rats approached the choice point in the maze.
Our data further shows that the alternating expression of spatial representations in the lateral septum is task dependent, such that it is strongest when the task also requires the animals to alternate between rewarded goal arms. These data suggests that task demands and experience shape which representations are activated near a choice point. The lateral septum receives strong input from hippocampal place cells, and while there may be integration and transformation of incoming spatial signals, our findings support the conclusions that hippocampal spatial representations and their temporal dynamics are conveyed to subcortical projection areas.

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**Title:** Complementary dynamics of two central complex local neuron populations support goal-directed olfactory navigation.

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**Abstract:** During plume navigation, insects use stochastic sensory cues to navigate towards the unknown location of an odor source. Recent genetic and optogenetic perturbations have implicated the fan-shaped body (FB)— a part of the central complex— in this behavior. However, little is known about the dynamics of neural activity in this structure during ongoing odor-guided navigation. Here, we developed a virtual olfactory navigation paradigm to investigate neural dynamics during this behavior using 2-photon imaging. Using this paradigm, we identified two populations of FB local neurons that show complementary encoding of odor and self-motion during olfactory navigation. One population of dorsal local neurons (hΔCK) showed a slow and persistent bump of activity in response to odor and also encoded segments of straight running outside the odor period. A second population of ventral local neurons (FC1) showed more transient responses to odor, and preferentially responded during turns. By simulating a virtual odor plume in closed-loop, we derived linear filters relating neural activity to both odor and self-motion history in each population. hΔCK and FC1 showed distinct filter shapes, consistent with the idea that hΔCK integrates odor evidence over tens of seconds and encodes straight running, while FC1 encodes more transient odor encounters and turns. Despite these different encoding dynamics, both populations showed slow modulation across trials related to upwind displacement during odor— a measure of task engagement. Silencing of both neural populations together, but not each separately, profoundly impaired olfactory navigation in a wind tunnel paradigm. Together, our data suggest that olfactory navigation requires internal representations that integrate sensory evidence (odor encounters) with self-movement on different timescales. Our work provides insight into the laminar organization of the central complex and the dynamics of neural representations required for goal-directed navigation in noisy and uncertain sensory environments.

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**Title:** Regional specialization of retrosplenial cortices in visuospatial coding along the anterior-posterior axis

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**Abstract:** The retrosplenial cortex (RSC) is a multimodal association hub which serves crucial roles in navigation, learning, and memory. In rodents, RSC integrates sensory visual and spatial contextual signals and shows landmark and place-selective responses that depend on the

hippocampus (Mao et al., 2018, 2020; Fisher et al., 2020). However, RSC inputs and response properties are anatomically and functionally diverse and have rarely been characterized, particularly along the anterior-to-posterior axis.We investigated functional and anatomical regional specialization in mouse RSC using in vivo cellular imaging and genetic brain-wide input mapping. Focusing on neural populations in the anterior and posterior RSC regions (aRSC, ~1.2-2.2 mm; pRSC, ~2.7-4.2 mm from bregma), we recorded in head-fixed mice (N=4 for Thy1-GCaMP6s mice, N=4 for CaMKII-tTA x TRE-GCamp6s mice; 100-400 um in depth), cellular calcium response during running and in response to visual stimulation. In a separate set of animals (N=3), we used retro-AAV tracing and whole-brain reconstruction to identify the distribution of neurons providing inputs to two regions. While both aRSC and pRSC show position-related and visually evoked activity, data indicate a specialization of aRSC and pRSC for signals of distinct modalities. Position tuning is vastly more pronounced and reliable in aRSC than in pRSC (mean ± sem, fraction of positioned tuned cells, 0.53 ± 0.05 vs 0.31 ± 0.06; $p<0.01$; spatial information, 0.28 vs 0.18 bits; $p<0.01$; Bayesian decoding error in cm, 4.4 ± 0.8 vs 8.9 ± 0.6; $p<0.01$. N=8, n=4149 vs 1922 cells in aRSC, pRSC). This enhanced position activity correlates with denser inputs from cortical areas carrying position-tuned signals (~20% from motor and entorhinal cortex). In contrast, visual responses are more pronounced and more reliable in pRSC than in aRSC (fraction of visually-responsive cells, 0.48 ± 0.06 vs 0.19 ± 0.03 in pRSC vs aRSC, N=8 animals, n=1568 vs 3197 cells; $p<0.01$). Enhanced pRSC visual responsiveness correlates with denser inputs from visual cortex (~20% labelled cells). Notably, aRSC and pRSC also differ in their visual spatiotemporal tuning, with pRSC responding preferentially to lower temporal and higher spatial frequencies and aRSC showing the opposite preference.Altogether, the results indicate regional specialization suggesting complementary functional roles of aRSC and pRSC in integration of visuospatial information. aRSC could be implicated in the rapid processing of visual cues and landmarks, enabling efficient route planning and execution. pRSC may be more involved in the integration of contextual information and the formation of cognitive maps.

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**Topic:** H.09. Spatial Navigation

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**Title:** Dynamic cognitive representations in the adult zebrafish telencephalon

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**Abstract:** Cognition relies on internal models of the world that allow the brain to interpret sensory information, to predict future events, and to optimize behavior. Such models are thought to involve the representation of relevant information in cognitive maps, which have been analyzed extensively in rodents in the context of spatial navigation. To explore whether cognitive maps of structured environments also exist in teleosts we performed *in vivo* two-photon calcium imaging of the dorsal telencephalon of head-fixed adult zebrafish behaving in a virtual reality

(VR). Fish performed linear traversals (trials) of a 3D virtual corridor with naturalistic background textures. Sets of complex landmarks (2D images of richly structured objects) were either absent or present along the walls of the corridor. We found many neurons with spatially selective activity under both conditions. In the presence of complex landmarks, individual neurons often had one or more firing fields that could be associated with landmarks. Collectively, the firing fields of the neuronal population tiled the environment. Spatially modulated activity of individual neurons evolved even under a constant environment, often towards fewer, more selective firing fields. To further explore the assumption that spatially modulated activity of individual neurons in our structured environment reflects a cognitive map we introduced perturbations into the VR. Complex landmarks were manipulated for a few (typically 5) consecutive trials (perturbation window). When all landmarks were deleted, some neurons continued to be active near specific landmark positions for one or a few trials before landmark-associated activity disappeared. These firing patterns imply that neuronal activity can be driven from a memory of the environment. Other neurons showed elevated activity with low spatial selectivity that contained information about the mismatch between the previous and present set of landmarks, indicating that the experienced environment is compared to a memory-based representation of the previous environment. Taken together, our results provide strong evidence for cognitive representations of structured environments in the telencephalon of an adult teleost and highlight their dynamic nature. These findings provide the opportunity to study cognitive representations in a model organism that offers unique opportunities for deep mechanistic analyses of information processing by large-scale activity measurements and connectomics.

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**Nanosymposium**

**NANO71: Optical Methodology: Application**

**Location:** WCC 147B

**Time:** Tuesday, November 14, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO71.01

**Topic:** I.04. Physiological Methods

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**Title:** Signal propagation atlas in C. elegans reveals contributions from extrasynaptic signaling

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**Abstract:** A fundamental problem in neuroscience is understanding how a network's properties dictate its function. Connectomics provides one avenue to predict nervous system function. To test this explicitly, we systematically measure signal propagation in 23,427 pairs of neurons across the head of the nematode *Caenorhabditis elegans* by direct optogenetic activation and simultaneous whole-brain calcium imaging. We measure the sign (excitatory or inhibitory), strength, temporal properties, and causal direction of signal propagation between these neurons to create a functional atlas. We find that signal propagation differs from predictions based on anatomy. Using mutants, we show that extrasynaptic signaling not visible from anatomy contributes to this difference. We identify many instances of dense-core-vesicle dependent signaling on seconds-or-less timescales that evoke acute calcium transients— often where no direct wired connection exists but where relevant neuropeptides and receptors are expressed. We propose that here extrasynaptically released neuropeptides serve a similar function as that of classical neurotransmitters. Finally, our measured signal propagation atlas better predicts neural dynamics of spontaneous activity than does anatomy. We conclude that both synaptic and extrasynaptic signaling drive neural dynamics on short timescales and that measurement of evoked signal propagation are critical for interpreting neural function.

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**Presentation Number:** NANO71.02

**Topic:** I.04. Physiological Methods

**Title:** Photoactivation of individual synapses in vivo with covalent photoswitches targeting endogenous glutamate receptors

**Authors:** A. GARRIDO-CHARLES[1,2], ***M. BOSCH**[3,2], H. LEE[2], X. ROVIRA[4,2], S. PITTOLO[5,2], A. LLOBET[6], H.-W. WONG[7,8], A. TRAPERO[2], C. MATERA[9,2], C. PAPOTTO[2], C. SERRA[10], A. LLEBARIA[11], E. SORIANO[12], M. SANCHEZ-VIVES[13,14], C. HOLT[8], P. GOROSTIZA[2,15];
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**Abstract:** Glutamate receptors play key roles in neurotransmission at excitatory synapses and in the regulation of synaptic plasticity. We have developed a targeted covalently-attached

photoswitch (TCP) that allows the remote control of endogenous ionotropic glutamate receptors (iGluRs) using light. We here combined this photopharmacological effector with genetic and chemical calcium sensors to demonstrate all-optical reversible control of iGluRs at multiple levels of spatial resolution in the brain: we achieved the photoactivation of multiple neurons, individual neurons, and single synapses in rat hippocampal slices and in intact Xenopus laevis brain in vivo, which is challenging using other methods. We show that this compound selectively targets AMPA and kainate receptors. Labeled receptors remained functional for long periods of time (>8 hours). This allowed us to longitudinally track endogenous iGluR physiology during events of synaptic plasticity, such as long-term depression (LTD). We could monitor the loss of functionality of AMPA/kainate receptors during NMDAR-dependent LTD in hippocampal neurons. TCPs are therefore a unique optical tool to label, photo-control and functionally track endogenous receptors in brain tissue without genetic manipulation.

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**Presentation Number:** NANO71.03

**Topic:** I.04. Physiological Methods

**Title:** Imaging penetrating arterioles and venules in mouse cortex using 14T single vessel fMRI and optical microscopy

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**Abstract:** In this study, we aim for the first time to image the same penetrating cortical vessels in a mouse using both ultrahigh field single vessel fMRI at 14 T and high-resolution optical imaging, namely 2-photon microscopy (2PM) and optical coherence tomography (OCT). We developed a method for craniotomy surgery to install an MRI coil surrounding a chronic sealed cranial window for optical access to the barrel cortex in 6–12-month-old C57/BL6 mice. For each mouse ($n$=3), we performed separate imaging sessions on different days for fMRI, 2PM, and OCT; mice were anesthetized with the same level of isoflurane for each imaging session. We obtained echo-planar MRI images (EPI) with 40 μm lateral resolution and 200 μm axial resolution in the mouse cortex, as well as single vessel fMRI velocity maps of the cortex. We also performed *in vivo* 2PM micro-angiography; we labeled the blood plasma with dextran-conjugated Alexa-680 and imaged microvasculature over the entire 3-mm-wide cranial window with a low-magnification objective to an axial depth of at least 600 μm below the cortical surface using 2 μm axial steps. Finally, we obtained volumetric images of the intravascular blood flow velocity projection to the optical axis by using Doppler OCT over the field of view of the cranial window. MRI EPI images were first coregistered with the 2PM microvascular angiograms and subsequently coregistered with the fMRI velocity maps and Doppler OCT velocity maps. We were able to compare blood flow velocity maps in the penetrating arterioles and surfacing venules in the mouse cortex measured by single-vessel fMRI and Doppler OCT at rest. Our

findings demonstrate the possibility of combining ultrahigh field single-vessel fMRI and high-resolution optical methods (e.g., 2PM and OCT) for studying brain structure and function at the single microvessel scale. Future work will aim to perform functional measurements in awake mice and simultaneous fMRI and optical measurements.

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**Topic:** I.04. Physiological Methods

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**Title:** Simultaneous tracking of many neuromodulatory signals in awake, behaving mouse brains

**Authors: *P. N. KALUGIN**[1,2,3,4], P. A. SODEN[2], C. I. MASSENGILL[1], O. AMSALEM[1], D. C. GUARINO[1], M. PORNIECE KUMAR[1,2], D. TINGLEY[1], Y. LI[5], M. K. LEHTINEN[2,3], M. L. ANDERMANN[1,3];
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**Abstract:** Most neurons express receptors for dozens of neuromodulatory molecules. Concentrations of these molecules vary widely in the brain interstitial space across time and space, and their influences overlap and combine to modulate neural activity. While current tools allow for small numbers of such signals (usually one or two) to be tracked at the same time, no methods exist to scale up this molecular dimensionality and reach a complete accounting of local neuromodulator concentrations in real time, in an awake, behaving animal. To address this gap, we have developed a probe to track a dozen or more neuropeptide and neuromodulator concentrations in various brain regions. Our probe consists of an emerging family of genetically encoded fluorescent sensors, the G protein-coupled receptor activation-based (GRAB) sensors, expressed in cultured cells and immobilized at the front of a gradient refractive index (GRIN) lens for 3D two-photon imaging. We have validated this probe both *in vitro*, by sequentially placing it cells-first into small volumes (≤20 μL) of freshly harvested and unprocessed CSF and serum from mouse and rat while imaging the cells through the lens, as well as *ex vivo* by pressing it against a brain slice and evoking neuromodulator release, detecting parallel molecular concentrations down to nanomolar levels. We have also tested it *in vivo* by acutely implanting it in several brain regions of awake, behaving mice, where we observed rapid shifts in

concentrations on a timescale of seconds in response to peripheral drug administration as well as acute behavioral stimuli such as feeding and unexpected pain. Our approach provides a novel way to rapidly profile a panel of molecules of interest in very small fluid samples and tissue regions *in vivo* and *ex vivo*. In future, this method can be used to study how state-related information is encoded and transmitted by a rich pattern of time-varying concentrations of multiple chemical signals.

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**Topic:** I.08. Methods to Modulate Neural Activity

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**Title:** Remote Neuromodulation of Visual Cortex with Transcranial Focused Ultrasound

**Authors: *K. YU**[1], S. SCHMITT[1], Y. NI[1], E. CRANE[1], M. A. SMITH[1,2], B. HE[1,2];
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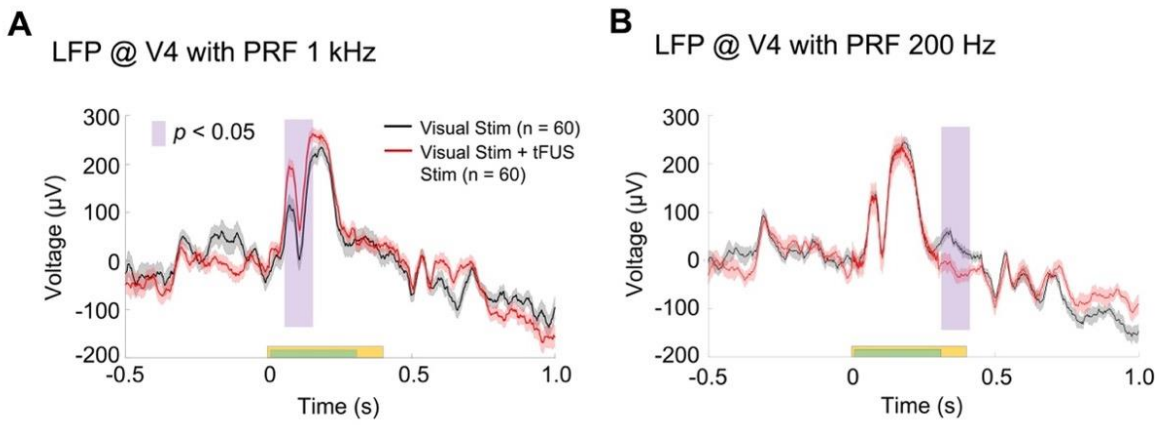
**Abstract:** Transcranial focused ultrasound (tFUS) is a potent neuromodulation tool featured with spatial specificity and penetration to the brain. It provides unprecedented capability in noninvasively modulating neuronal activities, and impacting the behavior outcome. In this work, we applied low-intensity tFUS stimulation with a customized 128-element random array ultrasound transducer ($f_0$: 700 kHz) in a rhesus macaque monkey engaged in a simple visually-guided saccade task. The stimulation was guided stereotaxically to the frontal eye field (FEF), a region of frontal cortex that guides eye movements. We recorded simultaneously from a 96-channel Utah array implanted in V4, a region of extrastriate visual cortex that receives feedback connections from FEF.
The subject was trained to perform tasks while seated in front of a computer screen. A visual stimulus (a natural image) was delivered for 400 ms while the subject maintained fixation. A reward was provided if the subject maintain fixation for the full trial and then followed the fixation dot when it moved to a peripheral location. For each recording session, 60 trials only with visual stimulation (400 ms) and 60 trials with concurrent visual and tFUS (300 ms) stimulation were mixed and randomly presented. We used two types of tFUS stimulation (same duty cycle) that were targeted for FEF with an estimated depth of 4 mm from cortical surface. Applying 1 kHz pulse repetition frequency (PRF) with a pulse duration of 0.5 ms, the local field potential in V4 elicited by the hybrid stimulation showed a statistically significant enhancement amid the sonication period (Fig. 1A). Specifically, the neurons significantly increased their activities comparing to those in the visual stimulation alone. On the contrary, an inhibitory effect of 200 Hz PRF tFUS at the FEF was observed close to the end of sonication (Fig. 1B). Consistently, the neurons also show reduced spiking activities. Overall, by tuning the ultrasound PRF, we demonstrated remote excitatory/inhibitory neuromodulation effects of tFUS to the extrastriate cortex in the macaque monkey model.

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**Presentation Number:** NANO71.06

**Topic:** I.08. Methods to Modulate Neural Activity

**Title:** Nanomaterial-enabled optical modulation of neural activity

**Authors: *Y. WANG**[1], D. RANKE[1], M. PREISEGGER[3], B. CHACON[4], G. MICHAEL[3], Y. GOGOTSI[4], T. COHEN-KARNI[1,2];
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**Abstract:** Controlling neural activity at the cellular level is a powerful tool to study the communication between individual neurons and understand brain functionality. Light-responsive nanomaterials (e.g., Au nanoparticles, Si nanowires and conductive polymers) have shown promise for non-genetic, high spatiotemporal resolution, and minimally invasive neural modulation. These nanomaterials convert incident electromagnetic radiation into a localized heat release and/or an electric field which in turn affects the membrane properties in the interfaced cells and tissues. However, such approach is limited due to low near-infrared (NIR) absorption, limited energy conversion efficiency, tissue penetration depth, high incident energy requirement, and potential cytotoxicity or phototoxicity. Here, we present a safe neural modulation approach with sub-μJ incident energy using engineered nanostructured Si, C (out-on-plane grown graphene), and two-dimensional (2D) transition metal carbides nanoflakes without generating cellular stress. We demonstrate neural excitation with low incident light energy with heterojunction of Si and out-of-plane grown graphene with 1 ms (635 nm) light pulses by optimizing the thickness, Si crystallinity and doping type. We also show rat dorsal root ganglion (DRG) neuron excitation with 2D metal carbides ($Ti_3C_2T_x$ (MXene)) thin film and flakes illuminated by a single 1 ms (635 nm, 2 μJ) short laser pulses (22 neurons across 3 cell cultures). We further systematically investigate the cytotoxicity and phototoxicity of the $Ti_3C_2T_x$-based excitation of rat DRG neurons across multiple assays, including plasma membrane integrity, mitochondria stress, and oxidative stress, and shown that the light condition typically used for neural excitation did not generate detectable irreversible damage to the neurons. Our approach

serves as a powerful toolset for studies of cell signaling within and between tissues and can enable therapeutic interventions. Expanding the material library for safe optical modulation and the systematic evaluation of their biosafety will inspire future clinical translation to achieve remote control of neurons in vivo.

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**Presentation Number:** NANO71.07

**Topic:** I.08. Methods to Modulate Neural Activity

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**Title:** Towards clinical advancement of non-invasive circuit modulation via ultrasound-mediated molecular control.

**Authors: *P. M. JOHNSON**, G. AYDEMIR, M. AGHILIBEHNAM, M. S. OZDAS, W. VON DER BEHRENS, M. YANIK;
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**Abstract:** Deep brain stimulation has demonstrated the remarkable potential of focal circuit modulation for multiple diseases, however, such an approach is neither scalable nor suitable for the most common neuropsychiatric disorders, and the most common alternative treatments (medications) come with severe and often debilitating side effects due to their systemic delivery. In an effort to maintain the benefits of both approaches, while eliminating their drawbacks, we have developed a technology capable of molecularly-precise focal circuit modulation using systemically administered Ultrasound-Controllable drug carriers (UC-carriers) that release their cargo with millimeter precision inside the brain through the application of a unique two-component Aggregation and Uncaging Focused Ultrasound Sequence (AU-FUS). This sequence first aggregates UC-carriers in the desired region by orders of magnitude and subsequently uncages their cargo, which crosses the intact blood-brain barrier. We demonstrate that this technique affords circuit-specific manipulation of cortical activity through suppression of sensory-evoked activity in the motor cortex using a GABA-A receptor agonist loaded into our UC-carriers in anesthetized animals. Critically, our approach uses orders of magnitude (1300x) less drug than is otherwise required by systemic injection and requires very low ultrasound pressures (20-fold below FDA safety limits for diagnostic imaging). In preparation for clinical application, we demonstrate scalable, high-purity (polydispersity index <0.1) synthesis of components of our UC-carriers using microfluidics, enabling tuning of carrier size (Z-Average ~ 70-250 nm) and dosing (nanogram to microgram quantity). These results demonstrate a viable translational platform approach towards treating a variety of brain disorders through precise molecular control of circuit activity.

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**Topic:** I.08. Methods to Modulate Neural Activity

**Support:** McGovern Addiction Initiative

**Title:** An integrated bidirectional neural probe for optical, electrical, and chemical investigation of dopamine dynamics in response to cocaine and fentanyl exposure

**Authors: *N. DRISCOLL**[1], M.-J. ANTONINI[1], P. MARETICH[1], K. NAGAO[1], S. HUNT[2], M. HUMMEL[1], E. VARGAS[1], A. SAHASRABUDHE[1], P. ANIKEEVA[1];
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**Abstract:** Within the brain, billions of neurons communicate via diverse electrical and chemical signaling mechanisms. To unravel how the function of specific neuronal subtypes within neural circuits gives rise to behavior, methods of recording and modulating neural activity across electrical, optical, and chemical modalities have been developed. Each technique has its own set of advantages and constraints, especially regarding spatial and temporal resolution, cell-type specificity, and the necessity of genetic manipulation. Combining multiple neural interfacing modalities into a single probe can leverage their unique advantages to reveal new insights into neural circuit dynamics. Here, we report a bidirectional, multifunctional neural probe that, for the first time, combines six modalities of neural interfacing: electrophysiology, electrical stimulation, fiber photometry, optogenetic stimulation, fast-scanning cyclic voltammetry (FSCV), and drug/gene delivery. We employed convergence thermal drawing to fabricate meters-long 300 μm diameter flexible fibers containing a polymer optical waveguide for fiber photometry and optogenetics, a microfluidic channel for drug/gene delivery, and carbon nanotube (CNT) electrodes for electrophysiology, electrical stimulation, and FSCV recording of dopamine (DA). We used our multifunctional neural probe to investigate dopamine dynamics *in vivo* in the mesolimbic pathway of mice after exposure to intraperitoneal (IP) doses of cocaine and fentanyl. We implanted fiber probes into the ventral tegmental area (VTA) and nucleus accumbens (NAc) of adult mice. During implantation, the embedded microfluidic channel was used to deliver the excitatory opsin ChrimsonR into the VTA and the DA fluorescent indicator dLight1.1 into the NAc. We recorded DA dynamics in the NAc via fiber photometry in awake, freely-behaving mice before and after IP doses of cocaine or fentanyl and observed changes in DA dynamics that differed between substances. We also performed photometry recordings of DA with simultaneous electrophysiology in anesthetized mice, using either electrical stimulation or optogenetic stimulation to evoke DA transients before and after drug dosing. These multimodal experiments, enabled by our integrated fiber neural probe, offer insights into how DA dynamics in the mesolimbic pathway are altered following exposure to cocaine and fentanyl.

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**Nanosymposium**

**NANO72: Pluripotent Stem Cells: Developmental Mechanisms and Disease Models**

**Location:** WCC 150

**Time:** Wednesday, November 15, 2023, 8:00 AM - 11:30 AM

**Presentation Number:** NANO72.01

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** R01-MH112940
Stanley Center for Psychiatric Research
U01-MH115727

**Title:** Multi-donor human cortical Chimeroids reveal individual susceptibility to neurotoxic triggers

**Authors: *N. ANTON BOLANOS**[1,3], I. FARAVELLI[2,3], T. FAITS[2,3,5], S. ANDREADIS[2], S. TRATTARO[2,3], R. KASTLI[2,3], X. ADICONIS[3], D. J. DI BELLA[2,3], M. TEGTMEYER[2,6], R. NEHME[2,3], J. Z. LEVIN[3,4], A. REGEV[7], P. ARLOTTA[2];
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**Abstract:** Inter-individual genetic variation affects the susceptibility to and progression of many diseases. Efforts to study the molecular mechanisms mediating the impact of human genetic variation on normal development and disease phenotypes are limited, however, by the paucity of faithful human-derived models, and the difficulty of scaling current systems to represent many individuals. Here, we present human brain “Chimeroids”, a highly reproducible, multi-donor brain organoid model that allows co-development of human cerebral cortex from a panel of individual donors in a single organoid, while maintaining fidelity to endogenous tissue. By re-aggregating cells from multiple single-donor organoids at the neural stem or progenitor stage, we generate Chimeroids in which each donor produces all cell lineages of the cerebral cortex, even when using pluripotent stem cell lines with notable growth biases. We leveraged Chimeroids to investigate inter-individual variation in susceptibility to neurotoxic stressors that exhibit high clinical phenotypic variability: ethanol and the anti-epileptic drug valproic acid. Individual donors varied in both the penetrance of the effect on target cell types, and the molecular phenotype within each affected cell type. Our results show that human genetic background is an important mediator of neurotoxin susceptibility and introduce Chimeroids as a scalable system for high-throughput investigation of the contribution of human genetic variation to brain development and disease.

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**Tegtmeyer:** None. **R. Nehme:** None. **J.Z. Levin:** None. **A. Regev:** A. Employment/Salary (full or part-time):; Genentech, Inc.. **P. Arlotta:** None.

**Presentation Number:** NANO72.02

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** SSADH Foundation

**Title:** Using human iPSC-derived neurons as a platform to investigate subtype specific alterations in neurodevelopmental disorders: our progress on SSADH Deficiency

**Authors: *W. AFSHAR SABER**[1], N. TEANEY[2], K. D. WINDEN[3], P. PEARL[1], M. SAHIN[3]; [1]Boston Children's Hosp. - Harvard Med. Sch., Boston, MA; [2]Boston Children's Hosp., Brookline, MA; [3]Boston Children's Hosp., Boston Children's Hosp., Boston, MA

**Abstract:** Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessive neurometabolic disorder caused by bi-allelic mutations in the ALDH5A1 gene. It is the most prevalent inherited disorder of GABA metabolism and is characterized by accumulation of two neuromodulators, gamma-aminobutyric acid (GABA) and gamma-hydroxybutyric acid (GHB), in the CNS. Previous studies using rodent models have shown that disruption in GABA signaling can lead to dysregulation of mitochondria numbers, turnover, and function. Over the last 30 years, an expanded understanding of pathophysiology based on the corresponding animal model (Aldh5a1-/- mice) has emerged, but effective pharmacotherapy remains elusive. Alternative models and therapies that address the accumulation of GABA and GHB, and their downstream effects, are needed. In this study, we used fourteen iPSC lines: three patient lines and sex matched parental controls and CRISPR corrected lines each transduced with hNGN2 and hDLX2-hASCL1 respectfully generate excitatory neurons and GABAergic neurons. We show that hiPSCs can differentiate into excitatory neurons and GABAergic neurons regardless of the allelic dosage of ALDH5A1. We found that hiPSC-derived excitatory neurons display altered neurite outgrowth and synaptic development which leads to hyperactivity of the developing excitatory neuronal network. Moreover, we showed that the CRISPR correction ALDH5A1corr/corr shows similar network activity to the parental control ALDH5A1+/- suggesting that hiPSC-derived excitatory neurons network's hyperactivation is linked to the ALDH5A1 mutation. Additionally, we identified neuron subtype-specific metabolic and gene expression changes linked to SSADH deficiency. Furthermore, we showed that similarly to clinical presentation, SSADHD results in increased GABA and GHB levels in hiPSC-derived GABAergic neurons. Furthermore, we developed an imaging platform based on calcium imaging and optogenetics to manipulate the network of neurons formed by hiPSC-derived GABAergic and excitatory neurons *in vitro* in a high-throughput fashion. Finally, we demonstrated we rescued these phenotypes using ALDH5A1 mRNA demonstrating the potential of the mRNA-based therapeutics in SSADH deficiency.

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**Presentation Number:** NANO72.03

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** NIH Grant EY033022
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**Title:** A microfluidic culture platform to explore neuronal compartmentalization and glial orientation in a human pluripotent stem cell model of neurodegeneration and neuroinflammation

**Authors:** C. GOMES[1], K.-C. HUANG[3], S. LAVEKAR[4], ***J. S. MEYER**[2];
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**Abstract:** Neurons are highly compartmentalized cells, and previous studies have demonstrated that the degeneration of neurons in some diseases occurs in a compartmentalized manner, with responses to injury occurring through different mechanisms in axonal vs. somatodendritic compartments. Thus, the goals of this study were to establish a novel, microfluidic-based platform for the analysis of neuronal compartmentalization in health and disease states. hPSC-derived neurons were seeded into microfluidic chips to recruit and isolate axons apart from the somatodendritic compartment. Initial studies explored axonal outgrowth as well as the compartmentalization of axons and dendrites via immunocytochemistry. Next, we explored the differential response between isogenic control and disease-associated cells in their respective axonal and somatodendritic compartments, followed by an analysis for changes in axonal transport. Further, we explored the axonal transcriptome via RNA-seq, including differences in the axonal transcriptome in disease states. Finally, we established models to uniquely orient astrocytes along the axonal compartment, including modulation of astrocyte reactivity as a pathological feature of neurodegeneration. Overall, growth within microfluidic chips allowed for more robust growth and maturation of neurons, including long-distance axonal projections as well as proper compartmentalization, based upon expression of SMI-312 and MAP2, respectively. Neurons derived from patient-specific cell lines exhibited a specific deficit in axonal outgrowth compared to isogenic controls, which was also associated with a decreased rate of axonal transport. Finally, upon introduction of astrocytes onto the proximal axonal compartment, the induction of astrocyte reactivity led to the onset of neurodegenerative phenotypes. These results represent the first application of hPSC-derived neurons in a manner that effectively recapitulates their highly compartmentalized properties. Taken together, these results should profoundly impact future studies, providing a much more physiologically-relevant in vitro model for neuronal development and degeneration.

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**Presentation Number:** NANO72.04

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** NIH (NINDS) U54NS117170 (JMP)
NSF Graduate Research Fellowship Program (MCV).

**Title:** Migratory deficits in GABAergic interneurons associated with SLC6A1-related neurodevelopmental disorder

**Authors: *M. C. VARELA**, T. N. THENSTEDT, M. D. UHLER, J. M. PARENT;
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**Abstract:** Epileptic encephalopathies and other neurodevelopmental disorders including autism have been linked to variants in the *SLC6A1* gene which encodes for the most abundant GABA transporter in the brain, GAT-1. GAT-1 is responsible for the reuptake of GABA at the synapse and, in the cortex, is expressed primarily in inhibitory neurons. While GAT-1 loss of function (LOF) results in epilepsy, developmental delays, and intellectual disability, how GAT-1 knockout (KO) and haploinsufficiency (HI) affect early human cortical development remains elusive. In this study, we use a novel 3D self-organizing single rosette cortical organoid (SOSR-CO) model, derived from human induced pluripotent stem cells (iPSCs), to test the hypothesis that GAT-1 LOF alters interneuron development, migration, and subsequent network formation. We first reprogrammed human foreskin fibroblasts into iPSCs with concurrent CRISPR/Cas9 gene editing targeted to generate out-of-frame insertions or deletions in the *SLC6A1* gene. Compound heterozygous (KO), heterozygous (Het), and isogenic control (WT) iPSC lines were generated. *SLC6A1* patient-derived (PT) iPSC lines and sex-matched controls were also generated. Cell lines from both males and females were used in this study. All CRISPR gene-edited, PT and relevant control iPSC lines were differentiated into dorsal SOSR-COs and also ventral ganglionic eminence patterned COs (SOSR-GEOs). We used a combination of immunocytochemistry, Western blot, and RT-qPCR of SOSR-GEOs cultured for 2-36 weeks to investigate interneuron specification and migration. As LOF *SLC6A1* variants are predicted to increase ambient GABA levels in early brain development, and ambient GABA levels influence the migration of developing interneurons, we also fused SOSR-COs and SOSR-GEOs to investigate interneuron migration and integration into the cortex. GAT-1 expression was markedly decreased by 80-97% in our KO and PT, and by 10-70% in our HET SOSR-GEOs versus WT (n=3 lines each). We observed delayed migration in mutant *SLC6A1* SOSR-GEOs compared to controls, with KO, Het, and PT lines exhibiting reduced cell migration (p=&lt 0.0001) and diminished process extension (p=0.002-0.03). These migratory deficits were partially rescued by the inducible expression of WT *SLC6A1* in KO lines. Our findings uncover a previously unidentified role of *SLC6A1* in GABAergic cell migration in early human cortical development which likely leads to aberrant network formation. Further investigation into the mechanism of this phenotype could provide treatment targets for *SLC6A1*-related neurodevelopmental disorders.

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**Presentation Number:** NANO72.05

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** NIH Training Grant in Integrative Biology: Neuroscience (T32NS096050)

**Title:** Impact of Cortical Activity Modulation on Interneuron Migration and Specification in a Human Forebrain Assembloid Model

**Authors: *A. SARKISSIAN**, C. EISENBERG, F. BIREY;
Human Genet., Emory Univ., Atlanta, GA

**Abstract:** Establishment of excitation/inhibition (E/I) balance depends on functional integration of GABAergic interneurons migrating from the ventral forebrain (subpallium) into glutamatergic neuronal networks in the cortex during development. Identifying the rules governing interneuron migration and maturation is critical to understanding E/I balance in health and disease. Even though cell-intrinsic transcriptional programs underlying interneuron development have been studied in detail, instructive roles of cell-extrinsic factors—such as attractive cues from the cortex—remain understudied.

Despite the importance of E/I imbalances in a number of neurological disorders such as autism, schizophrenia and epilepsies, our understanding of how E/I emerges is limited in humans due to lack of access to functional human tissue. To investigate the role of early cortical activity levels on interneuron migration and specification, we employed the human forebrain assembloid model—derived from human induced pluripotent stem cells (hiPSCs)—consisting of human cortical spheroids (hCS) fused to human subpallium spheroids (hSS), the source of GABAergic interneurons. Combining chronic optical stimulation or inhibition of excitatory cortical neurons with single-cell RNA sequencing and live cell imaging of interneuron migration, we asked how interneuron migration and maturation is modulated by cortical activity levels *in vitro*. Our analysis revealed gene sets differentially expressed between migrated versus dormant interneurons—as well as genes differentially expressed between migrated interneurons across our stimulated, control, and inhibited assembloids. Utilizing calcium imaging, we also compared assembloid network activity 12 weeks after assembly. We found that differences in interneuron migration rates and interneuron specification may explain long-term network activity differences revealed by calcium imaging. Our initial results have pointed to a differential role of early cortical activity modulation on unique aspects of interneuron migration, subtype specification, and maturation.

The findings shed light on the impact of early cortical activity levels on emergent cortical network function—potentially driven by intermediary effects on interneurons. Specifically, abnormal cortical activity may disrupt activity-dependent aspects of interneuron migration from the subpallium to the cortex, leading to an abnormal number or diversity of interneuron subtypes reaching the cortex, resulting in abnormal E/I balance in cortical networks.

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**Presentation Number:** NANO72.06

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** NSF RECODE Grant SFP_300690

**Title:** Synthetic Morphogenesis to Pattern Early Brain and Central Nervous System Development

**Authors: *C. HAMANN**, E. LIPPMANN, J. BRUNGER;
Vanderbilt Univ., Nashville, TN

**Abstract:** During development, signaling centers organize cellular differentiation via coordinated morphogen production. As ectodermal cells undergo neurulation, morphogen

networks shape the neural tube and early structures of the central nervous system. Notochord-derived sonic hedgehog (SHH) specifies formation of the floor plate, which serves as a source of SHH to pattern cells of the neural tube and define the dorsal-ventral axis. To elucidate key human developmental events, protocols that enable derivation of floor plate from human pluripotent stem cells (hPSCs) have been developed, and resultant floor plate replicas demonstrated the capacity to ventralize primary mouse neural precursor cells. Leveraging artificial cell circuits, we take a synthetic morphogenesis approach to uncover the design rules required to program hPSCs to autonomously form a floor plate region that serves as an organizing center to further elaborate neural tube formation. Thus, we engineered hPSCs that produce SHH under the control of the customized signaling receptor synNotch. Like native Notch, synNotch responds productively to artificial juxtacrine cues, such as transmembrane ligands on neighboring cells, leading to highly localized transgene expression. We engineered synNotch hPSCs to produce SHH, and our results show that successful juxtacrine binding of synNotch hPSCs potently upregulates SHH in response to the synNotch transmembrane ligand, GFP. SynNotch induction results in domains of high FOXA2 expression (floor plate marker) while also showing low PAX6 (dorsal tissue marker), as assessed by immunocytochemistry. Gene expression profiling also revealed also revealed a 470-fold increase in *FOXA2* along with concomitant up-regulation of *NETRIN-1* and *F-SPONDIN* and down-regulation of *PAX6* relative to controls by the end of the 11-day differentiation protocol. These results display a robust increase in a prominent floor plate markers while also exhibiting a decrease in dorsal markers, suggestive of cell patterning typically seen during development. Future work will expand on this synthetic morphogenesis approach to build a floor plate organizing center that can mimic functional properties of primary floor plate through a combination of stem cell technology and artificial signaling networks.

**Disclosures: C. Hamann:** A. Employment/Salary (full or part-time):; Vanderbilt University. **E. Lippmann:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Vanderbilt University. **J. Brunger:** A. Employment/Salary (full or part-time):; Vanderbilt 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.; Vanderbilt University.

**Presentation Number:** NANO72.07

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** Stanley Center for Psychiatric Research

**Title:** Biological potential and plasticity of very long-term brain organoids

**Authors: *I. FARAVELLI**[1,2], N. ANTÓN-BOLAÑOS[1,2], T. FAITS[2], A. WEI[2], S. ANDREADIS[1], M. MONTERO CRESPO[1,2], Y. SUNG MIN[1], X. ADICONIS[2], B. PAULSEN[1], G. QUADRATO[3,1], J. W. LICHTMAN[1], E. S. BOYDEN[4], A. REGEV[2], J. Z. LEVIN[2], P. ARLOTTA[1,2];

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**Abstract:** Human brain organoids offer a unique opportunity to understand developmental milestones, but we still have limited knowledge about the intricate molecular mechanisms involved in long-term processes of maturation and maintenance of the tissue. In this study, we profiled brain cortical organoids cultured for periods from 180 to over 1000 days using single-cell RNA-sequencing. Our analysis revealed various neuronal populations with specific subclusters that gradually decreased in complexity over time, while astrocyte populations became more prominent. We hypothesized that spontaneous activity and the establishment of functional synapses might play a crucial role in maintaining neuronal identity over time. To investigate this, we transferred younger organoids to a culture medium that promotes synaptic maturation, and evaluated morphology and synapse formation using electron microscopy. We found increased synaptic markers and a rescue of specific neuronal populations, such as callosal projection neurons and corticofugal projection neurons. Finally, we explored the plasticity of committed progenitors using both cell-autonomous and non-cell autonomous approaches. Our findings provide insight into the developmental capabilities of long-term organoid cultures exploring fundamental processes that might contribute to the maintenance of neuronal identity and progenitor plasticity.

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**Presentation Number:** NANO72.08

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** Picower Institute Innovation Fund (PIIF)

**Title:** 3d-printed synthetic vasculature enhances the fidelity of organoids as human brain model systems

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**Abstract:** Human cerebral organoids (HCOs) are stem cell-derived in vitro 3D tissue models of the human brain that have proven to be useful models for aspects of neurogenesis, as well as neurological disorders. Conventionally grown HCOs lack a functional vasculature resulting in hypoxia and necrotic core formation. This limits the size of organoids, reduces fidelity to in vivo counterparts, and restricts immunological extensions due to the prevalence of stress signals in the system.
To solve this transport problem, we developed a synthetic vasculature platform which is a combination of a vasculature array 3D-printed using two-photon polymerization (TPP) and a perfusion platform. Custom resin composition and TPP conditions enable the printing of high-resolution structures with desired features such as mechanical robustness, porosity,

cytocompatibility, and perfusability to support the growth of biological tissues.
Imaging results of week 7 HCOs grown on this synthetic vasculature platform showed that early neurogenesis and cortical layer formation (hallmark features of HCOs) were maintained. The distribution of neural progenitor cells (SOX2+) within the ventricles and the presence of deep-layer neurons (TBR1+ and CTIP2+) around these ventricles was maintained suggesting that the organoid development was not affected. Imaging results of week 4 HCOs grown on this platform with and without active perfusion revealed a clear reduction in apoptosis and necrosis in perfused HCOs. In addition, proliferating neural progenitor cells were maintained throughout the perfused HCO tissue whereas they have a limited presence near the periphery in non-perfused HCOs. Single cell-RNA-sequencing of week 4 perfused and non-perfused HCOs was carried out (N=4 from 2 batches for each condition). Enrichment analysis showed a reduction in hypoxia and glycolysis stress signals in perfused HCOs. Oxidative phosphorylation was the top upregulated pathway in perfused HCOs, indicating a metabolism shift from glycolysis. Metabolomics analysis of week 4 HCOs (N=4 from 1 batch for each condition) confirmed this shift. Metabolites related to glycolysis and purine degradation were downregulated in perfused HCOs, whereas the metabolites related to the citric acid cycle and biosynthesis pathways were upregulated.
Currently, efforts are underway to leverage this technology for more accurate disease modeling and to study unperturbed immunological interactions in HCOs and other organoid systems. This synthetic vasculature platform can potentially accommodate high throughput drug screening and facilitate live organoid imaging for temporal analyses of diseases.

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**Presentation Number:** NANO72.09

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** TURSP-HC2023/5

**Title:** Effects of prenatal gabapentinoids exposure on human cortical neurons

**Authors: *W. F. ALSANIE**[1], S. ABDULRAHMAN[3], A. ALSHAREEF[1], V. THAKUR[4], A. GABER[2], H. HABEEBALLAH[5], A. ALAMRI[2], M. ALHOMRANI[2], C. HAUSER[3];
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**Abstract:** Prenatal substance exposure is a major public health concern associated with many detrimental fetal consequences. Unfortunately, polysubstance use in pregnancy is common. Gabapentinoids are widely used as treatments in psychiatry and neurology; however, they have been increasingly reported as having potential for misuse. Moreover, gabapentinoids can cross the placental barrier. Due to difficulties in accessing fetal brains exposed to gabapentinoids, we used the human embryonic stem cell (hESC) line H9 to generate early, intermediate cortical progenitors and cortical neurons to modulate prenatal gabapentinoid exposure *in vitro*. Since the cortex is responsible for cognition and behavior, we focused on cortical development. We analyzed treated (10uM) and untreated (control) cultures for gene expressions, neurogenesis, and morphogenesis. At the early patterning stage, there was a significant increase in Tbr2+

intermediate progenitors in pregabalin- and gabapentin-treated cultures. In addition, there was a significant increase in the expression of cortical related genes *Pax6*, *Foxg1*, and *Tbr2* in pregabalin-treated cultures, whereas gabapentin significantly increased *Tbr2* expression solely. At the maturation stage, the number of mature cortical neurons was unchanged in pregabalin-treated cultures. At early maturation, gabapentin significantly increased Tbr1+ neurons, but not Ctip2+ neurons. At the genetic level, we screened the effects of pregabalin on different cortical layer related genes. Pregabalin significantly increased expression of *Brn2* without significant effects on other screened genes. Meanwhile, gabapentin did not alter any cortical layer related genes. However, the exposure of cortical neurons to gabapentinoids, during the maturation stage only, has upregulated upper layers cortical related genes, such as Satb2. Regarding morphogenical analysis, both pregabalin and gabapentin significantly decreased neurite length, branches, and neurites of human cortical neurons. Our data also shows that the effects of pregabalin and gabapentin on the morphogenesis of cortical neurons differ based on the presence of maturation factors, such as GDNF and BDNF, suggesting a possible interaction mechanism. Our study demonstrates that exposure to gabapentinoids during early brain development may interfere with the neurogenesis and morphogenesis of various neuronal subpopulations. Currently, we are investigating gabapentinoids' effects on cortical neuron functionality as we have shown previously an effect of gabapentinoids on mouse dopaminergic and cortical neurons.

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**Presentation Number:** NANO72.10

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** TURSP-HC2023/5

**Title:** Effects of prenatal gabapentinoids exposure on mouse cortical neurons

**Authors: *H. HABEEBALLAH**[1], S. ABDULRAHMAN[2], M. ALHOMRANI[3], A. ALSHARIF[3], A. ALAMRY[3], A. GABER[3], R. FELIMBAN[4], H. KHATTABI[4], C. HAUSER[2], W. ALSANIE[3];
[1]King Abdulaziz Univ., Jeddah, Saudi Arabia; [2]KAUST, Thuwal, Saudi Arabia; [3]Taif Univ., Taif, Saudi Arabia; [4]KAAU, Jeddah, Saudi Arabia

**Abstract:** Though widely used as treatments in psychiatry and neurology, gabapentinoids have been increasingly reported as having potential for misuse. Due to a lack of safety studies, gabapentinoids at therapeutic doses are the last treatment option for various neurological diseases, such as neuropathic pain, during pregnancy. Taking this into consideration, gabapentinoid abuse in pregnant women, or even use at therapeutic levels, may impair fetal development. Here, we used primary mouse embryonic neurons to investigate whether prenatal exposure can impair fetal brain development. We focused on cortical and ventral midbrain development, as they are responsible for cognition and behavior. Early neurogenesis and morphogenesis were analyzed *in vitro* using cortical and ventral midbrain neurons isolated from E12.5 embryonic mouse brains (n=6) in 3D cultures (using ultra-short peptides). We investigated the expression of different cortical layers and ventral midbrain specific proteins in

gabapentinoid-treated 3D cultures (using different doses), comparing these with untreated 3D cultures. In addition, we studied the morphogenesis *in vitro* in the 3D cultures. In embryonic mouse cortical neurons, pregabalin and gabapentin (in all tested doses) did not alter early development of preplate and deep layer cortical neurons in treated cultures, in comparison with untreated cultures. However, pregabalin and gabapentin significantly decreased neurite length of both preplate neurons and other deep cortical layer neurons. Meanwhile, in pregabalin-treated cultures, the number of branches significantly increased only in the other deep layer neurons not belonging to the preplate population. Nevertheless, in both cortical populations, the number of neurites and branches significantly decreased in gabapentin-treated cultures. In ventral midbrain neurons, pregabalin and gabapentin significantly increased neurite length of dopaminergic neurons, while the number of branches significantly increased in non-dopaminergic neurons. Pregabalin, but not gabapentin, significantly increased the number of neurites in non-dopaminergic ventral midbrain neurons. Our study demonstrates that exposure to gabapentinoids during early brain development may interfere with the neurogenesis and morphogenesis of various neuronal subpopulations. We are currently investigating gabapentinoids' effects on the functionality of the aforementioned neuronal populations.

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**Topic:** A.03. Stem Cells and Reprogramming

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**Title:** Molecular programs of regional specification and neural stem cell fate progression in macaque telencephalon

**Authors: *N. MICALI**[1], S. MA[1], M. LI[1,2], S.-K. KIM[1], X. MATO-BLANCO[3], S. SINDHU[1], J. ARELLANO[1], T. GAO[1], M. SHIBATA[1], K. GOBESKE[1], A. DUQUE[1], G. SANTPERE[3], N. SESTAN[1,4], P. RAKIC[1,5];
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Medicine, Wu Tsai Institute, Program in Cell. Neuroscience, Neurodegeneration and Repair, and Yale Child Study Cente, New Haven, CT; [5]Yale Sch. of Med., Kavli Inst. for Neurosci., New Haven, CT

**Abstract:** Early telencephalic development involves patterning of the distinct regions and fate specification of the neural stem cells (NSCs). These processes, mainly characterized in rodents, remain elusive in primates and thus our understanding of conserved and species-specific features. Here, we profiled 761,529 single-cell transcriptomes from multiple regions of the prenatal macaque telencephalon. We defined the molecular programs of the early organizing centers and their cross-talk with NSCs, finding primate-biased signaling implicating *GALP* in the antero-ventral telencephalon. Regional transcriptomic divergences were evident along the fronto-temporal axis at early states of neocortical NSC progression and in neurons and astrocytes, more than in intermediate transitions. Finally, we show that neuropsychiatric disease- and brain cancer-risk genes have putative early roles in the telencephalic organizers' activity and NSC progression.

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**Title:** Dephosphorylation of the mTOR effector 4EBP1/2 induces quiescence entry in prenatal neural stem cells

**Authors:** L. GEBEN[1], A. A. BROCKMAN[2], M.-B. L. CHALKLEY[2], S. SWEET[2], J. E. GALLAGHER[2], A. L. SCHEUING[2], R. SIMERLY[2], K. C. ESS[4], J. M. IRISH[3], ***R. A. IHRIE**[3]; [1]Pharmacol., [3]Cell and Developmental Biol., [2]Vanderbilt Univ., Nashville, TN; [4]Vanderbilt Univ. Med. Ctr., Nashville, TN

**Abstract:** Adult neural stem cells are derived from a preserved group of embryonic cells that undergo a temporary quiescence. Adult neurogenesis is dependent upon this prenatal quiescence entry; and though this event is essential in establishing the postnatal niche, few molecular actors have been found that initiate this process. We have found that specific downstream targets of the

mechanistic target of rapamycin (mTOR) kinase are selectively and differentially modulated in embryonic neural stem cells and their immediate progeny. Phosphorylation of the translation regulator 4EBP1/2, but not the more widely assayed ribosomal S6 protein, is detectable exclusively in dividing embryonic NSCs. Inhibition of this phosphorylation is sufficient to induce quiescence in vitro and alter prenatal cortical development. This role is distinct from the mTOR kinase's reported functions in the postnatal niche, as well as functions assigned to mTOR based solely on S6 phosphorylation. Finally, first-, second-, and third-generation mTOR inhibitors differentially impact these downstream effectors, providing a potential explanation for their mixed performance to date in targeting neurodevelopmental disorders such as Tuberous Sclerosis Complex. These data reveal a requirement for selective activation of an mTOR-driven effector in regulating prenatal stem cell quiescence and have direct implications for many neurodevelopmental disorders where mTOR signaling, and thus its downstream targets, are affected.

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**Presentation Number:** NANO72.13

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** NIHM Grant MH125579-02
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**Title:** Precise Therapeutic Targeting of Divergent NRXN1$^{+/-}$Phenotypic Mechanisms

**Authors: *M. FERNANDO**[1], Y. FAN[1], Y. ZHANG[1], S. KAMMOURH[1], A. MURPHY[1], S. GHORBANI[2], R. ONATZAVITCH[1], A. PERO[1], C. PADILLA[1], L. CAO[1], S. WILLIAMS[1], G. FANG[1], P. SLESINGER[1], K. BRENNAND[2];
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**Abstract:** Neurexins are critical pre-synaptic cell adhesion proteins that organize synaptic connections in the brain. Complex patterns of *NRXN1* alternative splicing are fundamental to diverse neurocircuitry and are dramatically impacted by rare copy number variants (2p16.3) linked to a variety of neuropsychiatric disorders. We use human induced pluripotent stem cells derived from *NRXN1*$^{+/-}$ cases and controls to contrast the cell-type-specific impact of unique non-recurrent mutations predicted to impact *NRXN1* alternative splicing, neuronal activity, and synaptic function. Whereas induced *NRXN1*$^{+/-}$ glutamatergic neurons show persistently decreased synaptic activity throughout neuronal maturation, induced *NRXN1*$^{+/-}$ GABAergic neurons show transiently increased synaptic activity specifically in immature neurons. Isogenic analyses reveal that distinct loss-of-function (LOF) or gain-of-function (GOF) splicing defects independently decrease synaptic frequency in glutamatergic neurons and increase it in GABAergic neurons. Treatment with transcriptional activators increases *NRXN1* expression in glutamatergic neurons, while antisense oligonucleotides can knockdown mutant isoform expression across both glutamatergic and GABAergic neurons. Overall, we causally demonstrate that perturbations in *NRXN1* splicing lead to divergent cell-type-specific synaptic outcomes. Direct or indirect

manipulation of *NRXN1* splicing isoforms is a promising therapeutic strategy for 2p16.3 deletions.

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**Presentation Number:** NANO72.14

**Topic:** A.03. Stem Cells and Reprogramming

**Support:** Community Foundation for Greater Buffalo

**Title:** Human specific a7nAChR-dependent adaptation to mechanical properties of the extracellular environment

**Authors: *I. IHNATOVYCH**, R. P. DORN, E. NIMMER, Y. HEO, Y. BAE, K. SZIGETI; SUNY At Buffalo, Buffalo, NY

**Abstract:** *CHRFAM7A*, a human restricted gene associated with neuropsychiatric and neurodegenerative disorders, is expressed in 99.3% of the human population. It is present in different copy number (0-4) and orientation (direct or inverted alleles). The direct CHRFAM7A protein incorporates into the α7 nicotinic acetylcholine receptor (α7nAChR) leading to a hypomorphic receptor. Our results of multiomics analysis of post mortem brains from the ROSMAP dataset demonstrated that *CHRFAM7A* affects $Ca^{2+}$ signaling, small GTPases, and the actin cytoskeleton. We utilized a human isogenic CHRFAM7A iPSC model and polyacrylamide hydrogels corresponding to Young's modulus 2 kPa and 5 kPa, the shift between rodent and human brain, to study human specific adaptation to tissue stiffness. The results of immunocytochemistry, atomic force microscopy (AFM), and G-LISA revealed distinct differences in growth cone (GC) morphology, polarization pattern, small GTPases activity, and intracellular elastic modulus between the medial ganglionic eminence (MGE) progenitors generated from CHRFAM7A null (0 copy number) and CHRFAM7A knock-in lines. In response to an increased matrix stiffness, null MGE progenitors developed pronounced and multidirectional GCs filopodia, while CHRFAM7A GCs demonstrated lamellipodia. Null cells became predominantly multipolar, while CHRFAM7A_KI cells became mostly bipolar. MGE progenitors derived from the CHRFAM7A KI line responded more efficiently to the stiffness of the environment compared to null cells by activating both CDC42 and Rac1 and with limited RhoA activation. Ability of the CHRFAM7A KI MGE progenitors to enter the stiffer environment was associated with increased *MMP2* and *MMP9* expression suggesting increased ECM degradation as a potential mechanism. Inhibition of Rac1 led to a decrease in both *MMP2* and *MMP9* levels only in the CHRFAM7A KI line. Our results provide evidence that the presence of human specific *CHRFAM7A* positively affects neuronal ability to adapt to the matrix stiffness. *CHRFAM7A* may facilitate neuronal adaptation to changes in the brain environment in physiological and pathological conditions contributing to risk or recovery.

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**Nanosymposium**

**NANO73: Early Life Stress and Neural Circuit Remodeling**

**Location:** WCC 152A

**Time:** Wednesday, November 15, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO73.01

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH R01MH127850-01

**Title:** Microglia depletion during a discrete developmental timepoint prevents long term working memory impairments following early life adversity

**Authors: *M. FANIKOS**[1], K. GILDAWIE[2], S. KOHN[1], A. PARAKOYI[1], H. BRENHOUSE[1]; [1]Northeastern Univ., Boston, MA; [2]Tufts Univ., Tufts Univ., North Grafton, MA

**Abstract:** The dynamic relationship between the nervous and immune systems plays an integral part in the long-term, sex-dependent impacts of adversity. Microglia are the primary neuroimmune cell and are known for their role in the brain's response to stress as well as their tendency to cause neuronal damage when overactivated. Functional and morphological activation states have been found to differ in male and female animals exposed to early adverse events. Microglia influence neuronal activity through the release of cytokines, elimination of synapses, and phagocytosis of debris. For example, microglia are known to drive degradation and regulation of perineuronal nets (PNNs). PNNs are specialized structures of the extracellular matrix that preferentially enwrap parvalbumin (PV) expressing interneurons. Furthermore, early-life adversity (ELA) reduces adolescent and adult PV expression in the prefrontal cortex (PFC). As PV neurons in the PFC are important for cognition, it is possible that their reduction may lead to adversity-induced deficits in cognition. We have shown that early adverse experiences induce a long-lasting reduction to PV cell count and PV+ PNN structural integrity in females only.
To address if microglial activity in the postnatal period mediates the sex-dependent response to adverse experiences early in life, microglia were transiently depleted during two different time points during ELA: Postnatal day (P)2 or P10. Rats underwent ELA in the form of maternal separation (MS) from P2 to P20 and were left undisturbed from weaning on P21 until behavioral testing on P70. Rats were tested in the spontaneous alternation task to assess working memory. Following this behavioral task, the density and intensity of PV neurons and PNNs in the PFC were quantified.
Female rats that underwent MS displayed impaired working memory in the spontaneous alternation task, however this effect was prevented in female rats with P2 microglia depletion. Interestingly, the female specific MS-induced impairment in working memory was not prevented with P10 microglial depletion. Males did not display changes in working memory following MS nor with microglia depletion at any timepoint. These findings indicate that ELA alters female microglia early during the neonatal period which leads to impaired working memory later in life. Future studies will investigate the specific changes in microglial gene expression that lead to these long-lasting consequences of ELA.

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**Presentation Number:** NANO73.02

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** R00MH108719
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**Title:** Early life adversity causes fear overgeneralization by impairing the serotonergic modulation of the ventral dentate gyrus

**Authors: *L. MALAVE**[1,2], R. DIXON[1], N. CHUMA[1], S. RAYPORT[3], C. ANACKER[1,2]; [1]Columbia Univ., New York, NY; [3]Depts of Mol. Therapeutics, Clin. Neuroscience, and Psychiatry, [2]Res. Fndn. for Mental Hyg. at New York State Psychiatric Inst., New York, NY

**Abstract:** Having a history of early life adversity (ELA), such as physical or emotional trauma experienced during sensitive periods in development, increases risk for depression and anxiety while reducing the likelihood to respond to antidepressants later in life. ELA exerts long-lasting changes on the developmental trajectory of neural circuits in a way that may be different from the effects of adult stress or genetic predisposition. Understanding the neurobiological mechanisms underlying the specific contributions of ELA to behavioral abnormalities may be relevant for the study of psychiatric disorders, such as such as depression, anxiety, and post-traumatic stress disorder. One common feature of psychiatric disorders is the overgeneralization of fear, during which a previous experience of a fearful context is transferred to a novel context. Fear generalization is increased following ELA in both humans and mice and can lead to a heightened state of fear and chronic avoidance behavior. To study ELA in mice, we use the limited bedding and nesting paradigm from postnatal day (P) 3-10. We found that Female, but not male, mice exposed to ELA overgeneralize between a foot-shock associated context and a safe context in adulthood at P56 (2way ANOVA ELA vs. sex interaction $F(1,71)=4.99$, $p= 0.029$). Using *in vivo* fiber photometry, ELA-exposed females had impaired serotonin signaling and led to hyperactivity of the ventral dentate gyrus (vDG), a key region of the hippocampus involved in fear processing (2way ANOVA sex-effect $F(1,12)=8.12$ $p=0.01$). Using *ex vivo* physiology we found that ELA-exposed mice have reduced excitability of serotonin neurons in the medial raphe nuclei at P56 (2way ANOVA ELA vs. control effect $F(1,42)=6.43$ $p=0.015$) that is not observed in males. Increasing serotonin signaling in raphe neurons using a transgenic mouse model (Pet1-tTS; Htr1atetO/tetO mice) rescued fear overgeneralization in ELA-exposed adult females (2way ANOVA ELA vs. genotype interaction $F(1,55)=7.7$ $p=0.01$). Whereas, inhibiting hyperactivity of the vDG using DREADDs also rescued fear overgeneralization in ELA-exposed adult females (2way ANOVA hM4Di effect $F(1,16)=27.36$ $p<0.0001$). Together, these findings point to an impairment in serotonin neuron function following ELA exposure that leads to hyperactivation of the vDG in adulthood and importantly highlight a sex specific effect in ELA-induced fear overgeneralization. Overall, these results help us better understand how serotonin regulation of the vDG may mediate ELA-induced behavioral impairments, providing new potential treatment options for psychiatric disorders that have their origin early in life.

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**Presentation Number:** NANO73.03

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIDA R01 DA049837
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**Title:** Adversity early in life alters addiction-related behaviors and produces sex-specific transcriptional changes in the basolateral amygdala

**Authors: *A. CUARENTA**[1], R. KARBALAEI[2], A. HEHN[2], S. ROTH[2], C. DECKERS[2], A. INGRAM[2], M. WIMMER[2], D. BANGASSER[1];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Psychology and Neurosci., Temple Univ., Philadelphia, PA

**Abstract:** Adversity early in life is a risk factor for the development of psychiatric disorders including substance use disorder (SUD). However, most individuals exposed to early life adversity (ELA) do not go on to develop disorders later in life. Stress that is not overwhelming can have an "inoculating" effect and can promote resilience later in life. Thus, ELA has the potential to produce behavioral and neurological changes that may be adaptive in some circumstances and maladaptive in others. In our laboratory, we use the limited bedding and nesting (LBN) paradigm in rats to model mild ELA. We have previously shown that LBN reduces morphine self-administration in adult male, but not female rats. We are now extending this to work to determine whether changes in reinforcing efficacy for morphine also apply to another class of drug: cocaine. We are also beginning to explore molecular changes induced by LBN. The basolateral amygdala (BLA) is a region critical for behavioral responses to stress and important for the integration of cues. Thus, we used RNAseq to assess LBN-induced transcriptional changes in the BLA. Our behavioral analysis demonstrates that rats exposed to LBN do not self-administer doses of cocaine (0.5 mg/kg/infusion) different from controls. However, we do see a difference in incubation of craving for cocaine, with LBN animals lever pressing for cocaine at a higher rate than control animals. We also find that LBN induces sex-specific changes in transcription. Bulk RNA sequencing was conducted to delineate the effect LBN had on the transcriptional profile of the BLA in adult rats We used rank-rank hypergeometric overlap analysis to compare overall gene expression pattern in males and females induced by LBN. and found a hotspot of genes upregulated in males and downregulated in females due to LBN. We narrowed our analysis to genes showing a significant difference between control and LBN and found 209 differentially expressed genes (DEGs) in females and 149 DEGs in males, with only 11 overlapping. KEGG and REAC pathway analysis were used to examine the biological processes altered by LBN. Pathways altered by LBN in females include alcoholism, cocaine addiction, glutamatergic synapse pathways (binding & activation), and regulation of dopamine secretion. Most of the genes in these pathways were downregulated due to LBN. In males, LBN increased transcription of the MAPK signaling pathway, neurexins and neuroligins, and neuron projection morphogenesis. Altogether we are developing a nuanced understanding of how mild ELA alters the transcriptome and SUD-related behaviors which may lead to advanced therapeutic techniques.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH F31 MH131351-02

**Title:** Epigenetic Manipulation in Nucleus Accumbens to Mimic Priming Effect of ELS on Susceptibility to Negative Behavioral Phenotypes After Adulthood Chronic Social Stress Experience

**Authors: *R. L. RASHFORD**[1], L. W. HIRSCHFIELD[1], N. ARZOUNI[1], C. J. PENA[2]; [2]Princeton Neurosci. Inst., [1]Princeton Neurosci. Inst., Princeton, NJ

**Abstract:** Early life stress (ELS) is one of the strongest predictors for the risk of developing depression and/or anxiety disorders. Our definition of ELS includes neglect, abuse (physical/sexual/emotional), and other highly negative childhood experiences. However, the specific role ELS plays in increasing this risk for mood disorders in adulthood is not well understood. Previous work found that ELS increases sensitivity to stress both behaviorally and molecularly in the nucleus accumbens (NAc), a key region of the reward pathway implicated in stress response. Specifically, we have found that the priming of chromatin into an open but not-yet active state occurs in response to ELS in the NAc, which may prime the genetic landscape to be more reactive to future stressors. However, the sufficiency of this priming response of chromatin opening in NAc to cause stress hypersensitivity is not yet known. We are therefore developing both promiscuous and site-specific epigenetic priming tools based on the methyltransferase SETD7 and heterochromatin-promoting enzyme KRAB to mimic and prevent the priming effect of ELS, respectively. We have identified regions of differentially accessible chromatin and used bioinformatic tools to predict specific genes potentially primed by ELS. Here we test the hypothesis that priming chromatin through deposition of histone 3, lysine 4 monomethylation (H3K4me1) in the NAc by SETD7 is sufficient to drive behavioral changes in mice exposed to mild social stress experienced in adulthood. SETD7 or GFP-expressing AAVs are bilaterally infused into male and female mouse NAc at ~PND10 to molecularly mimic early life stress experience. In adulthood, mice then experience mild social stress, followed by behavioral testing. These experiments provide insight into how epigenetic changes mediate the response to ELS to increase sensitivity to future stress.

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**Presentation Number:** NANO73.05

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** Hellman Fellowship

**Title:** Alteration of maternal perinatal interoception after early childhood trauma exposure: links with depression

**Authors: *B. L. CALLAGHAN**[1], P. SAVOCA[2];
[1]Univ. of California Los Angeles, Los Angeles, CA; [2]UCLA, Psychology, CA

**Abstract:** Perinatal depression affects up to 20% of pregnant women and mothers, and is more common in those who experienced childhood adversity. Beyond the impact of perinatal depression on maternal health and wellness, this condition is also an adverse caregiving environment for the developing fetus/child. Thus, assessing the mechanisms by which childhood trauma can impact peripartum depression will help to address intergenerational cycles of adversity. One potentially important mechanism to examine in this respect is changes in interoception during the peripartum. Interoception refers to the perception of the internal physiological state of the body (e.g., heart rate, hunger, taste, muscle tension etc.). Interoceptive ability is required for physiological regulation, and is thus a critical component of homeostatic functioning. Interestingly, dysregulation of interoception is recognized to play an important role in mental health. While the peripartum period is characterized by vulnerability to mental illness, and tremendous physiological change, we still know very little about how interoception changes during this important stage of adult development. In the proposed talk I will highlight unpublished data from the first wave of our ongoing longitudinal study (Sensations of Motherhood), comparing interoception in never pregnant women (N = 116) and first-time mothers in the second trimester (N = 118). We report striking differences in interoception in pregnant women, characterized by higher interoceptive accuracy, greater interoceptive attention, and more worrying about interoceptive cues relative to the never pregnant group. We also see that within the pregnant group, there is a negative association between childhood trauma exposure and interoception, and across all individuals there is a negative relationship between interoception and depression. These data suggest that childhood trauma impairs a normative boost in interoceptive accuracy in pregnancy that may place mothers at risk for depression.

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**Title:** Early life adversity promotes heightened startle through enhanced activity of corticotrophin releasing hormone neurons in the central amygdala of female mice

**Authors: *C. DEMAESTRI**[1], K. G. BATH[2];
[1]Columbia Univ., New York, NY; [2]New York State Psychiatric Inst., New York, NY

**Abstract:** Experiencing early life adversity (ELA) increases the risk of anxiety disorders, such as generalized anxiety disorder and post-traumatic stress disorder, with disproportionally higher risk in women compared to men. A core feature of these disorders is a disturbance in titrating behavioral responses to real and perceived threat which has been linked to hyperreactivity of the amygdala. A key neurobiological target for understanding the underlying mechanisms driving behavioral reactivity to threat are neurons expressing corticotropin-releasing hormone (Crh) in

the lateral central amygdala ($CeAL_{Crh+}$). $CeAL_{Crh+}$ neurons have been implicated in a host of processes associated with responsivity to threat, including modulating the startle reflex, the acquisition of conditioned fear, and anxiety-like behavior. Further sex biases in risk and symptom presentation have been proposed to be related to sexual dimorphic signaling of Crh+ across the brain that differentially influences a variety of Crh-dependent behaviors. However, given the varied functions regulated by $CeAL_{Crh+}$, and sex differences in the signaling of Crh+ that are region-specific, the underlying mechanisms that give rise to specific endophenotypes and how factors such as sex and ELA bias how distinct processes are impacted is unclear. In this work, we used the limited bedding and nesting model of ELA in mice to test its effects on startle, a translationally relevant behavioral phenotype used to assess sensitivity to threat. We tested if $CeAL_{Crh+}$ neurons were differentially recruited as a function of ELA and sex using *in vivo* fiber photometry imaging in a $Crh^{IRES\text{-}Cre}$ mouse line. We found sex-dependent changes resulting from ELA on the activity of $CeAL_{Crh+}$ neurons. ELA reared females, but not males, exhibited sustained $CeAL_{Crh+}$ activity during the presentation of a tone that was previously conditioned to be associated with a shock that persisted in the absence of the tone. The activity of $CeAL_{Crh+}$ was associated with a greater startle response and was necessary for engaging in startle. This work highlights sex and ELA as important factors when considering the functional substrates underlying anxiety disorders and has strong potential for guiding individualized treatment strategies that manage specific symptoms at the core of disorder.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH grant HD091376

**Title:** Pubertal stress produces lasting alterations to postpartum behavior and stress axis regulation

**Authors:** L. A. M. LUTHER, A. T. KHAN, B. M. KAREM, B. D. ELLIOTT, C. BOONE, ***K. E. MORRISON**;
West Virginia Univ., Morgantown, WV

**Abstract:** Early life stress is associated with a variety of negative outcomes in adulthood, including stress dysregulation and dysfunctional parental behaviors. We have previously shown that stress during puberty resulted in an altered hypothalamic-pituitary-adrenal (HPA) axis stress response in peripartum humans and mice. In humans, women who have experienced pubertal adversity showed increased postpartum depression scores. Here we sought to expand our understanding of the impacts of pubertal stress into the postpartum window. We hypothesized that peripubertal stress would impact both pup-directed and general anxiety-like behavior in the postpartum period. We conducted chronic variable stress (CVS) during the onset of puberty, from postnatal days (PN) 21-34. Female mice were exposed to a variety of auditory, olfactory, and tactile stressors. As adults, all females were bred with naive males. Upon confirmation of a copulation plug, females were singly housed throughout pregnancy and postpartum. On PN3, we measured behavior during a home cage pup retrieval task in order to assess naturalistic maternal behaviors. On PN7, we exposed dams to a maternal separation test to activate the HPA axis. We

collected tail blood to measure the corticosterone response to separation stress. Two hours after the stressor, we collected tissues of the HPA axis for measurement of relevant gene expression via qRT-PCR. In another cohort of animals, we tested anxiety-like behavior that was not related to pups. Postpartum females were tested on a battery of behavioral tests that included the open field, light-dark box, and social preference tests. Pubertal stress had a lasting impact on pup-directed behavior. In the pup retrieval task, CVS females had an increased number of partial, or failed, retrievals, an increased incidence of grooming pups outside of the nest, and decreased time in the nest zone of the cage. Pubertally stressed dams also showed an increased number of rearing behaviors during the test, which may indicate increased anxiety-like state. Pubertal stress also altered gene expression in all tissues of the HPA axis. Pubertal stress resulted in altered gene expression in the postpartum window in the PVN (Crh and Avp expression), pituitary gland (Crhr1 expression), and adrenal gland (Mc2r expression). Overall, these data show that pubertal stress results in lasting changes to the brain that, when interacted with the dynamic changes of pregnancy and postpartum, lead to altered responses to stress and disorganized maternal behavior. These studies provide insight into the complex risk factors that interact in the lifespan to produce negative outcomes for females and their offspring.

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**Title:** Effects of Maternal Separation on Risky Decision Making across the Lifespan

**Authors: *N. W. SIMON**, G. MINNES, A. WIENER;
Univ. of Memphis, Memphis, TN

**Abstract:** Early life stress (ELS) is increasingly common in the United States, and is associated with a multitude of enduring neural and behavioral aberrations. To develop treatments to mitigate the effects of ELS, it is critical to determine which aspects of cognition are vulnerable and when these disturbances manifest across the lifespan. Here, we tested the effects of maternal separation, an established rodent model of ELS, on risky decision-making in both adolescence (25-55 days old) and adulthood (80-100 days old). Risk-taking was assessed with the Risky Decision-making Task, in which rats choose between a safe reward and a larger reward accompanied by an escalating risk of punishment (mild foot shock). We observed that rats exposed to maternal separation were more prone to risky choice than controls during adolescence. Interestingly, this augmented risk-taking was no longer evident in adulthood. On average, rats were less risky during adolescence than adulthood. Males and females displayed comparable levels of risk-taking during adolescence, then adult males shifted toward increased risk-taking in adulthood. Finally, adult rats exposed to maternal separation were less sensitive to foot shock than controls. Collectively, these data show that ELS engenders risk-taking in adolescence but not adulthood, and that the development of risk-taking across adolescence to early adulthood differs based on sex. This has important implications for the development of

both behavioral and biological treatments to improve decision-making during the vulnerable adolescent period.

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**Presentation Number:** NANO73.09

**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH R01MH127850-01

**Title:** Early life adversity alters development of corticolimbic innervation and threat responsivity

**Authors: *C. CODY**, J. LARDIZABAL, C. MCKNIGHT, H. BRENHOUSE;
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**Abstract:** Typical development of threat evaluation can be disrupted by exposure to early life adversity (ELA) due to the rich postnatal development of involved circuitry. Specifically, childhood trauma in humans reportedly leads to a heightened acoustic startle reflex, as well as a blunted potentiation of acoustic startle in response to social threat cues. Prior work has shown hyper-innervation of glutamatergic basolateral amygdala (BLA) projections to the prefrontal cortex (PFC) in the adolescent time period following rearing in an adverse early life environment, but how these ELA-induced connectivity changes alter the startle circuitry required to execute a startle response and whether the BLA-PFC pathway is responsible for previously reported blunted startle response remains unknown. We directly tested whether BLA activity during a discrete adolescent period altered later acoustic startle, and if behavioral changes could be correlated with altered PFC innervation. To test these questions, we exposed rat pups to an ELA model of maternal separation on postnatal days (P) 2-21. Rats then underwent an injection of an inhibitory DREADD in the BLA in order to silence the excitatory projections during the adolescent time period of P33-39. Finally, rats were tested in an acoustic startle paradigm in late adolescence to assess the effects of reduced BLA-PFC connectivity on the ELA-induced blunted startle response. Density, intensity, and volume of axonal boutons in the BLA projecting PFC neurons were then assessed to elucidate how BLA inhibition during adolescence affects innervation later in life. These results explore how altered early life environments may affect the development of anxiety-related circuitry and anxiety responses later in life, and how rescue of this maladaptive response may be sex and developmentally specific.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH Grant 5K12HD052896

**Title:** Chronic early-life resource deprivation disrupts the landscape and function of cortical inhibitory neurons

**Authors: *R. M. RAJU**[1], A. DAVISON[1], J. F. SANTOYO[1], N. MILMAN[4], S. J. BARKER[2], K. ABDELAAL[3], L.-H. TSAI[1];

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**Abstract:** Nearly half of all children in the United States are exposed to adverse childhood experiences, with up to 20% experiencing chronic exposure to toxic levels of multiple stressors. Chronic exposure to early life stress (ELS) has dramatic, life-long consequences, tripling the risk of dementia, quadrupling the risk of depression and increasing the risk of suicide 30-fold. To study the biological impact of chronic ELS in a controlled and mechanistic manner, we developed a mouse model of ELS in which wild type mice are deprived of nesting and bedding material from postnatal day 2 into adulthood. Exposure to the chronic limited nesting and bedding (cLNB) model led to decreased weight gain relative to controls. Both male and female adult mice exposed to cLNB stress also displayed higher levels of anxiety, hyperactivity, and sociability relative to control mice. To determine the neural basis of these behavioral disruptions, we chose to focus on cortical inhibitory interneurons, as this cell population is actively undergoing migration and maturation during the initiation of the chronic stress paradigm. We assessed the distribution of cortical inhibitory interneurons across multiple brain regions using RNA in-situ hybridization for the interneuron markers Parvalbumin (PV), Somatostatin (SST), and Serotonin receptor 3a (5HT3a), which collectively stain nearly 100% of cortical interneurons. cLNB stress selectively altered the density of PV neurons in the prefrontal cortex and amygdala. Transcriptomic analysis revealed that stress-exposed prefrontal PV neurons up-regulate their metabolic and mitochondrial activity. Chemogenetic and optogenetic modulation of prefrontal PV neurons rescued anxiety deficits in cLNB exposed mice. Overall, this work confirms that cLNB stress induces changes in the landscape and function of cortical PV neurons, and that this cell-type may be a key driver of neurobehavioral changes induced by stress experienced early in life.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** R01MH122712
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**Title:** Serotonergic dysregulation and social dysfunction following early life stress

**Authors: *S. WOODS**, A. KISNER, R. BAHR, M. WYNALDA, S. HAUGHLAND, L. ESCOBAR, A. POLTER;
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**Abstract:** Early postnatal life is a sensitive period for the highly plastic, immature brain. During this time, developing cognitive and emotional circuitry may be susceptible to external social and emotional environmental stressors. These early life stressors, such as neglect, low socioeconomic status, and instability of resources can lead to increased risk of neuropsychiatric disorders like anxiety and depression in adulthood. The brain's serotonergic system, which originates in the dorsal raphe nucleus (DRN) and exerts widespread neurotrophic and neuromodulatory effects, has been implicated in several social behaviors, such as sociability and dominance behavior. Our

preliminary electrophysiological data shows that early life stress (ELS) leads to reduced excitability of DRN serotonergic neurons in juvenile (PND 15) and adult (PND 80) mice, but this deficit is absent in adolescence (PND 35). In addition, our behavioral data suggests alterations in adolescent social play and a robust subordinate phenotype in a social dominance assay in adult male and female mice following ELS. Based on these findings, we aimed to investigate the links between ELS-induced alterations in the serotonergic system and changes in social behavior. To model ELS, we utilized the limited bedding and nesting (LBN) paradigm in Pet1-Cre x Ai14 reporter mice and C57/Bl6 mice for electrophysiological and behavioral experiments, respectively. We used whole-cell electrophysiology in acute DRN slices to examine regulation of excitability and action potential properties by extrinsic and intrinsic factors, as well as synaptic transmission. Our data shows that changes in synaptic tone may underlie reduced excitability following ELS. We also used acute treatment with the selective serotonergic reuptake inhibitory fluoxetine to investigate the role of serotonergic tone in ELS-induced changes in social behavior. Our preliminary results may indicate that ELS leads to decreased social play behavior that may be improved by increased serotonergic tone following fluoxetine administration. Finally, we examined adult dominance behavior after fluoxetine administration by using the dominance test tube approach to assess intra-cage and inter-cage hierarchies. Our findings suggest that there are separate changes in social behavior between adolescence and adulthood that may be affected by fluoxetine administration, and these behavioral data coincide with alterations in electrophysiological properties of DRN serotonergic neurons, likely driven by synaptic inputs. Our ongoing experiments aim to shed light on the relationship between these electrophysiological and behavioral findings.

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**Topic:** A.06. Synaptogenesis and Activity-Dependent Development

**Support:** NIH Grant R01MH-116869
UAB Psychiatry startup funds

**Title:** Long-lasting impact of adolescent stress on postpartum social cognition: Unraveling circuit mechanism and glucocorticoid signaling

**Authors: *J. FRANCIS-OLIVEIRA**, K. KIM, S.-I. KANO, M. NIWA;
Psychiatry and Behavioral Neurobio., Univ. of Alabama at Birmingham Heersink Sch. of Med., Birmingham, AL

**Abstract:** Adolescent stress has long-lasting effects on adult behavior, particularly during the postpartum period, affecting both mothers and their children's quality of life. However, the specific circuit mechanisms underlying these behavioral changes remain unclear. This study aimed to investigate how adolescent psychosocial stress alters behaviors related to social cognition in the postpartum period through changes in brain circuitry and glucocorticoid levels. To evaluate social novelty recognition, we used the three-chamber social interaction test. Exposing female C57BL/6J mice to three-week isolation stress during late adolescence (35 days old) produced a sustained elevation of plasma glucocorticoids, accompanied by social novelty

deficits, only when combined with pregnancy and delivery (stressed dams). This was observed at one week postpartum and persisted up to three weeks postpartum, but not immediately after delivery. Considering the prelimbic cortex (PL) and anterior insula (AI) are involved in mood and social cognition, we postulated that adolescent psychosocial stress disturbs these areas, leading to postpartum behavioral deficits. Viral tracing experiments confirmed a pathway from AI to PL. We then performed in vivo calcium imaging, revealing a hypofunction of the AI-PL pathway which correlated with impaired social novelty behavior. Ontogenetic experiments demonstrated that activating the AI-PL pathway ameliorated social novelty deficits in stressed dams. To examine whether adolescent stress affects the AI-PL pathway through glucocorticoid receptor (GR) signaling, we established mice with AI-PL pathway-specific GR deletion (GR-KO mice). GR KO mice showed resilience to postpartum social novelty deficits induced by adolescent stress. In conclusion, our data indicate that adolescent psychosocial stress leads to hypofunction of the AI-PL pathway, resulting in social novelty deficits during the postpartum period, which requires GR signaling and correlates with increased glucocorticoid levels. These findings provide insight into the long-term physiological effects of adolescent stress, which significantly impacts postpartum behaviors in adulthood.

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**Nanosymposium**

**NANO74: The Aging Brain: Molecular and Cellular Mechanisms**

**Location:** WCC 146C

**Time:** Wednesday, November 15, 2023, 8:00 AM - 10:00 AM

**Presentation Number:** NANO74.01

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIA/NIH 1ZIAAG000539-01 (MRC)
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**Title:** Divergent patterns of aging across human brain regions at single cell resolution reveals links to neurodegenerative disease

**Authors: *M. DUFFY**[1], J. DING[2], R. LANGSTON[1], S. I. SHAH[3], M. A. NALLS[3,4], D. T. WHITAKER[1], P. K. AULUCK[5], S. MARENCO[5], J. R. GIBBS[2], M. R. COOKSON[1];
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**Abstract:** Age is the primary risk factor for neurodegenerative diseases including Parkinson’s (PD), Alzheimer’s and related dementias (ADRD), and amyotrophic lateral sclerosis (ALS). Previous studies have reported that chronological age is associated with altered gene expression profile across different brain regions. However, prior datasets have not disambiguated whether expression associations with age are due to changes in cell numbers or gene expression per cell.

In this study, we leveraged the resolution of single nucleus RNA-sequencing (snRNAseq) to examine changes in cell proportions and transcriptomes in four different brain regions from 12 donors aged 20-30 years (young) and 60-80 years (old). We sampled a total of 155,192 nuclei from two cortical regions (entorhinal cortex and middle temporal gyrus), and two subcortical regions (putamen and subventricular zone) with relevance to neurodegenerative diseases or the proliferative niche. We demonstrate that there are no changes in cellular composition of different brain regions with healthy aging. Instead, each brain region and major cell type in the brain shows distinct age-associated expression changes, including loss of protein synthesis genes in cortical inhibitory neurons, axonogenesis genes in excitatory neurons and in oligodendrocyte precursor cells, enhanced gliosis markers in astrocytes and disease-associated markers in microglia in addition to genes critical for neuron-glia communication. Importantly, we find cell type-specific enrichments of age associations with genes nominated by AD and PD GWAS, such as positive associations of apolipoprotein E (APOE; coefficient estimate=0.41, p=3.45x10-3) and leucine-rich repeat kinase 2 (LRRK2; coefficient estimate=0.33, p=5.78x10-5) specifically in microglia. Interestingly, these associations are independent of overall expression levels across cell types. Our data 1) highlight brain region and cell type-specific transcriptomic changes in aging that may contribute to selective vulnerability and 2) provide context for testing GWAS-nominated disease risk genes in relevant subtypes preclinically and subsequently, for developing more targeted therapeutic strategies.

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**Presentation Number:** NANO74.02

**Topic:** C.01. Brain Wellness and Aging

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**Title:** Spatial mapping of senescent cells in the aged mouse brain

**Authors: *C. M. CARVER**, P. GOMEZ, M. J. SCHAFER;
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**Abstract:** Cellular senescence is a conserved mechanism of aging characterized by cell cycle arrest and an inflammatory senescence-associated secretory phenotype. In the brain, molecular

heterogeneity, subregion diversity, and low abundance challenge the identification of senescent cells. Through single-cell RNA sequencing, we previously discovered that myeloid cells accumulate in the aged brain that exhibit overlapping signatures of senescence and disease-associated microglia (DAM), including downregulation of homeostatic genes and upregulation of chemoattractant factors, neurodegenerative risk factors, markers of lysosomal stress, cell cycle regulatory genes, and anti-apoptotic factors. However, the spatial location of these and other senescent cells and their influence in distinct brain microenvironments remains unclear. Here, we combined imaging and molecular biology methods with emerging spatial mapping technologies, to phenotype senescent cells in aged mouse brain. Through gene expression profiling of hippocampus, white matter, cortex, and cerebellum, we discovered that aged white matter tracts harbored greater p16, pro-inflammatory SASP, and DAM transcript levels relative to grey matter regions. Through immunofluorescent imaging we detected abundant cells co-expressing IBA1 (microglia marker) and GAL3 (senescence and DAM marker) in aged white matter regions, and senotherapeutic elimination of senescent cells reduced such senescent/DAM cells. We applied imaging mass cytometry to simultaneously map 35 cell-type and senescence markers and identified distinct populations of myeloid and non-myeloid cells expressing senescence markers in hippocampus, cortex, and adjacent white matter. We used GeoMx digital spatial profiling to map microenvironment-defined senescent myeloid transcriptional profiles and discovered that IBA1+GAL+ cells had a distinct gene expression signature from that of IBA1+ or GAL3+ cells, marked by increased DAM gene expression. Our findings demonstrate that white matter is a unique niche that accumulates senescent and DAM microglia in aging. Importantly, senescent/DAM cells in this niche are sensitive to senotherapeutic modulation, which may offer an important translational avenue for maintenance of healthy brain aging.

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Tel Aviv University President Award for women postdoctoral scholars (T.I.)

**Title:** Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17

**Authors: *T. IRAM**[1], T. WYSS-CORAY[2];
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**Abstract:** Recent understanding of how the systemic environment shapes the brain throughout life has led to numerous intervention strategies to slow brain aging. Cerebrospinalfluid (CSF) makes up the immediate environment of brain cells, providing them with nourishing compounds. We discovered that infusing young CSF directly into aged brains improves memory function.

Unbiased transcriptome analysis of the hippocampus identified oligodendrocytes to be most responsive to this rejuvenated CSF environment. We further showed that young CSF boosts oligodendrocyte progenitor cell (OPC) proliferation and differentiation in the aged hippocampus and in primary OPC cultures. Using SLAMseq to metabolically label nascent mRNA, we identified serum response factor (SRF), a transcription factor that drives actin cytoskeleton rearrangement, as a mediator of OPC proliferation following exposure to young CSF. With age, SRF expression decreases in hippocampal OPCs, and the pathway is induced by acute injection with young CSF. We screened for potential SRF activators in CSF and found that fibroblast growth factor 17 (Fgf17) infusion is sufficient to induce OPC proliferation and long-term memory consolidation in aged mice while Fgf17 blockade impairs cognition in young mice. These findings demonstrate the rejuvenating power of young CSF and identify Fgf17 as a key target to restore oligodendrocyte function in the aging brain.

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**Title:** Single cell transcriptomics analyses of particulate matter exposure in killifish identify the molecular mechanisms of age-dependent environmental neurotoxicity

**Authors: *P. BHATTARAI**[1,2,3], F. KASTURY[4], N. NELSON[2], K. DESANTIS[5], M. I. COSACAK[6], E. YILMAZ[2,3], S. NICHOLAS[7], C.-K. HU[8], B. BOSTICK[9], C. KIZIL[2,3], B. L. PEARSON[5];
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**Abstract:** Iron (Fe) dis-homeostasis in the brain is related to neurodegeneration, where Fe2+ and Fe2+/Fe3+ are toxic species. The exposure to particulate matter (PM) from the environmental source could cause Fe particles to cross the blood-brain barrier and brain neurotoxicity, yet the underlying molecular mechanisms are poorly understood. Here, we used aging model African turquoise killifish (Nothobranchius furzeri) to assess the alterations in gene expressions upon exposure to industrial activity PM. Steel manufacturing impacted PM (<74 μm) was obtained from Taranto (Southern Italy), a region with significant associations between dust exposure and negative neurobehavioral effect in children. Young (1.5 months) and old (6 months) killifish were exposed to either 200 mg PM/L (treated) or system water (control) for 72 hours. To detect Fe particles in the brain, fish was euthanized, with brain cryosectioned and dehydrated, followed by detection of Fe hotspots and their speciation using X-ray absorption spectroscopy at the National Synchrotron Light Source II. We found significantly higher numbers of Fe hotspots in

the telencephalon of exposed killifish brain compared with unexposed fish, confirming that Fe-rich particles from the PM sample crossed the blood brain barrier and accumulated in the brain. Speciation analysis confirmed the presence of Fe2+ and Fe2+/Fe3+ in brain tissues. To determine the effects of particle treatment on brain cell types, we performed single cell transcriptomics in the telencephalon of control and treated killifish of both ages. After 10X sequencing, mapping to killifish genome assembly and clustering analyses, all major cell types present in the brain were investigated. We identified all major brain cell types, including neurons, glia, immune cells, and vasculature via clustering analysis. Differential gene expression and pathway analyses revealed age-related effects of particle toxicity, particularly in neurons, glia and immune cells. In older killifish, neurodegenerative and immune responses were more pronounced, whereas in young animals the detoxification and neurogenic pathways were enriched. To further validate the single cell sequencing data, immunohistochemical (IHC) analysis were performed and analysed qualitatively. We found that particle exposure resulted in reduced synaptic density, reduced stem cell proliferation and neurogenesis and increased immune responses. Our results provide the first single cell analyses on killifish brains with particle toxicity in relation to aging, and propose the promising use of this model for age-related environmental toxicity.

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**Topic:** C.01. Brain Wellness and Aging

**Support:** Christopher Newport University Startup Funds
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**Title:** The impact of Intestinal Barrier Dysfunction on Aging and Disease

**Authors:** S. LE, D. GRACE, S. SABA, C. AUBY, ***A. SALAZAR**;
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**Abstract:** Aging is a process marked by a continuous decline in multiple physiological functions, including intestinal barrier function, which is tightly linked to longevity in *Drosophila melanogaster* and other organisms. We have previously shown that altered expression of occluding junctions in the guts of fruit flies can lead to various hallmarks of aging, including modulation of intestinal homeostasis, variations in microbial dynamics, changes in immune activity, and alterations in lifespan. Loss of a specific occluding junction, 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 following oral infection with pathogenic bacteria. Furthermore, intestinal up-regulation of Ssk improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. Additionally, perturbing barrier function in the gut has non-cell-autonomous impacts, including alterations in the brain and muscle. This work adds more information about the impact of the gut on tissue outside of the gut

and begins to address communication between the gut and the brain and muscles in disease models. 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.

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**Topic:** C.01. Brain Wellness and Aging

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**Title:** Implication of normal aging on inflammation, perineuronal nets, and parvalbumin interneurons in the dorsal striatum.

**Authors: *Z. A. COLON**[1], K. MAGUIRE-ZEISS[2];
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**Abstract:** Neurodegenerative disorders like Parkinson's disease (PD) are multifactorial diseases with the largest risk factor being age. Despite this many studies fail to take into consideration normal aging processes when studying age-related neurodegenerative disorders. This preliminary study aims to quantify the effects of normal aging on a subset of inflammatory markers known to alter extracellular matrices like perineuronal nets (PNNs) and synaptic connections of parvalbumin (PV) interneurons. We hypothesize that neuroinflammatory processes affect PNN homeostasis and PV function during age-related neurodegenerative disorders. Using cohorts of C57BL/6 male mice (N=5/group) that were aged to 4-months old (mo; Young) and 22-mo (Old). we focused on changes in the dorsal striatum, an area known to be affected in PD. RT-qPCR analysis of mRNA expression showed significant differences in the expression of complement-related proteins, matrix metalloproteinases (MMPs), CD68, interleukin-(Il)-6, and CCL5. These findings suggest an increase in complement-dependent inflammation, synaptic pruning, and phagocytosis during normal aging. Immunostaining analysis of the total PV cells (Anti-*PV*), PNNs (*Wisteria floribunda agglutinin*), and colocalization of these two markers was quantified in subregions of the dorsal striatum [dorsomedial (DMS) and dorsolateral (DLS)]. There were no significant differences between the groups for total cell numbers, however, the DLS of the young mice showed a significantly higher portion of PV cells surrounded by PNNs (39.44%) compared to the DLS of old mice (27.41%) ($F(3,16)=8.030$, $p=0.0017$) and DMS of both young (22.07%) mice. Together, these findings show that while there are no significant changes in the number of PNNs and PV interneurons there are several inflammatory changes that could alter PNN structure and synaptic connections. This preliminary study provides important information to inform our power analyses for future studies and establishes that there are baseline inflammatory differences in the dorsal striatum in old age.

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**Title:** Changes in glia-neuron interaction mediated by fractalkine are associated with aging

**Authors: *R. VON BERNHARDI**[1], C. METZ[2], J. L. EUGENIN[3];
[1]Univ. San Sebastian, Fac. for Hlth. Care Sci., Santiago, Chile; [2]Univ. San Sebastian, Santiago, Chile; [3]Biol., Univ. de Santiago, Santiago, Chile

**Abstract:** Aging is associated with changes in microglial cells activation, resulting in increased oxidative stress and neuroinflammation. Furthermore, it has been reported an increased neurotoxicity in conditions associated with an increased inflammatory activation of aged microglia. Given the fact that aging is the major risk factor for several neurodegenerative diseases leading to dementia, changes observed in age-related microglial cell activation could be key players in neuronal dysfunction and processes leading to CNS injury. The various mechanisms involved in the regulation of microglia by neurons, their age-related changes, and their involvement in the regulation of synaptic function, and glia-mediated neuroprotection and -degeneration, are not well understood. Age-related changes in glial cell activation involve differences in glial cell activation and cell signaling depending on inflammatory and regulatory cytokines. They depend on various factors, including cytokines like TGFβ, and Scavenger Receptor A (SR-A), which participate in the regulation of glial cell activation, and the Fractalkine/CX3CR1 signaling involved in the regulation of microglia by neurons. We have evidence that aging-related changes result in the modification of glial cells regulation at various levels. Here, we will characterize and discuss age-dependent changes of various pathways in the brain of wild type (WT) and an inflammatory mouse model (knockout for the scavenger receptor A, SRA-KO). WT and SRA-KO mice of 3-6, 12- and 20-month-old were analyzed. To evaluate the effect of inflammation, mice were administered intraperitoneally 1 mg/kg of LPS or vehicle. 24 hour later, the relative presence of various receptors was analyzed by qRT-PCR and western blot. Brain localization and distribution of receptors and signaling pathways were assessed by immunohistochemistry. We observed that changes on TGFβ, activation of inflammatory signaling pathways, SR-A and fractalkine were profoundly affected by aging, even in the absence of exogenous inflammatory conditions. Several changes are already observed at 12 months, including a 3-fold increase of Fractalkine compared with that of young mice, with predominance of the soluble fraction. Our findings suggest that aging favors the presence of Fractalkine in their soluble (cleaved) forms, promoting microglial inflammatory activation and a neuroinflammatory environment.

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**Title:** Microglia from cognitive SuperAgers exhibit downregulation of disease associated and oxidative burst related genes and upregulation of genes involved in DNA repair

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**Abstract: Rationale:** SuperAgers are individuals over age 80 with superior episodic memory, at least as good as individuals 20 to 30 years their junior. We have observed significantly higher rates of proliferation of microglia, the innate immune cells in the brain, isolated from SuperAger brains when compared with their cognitively average peers. We hypothesized that microglia transcriptional profiles could contribute to the SuperAging phenotype. **Methods:** RNA from microglia of 3 SuperAgers and 4 cognitively normal controls cultured to passage 4 were isolated and purified. RNASeq libraries were prepared using the Lexogen QuantSeq 3' RNA Library kit. Libraries were assessed using the Agilent High Sensitivity DNA chip. All the libraries were pooled and diluted to 1.8 pM and sequenced on an Illumina Miniseq instrument set to generate fastq files as output. Bioinformatic analysis was performed using the BlueBee integrated data analysis pipeline which trims sequences (using *Bbduk)*, aligns sequences to the Human genome (using STAR aligner) and counts genes (HTSeq-count). Differential expression was obtained by running read counts through DE-Pipeline in BlueBee. **Results:** A total of 21,699 human genes were analyzed. When initially isolated cells were compared with microglia cultured to third passage, known microglia genes were expressed in both while genes known to not be expressed in microglia were not expressed in either. Additionally, no expression was observed in genes expressed in other cell types, including astrocytes. Of the total genes analyzed, 434 were differentially expressed ($p<0.05$). SuperAger microglia exhibited downregulation of several transcripts involved in aging-related disease pathogenesis. These include Adap2, MAP2, RBM20, CNTN3 among others. SuperAgers also exhibited downregulation of several transcripts encoding for mitochondrial proteins that regulate the oxidative burst specifically genes that regulate NADPH dehydrogenase involved in mitochondrial electron transport. Diseases associated with these genes include encephalopathy and stroke like episodes. In contrast, the most highly upregulated genes in SuperAgers include genes involved in DNA repair and membrane trafficking. **Conclusions:** These results confirm that human microglia maintain their phenotype in culture. SuperAgers appear to have downregulation of genes involved in disease pathogenesis and mitochondrial oxidative burst that could injure the tissues and cells. In contrast genes involved in DNA repair appear to be upregulated. These data could provide a basis for the biological features that contribute to the SuperAgers phenotype.

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**Nanosymposium**

**NANO75: Tauopathy: Cellular and Molecular Mechanisms**

**Location:** WCC 144

**Time:** Wednesday, November 15, 2023, 8:00 AM - 10:45 AM

**Presentation Number:** NANO75.01

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

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**Title:** Microscale instability of neural dynamics drives pathological behavior

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**Abstract:** While emerging data suggest that pathological forms of tau may affect the circadian control of sleep in Alzheimer's disease (AD) patients, the biophysical mechanisms underlying this relationship are poorly understood. The identification of disease-relevant biophysical models can provide a quantitative understanding of the pathophysiological mechanisms underlying the onset and progression of AD, which will provide a framework for the development of AD-modifying therapies in the future. In the present study, we sought to characterize the mechanistic biophysical framework to explain how pathological tau affects circadian clock-driven neuronal computations in a Drosophila model of AD by ectopically expressing Tau4RΔK, a proaggregant human tau mutation, in DN1p circadian clock neurons. We characterize the electrophysiological properties of DN1p neurons expressing Tau4RΔK and find that the variability of depolarization onset speed during the action potential initiation process is significantly increased due to increased dynamic instability in the membrane potential dynamics of DN1p neurons. We identify increased noise variability of voltage-gated sodium channels during their non-stationary inactivation process as the biophysical origin of this increased dynamic instability in the membrane potential dynamics of DN1p neurons. We also found that flies expressing Tau4RΔK in DN1p neurons have increased sleep fragmentation and reduced sleep amount, especially during the night. Importantly, we show that Tau4RΔK expression in the brain significantly reduces lifespan in Drosophila. We show that antiepileptic drug administration extends the shortened lifespan of Tau4RΔK flies via stabilizing effects of dynamic instability suppression of membrane potential dynamics of DN1 neurons expressing Tau4RΔK. Further, we find that human iPSC-derived neural cultures from Alzheimer's disease patients show similar instability of neuronal dynamics, which can be suppressed by antiepileptic drug administration. Taken together, our data reveal the circumstances under which vulnerable phenotypes emerge to influence macroscopic phenotypes.

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**Presentation Number:** NANO75.02

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

**Support:** 5R01AG057962

**Title:** Regions with higher functional connectivity to the medial temporal lobe have higher tau accumulation even after controlling for their Euclidean distance

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**Abstract: Introduction:** Tau protein (one of Alzheimer's disease pathologies) seems to spread through inter-synaptic neural connection highlighting the possibility that functional connectivity between regions could facilitate its spread from one region to next. While recent neuroimaging studies provides evidence for such facilitation, the physical distance between two regions could be considered as a confounding factor for this analysis. In this study we aim to show that the effect of FC on the spread of tau is independent on the distance between two regions. **Method:** We identified 211 participants in which the number of regions with elevated tau (SUVR>1.25) were more than 10, but less than 100 regions. We also computed averaged functional connectivity of the MTL regions with the rest of the brain regions in these participants. In addition, we have computed the Euclidean distance between the center of the MTL regions and the rest of the brain regions. Multiple linear regression is used to model the tau accumulation in the cortical regions in respect to their functional connectivity to the MTL regions controlling for their Euclidean distance from the MTL regions. **Results:** As expected MTL regions shown to have the highest frequency of observing elevated tau (>50%). Fig. 1 shows the association of all regions FC with MTL regions versus their tau accumulation. As seen, the functional connectivity of the regions with MTL were significantly ($\beta=0.65$ $p<10^{-14}$) associated with the tau accumulation in those regions after controlling for the distance between the regions and the MTL. **Conclusion:** Our results indicates that the inter-regional functional connectivity could be considered as a facilitating mechanism in spreading tau from MTL to the rest of the brain and this facilitation is independent of the Euclidean distance between MTL and other regions.

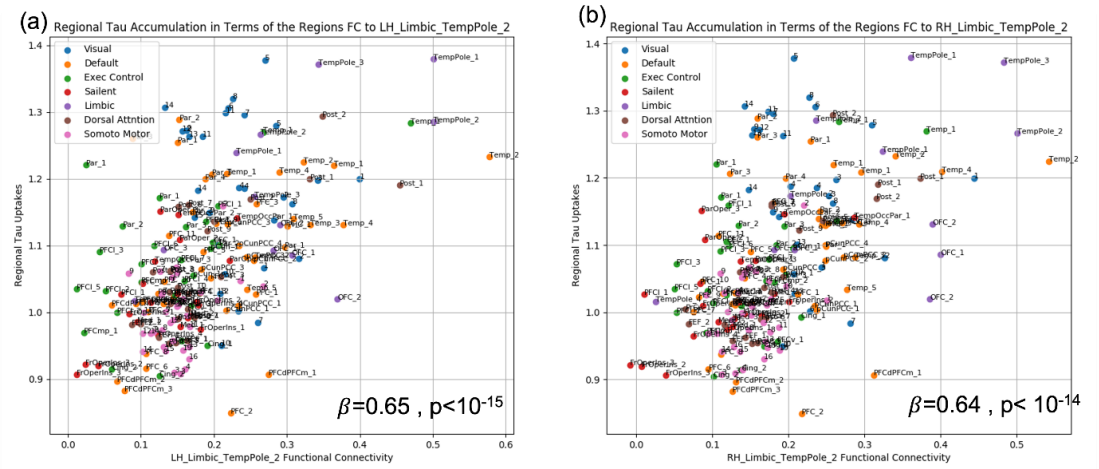


Figure 1: Regional tau accumulation is strongly associated with the functional connectivity of the region and MTL regions a) left hemisphere temporal pole 2 (closest to entorhinal) b) right hemisphere temporal pole 2 (closest to entorhinal) , controlling for the inter-regional Euclidean distance. Regions of different functional connectivity networks are depicted with different color.

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

**Support:** FIL Foundation
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**Title:** Frontotemporal dementia due to the MAPT 10+16 mutation is associated with changes in synaptic gene expression

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**Abstract:** Mutations in the *MAPT* gene encoding tau protein cause autosomal dominant neurodegenerative tauopathies including frontotemporal dementia (often with Parkinsonism) and progressive supranuclear palsy. In Alzheimer's disease, the most common form of tauopathy, synapse loss is the strongest pathological correlate of cognitive decline. Recently, PET imaging with synaptic tracers revealed clinically relevant loss of synapses in primary tauopathies including frontotemporal dementia with tau pathology; however, the molecular mechanisms leading to synapse degeneration in primary tauopathies remain largely unknown. In this study, we examined post-mortem brain tissue from people who died with frontotemporal dementia with tau pathology caused by the *MAPT* intronic exon 10+16 mutation (n=12), which increases splice variants containing exon 10 resulting in higher levels of tau with four microtubule binding domains. We used RNA sequencing and histopathology to examine temporal cortex and visual cortex, to look for molecular phenotypes compared to age, sex, and RNA integrity matched participants who died without neurological disease (n=12). RNAseq reveals substantial downregulation of gene expression involved in synaptic function including downregulation of pathways involved in synaptic transmission, both short and long-term synaptic plasticity,

regulation of NMDA receptor activity, synapse assembly and learning. Upregulated biological pathways included those involved in transcriptional regulation, histone deacetylation, and DNA damage response. Histopathology confirmed increased pathological tau accumulation specifically in temporal cortex (t=-5.4, p=0.0001 post-hoc Tukey test after linear mixed effects model), a trend toward loss of presynaptic protein staining (ANOVA after linear mixed effects model on Tukey transformed data: F[1,19.2]=3.13, p=0.09), and region-specific significantly increased colocalization of phospho-tau with presynapses and excitatory post synapses in temporal cortex. In contrast to our recent study of Alzheimer's disease, in *MAPT* 10+16 carriers with frontotemporal dementia we do not see differences in the burdens of synaptic proteins colocalised with astrocyte or microglial staining. Our data indicate that synaptic tau pathology likely contributes to pathogenesis in frontotemporal dementia with tau pathology caused by the *MAPT* 10+16 mutation, and that glial phagocytosis of synapses is not driving the phenotypes at least at the end stage of disease.

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

**Support:** NIH Grant R01AG066211

**Title:** Xbp-1s transcriptional targets in the endoplasmic reticulum unfolded protein response ameliorate tauopathy

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**Abstract:** Protein homeostasis (proteostasis) mechanisms fail with aging and disease, promoting toxic protein accumulation. Neurons are particularly vulnerable to proteostatic disruption leading to aging related neurodegeneration. Abnormal activation of the endoplasmic reticulum unfolded protein response ($UPR^{ER}$) is implicated in tauopathies, a group of neurodegenerative diseases characterized by pathological accumulation of the microtubule-associated protein tau. The $UPR^{ER}$ contains three branches with PERK, IRE1α, and ATF6 acting as ER unfolded protein sensors, which collectively regulate translational capacity and ER-associated degradation in response to ER stress. Previous work showed neuronal overexpression of IRE1α branch $UPR^{ER}$ transcription factor XBP-1s suppresses tauopathy in *C. elegans*. Whole-genome RNA sequencing of *C. elegans* overexpressing XBP-1s showed upregulation of the following genes with human homologs compared to non-transgenic controls: *csp-1, F42G8.7, F41E7.6, Y19D10A.16, C01B4.6, dnj-28, hsp-4, ckb-2, mct-2, lipl-3,* and *eol-1*. Surprisingly, each one of these genes is required for *xbp-1s*-mediated suppression of tauopathy, suggesting that XBP-1s activates a broad and non-redundant network of cellular mechanisms to reduce tau pathology. Of these, we examined the critical $UPR^{ER}$ regulator BiP/hsp-4, the ER resident HSP70 homolog. *Hsp-4* loss of function, but not loss of the cognate ER resident DNAJ protein *dnj-28,* eliminates *xbp-1s*-mediated suppression of tauopathy. While *hsp-4* loss of function exacerbates tau-induced

behavioral deficits, tau protein level and phosphorylation are unaffected. High level overexpression of *hsp-4* exacerbates tau-induced behavioral deficits and protein accumulation, while moderated *hsp-4* overexpression ameliorates this phenotype. Furthermore, caspases also appear to play an important role downstream of XBP-1s: both the XBP-1s target *csp-1* and its non-XBP-1s target gene family member *ced-3* are required for *xbp-1s*-mediated suppression of tauopathy. In summary, we present a dataset illuminating the mechanism of *xbp-1s*-mediated suppression of tauopathy involving a suite of diverse transcriptional targets. This dataset provides new therapeutic ideas that may be leveraged into novel treatments for tauopathy disorders.

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

**Support:** NIH Grant R01AG067048

**Title:** Stimulation of the NADK2-NADKH-PYCR1-proline pathway by tau oligomers rewires mitochondrial metabolism and controls its uptake by human neurons.

**Authors: *E. PARDO**[1], T. KIM[2], X. SUN[3], G. MINGLEDORFF[1], G. S. BLOOM[4], A. NORAMBUENA[5];
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**Abstract:** Transcellular spread of pathological forms of the microtubule-associated protein tau, mitochondrial dysfunction, and neuronal loss are key signatures of Alzheimer's disease (AD). However, how these processes are mechanistically connected remains elusive. Mitochondria are the main producer of ATP, and this energy-providing function directly depends on the coenzymes contained within the organelle. Two important coenzymes are the pyridine nucleotide NADH, and its phosphorylated form NADPH. Although an optimal NADH concentration is essential for mitochondrial function, recently it has been evident that the mitochondrial NADPH pool also has a critical role in regulating proline and collagen synthesis. Mutations in NADK2, the mitochondrial $NAD^+$ kinase responsible for maintaining the mitochondrial NADPH pool, have been shown to have profound neurological consequences in humans, but NADK2's role in AD has not been explored. Using NPC-derived human neuron cultures, CRISPR/CAS9 technology and two-photon fluorescence lifetime imaging of NAD(P)H, we found that sublethal doses of tau oligomers made from recombinant (rTauOs) or human brain-derived tau (bdTauOs), increased the mitochondrial content of NADPH, along with a significant decrease in ATP levels. Mitochondrial proline synthesis involves the action of three enzymes: NADK2, P5CS and PYCR1 (NNPP pathway); the last two catalytically consume NADPH with different affinities. We found that rTauOs increase neuronal expression of NADK2, P5CS and PYCR1, as well as expression of both proline and collagen VI in neurons. These effects were completely blocked when NADK2, P5CS and PYCR1 expression levels were reduced using lentiviral-mediated delivery of antisense-shRNA. Importantly, NADK2 and PYCR1 expression were upregulated in the human AD brain and iNeurons. To better understand the neuronal role of NADK2, we

analyzed the proteomic profile of CRISPR/CAS9 NADK2-KO human neurons using mass spectrometry. Among others, the expression of LRP1, a major TauO receptor at the plasma membrane, was highly compromised in NADK2-KO neurons. Remarkably, we found the expression of LRP1 to be increased in neurons treated with either rTauOs or bdTauOs. In addition, LRP1 expression was found to be higher in neuron cultures expressing the P301L and P301S tau mutants. Finally, the incorporation of biotin-labelled rTauOs was reduced by 50% in NADK2-KO human neurons. Altogether, these results suggest that TauO-mediated dysregulation of the NNPP pathway controls the expression of its own receptor, thus regulating a key aspect of TauO toxicity. Dysregulation of the NNPP pathway could be an early contributor to AD initiation.

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**Title:** Structural and functional damage to neuronal nuclei caused by extracellular tau oligomers

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**Abstract:** Oligomeric and filamentous tau are key pathogenic factors in tauopathies. These aggregates are released into the extracellular space and taken up by neurons, contributing to the spread of pathology. While the mechanisms of this prion-like spread have been extensively studied, the cell biological responses of neurons to aggregated tau have received much less attention. In this study, we investigated the impact of extracellular tau oligomers (xcTauOs) on nuclear structure and function in cultured primary mouse neurons. The cultures were stained with antibodies against lamin B1 or Lap2β, major subunit proteins of the nuclear lamina, which is a meshwork of intermediate filaments underlying the inner nuclear membrane. Within one hour of exposure to xcTauOs, neurons developed nuclear invaginations characterized by inward folds of the nuclear lamina that extend towards the deep interior of the nucleus. This deformation pattern was also observed in human and mouse brain tissues affected by tauopathies. Furthermore, we discovered that the impact of xcTauOs on nuclear shape depend on intracellular tau, as nuclear invaginations were not detected in cultured neurons derived from tau knockout mice unless tau expression was restored by lentiviral transduction. The nuclear lamina is involved in key nuclear activities, such as nucleocytoplasmic transport, chromatin organization and DNA transcription. To evaluate functional consequences of xcTauO-induced nuclear invagination, we performed

dextran exclusion assays in permeabilized neurons and examined cycling of Ran GTPase between the nucleus and cytoplasm in live neurons. Our results demonstrate that xcTauOs compromise the diffusion barrier maintained by nuclear pore complexes (NPCs) and disrupt active nucleocytoplasmic transport. Moreover, in cultured neurons exposed to xcTauOs, we observed an increase in H3K9Me3, a histone marker for heterochromatin. Based on this finding, we explored fluctuations in mRNA levels using the nanoString nCounter platform with the neuropathology panel for 760 genes, and by qRT-PRC. Differential gene expression analysis revealed that several of the genes up-regulated by xcTauOs are associated with transcription and gene splicing, and that the most highly up-regulated gene is *MAPT*, which encodes tau. Altogether, our findings implicate xcTauOs in altering nuclear architecture, damaging NPCs, and triggering a positive feedback loop that stimulates production of excess tau mRNA, and possibly by extension, more tau protein and toxic TauOs.

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**Title:** Low density lipoprotein receptor family members differentially regulate tau and apolipoprotein e

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**Abstract:** Alzheimer’s Disease (AD) is a leading cause of dementia and a major public health crisis impacting millions of people worldwide, and its etiology remains poorly understood. Multiple genetic mutations have been identified as risk factors for AD, including a) inheritance of the e4 allele of apolipoprotein E (ApoE4), which is the strongest genetic risk factor for AD, and b) several single nucleotide polymorphisms in the SORL1 gene, which encodes the sortilin related receptor 1 and shares structural homology with LDL receptor family members. Intriguingly, several AD-associated proteins bind and are regulated by members of the LDL-receptor family, including LRP1, VLDLr, and ApoER2. Furthermore, LRP1 was recently identified as major endocytic receptor for tau and regulates tau internalization, degradation, and seeding in a manner that is modified differentially by ApoE isoforms. Given the close link between LDL receptor family members and AD-associated proteins, we hypothesized that

additional members of this receptor family may also regulate tau trafficking, possibly in an apoE-specific manner, and here we investigate LRP1-, SORL1-, and VLDLr-dependent regulation of tau trafficking. Using surface plasmon resonance experiments we found that in addition to binding to LRP1, tau binds sortilin-related receptor (SORL1) and very low-density lipoprotein receptor (VLDLr) with high affinity. $^{125}$I-labelled tau uptake assays were used to investigate the role of SORL1 in endocytosis. When SORL1 expression was knocked down using siRNA in H4 cells we observed a significant increase in the LRP1-mediated degradation of $^{125}$I-labelled tau, revealing that SORL1 directs tau away from the lysosomal degradative pathways. The P301S FRET biosensor assay was used to assess SORL1's role in cytosolic tau seeding, and we found that SORL1 promotes cytosolic tau seeding induced by pathogenic forms of tau. Interestingly, a mutation in the SORL1 gene (N1358S) that is associated with increased risk of AD demonstrated a significant increase in tau seeding when comparted with WT SORL1. Together, these data suggest that SORL1 impacts the trafficking of tau to lysosomes and promotes tau seeding. We are currently investigating the role of VLDLr in tau processing. Collectively, our studies show that multiple members of the LDL receptor family interact with tau, differentially affecting the processes of tau uptake, degradation, and seeding. While LRP1 still appears to be the primary endocytic receptor for tau, understanding how each receptor modifies tau trafficking may be key to understanding AD pathology.

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

**Support:** TMU Strategic Research Fund

**Title:** Microtubule Affinity-Regulating Kinase 4 (MARK4) enhances tau toxicity via stress granule assemblies

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**Abstract:** Abnormal protein accumulation is associated with many neurodegenerative diseases, such as Alzheimer's disease. These proteins, including a microtubule-associated protein tau, undergo conformational changes to form neurotoxic oligomers and fibrils. Recently, the sequestration of these proteins into stress granules has been suggested to trigger the formation of pathological aggregations. The formation of stress granules can be induced by various stressors, and an RNA-binding protein TIA-1, which promotes the assembly of stress granules, has been reported to enhance tau toxicity. However, how stress granule assembly is enhanced in disease pathogenesis is not fully understood. Here we show that Microtubule affinity regulating kinase 4 (MARK4), which is known as a tau kinase and has been linked to elevated risks of AD, promotes stress granule formations. MARK4 expressed in HeLa cells was localized to stress granules with TIA-1. MARK4 expression significantly increased the number of stress granules. Kinase-inactive mutant MARK4 localized to stress granules and enhanced stress granule formation,

indicating that kinase activity is dispensable for this function of MARK4. Deletion of the C-terminal half after the kinase domain, including the spacer domain, abolished its stress granule localization and its effects on stress granule assembly. When tau was co-expressed, tau proteins were sequestered in stress granules with MARK4. MARK4 and TIA-1 synergistically increased tau phosphorylation at MARK4 target sites as well as tau protein levels, suggesting enhanced stress granule assemblies underlie the accumulation of phosphorylated tau. We also found that, in a fly model of tau toxicity, knockdown of the fly homolog of TIA-1 mitigated tau toxicity, while MARK4 expression suppressed this effect. Our results suggests that MARK4 enhances tau toxicity via stress granule assemblies. Since tau phosphorylation in the microtubule-binding repeats has been reported to enhance tau phase separation *in vitro*, we propose a model that MARK4 upregulation in AD brains promotes pathological tau formation via tau phosphorylation as well as enhanced assemblies of stress granules.

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

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**Title:** Mutant tubulins confer resistance to pathological tau

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**Abstract:** Misfolded and aggregated pathological tau is the primary component of lesions present in a number of age-related diseases (tauopathies) including Alzheimer's Disease (AD). With a rising aged population the prevalence of these diseases will become an enormous healthcare, economic and social burden. To-date there are no clinically proven disease altering treatments. The nematode, *C. elegans,* provides a powerful and uniquely genetically tractable tool through which to model human tauopathic neurodegeneration. The *C. elegans* models of tauopathy used here express human tau pan-neuronally and recapitulate a number of characteristic features of human disease: motility deficits indicative of neuron dysfunction, progressive tau aggregation, neuron loss and shortened lifespan. Forward genetic screening using our *C. elegans* models has led to the discovery or several genetic suppressors of pathological tau. α-tubulin and β tubulin heterodimers are the building blocks of microtubules, tube shaped polymer proteins that play key roles in the maintenance of cell structure and tracks for motor-driven transport. Microtubules exist in phases of dynamicity (growing and shortening) or stabilization (neither growing or shortening) and the balance between these phases within a cell is thought to be tightly regulated. As tau primarily functions to bind and stabilize microtubules, microtubule dysregulation has been hypothesized to play a role in the progression of tauopathic disease, though the specific toxic mechanistic dysfunction has remained unclear. Recently, we have discovered several mutant α-tubulin genes that modify tauopathy-like phenotypes in

transgenic *C. elegans* models. Mutations in *tba-1*, *tba-2* and *mec-12* suppress human tau-induced motility deficits and neurodegeneration. To begin to understand the underlying molecular mechanisms driving suppression of toxic tau phenotypes we tested levels of tubulin gene expression, total tau protein levels, phosphorylation and tau aggregation. Through these studies, we aim to elucidate whether the mechanisms of mutant tubulin suppression of tau induced pathology are based in changes to tau-microtubule interactions, contributing to greater understanding of the roles of the cytoskeleton in neurodegenerative disease.

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**Title:** Loss of tubulin tyrosine ligase is a novel feature of sporadic and familial AD and a regulator of tau hyperphosphorylation

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**Abstract:** Background: We have recently shown that loss of tubulin tyrosine ligase (TTL) and subsequent disruption of α-tubulin retyrosination is a feature of AD and a promoter of synaptic loss by inhibiting microtubule entries into dendritic spines. TTL+/- mice have cognitive deficits, altered LTP, synapse loss and increased levels of detyrosinated and Δ2 tubulin, two tubulin modifications that accumulate on non-dynamic microtubules. We also found that low levels of phospho-tau correlated with higher levels of detyrosinated and Δ2 tubulin in pyramidal neurons residing in the anterior hippocampal formation of human AD brains, raising the question of whether improper microtubule longevity drives early stages of tau hyperphosphorylation in AD. Here, we demonstrate that loss of TTL enhances both AMPK activation and tau phosphorylation in cultured rat hippocampal and human cortical neurons, and present preliminary evidence on the mechanisms by which this regulation may occur and contribute to TTL-loss dependent synaptic dysfunction.
Methods: Human cortical neurons derived from iPSC lines in which the London familial APP mutation V717I was knocked into one allele of the IMR90 control using CRISPR/Cas9, were used as a cellular model of familial AD. Lentiviral infection with shRNA against TTL was used to silence TTL expression. Microtubule dynamics were measured by confocal time-lapse microscopy in neurons expressing EB3-EGFP, a protein that tracks the growing plus ends of microtubules. Immunoblotting was performed to measure both unmodified and modified levels of TLL, tubulins, AMPK subunits, total tau, p262 and AT8 phospho-tau.
Results: In the APP neurons, we found that a reduction in TTL levels correlated with an increase in p262/AT8 phospho-tau variants and phospho-AMPK, the active state of one of the kinases that phosphorylates serine 262, a key residue in the binding of tau to microtubules. Knockdown

of TTL was sufficient to reduce microtubule dynamics and increase both active AMPK levels and phospho-tau.
Conclusions: Our results indicate that loss of TTL alone reduces dynamic microtubules, triggers tau kinase activation, and results in hyperphosphorylated tau. Together with our previously published data, these findings suggest that reduced levels of TTL act as one of the early drivers of tau hyperphosphorylation in AD.

**Disclosures: J. Parato:** None. **M. Pero:** None. **X. Qu:** None. **E. Richters:** None. **A. Sproul:** None. **F. Bartolini:** None.

**Presentation Number:** NANO75.11

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

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**Title:** The role of lysosomal progranulin-glucocerebrosidase complex in tauopathy

**Authors: *H. TAKAHASHI**[1], S. H. NIES[2,3], I. R. MACKENZIE[4], S. M. STRITTMATTER[2]; [1]Neurosci., Yale Univ., New Haven, CT; [2]Dept. of Neurosci., Yale Univ. Sch. of Med., New Haven, CT; [3]Univ. of Tübingen, Tübingen, Germany; [4]Univ. of British Columbia and Vancouver Gen. Hosp., Vancouver, BC, Canada

**Abstract:** Progranulin (PGRN), encoded by the *GRN* gene, is a secreted and lysosomal glycoprotein mainly produced by neurons and microglia. Although PGRN was initially linked to frontotemporal lobar degeneration (FTLD) with TDP-43 inclusions, subsequent genetic studies have suggested that *GRN* variations increase risk for Alzheimer's disease and Parkinson's disease. Additionally, accumulation of tau and/or α-synuclein in addition to TDP-43 is reported in FTLD *GRN* mutation carriers. In preclinical models, we and others have found an increase in tau accumulation in PGRN-deficient mice injected with AAV-human P301L tau and P301L tau transgenic mice with PGRN reduction. These studies suggest that PGRN regulates not only TDP-43 but also other proteinopathies, especially tauopathy and synucleinopathy. However, so far little is known about the mechanisms by which PGRN regulates other proteinopathies and whether PGRN reduction affects their symptoms.
In the present study, we investigated effects of PGRN reduction on tauopathy and tau-mediated phenotypes using the PS19 tauopathy mouse model overexpressing 1N4R human P301S tau on PGRN haploinsufficient and complete null backgrounds. We found that both complete loss and haploinsufficiency of PGRN increase tau inclusions, cause co-accumulation of α-synuclein, and exacerbate body weight loss, mortality, and disinhibited behaviors in PS19 mice. Unexpectedly, reduction of PGRN was protective against a spatial memory impairment and hippocampal atrophy and transcriptomic changes in PS19 mice. We also found that PGRN reduction in PS19 mice significantly decreases activity of β-glucocerebrosidase (GCase), a lysosomal enzyme previously associated with synucleinopathy and reportedly bound to PGRN, while increasing tau inclusions that are immunoreactive for GCase substrate glucosylceramide (GlcCer). In neuronal culture, GCase inhibition by conduritol B epoxide significantly increased tau aggregation

induced by Alzheimer's brain-derived tau fibrils. *In vitro* Thioflavin T assay showed that purified GlcCer directly promotes tau aggregation. Furthermore, neurofibrillary tangles in human tauopathy brains were found to be immunoreactive for GlcCer. Thus, our study reveals an unexpected role of GCase and GlcCer in tauopathy and demonstrates that PGRN regulates formation of tau and α-synuclein inclusions via GCase, which alters symptoms and neurodegeneration in tauopathy. A lysosomal PGRN–GCase complex may have a therapeutic target potential in tauopathy.

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**Nanosymposium**

**NANO76: Development of Novel Therapies for Neurodegenerative and Neuromuscular Diseases**

**Location:** WCC 201

**Time:** Wednesday, November 15, 2023, 8:00 AM - 11:00 AM

**Presentation Number:** NANO76.01

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

**Support:** Nant SRA
Acceleration Fund

**Title:** Discovery of an APP-selective BACE1 inhibitor for Alzheimer's disease

**Authors:** J. CAMPAGNA[1], B. JAGODZINSKA[1], D. WI[1], C. ZHU[1], J. T. LEE[1], W. COHN[1], Q. ZHANG[2], K. S. POKSAY[3], O. GOROSTIZA[3], O. DESCAMPS[3], ***P. SPILMAN**[1], D. E. BREDESEN[3], V. JOHN[1];
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**Abstract:** Alzheimer's disease (AD) is the most common age-related dementia, but currently approved treatments such as acetylcholinesterase inhibitors and NMDA receptor antagonists provide only temporary symptomatic relief or, for amyloid-directed antibodies, a modest decrease in the rate of cognitive decline. Therapies that target the mechanism(s) underlying the development and progression of AD are needed. The major component of amyloid is the peptide Aβ, which is generated by sequential cleavage of full-length amyloid precursor protein (APP) by β-site cleaving enzyme 1 (BACE1) and γ-secretase. Inhibition of BACE1 has been a target for AD therapeutic development, but has been hindered by off-target effects of clinically tested inhibitors, including inhibition of cleavage of non-APP substrates. Here, we report our identification of a BACE1 inhibitor that is not only selective for APP as the substrate, but also for BACE1 as the targeted enzyme. Our screening of a compound library for inhibition of BACE cleavage of a maltose binding protein (MBP)-conjugated-APPC125 substrate resulted in identification of a known drug as a weak (IC50 > 200 μM) inhibitor 'hit' of BACE1. Hit-to-lead optimization then led to a fluoro aminohydantoin (FAH) inhibitor with IC50 < 20 nM as

determined using the P5-P5' substrate assay. In multiple substrate and enzyme cell-free assays, lead compound FAH65 displayed high selectivity for inhibition of APP cleavage, with little activity against other BACE1 substrates including neuregulin 1, p-selectin glycoprotein ligand 1, and gp-130. FAH65 also shows little inhibitory activity against the enzymes cathepsin D or BACE2. In vitro, FAH65 inhibits production of BACE1 cleavage products soluble APPβ and the β C-terminal fragment (β-CTF), as well as Aβ1-40 and -42. FAH65 was demonstrated to be brain permeable in PK analysis and, in a murine model of AD, FAH65 improved memory based in the Novel Object and Novel Location Recognition memory testing paradigms. In brain tissue from the treated mice, β-CTF was decreased and the pro-cognitive, neurite-supporting peptide soluble APPα generated by the competing α-secretase APP cleavage pathway was increased. The active enantiomer - FAH65E(-) - is a potent BACE1 inhibitor that displayed target engagement in vitro and oral brain permeability in vivo. FAH65E(-) also has favorable in vitro ADME-T and drug-like physiochemical properties. Given FAH65/E(-)'s potency and selectivity as a BACE1 inhibitor, it merits additional pre-clinical development as a potential therapeutic that reduces Aβ formation and overcomes the deleterious effects of the non-selective BACE1 inhibitors that have failed in the clinic.

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**Presentation Number:** NANO76.02

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

**Title:** Fosgonimeton, a small molecule positive modulator of the neurotrophic HGF system, protects against amyloid beta-induced pathological alterations in Alzheimer's disease models in vitro and in vivo

**Authors: *S. E. SETTI**, A.-A. BERTHIAUME, S. M. REDA, W. WU, J. L. JOHNSTON, R. W. TAYLOR, K. J. CHURCH;
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**Abstract:** Amyloid beta (Aβ) protein pathology is a hallmark of Alzheimer's disease (AD), promoting tau pathology and neurodegeneration. Exposure to neurotoxic Aβ peptides results in deleterious consequences such as mitochondrial dysfunction, oxidative stress, and excitotoxicity. These pathological components make for promising therapeutic targets, as they are self-perpetuating and may contribute to or amplify the neurodegenerative cascade in AD. Pharmacological enhancement of the neurotrophic hepatocyte growth factor (HGF) signaling system is a multimodal approach that may be uniquely suited to address multiple aspects of Aβ toxicity, based on its neurotrophic, neuroprotective, anti-apoptotic, and anti-inflammatory effects. We have developed a series of small-molecule positive modulators of the neurotrophic HGF system, including fosgonimeton, as a potential therapeutic for neurodegenerative disorders, such as AD. In this study, we evaluate the ability of fosgonimeton to protect against Aβ-related pathology in vitro, as well as Aβ-induced cognitive deficits in vivo.
Primary rat cortical neurons exposed to Aβ1-42 for 24 hours exhibited neuronal death, neurite degeneration, and tau hyperphosphorylation. Pre-treatment with the active metabolite of fosgonimeton, fosgo-AM, significantly attenuated these outcomes. Exposure to Aβ1-42 for 4

hours also resulted in increased mitochondrial oxidative stress and cytochrome C release, effects that were significantly attenuated by fosgo-AM pre-treatment. Additionally, we found that fosgo-AM treatment increased expression of autophagy inducers, such as Beclin-1 and ULK1, suggesting that fosgo-AM may counteract Aβ-induced autophagic impairments to promote clearance of toxic proteins. We then assessed the ability of fosgonimeton to protect against Aβ1-42 pathology in aged mice. Bilateral intrahippocampal injection of Aβ1-42 resulted in significant cognitive impairment in the Y-Maze which was rescued by fosgonimeton treatment. Fosgonimeton treatment also significantly attenuated Aβ1-42-associated neurodegeneration, as indicated by reduced neurofilament light levels in CSF.
These data support the therapeutic potential of fosgonimeton in treating AD, as evidenced by mitigation of Aβ-induced pathological alterations through multiple mechanisms in vitro, as well as protection against Aβ-induced cognitive dysfunction in an aged mouse model of AD. Fosgonimeton is currently being investigated in clinical trials for treatment of mild-to-moderate AD (NCT04488419; NCT04886063).

**Disclosures: S.E. Setti:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **A. Berthiaume:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **S.M. Reda:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **W. Wu:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **J.L. Johnston:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **R.W. Taylor:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **K.J. Church:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma.

**Presentation Number:** NANO76.03

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

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Molecular & Cellular Biology at the University of Illinois Urbana-Champaign

**Title:** Targeting GPR3 using CRISPR-Cas12 improves memory in an Alzheimer's disease mouse model

**Authors: *M. ZEBALLOS C.**, H. MOORE, T. SMITH, T. GAJ;
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**Abstract:** Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by the extracellular accumulation of amyloid-beta (Aβ) plaques and intracellular aggregation of tau neurofibrillary tangles. The pathological Aβ and tau deposition is accompanied by neurotoxic effects, neuronal death, and cognitive decline. The orphan G protein-coupled receptor 3 (GPR3) has emerged as a potential therapeutic target for AD due to its ability to regulate γ-secretase activity without affecting Notch processing. Two main lines of evidence support this hypothesis: the genetic ablation of GPR3 in AD mouse models reduces amyloid pathology and mitigates cognitive deficits, and the increased GPR3 expression in postmortem brain tissue from patients with sporadic AD correlates with disease progression. Thus, reducing GPR3 expression could be a promising therapeutic strategy for AD. CRISPR platforms are a powerful genome editing platform effective in modulating the expression of target genes. One such platform is CRISPR-Cas12, which utilizes a CRISPR RNA sequence that directs the Cas12 nuclease to a target DNA locus by base complementarity. Upon DNA binding, Cas12 induces a double-stranded break that stimulates non-homologous end joining, an error-prone DNA repair pathway that introduces frameshift mutations that disrupt gene expression. We hypothesized that CRISPR-Cas12 could reduce GPR3 expression, reduce Aβ plaque deposition, and mitigate AD cognitive decline. In this study, we show that CRISPR-Cas12 can target GPR3 and improve spatial learning and memory in a mouse model of AD. We first developed a CRISPR-Cas12 system that efficiently targets GPR3 in Neuro2A mouse neuroblastoma cells. We then extended the validation of the GPR3-targeting strategy to the 5XFAD mouse model of AD, which expresses human Aβ precursor protein carrying the Swedish, Florida, and London mutations, along with the mutant presenilin 1. Homozygous 5XFAD mice present an accelerated cognitive decline that enables quick assessments of robust memory deficits. We utilized a PHP.eB adeno-associated virus for brain-wide delivery of the GPR3-targeting CRISPR-Cas12 system. Our results show strong cortical and hippocampus delivery via retro-orbital intravenous injections in one-month-old homozygous 5XFAD mice. Our CRISPR-Cas12 GPR3-targeting approach improved spatial memory in cued and acquisition training of the Morris water maze task at 3 and 5 months old compared to mice injected with a safe harbor gene-targeting control (n = 10 for both groups, female and male). Our results demonstrate the potential of CRISPR gene editing platforms for ameliorating neurologic disease phenotypes.

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**Presentation Number:** NANO76.04

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Support:** National Academy of Medicine

**Title:** Ultrasonic glymphatic manipulation based therapy for alzheimer disease

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**Abstract: Background**:Alzheimer's disease (AD) affects 6.7 million Americans aged 65+ and isprojected to increase to 14 million by 2060 without effective interventions.While commonly associated with older individuals, it can also affect people intheir 30s, 40s, or 50s. Current preventive measures are insufficient,necessitating the exploration of novel interventions. The glymphatic system, aglial cell-driven lymphatic system, plays a crucial role in removing brainwaste, such as free-form metabolites and amyloid plaque protein, which aremajor factors in AD and overall brain health.Ourresearch has a dual focus: understanding the role of the glymphatic system indisease progression and developing innovative interventions using low-intensitytranscranial-focused ultrasound (tFUS) to enhance glymphatic waste clearancefor potential AD management. Our recently invented ***tFUS sequence*** (650kHz at 0.2 MPa in-situ pressure, brain-wide exposure with 7.3% local dutycycle) significantly improves glymphatic transport [1]. The protocol is safe,with no parenchymal damage, significant neuronal degeneration, or astrocyticactivation observed 72 hours after the intervention. The required pressure iswell below FDA-approved limits, and existing clinical tFUS systems can achieveit, enabling smooth translation into clinical applications.In thisstudy, we are conducting further optimization of the ultrasound protocol toassess its effectiveness under diverse physiological conditions, consideringthe glymphatic system's sensitivity to the subject's physiological state. Thisoptimization is essential before implementing the protocol in AD intervention.**Methods**: Weexperimented on Sprague Dawley rats (N = 46), dividing them into three maingroups based on the level of anesthesia: Heavy-3% isoflurane, Moderate-2%isoflurane, and Light-1.5% isoflurane. We injected two imaging tracers ofdifferent sizes (IRDye-1kDa and IRDye-labeled IgG antibody 160 kDa)intrathecally and divided the subjects into two sub-groups: a Control Group andan Ultrasound Group. Throughout the experiment, we carefully monitored thesubjects' heart rate, respiratory rate, oxygen levels,perfusion,and body temperature. The ultrasound group was exposed to 650kHz at 0.2MPa for10 minutes to the entire brain.**Resultsand Conclusions**: The Mann-Kendall test showed significant monotonic increases inrespiratory rate over time for animals treated with ultrasound in Moderate andLight isoflurane groups, while in the Heavy group, there were significantdecreasing trends compared to Control Groups. Further, in the Ultrasound Group,respiratory rates significantly increased in the Light ($p = 0.024$) and Moderatecases ($p = 0.0412$), whereas it significantly decreased in the Heavy ones ($p =0.0377$). There were no significant differences in Control Groups at anyisoflurane levels. The physiological data indicate that ultrasound affectsrespiratory patterns differently depending on the animal's initialphysiological state. Specifically, animals that were lightly and moderatelyanesthetized became more wakeful with ultrasound treatment, while heavilyanesthetized animals became sleepier. Additionally, the wakeful brain showed ahigher diffusion of IRDyemoleculesfrom cerebral to interstitial space compared to sleepier ones in ex-vivo IVISimages. These results emphasize the importance of carefully regulatinganesthesia levels during ultrasound procedures to achieve the best outcomeswhen utilizing ultrasonic glymphatic manipulation-based applications. Futurework is to implement the ultrasonic glymphatic manipulation technique for ADmodel intervention.[1] **AryalM**. et al. Noninvasive

ultrasonic induction of cerebrospinal fluid flowenhances intrathecal drug delivery. J Control Release 2022;349:434-42

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**Presentation Number:** NANO76.05

**Topic:** C.06. Neuromuscular Diseases

**Support:** Genuv, Inc.

**Title:** Combined treatment with trametinib and riluzole improves motor function by enhancing autophagy in the SOD1-G93A mouse model of amyotrophic lateral sclerosis.

**Authors:** *S.-Y. LEE, **J. CHOIH**, Y. CHUN, M.-Y. KIM, S. HAN;
Genuv Inc., Seoul, Korea, Republic of

**Abstract:** Amyotrophic lateral sclerosis (ALS) is a neurological disease characterized by selective degeneration of motor neurons that causes muscle paralysis and eventual death. Riluzole, the first drug approved by the FDA for the treatment of ALS, shows modest improvement in symptoms, notably in survival, and is the standard of care for ALS. However, current treatments are far from providing a fundamental cure for the disease, and the need for more effective disease-modifying treatment is still strong. In a previous study, we confirmed that 0.1 mg/kg trametinib showed neuronal recovery in a neurodegenerative diseases model by inducing the autophagy-lysosomal pathway (ALP). Since lower doses of trametinib will be more tolerable to patients and many patients are already taking riluzole as a standard of care, we wanted to examine how treatment with low dose trametinib and riluzole would affect pathophysiology in an ALS mouse model. Hence, we evaluated the efficacy of trametinib and riluzole combination therapy in SOD1-G93A mice. The combined treatment of low dose 0.025 mg/kg trametinib and 8 mg/kg riluzole significantly improved neuropathological phenotypes in SOD1-G93A mice compared to the single treatment of trametinib or riluzole. Combination therapy delayed disease onset by 11 days (p value <0.01, vs vehicle) compared to the separate administration of trametinib (1 day) or riluzole (4 days). Trametinib+riluzole treatment improved disease score by up to 45 % (p value=0.000, trametinib: 12.5 %, riluzole: 25 %), rotarod by 1294 % (p value=0.001, trametinib: 215 %, riluzole: 313 %), grip strength by 94 % (p value <0.05, trametinib: -23 %, riluzole: 16 %), and survival by 9 days (p value=0.053, trametinib: -1 day, riluzole: 5 days) compared to vehicle. The data show that the efficacy of combined treatment was synergistic. Moreover, trametinib+riluzole treatment was shown to protect spinal motor neurons from damage. In motor neurons of trametinib+riluzole administrated SOD1-G93A mice, the level of cathepsin D and the co-localization of LC3 and LAMP1 increased, while abnormal p62 accumulation was significantly reduced, indicating improvement of autophagic flux. Altogether, these data suggest that low dose trametinib+riluzole combination therapy is pharmacologically superior to monotherapy in the ALS mouse model and could serve as a promising clinical combination for the improvement of current neuroprotective treatment strategies of ALS.

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**Presentation Number:** NANO76.06

**Topic:** C.06. Neuromuscular Diseases

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**Title:** Novel gene therapy for targeted clearance of pathological TDP-43 in amyotrophic lateral sclerosis and frontotemporal dementia

**Authors: Y. D. KE**[1], A. VAN HUMMEL[1], *L. M. ITTNER[2];
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**Abstract:** Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are characterized by cytoplasmic deposition of the nuclear TAR binding protein 43 (TDP-43). The TAR DNA-binding protein 43 (TDP-43) is a predominantly nuclear protein that regulates RNA processing and actively shuttles between the nucleus and cytoplasm. In 90% of ALS and 50% or FTD, TDP-43 is found to be re-distributed, undergo secondary modification like phosphorylation and fragmentation, and deposit in the cytoplasm of neurons. The mechanisms that regulate physiological subcellular distribution of TDP-43 and its conversion in disease, driving nuclear-cytoplasmic shuttling of TDP-43 remained elusive. Here, we identified a novel non-canonical interaction between a member of the 14-3-3 family and TDP-43 which regulates nuclear-cytoplasmic shuttling. Interestingly, neuronal 14-3-3 levels were increased specifically in sporadic ALS and FTD with TDP-43 pathology. Pathogenic TDP-43 or variants of TDP-43 showed increased interaction with 14-3-3 resulting in cytoplasmic accumulation, insolubility, phosphorylation, and fragmentation of TDP-43, resembling pathological changes in disease. Harnessing this increased interaction in pathological settings, we devised a novel gene therapy vector for FTD and ALS for targeted clearance of TDP-43 which mitigated functional deficits and neurodegeneration in several ALS/FTD mouse models expressing mutant or non-mutant

TDP-43. This new treatment shows preclinical promise and provides an exciting outlook for immediate translation into FTD/ALS therapy.

**Disclosures: Y.D. Ke:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patent. **A. Van Hummel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patent. **L.M. Ittner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patent.

**Presentation Number:** NANO76.07

**Topic:** C.06. Neuromuscular Diseases

**Support:** ALS Association

**Title:** Preclinical proof of concept of SOL-257, a gene therapy targeting misfolded TDP-43 in ALS

**Authors: *A. HISHIYA**;
Sola Biosci., NATICK, MA

**Abstract:** TDP-43 is a nuclear protein that plays an essential role in DNA and RNA processing. In 97% of ALS cases, TDP-43 becomes mislocalized to the cytoplasm, where it accumulates and forms aggregates within stress granules. These aggregates consist of pathologic forms of misfolded, phosphorylated, and C-terminal truncated TDP-43, which are resistant to further degradation. Both loss of function (nuclear TDP-43) and toxic gain of function (cytoplasmic TDP-43 aggregates) are believed to contribute to the causal pathophysiology of neurodegeneration in ALS.
We hypothesized that an engineered chaperone-based gene therapy specifically targeting misfolded TDP-43 could serve as an effective treatment strategy for the broader ALS population.
Methods/Results: SOL-257 is an AAV gene therapy that expresses a novel fusion protein consisting of two functional domains: a unique targeting domain that binds to misfolded TDP-43 and a common protein folding activation domain that binds to HSP70, a major cellular chaperone. The SOL-257 fusion protein acts as a co-chaperone, efficiently presenting misfolded TDP-43 to HSP70, which can either refold TDP-43 into its proper conformation or facilitate its degradation. After establishing proof of concept in vitro and demonstrating in vivo expression of the SOL-257 fusion protein, we conducted an in vivo proof of concept study using rNLS mice, a DOX-regulatable bigenic model of ALS with TDP-43-related pathology (NEFH-tTA × hTDP-43ΔNLS). Upon DOX removal at Week 5 of life, TDP-43ΔNLS expression leads to rapid clinical deterioration characterized by severe weight loss and death typically within 6-8 weeks. We evaluated disease progression after administering 6E10 vg of SOL-257 AAVrh10 or control AAVrh10 (no transgene) via ICV administration at P1. Compared to control mice, SOL-257-treated mice exhibited a substantial reduction in insoluble TDP-43 levels confirmed by Western blotting. Moreover, SOL-257-treated mice demonstrated statistically significant and clinically meaningful improvements in both weight gain and survival. Further behavioral tests are currently underway, and we will present the detailed results.
Conclusion: This in vivo proof of concept study demonstrated that SOL-257 gene therapy can

effectively prevent TDP-43-related toxicity in the rNLS mouse model of ALS. These results provide strong support for the ongoing preclinical development of SOL-257 for the treatment of ALS. Additionally, this versatile gene therapy platform, capable of expressing engineered co-chaperones with different target specificities, holds promising potential for the treatment of protein misfolding diseases in general.

**Disclosures: A. Hishiya:** None.

**Presentation Number:** NANO76.08

**Topic:** C.06. Neuromuscular Diseases

**Title:** NX210c drug candidate peptide improves motor function and prolongs survival in the SOD1$^{G93A}$ mouse model of ALS

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**Abstract:** NX210c is a short cyclic peptide derived from the thrombospondin repeats of the subcommissural organ-spondin that displays *in vitro* beneficial effects on several aspects of ALS pathogenesis such as on blood-brain barrier permeability and neuronal death induced by glutamate excitotoxicity or oxidative stress. Therefore, the aim of this study was to evaluate the therapeutic effect of NX210c in the SOD1$^{G93A}$ mouse model of ALS. Female SOD1$^{G93A}$ mice were treated daily with intraperitoneal injections of vehicle or different doses of NX210c (2.5, 5 or 10 mg/kg) from 90 days old. The static rods test was performed every other week to evaluate motor deficits. Briefly, mice were placed with their back facing the clamped end of the rod. The orientation time to turn back and the travel time to walk the 60 cm back to the edge of the rod were recorded (= total time). The smaller the rod diameter, the harder the task. The clinical score (see ALS Therapy Development Institute guidelines) was evaluated twice a week to determine overall survival. The orientation, travel and total times during the static rods test were higher in SOD1$^{G93A}$ mice compared with WT mice from 16 weeks old until disease end-stage ($p<0.001$). A dose-dependent improvement of motor performances was observed in SOD1$^{G93A}$ mice treated with NX210c. More particularly, the peptide at 10 mg/kg reduced ALS-induced increased orientation and travel times from 16 weeks old (WT: 1.3s and 3.2s, vehicle SOD1$^{G93A}$: 24.5s and 26.6s and 5.4s, NX210c SOD1$^{G93A}$: 2.6s and 4.8s for orientation and travel times, respectively; $p<0.01$) until disease end-stage on the largest diameter rod. Although no effect of NX210c at the lowest doses (i.e., 2.5 and 5 mg/kg) was observed on the overall survival of SOD1$^{G93A}$ mice ($p>0.05$), the median survival of SOD1$^{G93A}$ mice treated with NX210c at 10 mg/kg was increased by 11 days compared with that of vehicle-treated SOD1$^{G93A}$ mice (vehicle SOD1$^{G93A}$: 143d, NX210c SOD1$^{G93A}$: 154d, $p<0.01$). Overall, NX210c is a new promising drug candidate and its solid preclinical package (mechanism of action, proof of concept in ALS) should support the clinical development of NX210c in ALS patients. In addition, we have gathered several proofs of concept showing that NX210c restores cognitive functions (Lemarchant *et al.*, 2022; Le Douce *et al.*, 2021), which may represent a supplementary beneficial effect of NX210c for ALS patients, since 35% of them suffer from cognitive or behavioral impairments, with an additional 15% having FTD.

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**Presentation Number:** NANO76.09

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Title:** Novel small molecule poly-disaggregator therapeutics for ALS reduce TDP-43 oligomerization, aggregation, and pathology

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**Abstract:** TDP-43 pathology is a hallmark in most cases of Amyotrophic Lateral Sclerosis (ALS). TDP-43 pathology features cytoplasmic accumulation of insoluble TDP-43 aggregates. Notably, aggregates of several other amyloidogenic proteins appear alongside TDP-43 aggregates as co-pathology or multiple co-pathologies in the majority of ALS cases, including amyloid-β (>30%), α-synuclein (>10%), and tau (>80%). Furthermore, one amyloidogenic protein species can cross-seed aggregates or toxic oligomers of another distinct amyloidogenic protein species and accelerate disease progression. Together, these features underscore the potential therapeutic benefit of simultaneously targeting multiple amyloidogenic proteins. Using our novel machine learning platform, we identified small-molecule compounds that can disrupt toxic oligomers and aggregates of amyloidogenic proteins including TDP-43, amyloid-β, α-synuclein, and tau. We experimentally identified compounds that potently prevent *in vitro* aggregation and induce disaggregation of a minimal amyloidogenic TDP-43 (307-319) peptide as well as full length amyloid-β, α-synuclein, and tau using a dye-based aggregation assay. We find that one of the most potent compounds, ACE-339 dose-dependently reduced cytoplasmic TDP-43 aggregates in a human cellular TDP-43 aggregation model. TDP-43 and other amyloidogenic proteins can assemble into toxic oligomers as an intermediate step to aggregation. Using high resolution ion-mobility mass spectrometry to specifically identify small oligomers, we find that ACE-339 reduced oligomers and restored monomers and dimers formed by the TDP-43 peptide, suggesting that ACE-339 inhibits TDP-43 oligomerization. Furthermore, ACE-339 shows low plasma and brain homogenate binding, high metabolic stability, is orally bioavailable, and highly blood-brain barrier permeable. We tested ACE-339 in a doxycycline-repressible human iTDP-43$^{A315T}$ mouse model of ALS/FTD. Two months of daily oral dosing at two doses was well tolerated and ACE-339 was detectable at high concentrations in the brains of the mice at the end of the experiment. Notably, ACE-339 reduced TDP-43 pathology in the brain and showed partial behavioral rescue in iTDP43$^{A315T}$ mice. We found that ACE-339 reduced all markers of pathological forms of TDP-43 tested, including significantly reduced TDP-43 phosphorylation and TDP-43 insolubility, and cytoplasmic localization and ubiquitination trended lower with ACE-339 treatment. ACE-339 also improved motor performance and disinhibition deficits characteristic of iTDP-43$^{A315T}$ mice. Acelot's 'poly-disaggregators' have valuable therapeutic potential.

**Disclosures: M. Kokes:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment. **E. Shao:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment. **S. Arya:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment. **A. Van Hummel:** 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.; Collaborator with Acelot, Inc. **M. Sabale:** 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.; Collaborator with Acelot, Inc. **X. Liu:** 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.; Collaborator with Acelot, Inc. **K. Burke:** A. Employment/Salary (full or part-time):; Acelot, Inc, part-time employment. **L. Whitehead:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment. **J. Schwarz:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment. **K. Planey:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment. **M.T. Bowers:** 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.; Collaborator with Acelot, Inc. **Y.D. Ke:** 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.; Collaborator with Acelot, Inc. **A.K. Singh:** F. Consulting Fees (e.g., advisory boards); Acelot, Inc, Founder. **V. Mathur:** A. Employment/Salary (full or part-time):; Acelot, Inc, full-time employment.

**Presentation Number:** NANO76.10

**Topic:** C.06. Neuromuscular Diseases

**Support:** Lundbeckfonden LF-Experiment Grant (2020 call - project: Treating ALS disease by rescuing interneuronmotor neuron synaptic connectivity)

**Title:** Stabilization of V1 interneuron-motor neuron synapses ameliorates motor deficits in a mouse model of ALS

**Authors: *S. MORA**[1,3], R. VON HUTH-FRIIS[4], A. STUCKERT[5], G. NOES-HOLT[4], A. TOFT SØRENSEN[4], R. SELVAN[2], I. ALLODI[5], *S. MORA[3];
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**Abstract:** Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by progressive inability to execute movement. Although loss of spinal motor neurons (MNs) is a capital feature,

novel evidence from our laboratory points towards crucial a role of interneurons (INs) in the disease. V1 spinal inhibitory interneurons (positive for Engrailed 1 - En1) lose their connections to MNs at pre-symptomatic stages in the $SOD1^{G93A}$ mouse model. Such changes might play a pivotal role in MN hyperexcitability and death. We investigated whether forced overexpression of the protein Extended Synaptotagmin 1 (Esyt1), a presynaptic organizer known to play an important role in synaptic maintenance and neurotransmission which is downregulated in V1 spinal neurons early in disease, stabilized MN-IN connectivity and ameliorated associated motor deficits. Intraspinal injections of a *cre*-dependent AAV8-hSyn-DIO-hEsyt1-W3SL viral construct were performed at postnatal day 30 (P30) in lumbar segments 1-3 of $SOD1^{G93A}$ mice crossed with $En1^{cre}$ mice. Four genotypes (SOD1, SOD1;$En1^{cre}$, $En1^{cre}$ and wild-type) were investigated. Esyt1 overexpression was confirmed by RNAscope in situ hybridization. Treatment increased inhibitory synaptic density on lumbar MNs (measured by vesicular GABA transporter -VGAT- integrated density normalized by cell perimeter) in SOD1;$En1^{cre}$ mice compared to untreated littermates, and promoted MN survival, leading to an improvement of motor phenotypes: between P49-P112, SOD1;$En1^{cre}$ mice performed better compared to SOD1 littermates in parameters such as speed, step frequency, stride length, and hyperflexion of the hind-limbs, along with improved weight support. Together, our results suggest that interneurons can be a potential therapeutic target for ALS treatment and that ESYT1 might play a role in promoting MN survival.
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**Title:** Agonist of growth hormone-releasing hormone improves the disease features of Spinal Muscular Atrophy mice

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**Abstract:** Spinal Muscular Atrophy (SMA) is a neuromuscular disease characterized by motor neuron (MN) loss, associated with muscle atrophy, as well as other peripheral alterations. It is

due to the reduction of survival motor neuron protein levels. Nowadays the available therapies show several limitations: thus, new therapeutic strategies are needed and combined therapies with other drugs should be envisaged. Here we investigated the efficacy of the growth hormone-releasing hormone (GHRH) agonist MR-409 that was demonstrated to exert protective effects on muscle atrophy, cardiomyopathies, ischemic stroke, and inflammation, in other experimental models. Therefore, from postnatal day 2 (P2) to P12, we daily administered vehicle or MR-409 (1mg/Kg and 2mg/Kg) to SMNdelta7 mice, a severe SMA model. The treatment increased body weight and improved motor behavior in SMA mice, particularly at the highest dose tested. In addition, histological analyses revealed a dose-dependent increase in muscular fiber size and neuromuscular junction maturation, with an enhanced mono-innervation and a reduced denervation of the endplates, in both quadriceps and gastrocnemius muscles. Moreover, at molecular level, we observed an increased expression of several myosin heavy chain isoforms (Myh1, Myh2, Myh7 and Myh8) and of markers of myogenesis and muscular damage repairing (Myog and Myod1), as well as a significant downregulation of apoptosis markers correlated with muscular atrophy (MuRF1 and Atrogin-1) in the same muscles. Finally, the treatment with MR-409 delayed MN death and blunted neuroinflammation (decreased astrogliosis and downregulation of proinflammatory cytokines) in the spinal cord of SMA mice. In conclusion, the present study (Boido et al., Proc Natl Acad Sci U S A. 2023 Jan 10;120(2):e2216814120) demonstrated that MR-409 has protective effects in SMNdelta7 mice, suggesting that GHRH agonists are promising agents for the treatment of SMA, possibly in combination with SMN-dependent strategies.

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**Presentation Number:** NANO76.12

**Topic:** I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

**Support:** CHDI Foundation
FWO

**Title:** Resting state co-activation patterns are responsive to mutant huntingtin lowering in the LacQ140 mouse model of Huntington's disease

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**Abstract:** Huntington's disease (HD) is a monogenic neurodegenerative disorder caused by an expanded CAG repeat in the mutant huntingtin gene (mhtt). Lowering mhtt expression is an active area of HD therapeutic development, and there is an immediate need for responsive biomarkers. We recently reported that resting state (RS) functional MRI (fMRI) co-activation patterns (CAPs) are altered in the Q175 mouse model of HD. Utilizing a conditional mhtt expression system in the LacQ140 mouse model, here we investigate if RS-CAPs are responsive to mhtt expression lowering. We acquired RS-fMRI data (9.4T Bruker Biospec with cryo-coil; TR 500ms, 1200 repetitions) in three groups of mice under anesthesia (n=17/group, males & females): wild-type (WT), LacQ140 in which mhtt was allowed to express fully, and LacQ140 (2M) in which mhtt expression was lowered from 2 months onward. We concatenated preprocessed (filtered 0.01 - 0.2 Hz, global signal regressed) voxel-level images from all subjects before clustering the timeframes using K-means++. We then identified the optimal number of CAPs and investigated their temporal (occurrence, duration, entropies of transition probabilities) and spatial (voxel-level and regional activations) properties for group and sex effects using 2-way ANOVA. Finally, we assessed the cross-validated, predictive ability of CAP properties to classify the three groups. Out of the five optimal CAPs, one (LCN CAP) showed simultaneous activation and deactivation of the lateral cortical network (LCN) & default-mode-like network (DMLN) regions respectively, and another (DMLN CAP) the reverse pattern. At the voxel level, compared to two other groups, LacQ140 (2M) males showed higher activation magnitude in somatosensory, motor cortices, and caudate putamen (CPu) in the LCN CAP. Activation magnitude in the CPu in the DMLN CAP was lower in LacQ140 females compared to WT & LacQ140 (2M) females but no significant difference was found between the WT and LacQ140(2M) females. Regional activation magnitudes were higher in females compared to males. Reductions in the motor cortex activation in the LCN and DMLN CAPs and in the CPu activation in the DMLN CAP, found in the LacQ140 group, were reversed in the LacQ140(2M) group. CAP spatial properties predicted the LacQ140 (2M) group most accurately and misclassified LacQ140 (2M) mice were more likely to be predicted as WT and vice-versa. These findings demonstrate that genotypic changes in HD-relevant areas in prominent CAPs in this mouse model were reversed upon conditional lowering of mhtt expression. Accurate prediction of mhtt lowered group in a three-class classification shows the responsiveness of CAPs to mhtt lowering.

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**Nanosymposium**

**NANO77: Mechanisms in Multiple Sclerosis**

**Location:** WCC 152B

**Time:** Wednesday, November 15, 2023, 8:00 AM - 10:15 AM

**Presentation Number:** NANO77.01

**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** R01AG061708

**Title:** High density multi-electrode array recordings help investigate the upper motor neuron health and connectivity in ALS

**Authors:** C. A. QUINTANILLA[1], O. KASHOW[1], M. S. RADOJICIC[3], Z. FITZGERALD[1], D. BITLIS[1], B. GENC[1], E. ULUPINAR[1], M. MARTINA[4], P. R. ANDJUS[5], ***P. OZDINLER**[1,2]; [1]Neurol., Northwestern Univ., Chicago, IL; [2]Chem. of Life Processes Institute, Dept. of Mol. Biosci., Northwestern Univ., Evanston, IL; [3]Physiol., Univ. of Belgrade, Fac. of Biol., Belgrade, Serbia; [4]Neurosci., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [5]Physiol. and Ctr. for laser microscopy, Univ. of Belgrade Fac. of Biol., Belgrade, Serbia

**Abstract:** Cortical hyperexcitation is one of the underlying causes of upper motor neuron (UMN) vulnerability in amyotrophic lateral sclerosis (ALS), and early cortical connectivity defects contribute to disease pathology. However, the details of how cortical hyperexcitation contributes to disease pathology is not fully understood. Here, we introduce a high-density multi-electrode recording system to investigate cortical connectivity and cortical activity both within the context of health and disease. We utilize well-characterized ALS mouse models that are developed based on mutations detected in ALS patients, and that display progressive UMN loss. Crossing hSOD1$^{G93A}$, hPFN1$^{G118V}$ and TDP-43$^{A315T}$ mouse models, with UCHL1-eGFP mice helped generate ALS disease models in which UMNs are genetically labeled with eGFP, so that multi-array electrophysiological recordings can be performed with cellular precision and resolution, which was not possible before.Either mixed cortical cultures are established, or acute brain slices are prepared on the high-density multi-electrode arrays (HD-MEAs), which have 4096 electrodes arranged in a 64x64 grid on a complementary Metal-Oxide Semiconductor chip. Active Pixel Sensor technology enables 3-5 min recordings controlled by the BrainWave 5 software (3-Brain) at a sampling rate of 17kHz. Raw voltage traces are analyzed to detect and sort spiking events, performed with the Precise Timing Spike Detection algorithm at a differential threshold set to 10 times the standard deviation followed by spike sorting utilizing principal component analysis. Further filtering and de-noising continued with MATLAB. This assay reveals whether neurons of interest are receiving input and whether they have functional output in the form of field potentials of select areas, peak amplitude, frequency of action potentials at an individual neuron/cell level.Our ongoing studies begin to reveal the connectivity and activity patterns of cortical neurons and how they may be affected in the disease. After we understand the basis of cortical connectivity defects based on altered activity patterns, and how that relates to disease progression, this information can be used in drug discovery/verification platforms to identify compounds that enable functional improvement and enhanced cortical connectivity in ALS as a potential treatment strategy.

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**Presentation Number:** NANO77.02

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Title:** Identification of the protein interactome of nuclear phosphorylated tau in C9ORF72-amyotrophic lateral sclerosis.

**Authors: *T. PETROZZIELLO**, S. S. HUNTRESS, A. L. CASTILLO-TORRES, R. JAYAKUMAR, S. R. SUNDERESH, P. KIVISÄKK, S. E. ARNOLD, B. T. HYMAN, S. DAS, M. A. GARRET, M. E. CUDKOWICZ, J. D. BERRY, G. SADRI-VAKILI;
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**Abstract:** Recently, we have demonstrated that tau phosphorylated at S396 (pTau-S396) was mis-localized to the synapses and contributed to mitochondrial dysfunction in amyotrophic lateral sclerosis (ALS). Additionally, we demonstrated a significant increase in pTau-S396 in C9ORF72-ALS post-mortem motor cortex (mCTX), suggesting that phosphorylated tau may play a role in ALS cases harboring expansions in C9ORF72 consistent with alterations in protein misfolding and homeostasis widely described in C9ORF72-ALS. Here, we further investigated the role of tau in C9ORF72-ALS with a focus on the nucleus, given that increases in nuclear pTau have been linked to disruption of nuclear transport, a pathogenic mechanism contributing to protein aggregation. Our findings indicate that although there were no significant alterations in nuclear total tau, pTau-S396 as well as tau phosphorylated at S404 (pTau-S404) and T181 (pTau-T181) in ALS mCTX, there was a significant shift of only pTau-S396 from the cytosol to the nucleus in ALS. Importantly, there was a significant increase in pTau-S396 levels in C9ORF72-ALS mCTX nuclear fractions compared to controls. Further proteomic analysis of C9ORF72-ALS mCTX nuclear fractions immunoprecipitated with an antibody against pTau-S396 revealed aberrant interactions between pTau-S396 and its nuclear targets. Specifically, we identified 12 proteins with an increased interaction, including CD9, enolase 1 (ENO1) and ATPase $Na^+/K^+$ transporting subunit beta 2 (ATP1B2), as well as 13 proteins with decreased interactions with pTau-S396, including cell cycle exit and neuronal differentiation 1 (Cend1), aldehyde dehydrogenase 2 family member (ALDH2) and chaperonin containing TCP1 subunit 5 (CCT5). Ongoing studies are investigating plasma and cerebrospinal fluid (CSF) tau in longitudinal familial ALS samples, specifically C9ORF72-ALS, to determine whether tau could serve as viable biomarker in ALS.

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**Presentation Number:** NANO77.03

**Topic:** C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

**Support:** NIH Grant 1R21NS123845

**Title:** Enhanced extracellular vesicles release in a cerebral organoid model with loss of C9orf72

**Authors: *I. MARTORELL SERRA**, M. E. CICARDI, K. KRISHNAMURTHY, M. SINGER, D. TROTTI;
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**Abstract:** ALS and FTD, two neurodegenerative disorders on a shared continuum, exhibit progressive pathology that spreads throughout the CNS. This spreading has been associated with gliosis, indicating neuroinflammation as a contributing factor. The most common genetic cause of ALS/FTD is a hexanucleotide repeat expansion (HRE) in the *C9ORF72* gene. Three potential

pathogenic mechanisms associated with *C9ORF72*-HRE have been identified: reduction of C9orf72 protein (C9) levels, formation of nuclear RNA foci, and aberrant translation leading to the production of toxic dipeptide protein repeats. The precise impact of C9 haploinsufficiency on disease development is still being investigated due to ongoing exploration of the physiological functions of the C9 protein. Impaired C9 function induces lysosome aggregation, altered trans-Golgi vesicle trafficking, and aberrant extracellular vesicles (EV) secretion. EVs, bilayer membrane vesicles released by cells under various conditions, play critical roles in intercellular communication and inflammation within the CNS. Our hypothesis posits that C9 haploinsufficiency leads to abnormal EV production, thus contributing to the initiation and progression of neuroinflammation. To examine this, we generated cerebral organoids (COs) from iPSC-derived healthy controls with normal C9 levels ($C9^{+/+}$), C9-linked ALS/FTD patients ($C9^{+/-}$), and engineered C9 KOs ($C9^{-/-}$). We observed comparable rates of CO growth, assessed by surface area over time, and basal neural activity across all genotypes at 2 and 6 months *in vitro*, suggesting that C9 loss does not impede CO maturation *in vitro*. As expected, C9 protein levels were significantly reduced in $C9^{+/-}$ COs compared to controls and undetectable in $C9^{-/-}$ COs. Evaluation of neurodegeneration and EVs production at 6 months *in vitro* revealed a significant increase in cleaved caspase 3 (CC3) protein in $C9^{-/-}$ COs compared to their isogenic controls, along with an augment in EVs production. Our immediate objective is to validate the increased presence of CC3 in $C9^{-/-}$ COs using immunostaining and confocal imaging analysis. Additionally, we will assess the production of EVs in $C9^{+/-}$ COs compared to the control group. By conducting these experiments and combining them with our current findings, we aim to gain a deeper understanding of the role played by C9 in disease progression. Furthermore, we will utilize iPSC lines to derive microglia progenitors and subsequently introduce them into the corresponding background of COs before the maturation stage. This innovative approach will enable us to explore the potential involvement of EVs in the initiation and progression of neuroinflammation

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**Topic:** C.06. Neuromuscular Diseases

**Support:** Natural Science foundation of Jiangsu Province (BK20221155)

**Title:** The GPT hSOD1 G93A mouse model can replicate progressive ALS-like phenotypes

**Authors:** H. QI[1], Q. SONG[1], F. LIU[1], Z. LI[2], Z. YU[1], ***M. MOORE**[2], C. JU[1], J. ZHAO[1], X. GAO[1];
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**Abstract:** Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease that lacks effective therapeutic strategies to date. GemPharmatech (GPT) generated the GPT hSOD1 G93A transgenic mouse strain to replicate ALS-like phenotypes in an animal model, in line with the demands of medical research and drug development. The hSOD1 G93A mouse model carries the G93A mutation in human SOD1 gene and has widespread expression in mice. We observed an apparent body weight decrease in 25-week-old male mice but not in females. We further

performed a range of behavioral tests to identify the disease onset timing in GPT hSOD1 G93A mouse model, revealing that male mice experience onset at 25 weeks while female mice experienced onset about 2 weeks later. Pathological findings demonstrate gastrocnemius muscle degeneration and detectable immune cell infiltration following disease onset. We detected transcriptome alterations in the spinal cord tissue of GPT hSOD1 G93A mice and found that immune responses were activated after disease onset. Finally, the mice can survive for almost 8 months and die quickly Our data suggest that the GPT hSOD1 G93A transgenic mouse model can serve as a crucial tool for neurodegenerative disease research, providing valuable insights into disease mechanisms and supporting the development of disease-modifying therapies for ALS.

**Disclosures: H. Qi:** None. **Q. Song:** None. **F. Liu:** None. **Z. Li:** None. **Z. Yu:** None. **M. Moore:** None. **C. Ju:** None. **J. Zhao:** None. **X. Gao:** None.

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**Topic:** C.06. Neuromuscular Diseases

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**Title:** Triple mutant TDP-43 inducible mouse displays both the behavioral and pathological phenotype seen in ALS

**Authors: *M. DOPLER**[1], K. COX[1], T. PETERSEN[1], C. PREDDIE[1], L. WELLINGTON[1], W. SMITH[1], I. BROOKS[1], S. MCGRIFF[1], S. AREZOUMANDAN[1], M. A. GITCHO[2];
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**Abstract:** Amyotrophic lateral sclerosis (ALS) is a rare progressive neurodegenerative disease that is characterized by the death of upper and lower motor neurons with average survival being only 3-5 years. In approximately 95% of all ALS cases, hyperphosphorylated TDP-43 pathology contributes to motor neuron death though only 5-10% of familial cases are caused by mutations in TDP-43. With no current therapy targeting TDP-43 proteinopathy available, the development of new mouse models that recapitulate behavior, pathology and the biochemical change in solubility are needed. We have characterized a TDP-43 mouse model with 3 familial mutations (3X-TDP-43) under control of the tetracycline response system (Tet-off). The transgene is driven by the neurofilament heavy promoter (NEFH-tTA); specifically expressed throughout the brain and spinal cord. The transgene is not expressed until after weaning (P21-24). Age dependent severe motor deficits (rotor rod) are observed in mice starting at approximately 35 days of age for males (n=21, p=0.0003) with females at approximately 42 days of age (n=27, p=0.0001). The 3X-TDP-43 expressing mice had a significant decrease in survival with males living 48-80 days (n=18, p=<0.0001) and females living 44-113 days (n=9, p=<0.0001). Interestingly, there is 20% more males than females with ALS. Age-dependent pathological accumulation of phosphorylated TDP-43 is seen in both the nucleus and cytoplasm with a distinct age-dependent reduction in solubility. Mice also show a significant increase in astrocyte activation and deficits

in the inflammasome pathway. We hope that this model will provide insight into the development of therapeutics and increase our understanding of ALS and other TDP-43 proteinopathies.

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**Presentation Number:** NANO77.06

**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Proteome alterations in multiple sclerosis lesions

**Authors: *J. WILKINS**[1], K. K. MANGALAPARTHI[1], B. C. NETZEL[2], W. A. SHERMAN[1], Y. GUO[1], A. KALINOWSKA-LYSZCZARZ[3], A. PANDEY[1], C. F. LUCCHINETTI[4]; [1]Mayo Clin., Rochester, MN; [2]Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Med. Sci., Poznan, Poland; [4]The Univ. of Texas at Austin, Austin, TX

**Abstract:** Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Progressive damage to the central nervous system results in injury making MS a leading cause of non-traumatic disability in young adults. While the exact cause of MS remains unknown, mitochondrial dysfunction, iron dyshomeostasis, metabolic alterations, and immune-mediated processes are common factors associated with axonal injury and disease progression. To further help gain insight into underlying molecular mechanisms that may be driving progression in MS, we utilized laser capture microdissection and proteomics to interrogate changes in chronic inactive lesions of formalin-fixed paraffin-embedded (FFPE) brain tissue. We analyzed tissue from three chronic MS and three control individuals by liquid chromatography tandem mass spectrometry. Over 3,000 proteins were identified. Differentially expressed proteins in chronic inactive MS lesions confirm the loss of myelin-associated factors and changes in proteins implicated in metabolism, cytoskeletal organization, and myelin assembly. Comparison of alterations identified in MS white matter may suggest lipid metabolism is perturbed in the periplaque white matter. Our study highlights the feasibility to perform laser capture microdissection and proteomics analysis in FFPE MS brain tissue. Furthermore, our findings suggest myelinogenesis, bioenergetics, and focal adhesions are perturbed in chronic MS brain tissue providing additional insight that may aid in our understanding of pathophysiological mechanisms driving the disease.

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**Presentation Number:** NANO77.07

**Topic:** B.10. Demyelinating Disorders

**Support:** NMSS Grant RG-2110-38554

**Title:** Small molecule induced epigenetic rejuvenation overcomes myelinogenic barriers and stimulates myelin repair

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**Abstract:** Remyelination failure underlies symptoms of demyelinating diseases such as multiple sclerosis (MS). We found that oligodendrocytes present in the demyelinating MS lesions are epigenetically silenced and fail to produce myelin sheaths. Despite extensive studies on the differentiation of oligodendrocyte precursors, few small molecules that modulate myelinogenesis from differentiated oligodendrocytes have been identified. Here we developed an oligodendrocyte transgenic reporter for chemical screening and identified a small-molecule epigenetic modulator that enhances myelin production and ensheathment. In animal models of MS, this compound promoted remyelination and *de novo* myelination of regenerated axons after optic nerve crush, while increasing myelin sheath lengths in human iPSC-derived organoids.Multi-omics analyses (transcriptomics, epigenomics, 3D Hi-ChIP profiling, and metabolomics) revealed that treatment with the hit compound stimulated long-range enhancer-promoter interactions that upregulated crucial myelinogenesis-associated pathways, including RRAS2-AKT signaling, and reprogramed metabolic profiles by enhancing lipid and cholesterol biosynthesis for myelin production. Together, our study uncovers that small-molecule-modulated epigenome rejuvenation through relieving epigenetic barriers may serve as a potential myelin repair strategy for treating demyelinating diseases.

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**Topic:** B.10. Demyelinating Disorders

**Support:** Fondation Recherche Medicale - Fondation Acantha

**Title:** Investigating Sonic Hedgehog signalling during remyelination

**Authors: M. RUSSO**[1], H. FAURE[1], A. KASSOUSSI[2], A. ZAHAF[2], E. TRAIFFORT[2], *M. RUAT[1];
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**Abstract: Investigating Sonic Hedgehog signalling during remyelination** M. Russo, H. Faure, A. Kassoussi, A. Zahaf, E. Traiffort and M. Ruat.In the mature rodent brain, Sonic Hedgehog (Shh) signalling regulates stem and progenitor cell maintenance, neuronal and glial circuitry, and brain repair including remyelination. Pharmacological inhibition of Gli1, a transcription factor associated with Shh pathway, enhances remyelination via neural stem cells recruitment (Samanta et al., 2015). We have recently investigated the pro-myelinating properties of GSA-10, a small molecule developed by our group and which inhibits Gli1 transcription (Manetti et al., 2016). Using thelysophosphatidylcholine-induced focal demyelination mouse model, we have demonstratedthat GSA-10 promotes the recruitment and the differentiation of

Olig2$^+$ and CC1$^+$oligodendrocytes into the demyelinated corpus callosum, and represents a novel potentialremyelinating agent (Del Giovane et al., 2022). By single molecule fluorescent *in situ* hybridization, we have further identified for the first time Shh transcripts in a subset of oligodendrocytes expressing Olig2 and Sox10 mRNAs, throughout the mouse brain. Interestingly, using the C9C5 monoclonal antibody, which recognizes Shh peptides, we reported a broad expression pattern of Shh in a subpopulation (11-12%) of CC1$^+$ mature oligodendrocytes. These cells also express Olig2 and Sox10, two oligodendrocyte lineage-specific markers (Tirou et al., 2020), suggesting a role of Shh in myelinating activity. Moreover, we have investigated Shh mRNA and protein during post-natal myelination of mouse brain and we identified Shh-C9C5$^+$ cells to be upregulated from P4 toP20, in parallel with the expression of the Myelin Basic Protein. Experiments are in progress to further characterize Shh role and its regulation in primary cultures of rodentoligodendrocytes and during remyelination.

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**Topic:** B.10. Demyelinating Disorders

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ANSES Madonna

**Title:** Deleterious functional consequences of perfluoroalkyl substances accumulation into the myelin sheath

**Authors: *B. ZALC**[1], L. BUTRUILLE[2], P. JUBIN[2], E. MARTIN[2], M.-S. AIGROT[2], M. LHOMME[3], J.-B. FINI[4], B. DEMENEIX[4], B. STANKOFF[2], C. LUBETZKI[2], S. REMAUD[4]; [1]Sorbonne Université; Inserm, CNRS, ICM, Paris Cedex 13, France; [2]Paris Brain Inst. (ICM), Paris, France; [3]IHU ICAN, Paris, France; [4]CNRS UMR 7221, Paris, France

**Abstract:** Over the past 30 years an unexplained increased incidence in multiple sclerosis (MS), a demyelinating disease of the central nervous system, is observed in developed countries, suspected to be exacerbated by environmental factors. Concomitantly over 300 new chemicals have been synthesized and released in the environment. We questioned whether exposure to amphiphile perfluoroalkyl fluorosurfactant substances (PFAS) such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), could interfere with the process of myelin formation and remyelination. We first demonstrated by LC-MS/MS that PFOS, and to a lesser extent PFOA, accumulated into the myelin sheath of weaning pups, whose mothers were exposed to PFAS *via* drinking water during late gestation and lactation. Using *ex vivo* and *in vivo* approaches, we showed that PFOS, but not PFOA, affected remyelination. In rodents, demyelination was induced *ex vivo* by lysophosphatidylcholine exposure of mouse cerebellum explants and the effect of PFAS was evaluated during the remyelination period. *In vivo* studies were performed using the Xenopus leavis line Tg(*mbp:gfp-NTR*), a model of inducible-demyelination, showing that, at the end of demyelination, introduction of PFOS into the swimming water altered significantly spontaneous remyelination. This was associated with a

functional impact evaluated by distance travelled, speed of swimming and visual avoidance test. Our study, taking advantage of inter-species demyelination models, brings in-depth knowledge on the links between PFAS exposure, myelin integrity, generation of remyelinating oligodendrocytes and functional repair of brain lesions, and might pave the way to increased knowledge on the impact of environmental factors in multiple sclerosis.

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**Nanosymposium**

**NANO78: Stroke Recovery: Approaches to Therapy**

**Location:** WCC 207A

**Time:** Wednesday, November 15, 2023, 8:00 AM - 10:30 AM

**Presentation Number:** NANO78.01

**Topic:** C.09.Stroke

**Support:** The Ministry of Science and Technology of Taiwan, ROC, 111-2314-B-016 -051 -MY3
Medical Research Project grants TSGH-E110-213

**Title:** Augmenting brain myeloid cell phagocytosis by CDNF facilitates hematoma resolution and functional recovery after hemorrhagic stroke

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**Abstract:** During intracerebral hemorrhage (ICH), hematoma formation at the site of blood vessel damage results in local mechanical injury. Subsequently, erythrocytes lyse to release hemoglobin and heme, which act as neurotoxins and induce inflammation and secondary brain injury, resulting in severe neurological deficits. Accelerating hematoma resorption and mitigating hematoma-induced brain edema by modulating immune cells has potential as a novel therapeutic strategy for functional recovery after ICH. Here, we demonstrate that CDNF enhances microglial erythrophagocytosis accompanied by increasing CD36 and CD163 expressions, scavenger receptors on BV2 microglial cells exposed to erythrocytes. Then, we show that intracerebroventricular administration of recombinant human cerebral dopamine neurotrophic factor (rhCDNF) accelerates hemorrhagic lesion resolution, reduces peri-focal edema, and improves neurological outcomes in an animal model of collagenase-induced ICH. In line with *in vitro* results, CDNF can promote scavenger receptor expressions and facilitate erythrophagocytosis in microglia/macrophages, which subsequently increases reparative mediators while suppresses the production of pro-inflammatory cytokines within the hemorrhagic striatum. Administration of rhCDNF results in upregulation of the Nrf2-HO-1 pathway, but alleviation of oxidative stress and unfolded protein responses in the perihematomal area. Finally, we demonstrate that intravenous delivery of rhCDNF has beneficial effects in an

animal model of ICH and that systemic application promotes scavenging by the brain's myeloid cells for the treatment of ICH.

**Disclosures: K. Tseng:** None.

**Presentation Number:** NANO78.02

**Topic:** C.09.Stroke

**Support:** NIH Grant NS104117
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**Title:** Robust neuroprotection of ischemic penumbra by novel docosanoids targeting pro-homeostatic microglial and astrocyte genes

**Authors: *L. BELAYEV**[1], M. M. REID[1], A. OBENAUS[2], P. K. MUKHERJEE[1], M.-A. I. KAUTZMANN[1], G. ANDREW[1], L. KHOUTOROVA[1], J. X. JI[1], C. R. ROQUE[3], R. B. ORIA[3], B. F. HABEB[1], N. G. BAZAN[1];
[1]LSU Hlth. Sci. Ctr., New Orleans, LA; [2]Univ. of California Irvine, Irvine, CA; [3]Federal Univ. of Ceara, Fortaleza, Brazil

**Abstract:** Neuroprotection to attenuate or block the ischemic cascade and salvage neuronal damage has been extensively explored for treating ischemic stroke. However, despite increasing knowledge of the physiologic, mechanistic, and imaging characterizations of the ischemic penumbra, no effective neuroprotective therapy has been found. This study focuses on the neuroprotective bioactivity of docosanoid mediators: Neuroprotectin D1 (NPD1), Resolvin D1 (RvD1), and their combination in experimental stroke. Molecular targets of NPD1 and RvD1 are defined by following dose-response and therapeutic window. Physiologically-controlled male SD rats received 2h of middle cerebral artery occlusion (MCAo) by intraluminal suture. The behavior was evaluated on days 1, 2, 3, and 7, followed by an *ex vivo* MRI of the brains on day 7. In dose-response study, rats were treated with NPD1 (111, 222, and 333µg/kg), RvD1 (111, 222, and 333µg/kg), NPD1 + RvD1 or vehicle. All treatments were administered IV at 3h after the onset of MCAo. In the therapeutic window study, vehicle, NPD1, RvD1 (222 µg/kg), and NPD1+RvD1 were administered IV at 3, 4, 5, and 6 h after onset of MCAo. Treatments with NPD1 (111, 222, and 333µg/kg) alone greatly improved neurological scores (by 32, 28, 35%) and RvD1 (111, 222, and 333µg/kg) alone (by 35, 44, 31%) compared to the vehicle group. The neuroprotective effect was enhanced using the NPD1+ RvD1, which improved total neurological scores on days 1, 2, 3, and 7 by 25, 28, and 40% compared to the vehicle group. Ischemic core, penumbra, and total lesion volumes (computed from T2WI) were reduced with NPD1+RvD1 by 69, 70, and 67% compared to the vehicle. Combinatory NPD1+RvD1 treatment improved behavior when administered at 3, 4, 5, and 6 h by 56%, 23%, 26%, and 28%, respectively, compared to vehicles. Transcriptomic analysis revealed differentially regulated genes by DOC at 24h after treatment. We uncovered that protection after MCAo by the lipid mediators elicits expression of microglia and astrocyte-specific genes (*Tmem119, Fcrls, Osmr, Msr1, Cd68, Cd163, Amigo2, Thbs1,* and *Tm4sf1*). We have shown that treatment with NPD1 and RvD1 alone provides high-grade neuroprotection in the MCAo model. Combination therapy with NPD1 and RvD1 is more effective than the single therapy when administered up to 6 h after stroke. Uncovered genes are likely to enhance homeostatic microglia, modulate neuroinflammation,

promote DAMP clearance, activate NPC differentiation and maturation and synapse integrity, and contribute to cell survival. These treatments might provide the basis for future therapeutics for patients suffering from ischemic stroke.

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**Topic:** C.09.Stroke

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**Title:** Knockdown of NEAT1 prevents post-stroke lipid droplet agglomeration in microglia by regulating autophagy

**Authors:** Y. PAN[1], W. XIN[1], W. WEI[1], L. TATENHORST[1], I. GRAF[1], M. BÄHR[1], H. B. HUTTNER[2], ***T. R. DOEPPNER**[2];
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**Abstract:** *Background:* Lipid droplets (LD), lipid-storing organelles containing neutral lipids like glycerolipids and cholesterol, are increasingly accepted as hallmarks of inflammation. The nuclear paraspeckle assembly transcript 1 (NEAT1), a long non-coding RNA with over 200 nucleotides, exerts an indispensable impact on regulating both LD agglomeration and autophagy in multiple neurological disorders. However, knowledge as to how NEAT1 modulates the formation of LD and associated signaling pathways under stroke conditions is limited. *Methods:* In this study, primary microglia were isolated from newborn mice and exposed to oxygen-glucose-deprivation/reoxygenation (OGD/R). To further explore NEAT1-dependent mechanisms, an antisense oligonucleotide (ASO) was adopted to silence NEAT1 under in vitro conditions. Studying NEAT1-dependent interactions with regard to autophagy and LD agglomeration under hypoxic conditions, the inhibitor and activator of autophagy 3-methyladenine (3-MA) and rapamycin (RAPA) were used, respectively. In a preclinical stroke model, mice received intraventricular injections of ASO NEAT1 or control vectors in order to yield NEAT1 knockdown. Analysis of readout parameters included qRT-PCR, immunofluorescence, western blot assays, and behavioral tests. *Results:* Microglia exposed to OGD/R displayed a temporal pattern of NEAT1 expression, peaking at four hours of hypoxia followed by six hours of reoxygenation. After effectively silencing NEAT1, LD formation and autophagy-related proteins were significantly repressed in hypoxic microglia. Stimulating autophagy in ASO NEAT1 microglia under OGD/R conditions by means of RAPA reversed the downregulation of LD agglomeration and perilipin 2 (PLIN2) expression. On the contrary, application of 3-MA promoted repression of both LD agglomeration and expression of the LD-associated protein PLIN2. Under in vivo conditions, NEAT1 was significantly increased in mice at 24 hours post-stroke. Knockdown of NEAT1 significantly alleviated LD agglomeration and inhibited autophagy, resulting in improved cerebral perfusion, reduced brain injury and increased neurological recovery. *Conclusion:* NEAT1 is a key player of LD agglomeration and autophagy stimulation, and NEAT1 knockdown provides a promising therapeutic value against stroke.

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**Topic:** C.09.Stroke

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**Title:** Post-stroke loss of white matter tracts is associated with cognitive deficits in female Sprague Dawley rats and ameliorated by Mir-20a-3p treatment

**Authors: *D. SAMPATH**[1], J. M. SINGER[2], B. GOPALAKRISHNAN[2], D. A. HURST[2], R. J. MALONE[2], F. SOHRABJI[3];
[1]Neurosci. and Exptl. Therapeut., [3]Neurosci. and Exptl. therapeutics, [2]Texas A&M Univ., College Station, TX

**Abstract: Introduction:** Stroke is a leading risk factor for dementia. Our earlier studies show that the small non-coding RNA, mir20a-3p, is neuroprotective for stroke and reduces both sensory-motor impairment in the acute phase and long-term cognitive decline in female rats. Cognitive decline due to vascular diseases, such as stroke, is associated with deterioration of white matter. In this study, we examined fore brain white matter tracts in animals with characterized cognitive impairment due to stroke and the efficacy of micro RNA treatment. **Methodology:** Middle-aged (10-12-month-old) females were subjected to ischemic stroke using endothelin 1, injected adjacent to the left middle cerebral artery (MCA). Mir-20a-3p mimic or scrambled oligo was administered i.v. 4h, 24h and 70d post stroke. Animals were assessed periodically for cognitive performance up to 100d after stroke using both the cued fear conditioning test and the novel object recognition test (NORT). Thereafter rats were euthanized and perfusion fixed with 4% PFA. Brains were block-embedded, sectioned and processed for Weil myelin staining. Sections were imaged on a slide scanner, followed by quantification of the corpus callosum and internal capsule using the Q-path software. **Results:** Stroke resulted in impairment in both the cued fear conditioning test and the NORT, which was attenuated by mir-20a-3p treatment. Quantification of the corpus callosum and internal capsule volume (visualized by the Weil myelin stain) was measured in both hemispheres. We observed a significant reduction in volume of the corpus callosum and internal capsule in the ischemic hemisphere as compared to non-ischemic hemisphere in MCAo animals treated with the scrambled oligo. In contrast, sham (no-stroke) or MCAo+Mir-20a-3p animals displayed no differences in the volume of either tract between the two hemispheres. **Conclusion:** Cognitive changes measured with remote fear memory retrieval and NORT were associated with reductions in the volume of forebrain white matter tracts due to stroke. These stroke-induced deficits were attenuated in Mir-20-3p treated animals. **Supported by RFAG042189 to FS; AARF-21-849749 to DS.**

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**Topic:** C.09.Stroke

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**Title:** Endogenous zinc protoporphyrin is a promising therapeutic target for the treatment of intracerebral hemorrhage

**Authors: *R. PAN**[1], S. YU[2], H. ZHANG[2], G. TIMMINS[2], J. M. WEAVER, Jr.[2], Y. YANG[2], X. ZHOU[2], K. LIU[1];
[1]Cancer Ctr., Stony Brook Univ., Stony Brook, NY; [2]Dept. of Pharmaceut. Sciences, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM

**Abstract:** Hemorrhagic stroke, resulting from intracerebral hemorrhage (ICH), is a leading cause of disability and mortality. ICH exerts its deleterious action through several kinds of brain damage including blood-brain barrier disruption and infiltration of blood components into the brain parenchyma. The mechanisms of ICH-induced brain damage are thought to include: red blood cell lysis, heme release, and Fe overload, all leading to oxidative stress. However, these established mechanisms have not proven amenable to therapeutic intervention. Therefore, there is an urgent unmet need to discover other mechanisms that may be involved in ICH brain damage and to identify druggable targets that could lead to the development of interventional strategies with better outcomes for ICH patients. Our recent results provide compelling evidence that the accumulation of endogenously formed zinc protoporphyrin (ZnPP) critically contributes to ICH-induced brain damage. In ICH animal models, ICH caused a remarkable generation of ZnPP in brain tissue surrounding the hematoma, as evidenced by fluorescence microscopy and Autoflex MALDI-TOF mass spectrometry. Of particular note is that inhibiting ferrochelatase (FECH), an enzyme catalyzing insertion of $Zn^{2+}$ into protoporphyrin, not only substantially decreased ICH-induced ZnPP generation, but also significantly mitigated brain damage and improved neurobehavioral outcome, suggesting that endogenous ZnPP plays a key role in the brain damage following ICH. Furthermore, we have obtained preliminary evidence that ZnPP is generated in the brain of ICH patients. Thus, investigating endogenous ZnPP formation and its role in the mechanism of ICH brain injury is clinically relevant and significant. Our findings reveal a novel mechanism of ICH-induced brain damage through ferrochelatase-mediated formation of ZnPP in ICH tissue. Since ferrochelatase can be readily inhibited by small molecules, such as protein kinase inhibitors, this may provide a promising new and druggable target for ICH therapy.

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**Presentation Number:** NANO78.06

**Topic:** C.09.Stroke

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**Title:** Transcriptional analysis of regions undergoing post-stroke plasticity after photothrombotic stroke in mice

**Authors: *D. BETZ**[1], K. R. ZUURBIER[2], M. KENWOOD[4], E. J. PLAUTZ[3], P. M. DOUGLAS[5], M. P. GOLDBERG[4];
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**Abstract: Transcriptional analysis of regions undergoing post-stroke plasticity after photothrombotic stroke in mice Background:** Chronic disability affects over half of stroke survivors. After motor cortical stroke, robust plasticity occurs in multiple distinct regions, including the peri-infarct and contralesional motor (cM1) cortex. In rodent models of stroke, these regions exhibit overlapping cellular responses such as increased neuronal activity and immune activation. However, the overlap of molecular mechanisms between these regions has not been extensively studied. **Aim:** To compare the transcriptomes of peri-infarct and contralesional motor cortex undergoing post-stroke plasticity in mice. **Methods:** This study utilized 8-week-old male C57/Bl6 mice that received photothrombotic stroke (PT) (n=3) or sham surgery (n=3) targeted to primary motor cortex (M1). One week later, the peri-infarct and cM1 were dissected and processed for RNA isolation. Quality control, mRNA purification, and paired-end 150 bp Illumina sequencing were performed by Novogene, and analysis was conducted using Qiagen's CLC genomics workbench. **Results:** Differentially expressed genes (stroke vs sham) in the peri-infarct region greatly outnumbered the cM1 (911 vs. 182, p-adj<0.1). Top upregulated genes in the peri-infarct suggested high phagocytic activity and immune cell infiltration, while cM1 genes involved a wider spectrum of processes, including regeneration, angiogenesis, and leukocyte activation. Compared to peri-infarct, cM1 gene expression was enriched for pathways related to post-stroke plasticity, such as synaptic signaling and actin cytoskeletal reorganization (p-adj < 0.1). Transcriptional regulatory network analysis of the genes comprising plasticity-related pathways identified two transcriptional regulators, Restrictive element-1 Silencing Transcription Factor (REST) and specificity protein 1 (SP1), whose roles in the cM1 have not been previously established. **Conclusions:** At 7 days post-stroke, gene expression in peri-infarct cortex is dominated by inflammatory processes. In contrast, contralesional cortex includes differential pathway activation of neuronal regeneration and vascular proliferation. These results provide insights into transcriptional regulation in uninjured cortical regions that undergo post-stroke plasticity.

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**Topic:** C.09.Stroke

**Support:** VA grant I01BX005127

**Title:** Efficacy of miR-21 mimic as a post-stroke therapeutic following STAIR criteria

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**Abstract:** The miR-21 is a ubiquitous microRNA with high levels in CNS. As miR-21 targets many transcripts associated with inflammation and oxidative stress, it is thought to protect cells

following acute pathologic challenges like stroke and TBI. We observed that transient middle cerebral artery occlusion (MCAO) in adult rodents induces miR-21 expression in the ipsilateral cortex. Furthermore, induction of ischemic tolerance significantly increased miR-21 levels in rodent brain. Hence, we evaluated the therapeutic potential of miR-21 mimic following transient focal ischemia in rodents. We tested its efficacy with many combinations and permutations as recommended by Stroke Treatment Academic Industry Roundtable (STAIR). We previously showed that intracerebral administration of miR-21 mimic (2h prior to ischemia) decreased the infarct volume and promoted better motor function recovery in adult as well as aged male and female C57BL/6 mice subjected to transient MCAO. Mechanistically, miR-21 mimic treatment decreased the post-ischemic levels of pro-apoptotic and pro-inflammatory RNAs, which might be responsible for the observed neuroprotection. As post-ischemic treatment and intravenous (IV) administration are more translational, we further tested the efficacy of miR-21 mimic provided via retroorbital route after stroke. The miR-21 mimic provided IV at 5 min of reperfusion decreased the infarct volume, and improved the motor function recovery in adult male and female mice subjected to transient MCAO. However, miR-21 mimic provided IV at 2h of reperfusion failed to protect the post-stroke brain. Furthermore, miR-21 mimic also had no significant neuroprotective efficacy in male type-2 diabetic db/db mice. Overall, these studies indicate a limited neuroprotective potential of miR-21 following experimental stroke.

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**Title:** Mir-34a modulates outcomes after experimental stroke in mice

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**Abstract:** Ischemic stroke is one of the leading causes of death worldwide and causes significant morbidity among survivors. Studies have shown correlations between small noncoding RNAs (microRNAs, miRNAs) and stroke. We have previously reported that miR-34a depletion

decreases infarct volume and attenuates neurological deficits in stroke mice. To further investigate the role of miR-34a in stroke, we utilized inducible miR-34a overexpression (miR-34a$^{TG}$) mice. Transient middle cerebral artery occlusion (tMCAO) was performed on 6-month old doxycycline-induced miR-34a$^{TG}$ mice compared to wild-type (WT) controls. We confirmed that miR-34a$^{TG}$ mice had a ~5-fold increase of miR-34a in plasma at 24 hours post-stroke as detected by real-time PCR compared with WT naive controls ($p < 0.0001$) and WT-stroke controls ($p < 0.0001$). Following ischemia due to 60 min tMCAO, overexpression of miR-34a significantly exacerbated neurological deficit scores ($p = 0.0035$) and increased infarct volume compared to WT control mice at 24 hours of reperfusion ($p = 0.002$). Systemic delivery of a locked nucleic acid (LNA)-modified miR-34a inhibitor, antagomir-34a, in stroke mice substantially reduced infarct volume ($p = 0.0021$) and improved neurological deficits ($p = 0.0057$) at 24 hours post-stroke. Our post-stroke behavioral study revealed that antagomir-34a significantly improved functional outcomes including motor function assessed by accelerated rotarod tests at 2 weeks post-stroke ($p = 0.0046$) and memory deficits assessed by Morris Water Maze tests at 4 weeks post-stroke ($p = 0.0012$). These data demonstrate that miR-34a plays an important role in modulating acute stroke outcomes and suggest that miR-34a may be a promising therapeutic target for stroke therapy.

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**Title:** Intranasal administration of mesenchimal stem cell-derived exosomes promotes forelimb motor recovery in an experimental model of cortical stroke

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**Abstract:** Early treatment of ischemic stroke may reduce disability, complications and mortality. We recently demonstrated that in experimental models of neurological disorders intranasal (IN) administration of stem cell-derived exosomes restored neurotrophin- and synaptic plasticity-related signals along with rescuing the impairment of neurogenic niche proliferation (Spinelli et al., IJMS 2020, Natale et al., Stem Cells 2022). Here we investigated whether IN administration of exosomes derived from human bone marrow mesenchymal stem cells (hMSC-exo) could promote the recovery of forelimb motor function after stroke. We performed experiments on mice subjected to focal ischemia induced by rose bengal photothrombosis on the caudal forelimb primary motor cortex. The treatment started 48 hours after stroke and consisted in $50\times10^6$ exosomes/nostril, administered twice a week for 4 consecutive weeks. A battery of tests was used to assess forelimb motor function, before and 48 hours after ischemia and, subsequently,

every week up to end of treatment (week 4). Our results indicated that mice treated with hMSC-exo showed better performance compared to vehicle treated mice, starting from the second week after stroke, as revealed by higher score in the grid walking test (% foot faults: 8% *vs.* 12%; hMSC-exo *vs.* vehicle: p=0.003; n=17 mice/group); in the cylinder test (% touches with impaired forelimb: 62.3% *vs*. 44.5%; hMSC-exo [n=7] *vs.* vehicle [n=6]: p=0.005) and in the grip strength test (force(g)/body weight (g): 5.6 *vs*. 3.8; hMSC-exo *vs.* vehicle: p=0.01; n=6 mice/group). Interestingly, histopathological assessment revealed that at the end of treatment, infarct volume was reduced in exosome-treated mice (infarct volume: 0.49 $mm^3$ *vs*. 1.04 $mm^3$; hMSC-exo *vs.* vehicle: p=0.03; n=5 mice/group), suggesting a neuroreparative effect of exosomes, favouring functional recovery.

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**Title:** Unveiling the age-related metabolic shift of innate immune cells in response to stroke injury

**Authors: *C. AGBAEGBU IWEKA**[1], M. CABRERA[1], E. BLACHER[1], L. ALEXANDROVA[2], M. MCREYNOLDS[3], K. I. ANDREASSON[1];
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**Abstract:** Stroke is a multiphasic process that exhibits a secondary progression of ischemic injury due to an intense inflammatory response that worsens the stroke injury. The question is "*Why do innate immune cells contribute to stroke injury?*" Stroke occurs more frequently in aged individuals where severity and outcome are significantly worse than for young individuals. Aging is characterized by structural and functional alterations in the immune system, leading to constitutive low-grade inflammation, impaired immune function, immunosenescence and dyshomeostasis. Immune function is dependent on a homeostatic metabolism and recent studies have highlighted a fundamental role for cellular metabolism in programing immune responses. Our previous studies and others have demonstrated a significant decrease in nicotinamide adenine dinucleotide ($NAD^+$), a key factor for cellular metabolism, in aging innate immune cells. $NAD^+$ is known to be quickly depleted in an immune challenge thereby disrupting immune cell homeostasis and function. In this study, we hypothesized that *the metabolism of innate immune cells is impaired, leading to the maladaptive immune response that exacerbates stroke injury in aged individuals*. We performed a metabolic assessment of blood monocytes isolated from young (3-6 mo) and aged male mice (18-20 mo) at 4.5 hr, 24 hr and 72 hr after stroke. We observed significant age-dependent changes in the oxygen consumption rates (OCR) that correlated with the inflammatory status of blood monocytes from stroked mice. The OCR of blood monocytes from young mice was unchanged at 4.5 hrs, decreased briefly at 24 hrs and recovered by 72 hrs after stroke. However, the blood monocytes from aged mice exhibited an

increase in metabolic activity at 4.5 hrs and metabolic exhaustion by 24 hrs that persisted at 72 hrs after stroke. Additionally, the inflammatory status of the blood monocytes from young mice did not change impressively at any time point after stroke, whereas a significant increase in inflammation was observed in the blood monocytes from aged mice after stroke. Finally, the blood monocytes from the aged stroke mice had significantly lower $NAD^+$ levels and produced quinolinic acid, a neurotoxin that binds N-methyl-D-aspartate (NMDA) receptors to induce excitotoxicity in neurons. These results indicate that systemic blood monocytes are already metabolically depleted, neurotoxic and functionally impaired prior to infiltrating the brain parenchyma as early as 4.5 hours after stroke in aged mice. These deficits in the metabolism of blood monocytes from aged mice may account for the maladaptive immune response that exacerbates stroke injury in aged mice.

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**Nanosymposium**

**NANO79: Representations of Objects and Scenes**

**Location:** WCC 147A

**Time:** Wednesday, November 15, 2023, 8:00 AM - 10:15 AM

**Presentation Number:** NANO79.01

**Topic:** D.06. Vision

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**Title:** Optogenetic Stimulation of Inferotemporal Cortex is Perceived Earlier than Stimulation

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**Abstract:** Local stimulation in high-level cortical visual areas perturbs the contents of visual perception. We have previously demonstrated that visual percepts evoked by optogenetic stimulation in IT cortex can be reconstructed using a method we have termed perceptography. While perceptography informs about the shape and quality of stimulation induced perceptual events, we do not know when they are perceived with respect to the external physical events. Specifically, we do not know if a brief cortical stimulation impulse is perceived to be simultaneous, earlier or later relative to concurrent sensory input. In this study, we use high throughput behavioral optogenetics coupled with visual interference to measure the time course of the optogenetically evoked perceptual event. An adult macaque monkey was trained to behaviorally detect and report a brief optogenetic excitatory impulse delivered to its central IT cortex. The animal started each 1.6s trial by fixating on a randomly chosen computer-generated image (8 deg.). A ~1x1mm area of the IT cortex was optogenetically stimulated in half of the trials at random for 60ms half way through the image presentation using an implanted LED

array. To reveal the temporal profile of the stimulation induced visual percept, we covered the image with a high contrast dynamic noise pattern (12 deg.) for 100ms at one of eleven possible time points relative to the onset of stimulation. We hypothesize that by interrupting the image presentation at the proper time we are able to mask the stimulation-evoked visual percept. By looking at one of the two subsequently presented targets, the animals reported whether or not the trial included a cortical stimulation impulse and received liquid reward for correct reports. We find that the impact on the monkey's performance varies with the onset time of the visual noise. After training, in the absence of noise, the monkey had a baseline accuracy of 96%. Presentation of visual noise 200ms prior to stimulation elicited a significantly larger miss rate compared to baseline ($p<0.05$) and to other noise onset times ($p<0.05$). This is reflected by a significant decrease in the monkey's d' for the same noise onset time against baseline ($p<0.05$) and against other noise onset times ($p<0.05$). Furthermore, we find that perceptography with an image perturbation onset of 200ms prior to stimulation induces larger false alarm rate compared to image perturbation with an onset of 0ms relative to stimulation ($p<0.05$).

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**Title:** The neural representation of the fake objects

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**Abstract:** The inferotemporal (IT) cortex is crucial for object recognition. Earlier research has suggested that the IT cortex's functional structure can be understood through an object space model built using deep learning networks. However, category-specific regions in the IT cortex, such as areas dedicated to faces, scenes, and bodies, imply that its organization might also be based on semantic categories. To distinguish between these two hypotheses, we used fMRI to measure human subjects' responses to artificial images, referred to as "fake objects," which lack any semantic category information. The fake objects were created using a Generative Adversarial Network (GAN). We projected these generated fake objects onto the PC1-PC2 space using AlexNet, which was derived from a PCA analysis of the fMRI responses of human subjects when they were shown 500 real objects. We chose 100 fake objects based on their projections onto the PC1-PC2 space, resulting in a ring-like structure. Subjects were instructed to perform three tasks in separate scans: two image categorization tasks based on the images' projection onto the two orthogonal axes in the object space and a fixation color discrimination task. The study's results show that the IT cortex can be effectively modulated by these fake

objects, and the modulation of each voxel can be accurately represented by the object space model as the projection on the preferred axis. This holds true even for voxels located in category-selective regions, such as the Fusiform Face Area (FFA) and Extrastriate Body Area (EBA). Furthermore, the preferred axis of each voxel in the IT cortex remained consistent across the three tasks, although the absolute selectivity decreased in the fixation task. Additionally, the modulation of the two different image categorization tasks was more noticeable in the frontal and parietal cortex. Our results demonstrate that the functional organization of the IT cortex can be better explained by the object space model than the semantic model, and the representation of object space is relatively stable across different tasks, whose outputs can be read out by the later stages of the brain.

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**Title:** Fine-scale representation of eye-of-origin information throughout the visual hierarchy

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**Abstract:** It is generally believed that inputs from the two eyes converge beyond the primary visual cortex (V1). While some studies have reported successful decoding of eye-of-origin in the extrastriate cortex up to V3, these findings were largely attributed to large-scale nasotemporal difference in monocular responses. In this study, we investigated the presence of eye-of-origin information in the high-level visual cortex and frontoparietal areas using high-resolution (1.2 mm isotropic) functional magnetic resonance imaging (fMRI) at 7 Tesla. In an ocular-bias localizer, fifteen human participants viewed a counterphase flickering checkerboard monocularly, alternating between the two eyes every 24 seconds. They also engaged in a binocular rivalry task with a pair of red and green gratings dichoptically presented in orthogonal orientations. The association between eye and color changed across runs. We trained a linear classifier (SVM) on the localizer data to predict the stimulated eye at each time point. Surprisingly, significantly above chance-level decoding of eye-of-origin was found for nearly all tested visual areas, including V1, V2, V3, hV4, VO, LO, TO, V3ab, IPS, and FEF. Performance gradually declined as we averaged voxels into fewer features, but no difference was observed whether voxels were binned randomly or within hemisphere, suggesting that the results cannot be explained by nasotemporal bias. We were also able to cross-decode the eye-of-origin for the perceptually dominant stimulus during binocular rivalry in LO, V3ab, and IPS beyond the extrastriate cortex. Our results indicate that eye-of-origin information is more widely preserved throughout the visual hierarchy than previously believed. The distinct influences of each eye

persist into later processing stages, even when both eyes are stimulated, potentially providing a mechanism for top-down influence in binocular vision.

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**Presentation Number:** NANO79.04

**Topic:** D.06. Vision

**Title:** Comparing human and macaque functional organization using fMRI

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**Abstract:** The macaque is often used as a model of human visual perception and cognition. Although convergent results suggest clear homologies for early visual areas (V1, V2, V3, and MT), the degree to which higher-order cortical regions (IT and PFC) are similar has been more difficult to ascertain. Seeking a deeper understanding of the functional organization of these higher-order regions, we collected extensive data from ten human participants and two macaques using the same method (fMRI), the same visual stimuli, and highly similar behavioral tasks. We identified retinotopically organized areas such as V1 with Population Receptive Field (PRF) mapping, and functionally defined regions such as those of the ventral visual pathway with images of faces, bodies, objects, scenes, colors, and words. We then used a new version of "hyperalignment" (optimized for group comparison) to identify representations shared between species during the viewing of naturalistic stimuli. At a fine spatial scale, the organization of information across voxels is known to be highly idiosyncratic. Hyperalignment has been used extensively in humans to abstract beyond this level of idiosyncrasy, and relate activation patterns across individuals. Here, we first identified regions of interest (category and/or retinotopic), then used hyperalignment to compare their multivariate signatures between species.
We first confirmed that we could recover a multivariate representational space for regions activated by faces in the category localizer task. We found that between three and five dimensions were optimal for between subject (and species) movie segment decoding. We then tested the precision of this approach by examining the extent to which a "searchlight" analysis (which explored the entire monkey cortical sheet for information shared with human face-selective regions) could recover monkey face regions. The results differed by stimulus type. When using a novel stimulus, which minimized visual motion and required fixation, shared variance was largely constrained to face-selective patches. When using traditional, but more complex, movie stimuli, shared variance also included early visual areas (V2, V3d, V3v, MT, V4d, V4v), parietal cortex (IPS), and widespread regions of IT.
This proof of principle of the cross-species hyperalignment approach opens the door to investigating shared representational structure for retinotopically-organized regions and other categorically-selective regions (body, object, scene, color, and word-selective regions).

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**Title:** Object Space as the Foundation for Object Recognition in the Human Ventral Temporal Cortex

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**Abstract:** Object recognition, an essential cognitive function in the human visual system, depends on the ventral temporal cortex (VTC). However, the functional principles and neural mechanisms of the IT cortex remain largely unexplored. Earlier studies have proposed the use of the object space model to understand the functional organization of the IT cortex in macaques (Bao, et al. 2020), but its relevance to humans is still unclear. To investigate this, we performed a functional magnetic resonance imaging (fMRI) study on five human subjects who were shown 500 static object stimuli. The object space was defined using principal component analysis of the VTC's responses. Our results showed that the functional organization of the VTC can be represented by a low-dimensional object space, with the first two principal components accounting for 92% variance of the consistency of the representation space. These two principal components can be broadly characterized as face versus spiky objects and animal versus stubby objects. Additionally, to examine the consistency of object spaces among different participants, we used hyperalignment methods (Haxby, et al. 2020) to project responses of the VTC onto a common space and then back onto the cortex of one participant, creating a unified template. The high consistency across subjects was found not only in known category-selective areas but also in other parts of the VTC, suggesting a common space represented across different subjects. To further investigate the similarities in object representation between humans and macaques, we compared the object space between the two species. Comparisons with electrophysiological data from neurons showed that the space constructed by human VTC responses closely resembles that represented in the IT of macaques. This implies that the creation of object-specific space representations is a key aspect of object recognition and that the functional organization of the IT cortex is preserved across species.

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**Title:** Visual grouping and segmentation via corticocortical feedback: A causal study

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**Abstract:** Cortical analysis of visual scenes involves grouping and segmentation of image components, which is thought to be mediated by intra- and inter-areal interactions, but the causal role of corticocortical feedback remains unclear. In order to dissect the contribution of corticocortical feedback to visual grouping and segmentation, we trained monkeys in different figure-ground perceptual tasks. We examined the influences of V4-to-V1 feedback by comparing V1 neuronal responses before and after cooling or lesioning of V4, a mid-level visual area that is known to represent global forms and provide direct and indirect feedback connections to V1. Inactivation of V4 markedly impaired contour and surface perception; it also diminished or even abolished the global contour and surface signals contained in the late components of V1 neuronal responses. In contrast, the feature-dependent contextual modulations seen in V1 early responses were little affected, such as the effects of surround inhibition and local orientation contrast. The feedback modulation of the grouping and segmentation processes in V1 was characterized by superimposed facilitatory and inhibitory components with asynchronous onset times. Such a modulatory mode, which targeted the late components of V1 responses, was necessary for, and also shared by, distinct figure-ground segregation processes including not only contour grouping and surface segmentation but also singleton detection. The feedback influences tended to decouple correlated neural activities, as evidenced by an increase—after V4 inactivation—in the spike-spike coherence between V1 neurons. However, decoding analyses showed that the decoupling did not contribute to the neural population code for grouping and segmentation. Overall, our findings provide causal evidence that corticocortical feedback plays a critical and unified role in visual grouping and segmentation.

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**Title:** Probing the Dynamics Preferences of Object vs. Textural via Image Optimization

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**Abstract:** The neural basis of our object sense is an important question for both biological and artificial visual systems. To study it in visual cortex (including V1, V4 and pIT), we set out to optimize images for neurons in all these regions, applying a closed-loop evolutionary algorithm. Crucially, we used two generative image models: (1) DeePSim, which parametrizes irregular image patterns, and (2) BigGAN which parametrizes object identity and nuisance variables. We found that neurons could guide image optimization on both pattern- and object-based image manifolds, in two monkeys. Image optimization in DeePSim successfully increased the neuronal

firing rate in nearly all experiments for both V1 and V4. However, when it came to BigGAN, it failed consistently for V1; while for V4, it succeeded in about 50% of experiments. Interestingly, in IT, the success rate was around 60% for both image spaces. This suggests that neuronal tuning along the ventral stream became more aligned with object-based and less aligned with pattern-based parametrization.In V1 and V4, optimized DeePSim images evoked higher firing rates than BigGAN; in contrast, in IT, the optimized images from both spaces evoked comparable rates. Intriguingly, in convolutional neural networks (CNNs), optimized DeePSim images were more activating than BigGAN across the hierarchy. This suggests that CNN units were less object-aligned than IT neurons, corroborating the finding that CNN models are more texture-biased. Next, by analyzing neuronal dynamics, we found that IT response preference for BigGAN images emerged later in time, suggesting that "object preference" in IT required recurrent processing. To evaluate the images, we found that the V4 neurons guided the BigGAN images to become less object-like relative to baseline, while guidance from IT neurons led the BigGAN images to have higher objectness score than baseline. In contrast, guidance from both V4 and IT made DeePSim images more object-like. Visually, we could identify similar features synthesized in both spaces, and we found the feature similarity of the optimized images correlated more with the similarities of the response dynamic in two spaces rather than mean response. This suggests that the dynamics of the neuronal response encode critical sensory information.All in all, our results point to a dynamic preference for objects that gradually evolves over time in higher visual cortex, e.g., IT, while still retaining robust responses across pattern generating space. These results also highlight a gap of object alignment between the current computational models and biological visual system, which may be addressable by recurrent processing.

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**Topic:** D.06. Vision

**Title:** Spontaneous visual processing of non-rigid materials recruits intuitive physical inference regions and activates physics-based representations in the human brain

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**Abstract:** The visual world of fluids and non-rigid objects such as liquids and cloths poses a fundamental challenge to perception: Their shapes are either simply mutable or can deform endlessly under external forces, yet we form rich and persistent percepts by observing how they move. Previously, using psychophysics and modeling, we found that human soft object perception is best explained by a model that incorporates 'intuitive physics', as opposed to alternative models that only consider pattern recognition. Here, we hypothesize that spontaneous visual processing of soft objects, i.e., in the absence of a physics-related task, (i) activates overlapping brain regions as those implicated in intuitive physical inference and (ii) leads to representation of physical properties (e.g., stiffness, viscosity) in these regions. To investigate, we scanned participants (N=15) in fMRI using previously validated localizers for intuitive physical inference and soft material perception. We also scanned the same participants while they passively viewed naturalistic animations of cloths and liquids with different stiffness and

viscosity values and scene configurations. Despite the differences in various aspects of the two localizers (static images vs. dynamic videos and physical judgment vs. passive viewing), we found substantial overlap between the regions of interest (ROI) identified from the intuitive physics and the soft material perception localizers. The overlap of the two localizers lends support for our first hypothesis and occurs primarily in parietal (the postcentral, supramarginal, and angular gyri) and occipital temporal regions (inferior temporal gyrus and lateral occipital cortex). To test our second hypothesis, we obtain the univariate activity differences between soft vs. stiff cloths and runny vs. thick liquids, and compare these differences for V1 vs. the union of the two ROIs. In preliminary analysis, we find a significantly greater effect of physical property in the union of the two ROIs than V1 ($p=.03$, paired-sample t-test). These results suggest that the perception of fluids and soft objects is a form of intuitive physics, and indicate a common 'physics engine' in the brain supporting both how we reason about and perceive the physical world.

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**Title:** Bipartite invariance in mouse primary visual cortex

**Authors: *Z. DING**[1], D. TRAN[1], K. PONDER[1], E. COBOS[1], P. FAHEY[1], Z. DING[1], E. WANG[1], T. MUHAMMAD[1], J. FU[1], S. CADENA[2], S. PAPADOPOULOS[1], S. PATEL[1], E. WALKER[3], J. REIMER[1], K. FRANKE[1], F. SINZ[1], A. ECKER[4], X. PITKOW[1], A. TOLIAS[1]; [1]Baylor Col. of Med., Houston, TX; [2]Inst. of Computer Sci. and Campus Inst. Data Sci., Univ. of Göttingen, Göttingen, Germany; [3]Dept. of Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [4]Max Planck Inst. for Dynamics and Self-Organization, Göttingen, Germany

**Abstract:** A key challenge sensory systems have to solve is to robustly extract specific features despite large variations in their natural visual input. To understand how brains achieve this generalization, it is crucial to identify features that neurons exhibit selectivity and invariance towards. However, the high-dimensional nature of ecological visual inputs makes it challenging

to systematically characterize neuronal tuning. As a result, our knowledge of the invariances encoded by neurons is restricted to a handful of examples, such as phase invariance demonstrated by complex cells in the primary visual cortex when presented with grating stimuli. Here, we extended "inception loops" — a paradigm that iterates between large-scale recordings, neural predictive models, and *in silico* experiments followed by *in vivo* verification — to characterize neuronal invariances in the mouse primary visual cortex. Using a model trained to predict responses to arbitrary visual stimuli we synthesized Diverse Exciting Inputs (DEIs), a set of inputs that differ substantially from each other while each driving a target neuron strongly, and verified these DEIs' efficacy *in vivo*. We discovered a novel bipartite invariance: one portion of the receptive field encoded phase invariant texture-like patterns, while the other portion encoded a fixed spatial pattern. Our analysis revealed that the division between the fixed and invariant subfields matched object boundaries defined by spatial frequency differences in highly activating natural image patches, suggesting that bipartite invariance contributes to segmentation. We also replicated these bipartite invariances in the functional connectomics MICrONS dataset, which opens the way towards dissecting circuit-level mechanisms. Employing this method across cell types, visual areas, sensory modalities, and species can unravel how latent variables are robustly extracted from natural scenes, leading to a deeper understanding of generalization.

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**Nanosymposium**

**NANO80: Addiction: Genetics, Translational, and Clinical Studies**

**Location:** WCC 147B

**Time:** Wednesday, November 15, 2023, 8:00 AM - 11:30 AM

**Presentation Number:** NANO80.01

**Topic:** G.09. Drugs of Abuse and Addiction

**Title:** Rdoc-based approach to classify prevention interventions for substance use disorders in adolescents: a systematic review

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**Abstract: RDoC-based Approach to Classify Prevention Interventions for Substance Use Disorders in Adolescents: A Systematic Review**
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In contrast to treatment interventions viewing substance use disorder as a brain disease, thus far, little attempt has been made to convey neuroscience knowledge as a framework for prevention studies. In this review study, we first carried out a systematic review to synthesize the available evidence about the available school-based addiction prevention programs for adolescents. We then categorized these interventions within an RDoC-based (Research Domain Criteria) framework. Relevant search terms were used to identify intervention studies published from 1996 to August 2022. In this systematic review, we selected studies having a person-centered, drug/alcohol prevention as the main approach, written in English, and conducted as a school or college-based program for adolescents who were not clinically diagnosed with a disorder and were not considered as current drug users. The final review included 22 distinctive prevention interventions out of 101 selected published studies. Categorization of the interventions to the RDoC constructs (Negative Valence Systems, Positive Valence Systems, Cognitive Systems, Arousal and Regulatory Systems, and Social Processes) showed that the Social Processes was the most frequently targeted construct in the interventions (n=22, 100%), followed by the Positive Valence Systems (n=18, 81.81%), and the Cognitive Systems (n=15, 68.18%). The least frequently targeted constructs were found to be Regulatory Systems and the Negative Valence Systems (31.81% and 18.18%, respectively). Using an RDoC-based framework to process published interventions and develop new interventions will mobilize available neuroscience resources in different levels (molecular, cellular, neural, behavioral and self-reports) including large scale RDoC based studies/databases like ABCD and HBCD to be implemented in addiction prevention and delineate the gaps in considering important brain-based targets and the relevant interventions.

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**Topic:** G.09. Drugs of Abuse and Addiction

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**Title:** Translational Neuroscience Perspectives on the Prevention of High-Risk Behaviors

**Authors: *E. J. ROSE**;
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**Abstract:** High-risk behaviors that are relatively ubiquitous in adolescence and emerging adulthood, such as substance use, are associated with structural and functional adjustments in interconnected neural systems underlying neurocognitive phenotypes that are associated with self-regulation, i.e., altered ***reward*** *(*e.g., reward anticipation, learning, & receipt*; mesocorticolimbic dopamine pathway regions in the striatum and prefrontal cortex);* ***executive control*** *(e*.g., working memory, impulse control, decision-making*; dorsolateral, medial prefrontal, and orbitofrontal cortices)*, and ***affective processing*** *(amygdala).* Moreover, cross-sectional and longitudinal studies of the emergence of high-risk behaviors indicate that differentiation in these critical neurocognitive networks may not simply be a consequence of

risky behaviors *per se.* Rather, atypical neurodevelopmental trajectories in these neural systems may be an antecedent of maladaptive behavioral outcomes, especially in those with increased liability for such behaviors (e.g., individuals who experience adverse and traumatic experiences in childhood and adolescence). While a more comprehensive delineation of potential neurocognitive precursors of riskier patterns of behavior, which are characterized by less adaptive self-regulation, may provide a critical opportunity for more effective preventive interventions for high-risk outcomes, on the whole, prevention research has neither specifically focused upon nor sought to ameliorate these potentially malleable neurobiological mechanisms. This presentation will highlight neuroimaging evidence from our research group regarding neural antecedents and consequences of high-risk behaviors in at-risk young people, how they correspond to individual, social, and contextual risk factors (e.g., poverty, food insecurity, maltreatment), *and* how they might contribute to novel, neuro-informed preventive interventions that are better tailored and more effective than alternative strategies.

**Disclosures: E.J. Rose:** None.

**Presentation Number:** NANO80.03

**Topic:** G.09. Drugs of Abuse and Addiction

**Support:** 1R41MH133540-01

**Title:** An Emotion Recognition Training Program For Youth With Conduct Disorder

**Authors: *N. THOMSON**[1], L. HAZLETT[2];
[1]Surgery and Psychology, [2]Surgery, Virginia Commonwealth Univ., Richmond, VA

**Abstract:** Background: Conduct disorder (CD) remains one of the most common and impairing psychiatric disorders among youth. A subset of youth with CD display callous-unemotional (CU) traits. Youth with CD and CU traits are more likely to engage in chronic criminal behavior and develop psychopathology into adulthood. Although both CD and CU traits are inextricably linked to poor outcomes, there remains a scarcity of targeted interventions for CD and CU traits. Interventions that do exist largely focus on reducing antisocial behavior rather than disrupting the developmental mechanisms of CD and CU traits. The present study discusses the development process for virtual reality intervention specifically designed to target mechanisms of CD and CU traits. Further, we report on the acceptability of the intervention, and the feasibility and usability of Impact VR in schools, hospitals, and in the home.
Methods: Semi-structured interviews were conducted with youth with CD and their caregivers, teachers, and mental health professionals (*N*=60). Participants completed the interviews after participating in Impact VR and after using the VR headset in their homes, school, or mental health clinic.
Results: Overall, youth with CD reported a high level of acceptability for Impact VR. Youth participants stated that Impact VR was low burden (e.g., not too long, physically comfortable), culturally sensitive, had high intervention coherence (e.g., instructions and tasks were clear), and yielded a high score on opportunity cost (e.g., the intervention was a good use of my time). Further, youth reported a high level of self-efficacy and perceived effectiveness (e.g., understanding the skills being taught, and how the skills were useful). Lastly, caregivers, teachers, and mental health professionals agreed that the intervention was appropriate for its

intended use, and was easily implemented across different environments (i.e., schools and homes). All adult participants reported that they would like to have Impact VR available as a resource to youth with CD in their respective positions (i.e., teacher, mental health professional). Conclusions: The preliminary findings of this new and innovative intervention indicate a strong level of acceptability for Impact VR as an intervention for youth with CD. Further, the feedback from teachers, parents, and mental health professionals suggests Impact VR is suitable for deployment in a range of environments.

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**Title:** Development of a mobile cognitive resilience training program for substance use prevention

**Authors: *J. VASSILEVA**[1], T. REZAPOUR[3], K. MCLEAN[2], N. MALEKI[4], H. EKHTIARI[5]; [1]Psychiatry, [2]Virginia Commonwealth Univ., Richmond, VA; [3]Inst. for Cognitive Sci. Res., Tehran, Iran, Islamic Republic of; [4]Metacognium LLC, Murfreesboro, TN; [5]Univ. of Minnesota, Minneapolis, MN

**Abstract:** Despite revolutionary advances in understanding the neurobiological basis of addiction, these advances have not been translated into prevention and intervention programs for substance use disorders. To address this gap, we recently developed a mobile program for adolescents and young adults that integrates neuroscience-based psychoeducation and game-based cognitive training. The goal of the program is to enhance metacognitive awareness, build cognitive resilience, and promote the use of specific neurocognitive skills to help students cope with stress and prevent or reduce substance misuse. The program is based on a recently proposed RDoC framework for substance use prevention, which targets 5 neurofunctional domains of functioning (Rezapour et al., 2023). It incorporates neurocognitive games, videos, animations, and cartoons, and provides students an interactive psychoeducation on key neurocognitive functions implicated in substance use and cognitive components of resilience. It then instructs them on how they could gain better control of their thoughts, emotions, and behaviors by implementing metacognitive and compensatory strategies, presented within the context of real-life scenarios involving substance use. The app-based program consists of 4 self-administered 20-30 minutes-long sessions, each of which focuses on a specific neurocognitive function implicated in substance use and other addictive behaviors: (1) Attention, (2) Memory, (3) Cognitive Flexibility & Inhibitory Control; and (4) Decision-Making & Incentive Salience. We recently completed a pilot study to obtain preliminary data on the feasibility of delivering the program to college students. Participants were recruited from the Spit for Science Registry at Virginia Commonwealth University. Consenting participants were invited to complete a pre-intervention screening survey, 4 intervention sessions, and a post-intervention survey providing

feedback about the intervention. Of the 85 participants who completed the pre-intervention survey, 72 completed one session, 71 - two sessions, 69 - three sessions, and 67 - four sessions, all of whom gave post-intervention feedback. Preliminary results reveal high acceptance and satisfaction with the program, including the length and number of sessions. From the different intervention components, the neurocognitive games were liked the most (70.8%) and the brain training strategies were liked the least (33.8%). These preliminary results will be used to revise the intervention and conduct a randomized controlled trial (RCT) to evaluate its efficacy.

**Disclosures: J. Vassileva:** None. **T. Rezapour:** None. **K. McLean:** None. **N. Maleki:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Metacognium LLC. **H. Ekhtiari:** None.

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**Title:** Bridging the Gap between Genetic Research and Prevention: The Development of a Novel Personalized Feedback Program for Substance Use

**Authors: *D. DICK**[1], M. CHOI[2], M. N. DRIVER[4], E. BALCKE[1], T. SAUNDERS[5], J. M. LANGBERG[3];
[1]Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ; [2]Dept. of Psychology, [3]Grad. Sch. of Applied and Professional Psychology, Rutgers Univ., Piscataway, NJ; [4]Dept. of Human and Mol. Genet., [5]Dept. of Recreation and Well-Being, Virginia Commonwealth Univ., Richmond, VA

**Abstract:** Background: Risky substance use among college students is widespread. Current interventions focus primarily on students' current substance use. We hypothesized that shifting focus from *current use* to *underlying risk factors* that have been identified in the genetic epidemiological literature, would be a complementary approach for prevention programming, that also aligns with the personalized medicine movement, which aims to provide individuals with information about their risk profile, along with personalized recommendations, to enable better self-regulation of health.
Methods: Our group developed an online Personalized Feedback Program (PFP) for college students that provides feedback about individuals' underlying genetically influenced externalizing and internalizing risk factors for substance use, along with personalized recommendations/resources. An open trial (n=286) was conducted to assess preliminary responses to the PFP and evaluate intentions related to campus resource use. A randomized controlled trial subsequently was initiated to further evaluate the efficacy of the PFP. Participants (n= 251) completed one of four conditions: (1) campus resource list, (2) online PFP, (3) online

Substance Use Feedback Program (based on brief motivational intervention principles), and (4) PFP + Substance Use Feedback Program. A baseline survey was administered that assessed substance use, mental health, risk comprehension, and utilization of campus resources, and two follow-up surveys were administered at 30-days and 3-months post-intervention.
Results: The open trial and RCT indicated high acceptance of the program: 81% of students in the open trial and 89% of RCT participants reported they enjoyed the PFP. After completion of PFP in the open trial, students reported intending to use 1.2 more resources ($t$=-9.2, $p$<.001). Among drinkers, 39% of open trial participants and 38% of RCT participants reported intending to drink less after completion of the PFP, as compared to only 22% who reported intentions to reduce drinking prior to completion of the PFP. There were significant reductions in cannabis use 30 days post-completion of the PFP compared to the other groups; trends for alcohol were also in the expected direction.
Conclusions: The provision of personalized information about risk, coupled with personalized recommendations and resources, may serve as a complementary method to reduce risky substance use among college students.

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**Topic:** G.09. Drugs of Abuse and Addiction

**Title:** Neuroscience-informed Framework of Prevention Intervention in Substance Users

**Authors: *H. EKHTIARI**[1], T. REZAPOUR[2], J. VASSILEVA[3];
[1]Laureate Inst. For Brain Res., Minnepolis, MN; [2]Inst. for Cognitive Sci. Studies (ICSS), Tehran, Iran, Islamic Republic of; [3]Virginia Commonwealth Univ., Richmond, VA

**Abstract: Neuroscience-Informed Framework of Prevention Interventions in Substance UseDisorders**Hamed Ekhtiari[1], Tara Rezapour[2], Jasmin Vassileva[3]1. Department of Psychiatry, University of Minnesota, Minneapolis, MN, USA2. Department of Cognitive Psychology, Institute for Cognitive Science Studies (ICSS), Tehran, Iran3. Department of Psychiatry, Virginia Commonwealth University, Richmond, VA, USANeuroscience has contributed to uncover the mechanisms underpinning substance use disorders(SUD). The next frontier is to leverage these mechanisms as active ingredients to create moreeffective interventions for SUD. Recent large-scale cohort studies are generating multiple levelsof neuroscience-based information with potential to inform the development and refinement offuture preventive strategies. However, there is still no available well-recognized frameworks toguide the integration of these complex datasets into prevention trial protocols. The ResearchDomain Criteria (RDoC) provides a neuroscience-based multi-system framework that is wellsuited to facilitate translation of neurobiological mechanisms into behavioural domains amenableto preventative interventions. We propose a novel RDoC-based framework for prevention sciencethat organizes and advances the integration of technologies and findings from neuroscience intothe refinement of current and construction of future preventive and early interventions. Thisneuroscience-informed framework categorizes addiction risk factors within the dysfunction of thefive major RDoC domains *(Negative Valence Systems, Positive Valence Systems, CognitiveSystems, Arousal and Regulatory Systems, and Social Processes)*. By using

this neuroscience-informed framework, distinct neurocognitive trajectories which have been recognized asprecursors or risk factors for SUDs, can be targeted, and more importantly, the change processescan be evaluated to inform causal hypotheses. This framework can also inform individualizedassessment, intervention development and outcome measurement in preventive interventions.

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**Title:** Multi-modal prediction of successful smoking cessation: Insights from neural, computational, and ecological momentary assessments data

**Authors: *M. KWON**[1], J.-H. LEE[1], H. KIM[1], E. LEE[1], J. J. IM[1], H. DOH[1], J. YANG[1], M. A. PITT[4], J. I. MYUNG[4], H. PARK[5], H. JOH[2], W.-Y. AHN[1,3];
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**Abstract:** A myriad number of factors influence smoking cessation, which makes it difficult to predict its outcomes. Despite recent studies utilizing machine learning techniques to predict smoking cessation outcomes and to identify their predictors, they primarily focused on a single type of data, such as self-report surveys, computational markers, or neural measures, which could suffer from low test-retest reliability. To address this gap, we gathered multi-modal data before and throughout smoking cessation intervention. The pre-intervention measures included functional magnetic resonance imaging data obtained during a decision-making task, assessments of distress tolerance, subjective pain ratings, and facial expression in a socially and physically stressful environment, and demographic and clinical self-report surveys. Throughout the intervention, we utilized ecological momentary assessment (EMA) techniques for daily self-reports on psychological variables, such as depression, anxiety, smoking craving, mood, and stress over the course of the smoking cessation program. We also assessed computational markers of risk-taking, ambiguity-aversion, and delay discounting on a daily basis by using app-based decision-making tasks: choice under risk and ambiguity and delay discounting task. We applied a machine learning model (i.e., elastic net) to the multi-modal dataset to predict the outcomes of the smoking cessation program. The area under the curve of the ROC curve was 0.82 in the test set. The results indicate that adherence to treatment, including the use of prescribed medication and active participation in weekly clinics, emerged as important predictors of smoking cessation. Furthermore, self-report measures and computational markers collected through EMA significantly contributed to the prediction of smoking cessation. Notably, lower levels of craving, depression, and delay discounting, as well as consistent sleep patterns during the program, were indicative of successful smoking cessation. These results highlight the

importance of EMA measures in reliably capturing dynamic states of individual characteristics that contribute to smoking cessation. By encompassing a range of neural, computational, and behavioral features through various modalities, our study underscores the significance of employing reliable measurements for accurately predicting smoking cessation success.

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**Title:** Proteomic insight to inform drug discovery for problematic alcohol consumption

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**Abstract: Background**Alcohol use disorder (AUD) is a common neuropsychiatric disorder that is a leading cause of morbidity and mortality worldwide; however, only a few pharmacological treatment options are currently available, highlighting the need for novel and safe drug development. While protein biomarkers with causal genetic evidence are promising novel drug target candidates for AUD, systematic scans of brain proteins have not been performed. **Methods**We integrated genome-wide association summary statistics (GWAS) for AUD and alcohol consumption behaviors, i.e., problem items from the Alcohol Use Disorders Identification Test (AUDIT-P), binge drinking, and total drinks per week (DPW), and applied *cis*-instrument Mendelian randomization (MR) to perform a proteome-wide MR with data from >1,700 brain proteins within the dorsolateral prefrontal cortex (dlPFC) to investigate their relationships with the AUD and alcohol consumption behaviors. We performed replication and validation analyses in independent proteomic and transcriptomic datasets. **Results**
We identified 34 unique brain protein-alcohol associations that emerged as causal mediators of AUD and alcohol consumption behaviors. Novel proteins not previously implicated in alcohol consumption behaviors included *CAB39L*, *TESC* (P-value=$1.9910^{-7}$ (AUD)), *ERLIN1* (P-value=$2.3110^{-12}$ (AUDIT-P)), *CPS1* (P-value=$6.910^{-6}$ (binge drinking)), *HDGF* (*SLC5A6* (P-value=$1.5310^{-7}$ (DPW)). *CAB39L* was consistently associated with increased drinking across alcohol phenotypes (with P-values ranging from $8.6610^{-10}$ (AUD) to $1.6910^{-114}$ (DPW)). We were able to replicate proteins using independent dlPFC protein and gene expression datasets. 11 of the proteins also showed evidence of a shared causal variant between the brain protein and respective alcohol consumption behavior, including *CAB39L*, *HDGF*, and *SLC6A5* with DPW and *CPS1* with binge drinking. Single-cell enrichment was predominantly in excitatory neurons within the dlPFC. MR identified corresponding associations for 31 of the 34 alcohol-related proteins with 22 neuropsychiatric endpoints, highlighting pleiotropic associations. *TESC*, *SLC6A5*, *HDGF*, and *CPS1* were not associated with other neuropsychiatric endpoints, suggesting potentially specific roles in alcohol consumption **Conclusions**Our findings highlight

the power of integrating genetics, proteomics, and transcriptomics in elucidating the underlying biology of AUD and alcohol consumption behaviors, linking them with neuropsychiatric disorders and identifying novel drug targets that may aid the development of new therapeutics aimed at reducing problematic drinking.

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**Title:** Molecular signaling pathways in the hippocampus of rhesus monkeys with chronic alcohol use

**Authors: *T. PAREEK**[1], X. ZHANG[3], M. ELADAWI[3], D. M. PLATT[1,2], K. A. GRANT[4,5], B. GISABELLA[1,2], S. O'DONOVAN[3], H. PANTAZOPOULOS[1,2];
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**Abstract:** Aim: Context-induced relapse is a major problem limiting recovery from alcohol use disorder (AUD). The hippocampus is critically involved in contextual reward memories. However, the understanding of molecular pathways in the hippocampus of human and non-human primates with chronic alcohol use is limited, limiting the development of novel pharmacotherapies for context-induced relapse. In the present study, we examined the molecular pathways altered in the hippocampus of rhesus monkeys with chronic alcohol use. Methods: RNAseq profiling was conducted on hippocampal samples from adult, male rhesus monkeys with a history of chronic alcohol use (n=7) or no alcohol use (n=5) obtained from the Monkey Alcohol Tissue Research Resource. Differential gene expression and gene ontology pathway analyses were conducted using full transcriptome GSEA pathway analysis, leading edge analysis, and targeted pathway Enrichr analysis to identify differentially expressed pathways and leading-edge genes. We conducted perturbagen analysis using the LINCS database to examine drugs and mechanisms of action (MoAs) with concordant or discordant signatures compared to the gene signatures identified in the hippocampus of monkeys with chronic alcohol use. Results: We identified 2291 differentially expressed genes (DEGs) in monkeys with chronic alcohol use including genes implicated in GWAS studies such as GLP2R and GABBR2. Downregulated pathways included chemical synaptic transmission, brain development, glutamatergic synapse and circadian rhythm regulation. Upregulated pathways implicated mitochondrial function. Targeted pathway analysis identified downregulated GABA and calcium signaling pathways. Leading edge analysis identified CACNA1C, CACNA2D1, glutamate and cholinergic receptors

as downregulated leading-edge genes. Further, Enrichr analysis detected 417 significantly altered pathways including calcium ion-regulated exocytosis of neurotransmitter and GABAergic synapse. We identified 282 unique concordant (e.g., GABAA receptor) and 584 discordant MoAs (e.g., calcium channel blockers) using LINCS analysis. Overlap across all datasets indicates shared downregulation of pathways involved in neurotransmitter receptor activity and calcium ion transmembrane transporter activity. Conclusion: The current results identify molecular pathways that may serve as future targets for development of pharmacological therapies for context-induced relapse in AUD and point to pathways involved in GABA signaling, mitochondrial function, synaptic regulation and circadian rhythm regulation in the hippocampus of subjects with chronic alcohol use.

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**Topic:** G.09. Drugs of Abuse and Addiction

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**Title:** Adolescent alcohol exposure increases m6A methyltransferase Mettl3 in the central amygdala

**Authors: *J. TANDUKAR**[1], E. MALOVIC[2], S. PANDEY[2], R. GAO[1,3];
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**Abstract:** Adolescents exposed to alcohol are at an increased risk for developing alcohol use disorder (AUD). To date, studies on the interplay between epigenetics and AUD have primarily focused on DNA and histone modifications such as DNA methylation and histone acetylation. The effects of AUD on RNA epigenetics, however, remain understudied. METTL3 is an RNA methyltransferase that co-transcriptionally methylates the internal adenosine residues of eukaryotic mRNAs in an epigenetic modification called m6A methylation. Here, we aim to understand how the level of METTL3 can be affected by alcohol. We used rat models (males) and divided them into two groups - adolescent intermittent ethanol (AIE, experimental) and adolescent intermittent saline (AIS, control). AIE rats (n=5) were intraperitoneally injected with ethanol whereas AIS rats (n=5) were injected with saline, 2 days on and 2 days off, from ages 28-41 days. At 95 days of age, the animals were euthanized, and the brain tissues were collected. We used immunohistochemistry to label the METTL3 protein and fluorescence *in situ* hybridization (FISH) to label *Mettl3* RNA for simultaneous visualization. However, sensitive quantification of both the METTL3 protein and its mRNA requires high spatial resolution and near single-molecule sensitivity. For this purpose, we developed a new expansion microscopy pipeline named RESOLution enhanced Visualization using Expansion-coupled FISH (RESOLVE-FISH) to resolve the transcripts and its encoding proteins at subcellular level, while preserving the original spatial context of the brain tissue. Using this approach, we were able to resolve fluorescent puncta corresponding to single *Mettl3* mRNA and single METTL3 protein clusters in the central amygdala, which is known to be affected by AUD. We found that *Mettl3* RNA (p= 0.0063) was increased 1.8-fold in AIE rats while the METTL3 protein level also

increased but was not significant (p=0.3638). The higher level of *Mettl3* and METTL3 in AUD central amygdala suggests an increased level of m6A methylation, which may be associated with specific differential gene regulation that could be responsible for increased anxiety in AIE exposed rats. Our study will thus provide a more complete picture of the links with alcohol consumption, RNA epigenetic modifications (RNA methylation), and anxiety.

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**Title:** Dependence escalated alcohol self-administration is regulated by the novel mechanism TARP γ-8

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**Abstract:** Alcohol dependence and multiple withdrawal experiences are related to increased severity of alcohol use disorder (AUD), craving, and resistance to treatment. Alcohol gains control over behavior, in part, through pathological adaptations of glutamatergic AMPA receptor (AMPAR) mechanisms that regulate plasticity in reward pathways. The protein, transmembrane AMPAR regulatory protein (TARP) γ-8, regulates AMPAR trafficking, activity, and CaMKII-dependent plasticity, making it critical for AMPAR mediated neural transmission. TARP γ-8 also has a highly restricted expression limited to regions known to regulate glutamatergic response to alcohol including the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and the hippocampus (HPC). Evidence indicates chronic alcohol increases glutamate levels, which in turn promotes the influx of calcium, and initiates a cascade where CaMKII phosphorylates AMPARs to increase and sustain AMPAR activity. Since AMPAR activity is required for the development of new behavior and retention of actions, this fundamental neural process may underlie the development, maintenance, and critically, dependence-escalated self-administration of alcohol. Our studies with TARP γ-8 hetero- and homozygous knockout mice (M&F) suggest that TARP γ-8 is required for operant alcohol self-administration and reducing TARP γ-8 impairs the acquisition of this behavior. Similarly, these mice do not experience dependence escalated alcohol self-administration suggesting this physiologically driven escalation requires TARP γ-8. This effect appears to be driven by TARP γ-8 in the BLA, which was targeted with the highly selective negative modulator, JNJ-55511118. In contrast, over-expression of TARP γ-8 in the BLA results in increased alcohol responding in an AMPAR-activity dependent manner.

Our data also suggest TARP γ-8 is a target of alcohol dependence as gene expression is altered in a region-specific manner. Finally, preliminary data of BLA $Ca^{2+}$ signaling using a highly novel multi-spectral, fiber photometry platform shows increased $Ca^{2+}$ in response to an ethanol reinforced response that does not occur during a non-reinforced response. Future work will extend these results to include simultaneous measurement of TARP γ-8 dependent Ca2+ signaling in reward-related regions including the mPFC, BLA, HPC and NAc. This work provides fundamental insights into TARP γ-8 dependence-escalated alcohol self-administration which has high translational value for AUD and has potential to inform the development of pharmacotherapeutic strategies that target AMPAR function in a highly selective, brain region-specific manner.

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**Title:** The glycine transporter-1-inhibitor Org 24598 facilitates the alcohol deprivation abolishing and dopamine elevating effects of bupropion + varenicline in rats

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**Abstract: Objectives:** Alcohol Use Disorder (AUD) involves perturbations of dopamine (DA) systems in the brain reward circuitry. Available pharmacotherapies for AUD present modest effects but may be improved by targeting mechanisms by which alcohol accesses the DA reward pathway and by counteracting brain hypodopaminergia following chronic alcohol intake. Previous studies suggest that alcohol targets glycine receptors (GlyR) in the nucleus Accumbens (nAc) that inhibit γ-aminobutyric acid (GABA)-ergic projections to the ventral tegmental area, ultimately leading to increased nAc DA release. Indeed, direct and indirect glycine receptor (GlyR) agonists raise basal DA, attenuate alcohol-induced DA release in the nAc and reduce

alcohol intake in rats. Interestingly, combined treatment with varenicline+bupropion produces additive effects on accumbal DA output and abolishes the alcohol deprivation effect (ADE) in rats, predictive of clinical efficacy in man. This study examines whether the glycine transporter-1(GlyT1)-inhibitor Org 24598, an indirect GlyR agonist, enhances the ADE-reducing and DA elevating action of varenicline+bupropion in lower doses than previously applied. **Methods:** Effects on voluntary alcohol consumption, the ADE and extracellular nAc levels of glycine and DA were examined following Org 24598 6 and 9 mg/kg i.p., bupropion 3.75 mg/kg i.p. and varenicline 1.5 mg/kg s.c., in monotherapy or combined, using a two-bottle, free-choice alcohol consumption model with an ensuing ADE paradigm, and *in vivo* microdialysis in male Wistar rats. **Results:** All treatment regimens appeared to abolish the ADE but only the effect produced by the triple combination (Org24598+varenicline+bupropion) was statistically significant compared to vehicle. Hence, addition of Org 24598 may enhance the ADE-reducing action of varenicline+bupropion and appears to allow for a dose reduction of bupropion. Org 24598 raised accumbal glycine levels but did not significantly alter DA output in monotherapy. Varenicline+bupropion produced a substantial elevation in accumbal DA output that was slightly enhanced following addition of Org 24598. **Conclusions:** Conceivably, the blockade of the ADE is achieved by the triple combination enhancing accumbal DA transmission in complementary ways, thereby alleviating a hypothesized hypodopaminergia and negative reinforcement to drink. Ultimately, combining an indirect or direct GlyR agonist with varenicline+bupropion may constitute a new pharmacological treatment principle for AUD, although further refinement in dosing and evaluation of other glycinergic compounds are warranted.

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**Title:** Microglial acid-sensing regulates AUD-associated outcomes in mice

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**Abstract:** Alcohol use disorders (AUDs) are prevalent and debilitating. Improving our understanding of how alcohol engages systems important for regulating behaviors, emotions and physiology could improve our ability to treat these disorders. Alcohol use is a potent threat to physiological homeostasis, inducing acidosis within 20 minutes which can last >15-24 hours in plasma/brain and associates positively with withdrawal severity. Maintaining physiological

homeostasis is critical for an organism's survival. Thus, alcohol's effects on behavior and physiology may result from the drive to maintain homeostasis. However, the role of acidosis in regulating alcohol's outcomes, and the specific acid-sensing receptors mediating these effects, need to be identified and investigated. T-cell death associated gene 8 (Tdag8) receptor is an acid sensor on microglia. Previous studies have shown TDAG8 regulates anxiety and fear-relevant behaviors and physiology (respiration/blood pressure) via neuroimmune signaling. Given emerging evidence of the role of neuroinflammation and acidosis in AUD-relevant outcomes, we sought to test the hypothesis that acid-sensing receptor TDAG8 regulates EtOH consumption, and behavioral and physiological responses to ethanol. Our data show that TDAG8 expression is upregulated within brain following EtOH injections. Male and female TDAG8 deficient mice (TDAG8-/-) drink less than their wild-type (TDAG8+/+) littermates in the drinking in the dark (DID) model of voluntary EtOH consumption. There were no effects on water or 10% sucrose consumption. TDAG8-/- mice also showed reduced EtOH-evoked microglial activation following DID. Further, TDAG8-/- mice showed enhanced EtOH-evoked ataxia and respiratory depression while there were no effects on locomotor stimulation. Together, these data suggest EtOH administration engages acid-sensing receptor TDAG8 and that TDAG8 may regulate EtOH drinking via microglia activation. These data also suggested TDAG8 regulates more aversive aspects of alcohol drinking (ataxia and respiratory depression), while having no effect on the more stimulating aspects of alcohol use (locomotor stimulation). Future studies will investigate the role of TDAG8 in mediating physiological outcomes during acute withdrawal, and after prolonged abstinence in previously dependent individuals.

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**Title:** The role of the central β1 adrenergic receptor in binge drinking and interactions with the ghrelin system

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**Abstract:** Alcohol use disorder (AUD) is a highly prevalent public health issue. Binge drinking is a very common and harmful step in AUD. We and other groups have shown that the stomach-derived peptide ghrelin is implicated in alcohol-related outcomes. Ghrelin receptors (GHSR) are expressed in the brain and the periphery. We previously found that both intraperitoneal and intracerebroventricular administration of GHSR antagonists reduced intake in the Drink-in-the-Dark (DID) mouse model of binge drinking, whereas sequestering circulating ghrelin by the means of a vaccine did not. Thus, our hypothesis is that binge-like drinking is reduced by central GHSR antagonism independently of peripheral ghrelin. To further investigate this hypothesis, we targeted β-1 adrenergic receptors (β1ARs), the activation of which are required for stress and fasting-induced ghrelin release. However, the involvement of β1AR has not been addressed a) in a model of alcohol binge drinking b) within the context of ghrelin-alcohol signaling c) through the lens of central *vs*. peripheral signaling d) in both male and female subjects. In this study, we tested the hypothesis that β1AR blockade, will reduce blood ghrelin levels, and this will have no effect on alcohol drinking in male or female mice. We administered two β1AR blockers intraperitoneally: atenolol (AT, peripherally restricted) and metoprolol (MT, brain permeable). We used 18 male and 18 female C57Bl6 mice of 11 weeks of age. Alcohol intake was measured in g/kg of body weight and results were analyzed by ANOVA. Results showed that MT but not AT decreased alcohol intake ($p = 0.096$). Also, the co-administration of AT or MT with JMV2959, a GHSR antagonist, decreased intake ($p < 0.0001$); with an additive effect seen with MT. Also, the co-administration of AT or MT with PF-5190457, an inverse agonist that blocks constitutive activity of GHSR, decreased intake ($p < 0.0001$). Finally, blood ghrelin measurements indicate that atenolol ($p < 0.0001$) and metoprolol ($p = 0.0076$) both decrease blood ghrelin levels. We observed no significant sex differences in the results. These results suggest that the blockade of central but not peripheral β1ARs decreases binge-like alcohol drinking. Also, β1AR blockade is sufficient to block ghrelin secretion in binge drinking mice, and β1AR blockade depletes ghrelin levels. Finally, the blockade of β1AR does not prevent GHSR antagonists from decreasing drinking. In conclusion, the ghrelin peptide itself may not drive binge-like drinking, but both β1ARs and GHSRs represent possible targets for therapeutic intervention for AUD. The potential additive effects of MT and GHSR blockade should be further investigated in AUD.

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**Nanosymposium**

**NANO81: Advancing Optical Microscopy Imaging for Neuroscience**

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**Time:** Wednesday, November 15, 2023, 8:00 AM - 9:45 AM

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**Title:** Nanoscale reconstruction of brain tissue with light microscopy

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**Abstract:** Brain tissue is a tantalizingly complex arrangement of neurons into an information processing network. Its cellular structure is too fine grained to be captured by conventional light microscopy. We have recently developed an optical super-resolution imaging/machine learning technology that breaks the intertwined limitations in resolving power, signal-to-noise ratio and tissue light exposure of classical super-resolution imaging. This allows reconstructing the tissue's cellular constituents at 3D nanoscale resolution in the living system, giving access to dynamic as well as molecular information in tissue reconstruction (Velicky *et al*., Nature Methods (2023), DOI: 10.1038/s41592-023-01936-6). We have further developed an extracellular labelling/super-resolution imaging approach to visualize the cellular architecture of brain tissue across spatial scales in fixed tissues (Michalska *et al.*, bioRXiv (2022), DOI: 10.1101/2022.08.17.504272), using either stimulated emission depletion (STED) or expansion microscopy to enhance resolution. We showcase application of these technologies in various preparations and brain regions, notably including the hippocampal mossy fiber circuitry. I will discuss how we develop such technologies further to visualize brain tissue architecture, place cells, synapses, and specific molecules into their tissue context, and quantitatively analyze the tissue's architecture. These technologies will be enabling for shedding light on fundamental questions of tissue organization both in healthy and diseased brains.

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**Topic:** I.03. Anatomical Methods

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**Title:** Uncovering Brain Tissue Architecture with Super Resolution Light Microscopy

**Authors: *M. R. TAVAKOLI**[1], J. LYUDCHIK[1], N. AGUDELO DUENAS[2], J. VORLAUFER[1], V. VISTUNOU[1], C. KREUZINGER[1], C. SOMMER[1], A. CENAMERI[1], J. G. DANZL[1];
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**Abstract:** Brain tissue comprises an extremely dense and complex arrangement of cells that make up the information-processing network that enables brain function. The connectivity of neurons underlie the unparalleled capabilities of our mind. Thus, mapping brain structure, which underlies brain function, has become a central focus in neuroscience. Electron microscopy provides extremely high resolution and comprehensive visualization of brain structure but requires correlative workflows to access molecular information. Light microscopy holds tremendous potential to analyze the ultrastructure of brain tissue together with its molecular makeup. However, conventional light microscopy (LM) provides limited resolution (~ 200 nm in xy and ~ 1000 nm in z-axis), far too coarse to precisely locate specific molecular players within sub-micrometer-sized structures, such as synapses. We have developed a light microscopy based approach for visualizing and studying the tissue's ultrastructure at ~16 nm lateral resolution. With its high speed, synapse-level resolution, and ability to leverage genetically targeted, cell type-, and protein-specific labeling, our technology fills a valuable niche between the high throughput of conventional optical pipelines of neuronal anatomy and the ultrahigh resolution of corresponding EM pipelines, which we showcase in various brain regions, including cortex and hippocampus.

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**Topic:** I.03. Anatomical Methods

**Title:** Aberration-corrected STED microscopy with MATRIX detection for neuroscience research

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**Abstract:** STimulated Emission Depletion (STED) microscopy routinely enables super-resolution imaging of nanoscale structures in biological specimens (Figure 1). Among the super-resolution modalities, STED microscopy stands out due to its compatibility with challenging specimens such as biological tissue, a prevalent specimen type in neuroscience despite its complexity. In tissue samples, acquiring STED images with high signal-to-noise is challenging for two reasons. First, improving resolution results in a smaller effective point-spread-function, which leads to dimmer images. In tissue samples, this can translate to a decrease in the signal-to-background ratio due to the high out-of-focus background contribution. Second, thick tissue

samples typically have non-constant refractive index maps that lead to optical aberrations that compromise the quality of the STED doughnut, and therefore resolution. This is especially true for three-dimensional (3D) STED.
We present two approaches that work in-tandem to improve STED imaging in tissue. The first approach is MATRIX detection, which harnesses the power of an array-based detector to discriminate in-focus signal from out-of-focus background. MATRIX detection can thus significantly increase signal-to-background ratios. MATRIX detection can also be combined with adaptive optics, which (in our implementation) relies on a deformable mirror to introduce phase distortions that negate those induced by the specimen. In this way, optical aberrations can be corrected, and image quality and resolution maintained even when imaging in aberrating samples. We will present the combination of MATRIX detection and adaptive optics for STED microscopy and demonstrate their application in a wide range of neurobiology specimens.

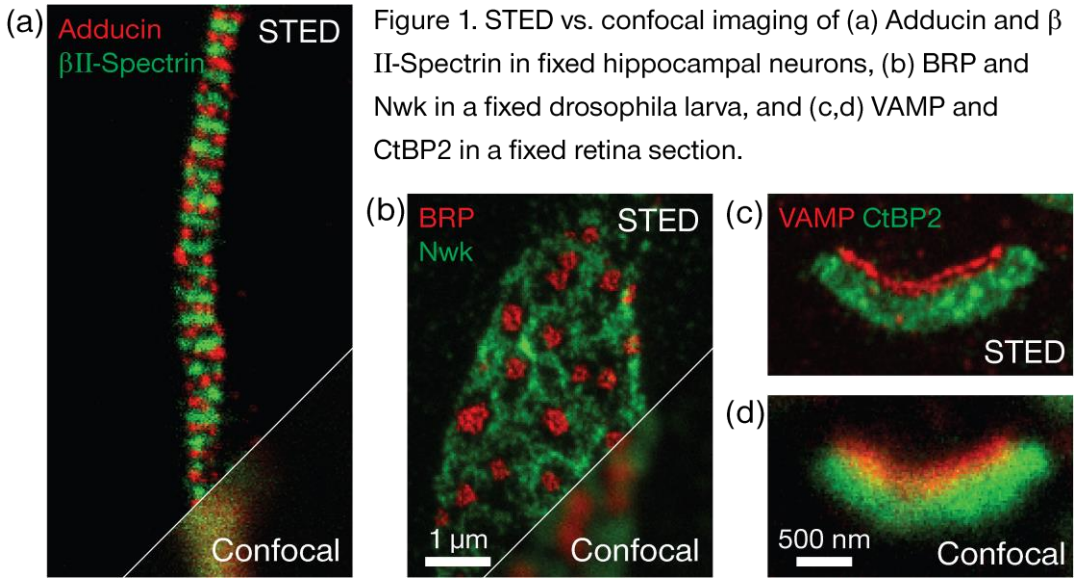


Figure 1. STED vs. confocal imaging of (a) Adducin and β II-Spectrin in fixed hippocampal neurons, (b) BRP and Nwk in a fixed drosophila larva, and (c,d) VAMP and CtBP2 in a fixed retina section.

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**Title:** Three-dimensional imaging of peptidergic sensory nerves and an examination of their function in healthy mouse prostate

**Authors: *H. XIA**, T. J. JERDE, J. C. FEHRENBACHER;
Pharmacol. and Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN

**Abstract:** Rationale
The prostate is known to be densely innervated. Sympathetic and parasympathetic innervation controls the normal physiological function of the prostate and has been shown to modulate prostate-associated pathologies. While sensory nerve-associated neuropeptides, such as tachykinins and calcitonin gene-related peptide (CGRP) are present in the prostate, no studies have demonstrated contiguous sensory innervation of the prostate, nor have they determined a functional role of peptidergic sensory nerves (PSNs) to maintain prostate homeostasis. Fundamentally, PSNs are known to be responsible for nociception. However, emerging research has shown that PSNs are involved in the regulation of vasculature, stem cells, and immune cells during development, homeostasis, or tissue repair. Therefore, we hypothesize that peptidergic sensory nerves are integral to the homeostatic regulation of the prostate.
Methods
To evaluate the microarchitecture of PSNs, prostates were harvested from transgenic mice expressing EGFP driven by the CGRP promoter (Calca-fEGFP) and fixed with 4% PFA. Tissues were immunolabeled and processed through a modified ethyl cinnamate-based optical tissue clearing protocol and imaged by confocal microscopy. To determine the functional significance of PSNs, we selectively ablated PSNs by administering diphtheria toxin to transgenic mice that specifically express the diphtheria toxin receptor on PSNs. Sensory nerve ablation was confirmed by deficiencies in response to nociceptive thermal stimuli and by immunofluorescence imaging of dorsal root ganglia (DRG). Prostates from PSN-ablated animals were harvested for tissue clearing and paraffin embedding followed by hematoxylin and eosin staining for histomorphological assessment.
Results
Continuous, tortuous, $GFP^+/CGRP^+$ nerves fibers are seen in 50-100µm thick volumes in immunolabeled, cleared, prostate lobes. Punctate CGRP signals are dispersed along continuous $GFP^+$ fibers indicative of large, peptidergic dense core vesicles in PSNs. PSN-ablated animals showed increased latency to thermal stimuli and decreased number of $CGRP^+$ DRG cell bodies compared to wildtype control animals.
Conclusions
Immunofluorescence labeling in cleared tissue has enabled us to clearly delineate the microarchitecture of PSN-neuronal tracts interwoven around prostatic acini. The highly organized structure of innervating PSNs proximal to the epithelial glands suggests that PSNs play a role in normal prostatic function. Our 3D imaging demonstrates that PSNs have a stromal localization with abundant and organized fibers in close apposition to the acini.

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**Title:** Optimized clearing and 3D imaging for large-scale characterization of the mouse and human enteric nervous system

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**Abstract:** The gut-brain connection influences health and disease by affecting systems ranging from metabolic, neurological, digestive and immunological. It is becoming increasingly relevant for neuroscience research to look beyond the central nervous system in order to fill sustained gaps in knowledge. Advances in 3D imaging techniques have added to neuroscientists' toolbox to interrogate the complexities of structural and functional changes in the nervous system under disease conditions, and to showcase the effectiveness of novel treatments. Here, we share a neuroscience-friendly pipeline for clearing, labelling and 3D imaging to enable the study of the enteric nervous system (ENS) in mouse and human gut tissues. In addition, we present the usefulness of this pipeline to identify morphological parameters with the potential to reflect changes in physiology that may be relevant biomarkers of gut-brain disorders. We establish a standard processing pipeline for 3D microscopy of ENS morphology in fixed, cleared and immunolabelled tissues originating from healthy mouse and human tissues. Across species, we image full-thickness colon samples with maintained tissue integrity, ranging in thickness from 200 micrometers in mouse to 4 millimeters in human tissues. In a single field of view and at a resolution of 0.42 micrometers, we acquire a volume of $3mm^3$ in multiple channels. These imaging capabilities are particularly relevant when biomarkers of physiological dysfunction are heterogeneously distributed within tissues. We validate immunolabeling targets, which include neuronal cell bodies (Hu C/D), neuronal fibers (Tuj1), enteric glia (S100β), synaptic vesicles (Synapsin 1) and more. We harness endogenous tissue autofluorescence to contextualize the enteric nervous system's morphology and identify a potential for label-free fluorescence observation of the myenteric plexus. In the case of the mouse, we can investigate the distribution of ENS biomarkers across 7 regions of the GI tract. By adapting image processing techniques for 3D microscopy datasets we can segment structures of interest, as shown by distinguishing the myenteric plexus from the mucosal and tertiary plexuses in a single channel. Thus, it is possible to analyze the state of the ENS in multiple species by observing its occupancy and distribution from lumen to serosa. Since ENS architecture restructuring is known to be symptomatic of gut-brain disorders, such as in Parkinson's disease, characterizing its morphology in large uninterrupted volumes of tissue can facilitate a better understanding of the local gut-brain environment and lead to future screening and treatment avenues.

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JSPS Postdoctoral Fellowship PE20032

**Title:** Characteristics of myelin and neuronal density distributions in the marmoset claustrum

**Authors:** T. GODFREY, N. ATAPOUR, B. GAMANUT, D. RESER, M. ROSA, ***A. GAMANUT**;
Monash Univ., Clayton, Australia

**Abstract:** The variations in the density of neuronal numbers, together with the amount of myelinated fibres and their orientations are used qualitatively in neuroanatomy to establish borders of many regions in the brain. In the current study we quantified these features in the claustrum complex of marmosets in order to have their detailed spatial distributions. Specifically, we created Voronoi tessellations of the mapped neurons from NeuN stainings, in order to obtain the maximum resolution of neuronal densities. We also analysed the densities of myelin fibres and their local orientations. After superimposing the results, we recovered the subdivisions of the claustrum complex with high precision. This method can be used successfully in other regions which require fine analysis of myelin and neuronal distributions.

**Disclosures: T. Godfrey:** None. **N. Atapour:** None. **B. Gamanut:** None. **D. Reser:** None. **M. Rosa:** None. **A. Gamanut:** None.

**Presentation Number:** NANO81.07

**Topic:** C.01. Brain Wellness and Aging

**Support:** NIH NINDS U54 NS100717-01
W.M. Keck Foundation
Alzheimer Association
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NRI-MCDB Microscopy Facility at UCSB

**Title:** Sub-cellular mapping of lipid metabolites through mid-infrared photothermal Imaging of vibrational probes

**Authors: *Y. BAI**, S. GLASAUER, X. TIAN, C. CAMARGO, A. LONGHINI, K. KOSIK;
Univ. of California Santa Barbara Neurosci., Santa Barbara, CA

**Abstract:** Metabolic heterogeneity plays a crucial role in human health, impacting brain development and disease progression. To accurately model brain metabolic heterogeneity and develop effective therapies, advanced metabolic imaging technologies are needed. While clinical imaging platforms offer powerful *in vivo* metabolic measurements, their spatial resolution is inadequate for single-cell analysis. In this work, we introduce an innovative optical metabolic imaging platform that enables sub-cellular mapping of lipid metabolites with high protein specificity. Our approach involves the use of a biocompatible and biorthogonal probe azide-

palmitic acid (PA) as a lipid metabolic precursor. The azide (-$N_3$) molecules are metabolically incorporated into newly-synthesized lipids such as membranes and lipid droplets. To selectively detect these newly-synthesized lipids, we have developed an optical photothermal infrared (OPTIR) microscope that targets the unique chemical bond stretching frequency of azide at 4.77 μm. Using this novel metabolic imaging platform, we investigated lipid metabolism in various human-relevant model systems. We first study the dynamics of lipid metabolism in neuroglioma cells following the azide-PA supplementation. Interestingly, we observed an initial increase in newly-synthesized lipids and total lipid signal for approximately 6.5 hr, followed by a gradual decrease in both signals, suggesting catabolism of lipids to fuel cellular functions. Furthermore, we validated the platform's ability to study disease mutation-related metabolic changes by comparing lipid metabolism in control and *GRN*-KD iPSCs. Intriguingly, we observed an increase in newly-synthesized lipids in *GRN*-KD stem cells, while the total lipids level remained identical between the two cell lines, indicating a potentially higher lipid turnover rate in *GRN*-KD stem cells. We also assessed lipid metabolism in iPSC-derived brain organoids, which involves the self-assembly of different cell types. By acquiring cell-type-specific fluorescence imaging and lipid metabolic imaging from the same field of view, we compared the spatial correlation of newly-synthesized lipids with different cell types. Our results revealed significantly higher lipid metabolism in astrocytes compared to neurons. In summary, our study presents a single-cell metabolic imaging platform capable of directly imaging lipid metabolic activity in human-relevant models with high resolution and specificity. We anticipate that the integration of OPTIR microscopy with infrared probes will facilitate profound neuroscience discoveries and lead to improved disease treatments.

**Disclosures: Y. Bai:** A. Employment/Salary (full or part-time):; Photothermal Spectroscopy Corp.. **S. Glasauer:** None. **X. Tian:** None. **C. Camargo:** None. **A. Longhini:** None. **K. Kosik:** F. Consulting Fees (e.g., advisory boards); Expansion Therapeutics, ADRx, Herophilus, Tau Consortium, Minerva Biotechnologies.

**Nanosymposium**

**NANO82: Comparative Neuroscience**

**Location:** WCC 140

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO82.01

**Topic:** A.10. Development and Evolution

**Title:** Molecular atlas of the adult zebrafish telencephalon reveals spatiomolecularly distinct nuceli homologous to cortical and subcortical structures of terrestrial vertebrates

**Authors:** B. A. BREDESEN-AA, ***E. YAKSI**;
Kavli Inst. for Systems Neurosci., Trondheim, Norway

**Abstract:** Adult of many teleost fish species, including zebrafish with well-developed pallium can perform cognitively demanding associative, spatial, social learning and working memory tasks. Hence, zebrafish must have evolved a version of pallial circuit elements and architectures

that can enable cognitively demanding behaviors, by using the raw cellular constituents and connectivity principles that were available to the ancestors of all vertebrates. While homologs of mammalian amygdala, olfactory cortex, and hippocampus in zebrafish pallium have been proposed, how exactly the cellular elements of teleost pallium relate to vertebrate cortical evolution, has not been answered. Here, we generated the first sub-cellular resolution Atlas of Zebrafish Transcriptomic Encephalic Cytoarchitecture (AZTEC) of 99 multiplexed genes for >300.000 neurons. Our results revealed multiple excitatory and inhibitory neuron types and non-neuronal cells across zebrafish forebrain and propose distinct marker genes. We observed that while some of these cell types are dispersed widely, several inhibitory and excitatory neuron classes are organized into spatially distinct forebrain nuclei. Spatiomolecular clustering of AZTEC data and 3D alignment onto adult zebrafish brain atlas confirm several previously proposed forebrain nuclei, but also reveal multiple novel substructures that were not described. Aligning AZTEC with single-cell transcriptome of zebrafish and other vertebrates revealed several cell types (non-neuronal, excitatory, inhibitory) and cortical/subcortical regions that are conserved across vertebrates. Evolutionarily identified zebrafish forebrain regions mapped by AZTEC exhibit spatiotemporally distinct resting-state activity and functional connectivity, measured by calcium imaging in adult and juvenile zebrafish.

**Disclosures: B.A. Bredesen-Aa:** None. **E. Yaksi:** None.

**Presentation Number:** NANO82.02

**Topic:** A.10. Development and Evolution

**Support:** GM144276
GM120782

**Title:** Single cell transcriptomic analysis of prefrontal cortex of tame and aggressive foxes

**Authors:** J. L. JOHNSON[1], E. E. HECHT[2], D. V. SHEPELEVA[3], A. V. KHARLAMOVA[3], R. G. GULEVICH[3], A. V. VLADIMIROVA[3], L. N. TRUT[3], Y. E. HERBECK[3,4], J. N. SAVAS[5], ***A. V. KUKEKOVA**[1];
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**Abstract:** Tame and aggressive populations of red foxes (*Vulpes vulpes*) represent one of the longest andwell-known selective breeding experiments in behavioral genetics. We performed single nucleusRNA sequencing (snRNA-seq) analysis of prefrontal cortex samples from six tame and sixaggressive foxes to determine effects of selection on this primary brain region for cognition andbehavior. Each population sample included three young female foxes (9 months of age) and threeold female foxes (7-9 years of age). Sequencing of individual 10x Genomics libraries generated~498 million reads per sample. After filtering, we had ~5,376 nuclei per sample and ~4,678genes per nucleus. Clustering analysis identified 12 clusters of excitatory neurons, nine clustersof interneurons, as well as astrocytes, oligodendrocytes, oligodendrocyte precursor cells, andendothelial cell clusters. Cell types were determined by integrating human and mouse data, withmost subclasses being represented in the fox cells. A larger number of differentially expressed(DE) genes were identified in the age group comparison than in the

population comparison.Population comparison at 9 months of age highlighted the role of excitatory neurons in cortexlayers 2/3. DE genes upregulated in tame foxes were most significantly enriched for KEGGpathways and Gene Ontology (GO) terms *glutamatergic synapse*, *plasma membrane*,*postsynaptic density*, *calcium ion binding*, and *axon*. In turn, DE genes upregulated in aggressivefoxes were most significantly enriched for GO terms related to *endoplasmic reticulum*, *ribosome*and *oxidative phosphorylation* and pathways related to neurodegenerative diseases. Thepopulation differences at the old age were less pronounced. Unexpectedly, we observed anoverlap between KEGG pathways and GO terms for DE genes upregulated in young aggressiveversus young tame foxes and those upregulated in old tame versus young tame foxes. The resultsof this study highlighted cortex layers, neuronal clusters and gene pathways differentiating tameand aggressive foxes and revealed population specific changes associated with aging.

**Disclosures: J.L. Johnson:** None. **E.E. Hecht:** None. **D.V. Shepeleva:** None. **A.V. Kharlamova:** None. **R.G. Gulevich:** None. **A.V. Vladimirova:** None. **L.N. Trut:** None. **Y.E. Herbeck:** None. **J.N. Savas:** None. **A.V. Kukekova:** None.

**Presentation Number:** NANO82.03

**Topic:** A.10. Development and Evolution

**Support:** NIH Grant R01NS108424-03S1
NIH Grant UF1NS115821
NIH Grant F31 NS131049-01

**Title:** The evolution of cell types in the amniote pallium

**Authors: *C. G. OROZCO**, D. P. MERULLO, A. KULKARNI, G. KONOPKA, T. F. ROBERTS;
Neurosci., Univ. of Texas, Southwestern Med. Ctr., Dallas, TX

**Abstract:** The 6-layered cortex of the mammalian brain enables our advanced cognitive behaviors. However, mammals haven’t cornered the market on advanced cognitive abilities and not all smart animals have a cortex. Birds have evolved to inhabit all corners of our planet and rival mammals in their cognitive abilities. Yet, birds lack a cortex. Most of the avian pallium is taken up by the dorsal ventricular ridge (DVR), a non-layered structure. Recent research using single-nuclei RNA sequencing in songbirds demonstrated that the transcription factor expression in areas of the DVR was most similar to the mammalian ventral pallium, which develops into the amygdala and olfactory cortex. However, when only examining effector gene expression, which confers cells their functionality, the excitatory neurons exhibited greater similarity to neurons in the mammalian cortex and less similarity to the ventral pallium. Building on these findings, we are now providing a more comprehensive accounting of cell types, their evolution, and the origins of avian telencephalic regions by extending our snRNA-sequencing to almost all areas of the pallium and subpallium. Our analysis compares the transcriptomic data in zebra finch to the Allen Institute's in situ database and large single-cell transcriptomic datasets from mice and reptiles. By examining these gene expression patterns, we aim to better understand the evolution of neuronal cell types in the vertebrate brain and the cellular programs harnessed by natural selection to build brain networks that underlie advanced cognition.

**Disclosures: C.G. Orozco:** None. **D.P. Merullo:** None. **A. Kulkarni:** None. **G. Konopka:** None. **T.F. Roberts:** None.

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**Topic:** A.10. Development and Evolution

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the Yamada Science Foundation
the KOSE Cosmetology Research Foundation
the Mitsubishi Foundation
the Astellas Foundation for Research on Metabolic Disorders

**Title:** The molecular mechanisms underlying the expansion of the primate subplate layer during the evolution of the cerebral cortex

**Authors: *C. OHTAKA-MARUYAMA**;
Developmental Neurosci. Project, Tokyo Metropolitan Inst. of Med. Sci., Setagaya-ku, Japan

**Abstract:** Subplate neurones (SpN) are first-born and matured neurons during cortical development and play an essential role in establishing the thalamocortical circuit and radial migration. The mammalian neocortex consists of a vast number of neurons elaborately arranged within a six-layer structure. SpNs function to ensure the precise arrangement of neurons and early axonal projections occur within the limited time of the embryonic period. Compared to mice, the SP layer of the primate is known to develop thickly during the fetal period but decreases in thickness after birth. At the same time, SpN is largely lost through cell death. However, it has also been reported that more SpN remains in diseased brains, such as autism. Therefore, in normal development, it seems essential that SpNs that have completed their role in the embryonic period disappear quickly. Why does the SP layer develop so thickly in primates? We performed spatial transcriptome analyses of marmoset and human fetal brains to explore how this contributed to the evolution of the primate brain. As a result, genes specifically highly expressed in the SP layer of primates were identified. In this presentation, I will discuss the role of these genes in SP layer expansion and brain evolution.

**Disclosures: C. Ohtaka-Maruyama:** None.

**Presentation Number:** NANO82.05

**Topic:** A.10. Development and Evolution

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K99 NS122085
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Johns Hopkins University Discovery Award

**Title:** Joint Decomposition Identifies Conserved Transcriptome Dynamics across Single-Cell Atlases of the Developing Neocortex that Map onto Genetics of Human Brain Structure and Disease

**Authors:** S. SONTHALIA[1], H. CHEN[4], J. LIU[1], G. STEIN-O'BRIEN[2], L. XIAO[6], B. CAFFO[5], S. ADKINS[7], J. ORVIS[7], R. HERTZANO[7], A. MAHURKAR[7], S. AMENT[7], A. CASELLA[7], N. MICALI[8], S. MA[8], N. SESTAN[8], P. RAKIC[8], ***C. COLANTUONI**[3];
[1]Biomed. Engin., [2]Neurosci., [3]Neurol. and Neurosci., Johns Hopkins Med. Institutions, Baltimore, MD; [5]Biostatistics, [4]Johns Hopkins Sch. of Publ. Hlth., Baltimore, MD; [6]Biostatistics, NC State, Raleigh, NC; [7]Inst. for Genome Sci., Univ. of Maryland, Baltimore, MD; [8]Yale Univ., New Haven, CT

**Abstract:** Single cell transcriptomic and epigenomic analyses of neurogenesis have been used to generate cellular atlases of mammalian neocortical development in rodent, primate and human brain tissue. Similar maps have been generated in cerebral organoid models, but understanding of the exact elements of development that are recapitulated in vitro is lacking. To harness the collective discovery power of these rich data resources, we have brought the atlases together in a single unified analytical environment to serve both computational biologists and cell biologists without coding expertise (nemoanalytics.org; see "Highlighted profiles" under "Select profile" menu). NeMO Analytics can be used to explore the expression of individual genes across the atlases, calculate differential expression across sample or cell types, and perform dimension reduction: nemoanalytics.org/p?l=NeocortexEvoDevo&g=FEZF2.
To leverage these assembled atlases, we have developed SJD (doi.org/10.1101/2022.11.07.515489 & chuansite.github.io/SJD) that decomposes biologically related collections of multi-omics data matrices. Using SJD, we have defined many transcriptomic elements that are conserved across mammalian neurogenesis and programs which are specific to human cortical development. By integrating GWAS analyses from the UK BioBank, we map these shared and human unique elements of neocortical neurogenesis onto the genetics of human brain structure and disease, revealing links between particular elements of neurogenesis and brain structure and specific disorders.
In addition, we have implemented our transfer learning framework, projectR (doi.org/10.1093/bioinformatics/btaa183), within NeMO Analytics so that gene signatures (e.g. lists of genes of interest, or weighted gene lists from a PCA, SJD, or similar analysis) can be visualized across the collections of datasets in NeMO Analytics. Within NeMO Analytics, projectR enables researchers without coding expertise to define transcriptomic signatures in a dataset of interest and explore these dynamics across the compendium of related datasets in the analytical environment. Our high resolution map of transcriptomic dynamics in neocortical neurogenesis can be leveraged to explore and design manipulations of precise cellular mechanisms underlying risk for common complex brain disorders in tractable in vitro systems. We invite the research community to explore this collection of public data resources along with

the transcriptomic elements of human neurogenesis that we have defined and their projection into in vitro stem cell models (nemoanalytics.org; see “Transfer Learning” link).

**Disclosures: S. Sonthalia:** None. **H. Chen:** None. **J. Liu:** None. **G. Stein-O’Brien:** None. **L. Xiao:** None. **B. Caffo:** None. **S. Adkins:** None. **J. Orvis:** None. **R. Hertzano:** None. **A. Mahurkar:** None. **S. Ament:** None. **A. Casella:** None. **N. Micali:** None. **S. Ma:** None. **N. Sestan:** None. **P. Rakic:** None. **C. Colantuoni:** None.

**Presentation Number:** NANO82.06

**Topic:** A.10. Development and Evolution

**Support:** Simons Foundation Center for the Social Brain

**Title:** The differential pallial transcriptome of juvenile and adult songbirds

**Authors: *A. NAIR**[1], I. BARRERA[2], F. CHEN[2], M. S. FEE[3];
[1]Biol., MIT, Cambridge, MA; [2]Broad Inst., Cambridge, MA; [3]Brain and Cognitive Sci., Massachusetts Inst. Tech., Cambridge, MA

**Abstract:** During development, the songbird brain develops an anatomically and molecularly distinct set of brain nuclei that play well defined computational roles in the learning and production of song. While prior work has characterized gene expression differences between these song nuclei and the brain matter in which they grow, it is unclear when these changes emerge, what the initial distinguishing features of song nuclei might be, or how gene expression changes throughout development. We used the spatial transcriptomic methodology Slide-seq to address these unresolved questions and present a set of genes differentially expressed between juvenile and adult male songbirds. These genes are candidate genes for understanding the molecular processes underlying the evolution of song nuclei and more broadly the molecular processes involved in the evolution of more complex behaviors from those achievable from existing neural circuitry.

**Disclosures: A. Nair:** None. **I. Barrera:** None. **F. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Curio Biosciences. **M.S. Fee:** None.

**Presentation Number:** NANO82.07

**Topic:** A.10. Development and Evolution

**Title:** Sulcal-driven cortical mapping reveals evolutionary divergences between old world monkeys, chimpanzees and humans.

**Authors: *K. LOH**[1], G. AUZIAS[2], W. D. HOPKINS[3], M. PETRIDES[4], J. SALLET[5], C. AMIEZ[6], O. COULON[2];
[1]Montréal Neurolog. Inst., Montreal, QC, Canada; [2]Inst. de Neurosciences de la Timone, Aix Marseille Université, CNRS, Marseille, France; [3]MD Anderson Cancer Ctr., Bastrop, TX; [4]Montreal Neurolog Inst. Mc Gill Univ., Montreal, QC, Canada; [5]Univ. of Oxford, Oxford, United Kingdom; [6]INSERM U1208, Bron, France

**Abstract:** Cortical folding in the primate brain adheres to a common topology and is strongly related to the underlying organization of cytoarchitectonic areas in the brain. By examining how each species' sulcal patterns differ, recent studies have revealed important insights about primate brain evolution in the frontal cortex (e.g. Amiez et al., 2019; 2023). Based on this principle, we developed a cross-species cortical mapping approach that implements: *first*, the alignment of individual cortical surfaces onto a species-common parametric domain defined by each species' sulcal organization, and *second*, the cross-alignment of each species' sulcal organization model based on known sulcal homologies. In this work, we applied this approach to compare MRI reconstructed cortical surfaces from two old world monkey species: macaque monkeys (n=21) and baboons (n=32), and two ape species: chimpanzees (n=36) and humans (n=100). To reveal the evolutionary divergences between species, we mapped individual surfaces between the four species, and computed vertex-wise relative cortical expansions between each pair of species. Our results revealed the distinct patterns of relative cortical expansions associated with the evolutionary divergences between old world monkeys, chimpanzees and humans, and sheds new light on human brain evolution: First, we show that *direct comparisons between human and chimpanzee brains do not provide the full picture of how the human brain has diverged from other primates*. Instead, we have to consider how the human and chimpanzee brain have *differentially* evolved from old world monkeys. For instance, we observed that although certain brain regions (e.g. mid-dorsolateral prefrontal cortex and lateral occipital-temporal cortex) might appear to have expanded in humans versus chimpanzees, this expansion was in fact driven by a greater contraction in chimpanzees versus humans, as both species diverged from old world monkeys. Second, we show that *not all brain regions expand or contract in a monotonic fashion going from old world monkeys to chimpanzees and to humans.* We observed that whereas some brain regions do show monotonic changes from old world monkeys to chimpanzees to humans (e.g. inferior frontal cortex, precuneus, and supplementary motor cortex), several other regions (e.g. lateral frontal pole and ventral temporal cortex) show non-monotonic changes going from old world monkeys to chimpanzees, and to humans (e.g. contracting from old world monkeys to chimpanzees, but expanding from chimpanzees to humans). This observation contradicts existing views that the human brain is a scaled-up version of the nonhuman primate brain.

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**Topic:** A.10. Development and Evolution

**Support:** NINDS Grant R15 090296
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Georgia State University 2nd Century Initiative Primate Social Cognition, Evolution, & Behavior Grant

**Title:** Sex differences in capuchin monkey brains

**Authors: *O. T. REILLY**[1,2,3], S. F. BROSNAN[2,3,4,5], M. E. BENÍTEZ[6,3], K. A. PHILLIPS[7,8], E. E. HECHT[1];
[1]Human Evolutionary Biol., Harvard Univ., Cambridge, MA; [2]Psychology, Georgia State Univ., Atlanta, GA; [3]Language Res. Center, Georgia State Univ., Atlanta, GA; [4]Ctr. for Behavioral

Neuroscience, Georgia State Univ., Atlanta, GA; [5]Neurosci. Institute, Georgia State Univ., Atlanta, GA; [6]Anthrop., Emory Univ., Atlanta, GA; [7]Trinity Univ., Trinity Univ., San Antonio, TX; [8]Southwest Natl. Primate Res. Center, Texas Biomed. Res. Inst., San Antonio, TX

**Abstract:** Several nonhuman primate species exhibit sex differences in behavior, suggesting that there may be underlying sex differences in the brain. It is important to investigate neuroanatomical variation found in other primate species to better understand the evolution of sex differences in the human brain. One neotropical nonhuman primate species that shares many behavioral characteristics with humans relevant to social organization is the tufted capuchin monkey (*Sapajus [Cebus] apella*). We conducted a whole-brain volumetric analysis of gray matter using voxel-based morphometry on T1-weighted magnetic resonance imaging scans from 20 capuchin monkeys (15 female, 5 male). Males showed significant expansion in areas of the hypothalamus, and females showed expansion in the early visual cortex, the cerebellum, and higher-order areas across the occipital and temporal cortex. Several of these regions were non-overlapping between males and females. We then extended this work to investigate sex differences in white matter tracts by conducting tract-based spatial statistical analysis on fractional anisotropy (FA) images from the T1-weighted scans. Females showed significantly higher FA than males in the right cerebral hemisphere, specifically in regions of frontal-parietal white matter. These values were also non-overlapping. We suggest that distinct socioecological niches which male and female capuchins occupy could be associated with the sex differences found in cortical gray and white matter. These sex differences in neuroanatomy appear more pronounced than those typically observed in humans. These differences could be associated with human adaptations for prolonged neurodevelopmental trajectories and increased plasticity.

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**Nanosymposium**

**NANO83: Glia-Neuron Interactions from Synapses to Networks**

**Location:** WCC 201

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO83.01

**Topic:** B.09. Glial Mechanisms

**Support:** HRD 1401026
IOS 1755341

**Title:** Synaptobrevin (Vamp2) dominant negative prevents glutamate exocytosis by astrocytes

**Authors: *T. AMANFO**[1], M. TEMBURNI[2], V. TALABATTULA[2], R. DZAKPASU[3], M. MOORE[2];
[2]Delaware State Univ., [1]Delaware State Univ., Dover, DE; [3]Dept. of Physics and Dept. of Pharmacol. and Physiol., George Washington Univ., DC, WA

**Abstract:** **Synaptobrevin (Vamp2) dominant negative prevents glutamate exocytosis by astrocytes**Tobenna Amanfo[1], Venkata Ajay Narendra Talabattula[1], Michael Moore[2], Rhonda Dzakpasu[3,4] and Murali Temburni[11]Delaware Center for Neuroscience Research and Department of Biological Sciences, Delaware State University, Dover DE 19901[2]Delaware Institute of Science and Technology, OSCAR Imaging Facility[3]Department of Physics and 4Department of Pharmacology and Physiology, Georgetown University, Washington, DC 20057
We established pure neuron only and mixed (astrocyte and neuron) cultures on multi-electrode arrays (MEAs) from the embryonic chick optic texture with the goal of testing the role of astrocytes in the development of neuronal network activity. Our preliminary results indicate that astrocytes are necessary for the synchronous oscillatory activity of neuronal cultures. Mixed neuron and astrocyte cultures show random spiking activity without synchronization. Astrocytes have been shown to modulate network activity by releasing gliotransmitters like glutamate, D-serine, and ATP. We hypothesize that glutamate sensing at tripartite synapses via mGluRs elevates local calcium within astrocyte processes. With sufficient activation, the localized calcium elevation crosses a threshold causing a calcium-induced calcium release (CICR) within the astrocyte leading to glutamate exocytosis. The literature is divided on the presence of SNARE proteins within Astrocytes. Hence our lab, is interested in exploring this uncertainty. We targeted the SNARE protein Synaptobrevin (Vamp2) within astrocytes as crucial for communication with neurons via its molecular induction of vesicle docking. We proposed to test this model by expressing a truncated Vamp2 subunit (Vamp2 DN) which acts as a dominant-negative to block exocytotic release. Astrocytes expressing the Vamp2DN are expected to release significantly less glutamate upon calcium elevation, thereby reducing synchrony of neuronal activity. We have generated primary astrocyte lines expressing the synaptobrevin dominant-negative (Vamp2DN along with the glutamate season iGluSnFR. We demonstrate that Vamp2DN expressing astrocytes have significantly reduced glutamate exocytosis when CICR is induced with Ionomycin. We will co-culture the Vamp2DN astrocytes with neurons and record network activity on MEAs (Multi-Channel Systems). With these tools, a more comprehensive molecular model for astrocyte involvement in generating neuronal synchrony can be developed.

**Disclosures: T. Amanfo:** None. **M. Temburni:** None. **V. Talabattula:** None. **R. Dzakpasu:** None. **M. Moore:** None.

**Presentation Number:** NANO83.02

**Topic:** B.09. Glial Mechanisms

**Support:** FONDECYT 1211359
FONDECYT 1221028

**Title:** Respiratory drives exerted by ATP and D-serine are reciprocally dependents

**Authors: *J. L. EUGENIN**[1], N. C. ABARCA[1], K. GÓMEZ[1], R. VON BERNHARDI[2]; [1]Biol., Univ. de Santiago, USACH, Santiago, Chile; [2]Fac. for Hlth. Sci., Univesidad San Sebastián, Santiago, Chile

**Abstract:** Breathing is regulated by central chemoreceptors located in the brainstem. Medullary astrocytes release ATP, D-serine, and glutamate in response to hypercapnia (high levels of $PCO_2/H^+$). These gliotransmitters drive the respiratory rhythm, increasing the respiratory

frequency (fR). Notoriously, the metabolic inhibition of astrocytes or the single and independent functional elimination of ATP or D-serine (enzymatic degradation or receptor blockade) almost completely inhibited the respiratory response to hypercapnia. Why the sole elimination of one gliotransmitter could affect so profoundly the global response induced by all of them? Here, we addressed whether the respiratory responses induced by the single application of one gliotransmitter depends on the preserved functionality of a second gliotransmitter. In caudal brainstem slices obtained from neonatal CF1 mice (P0-P4), fictive respiration was recorded from the ventral respiratory column (VRC) with glass suction electrodes. Slice superfusion was performed with artificial cerebrospinal fluid (aCSF, containing in mM: 129 NaCl, 3 KCl, 1.5 CaCl2, 1 $MgSO_4$, 23.5 NaHCO3, 0.5 NaH2PO4, 30 dextrose) equilibrated with O /CO = 95%/5%, (pH 7.4, 30 ± 1°C). Concentration-response curves for the increase in fR induced by superfusion of aCSF medium containing 10-1000 µM ATP or 1-100 µM D-serine were performed in the presence and absence of D amino acid oxidase (DAAO), an enzyme that degrades D-serine, or purinergic blockade with 50 µM suramin or 5 µM MRS2179, respectively. D-serine concentration-response curves were also performed in 9mM (high) potassium medium to evaluate the role of excitability in the observed responses. Both, ATP and D-serine, induced a concentration-dependent increase in fR. The maximum increase in fR, expressed as percentage of basal values, were 208.9 % ± 20.2 % (n= 9) for 1 mM ATP and 135% ± 3.9% (n = 4) for 100 µM D-serine. DAAO superfusion for 45 min flattened the ATP concentration-response curve. Similarly, the purinergic blockade also flattened the D-serine concentration-response curve. Furthermore, high potassium increased basal fR, but did not modify the fR increases induced by D-serine in presence or absence of purinergic blockade. Our results indicate that the increases in fR induced by ATP depend on the concomitant presence of D-serine, and vice versa. Increase in excitability of the network does not recover the responses in fR induced by D-serine in presence of purinergic blockade. These results suggest a complex functional interaction between both gliotransmitters. Changes in their interaction could be relevant in respiratory neural network dysfunctions.

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**Topic:** B.09. Glial Mechanisms

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Howard Hughes Medical Institute

**Title:** A feedforward inhibitory motif in the chemical connectome

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**Abstract:** The precise synaptic connectivity among all neurons in a brain defines its connectome, and specific sets of connections form neural circuit motifs that implement different computations essential for behavior. In addition to synaptic signaling, neurons also communicate through the release of diffusible neuromodulators that, with their cognate receptors, form the chemical or chemo-connectome. The chemical connectome is thought to modulate fast synaptic transmission over relatively long timescales. We challenge the framework of the chemical connectome as a slow modulatory layer by uncovering a biochemical circuit, downstream of the neuromodulator norepinephrine, that implements feedforward inhibition over fast timescales during futility-induced behavioral state transition in the larval zebrafish. Norepinephrine released during futility drives a short-term elevation in motor vigor (the excitatory phase) followed by a long-term suppression of swimming (the inhibitory phase). Combining whole-brain imaging of neural activity and neuromodulator release with behavioral pharmacology, we find that norepinephrine causes adenosine triphosphate (ATP) release from astroglia sufficient for passivity. ATP does not act directly on downstream neurons but is instead first metabolized into adenosine, and the inhibition of this biochemical pathway, or of adenosine receptors, inhibits futility-induced passivity. Remarkably, while broad inhibition of noradrenergic signaling in both neurons and astroglia suppresses both the excitatory and inhibitory phases of the futility response, inhibition of the astroglial purinergic signaling pathway suppresses only the inhibitory phase, suggesting that neurons and astroglia play different roles in mediating norepinephrine's behavioral effects. In sum, our work uncovers a feedford inhibitory circuit motif implemented by the chemical, instead of anatomical, connectome and, together with other work in flies and rodents, identify an evolutionarily conserved and behaviorally relevant noradrenergic-to-purinergic signaling channel in astrocytes.

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**Topic:** B.09. Glial Mechanisms

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**Title:** Microglial expression of the circadian gene, Bmal1, regulates density and function of hippocampal excitatory synapses during adolescent brain development

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**Abstract:** Sleep disturbances are prevalent in neurodevelopmental disorders (NDDs), and animal studies show that early life circadian disruptions can exert lasting, maladaptive impacts on social and mood-related behaviors. Despite the correlation between NDDs and circadian disruption, it is unclear whether the circadian system directs developmental processes and if disrupted rhythms play a role in NDD pathophysiology. The neuroimmune system may serve as one interface where circadian rhythms influence brain development. Microglia, the brain's resident immune

cells, sculpt neural circuitry by strengthening useful synapses and pruning unnecessary ones. Aberrant developmental pruning can impair synaptic functioning, and over- or under-active pruning is implicated in schizophrenia and autism spectrum disorders, respectively. In adult mice, the core circadian gene, *Bmal1*, regulates microglial phagocytosis of synaptic elements, but it is unknown whether *Bmal1* influences the maturation or pruning of synapses early in life. Here we used a tamoxifen-inducible, microglia-specific, *Bmal1* knockout mouse to test whether the microglial clock influences synaptic structure and function in early adolescence. We injected Tmem119:CreER2::Bmal1$^{fl/fl}$ (cKO$^{MG}$) mice and littermate controls with tamoxifen at P3-P5 and performed in vitro electrophysiological recordings on CA1 hippocampal pyramidal neurons at P24-P32. Recorded neurons were additionally filled with neurobiotin for *post hoc* immunohistochemical processing and morphological analysis. Adolescent cKO$^{MG}$ mice exhibited a reduced frequency of miniature excitatory postsynaptic currents (mEPSCs), but an increased density of dendritic spines, where excitatory synapses are located. Increased spine density was region-specific and restricted to the proximal dendritic trunk in *stratum radiatum*. The elevated spine density in cKO$^{MG}$ mice was due to increased density of thin, immature spines with no changes in the density of thicker spines. Taken together, these findings suggest that microglia-specific *Bmal1* knockout may produce an overabundance of immature, "silent" spines that lack AMPA receptors. Alternatively, presynaptic release might be altered in cKO$^{MG}$ mice. These data demonstrate that microglial *Bmal1* informs developmental synapse maturation and pruning. The microglial clock, therefore, might serve as a novel therapeutic target to address synaptic dysfunction in NDDs.

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**Title:** Astrocytes prevent synaptic depotentiation by limiting repetitive dendritic activity during motor learning

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**Abstract:** Many lines of evidence suggest that astrocytes have important functions in regulating activity-dependent synaptic plasticity, but their role in learning-related synaptic changes in the living brain remains unclear. Here we show that motor training induced synaptic potentiation on apical dendrites of layer 5 pyramidal neurons, as well as a robust increase of $Ca^{2+}$ in the processes and somas of astrocytes in the mouse motor cortex. Blocking astrocytic $Ca^{2+}$ activities by either the suppression of α1-IP3R2 signaling or the activation of Gq-DREADD receptors in astrocytes led to synaptic depotentiation during motor learning and impairment of performance improvement. Notably, synaptic depotentiation occurred on a fraction of dendrites with repetitive

dendritic $Ca^{2+}$ spikes. On those dendrites, spines active before the generation of dendritic $Ca^{2+}$ spikes underwent CaMKII-dependent synaptic depotentiation during motor learning. In addition, activating adenosine receptors reversed repetitive dendritic $Ca^{2+}$ activity and synaptic depotentiation caused by astrocytic $Ca^{2+}$ reduction, indicating the involvement of ATP released from astrocytes and adenosine signaling in the processes. Together, these findings reveal an important function of astrocytes in preventing synaptic depotentiation by limiting repetitive dendritic $Ca^{2+}$ activity, thereby contributing to learning and memory formation.

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022-105

**Title:** Screening Human Astrocyte Matrisome Components upon Synaptic Networks within Neural Organoids

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**Abstract:** Astrocytes promote neural network maturation in the nervous system in part through secretion of matrisome components such as pro-synaptogenic proteins. Which specific astrocyte-derived components are necessary and sufficient for synapse network activity in human neurons? To identify and test potential candidates, we utilized our previously established bioengineered neural organoid approach (Asteroids) composed of astrocytes and excitatory neurons directly induced from human pluripotent stem cells (iPSCs, hPSCs) (Cvetkovic et al.). Mining a combination of RNAsequencing, immunostaining, and proteomics of conditioned media from these cells, we identified specific components that are restricted to astrocytes and have potential to modulate synapses. To test these components, we treated neuron organoids with specific proteins and measured synaptic networks defined by spike dynamics during multi-electrode array (MEA) recordings, synchronous burst activity during live GCaMP-based calcium imaging, and density of synapses. Next, to determine whether factors derived directly from astrocytes are capable of modulating synapses within organoids, we developed and optimized a novel approach consisting of encapsulating mature astrocytes in sodium alginate hydrogels for indirect coculture with neuronal organoids. This approach revealed that astrocytes maintain viability within the porous capsules, continue to secrete proteins into the extracellular space, and become reactive upon application of exogenous inflammatory cytokines to model neuropathology. Overall, these studies reveal human astrocyte matrisome components that modulate neural networks and deliver novel technologies to investigate astrocyte-neuron interactions in healthy and diseased states.

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**Title:** Astrocytes of lateral parafacial region play a key role in the breathing control of mice under high $CO_2$ levels

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**Abstract:** A growing body of evidence suggests that astrocytes located in medullary respiratory chemoreceptive areas are both sensitive to CO2, pH and O2 and able of regulating breathing. Astrocytes might also be important in processing ventilatory responses to hypercapnia in other medullary respiratory networks, such as the lateral parafacial region (pFL). In this study, we tested the hypothesis that astrocytes residing in the pFL, which contains a cluster of expiratory neurons involved in recruiting abdominal muscles during hypercapnia, modulate the ventilatory response to hypercapnia in mice. For this purpose, Aldh1L1-Cre/ERT2 transgenic mice ($Aldh^{cre/+}$) 17-25 weeks age (n=10), which have tamoxifen-inducible Cre recombinase expression in astrocytes were used. The animals received bilateral intracranial injections of pAAV-DIO-hM4D(Gi)-mCherry into $pF_L$. Ventilatory parameters, such as respiratory frequency (fR), tidal volume (VT) and minute ventilation (VE) were acquired by whole body plethysmography in conscious mice. The role of $pF_L$ astrocytes was assessed by activating DREADDs-Gi using intraperitoneal injections of an agonist JHU37160 (0.1 mg/Kg) during normocapnia and hypercapnia ($CO_2$ 7%). Wild type ($Aldh^{+/+}$, n=10) mice were used as control. The effects of hypercapnia on the $pF_L$ astrocytes intracellular [$Ca^{2+}$] were also measured using medullary slices, multiphoton microscopy and genetically encoded calcium indicator ($Aldh^{cre/+}$/$Gcamp6^{flox/+}$). This study was approved by the Institutional Ethical Committee (1079/2022). Three important data were obtained in the *in vivo* experiments: 1) at normocapnia, both $Aldh^{cre/+}$-hM4D(Gi) and $Aldh^{+/+}$ mice exhibit similar fR, VT and VE; 2) at normocapnia, the JHU had no effects on ventilatory parameters of $Aldh^{cre/+}$-hM4D(Gi) mice, and; 3) JHU attenuated the fR response to hypercapnia of $Aldh^{cre/+}$-hM4D(Gi) mice: ΔfR at 30 min: 96 ± 42 cpm ($Aldh^{+/+}$) vs 45 ± 44 cpm [$Aldh^{cre/+}$- hM4D(Gi)], p = 0.04; at 45 min: 77 ± 47 cpm ($Aldh^{+/+}$) vs 16 ± 53 cpm [$Aldh^{cre/+}$-hM4D(Gi)], p = 0.01. VT and VE responses were not affected by JHU. Hypercapnia also increased intracellular [$Ca^{2+}$] of $pF_L$ astrocytes (p=0.001; 35 astrocytes from 5 independent experiments) *in vitro*. The data shows that $pF_L$ astrocytes play a key role in the fR response to hypercapnia in mice.

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**Title:** Astrocyte-secreted Neurocan controls inhibitory synapse formation and function

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**Abstract:** The development of functional neuronal circuits depends on the proper balance between excitatory and inhibitory synaptic inputs. Astrocytes shape the development and maturation of excitatory and inhibitory synapses by the secretion of synaptogenic proteins. Several astrocyte-secreted factors that control excitatory synaptogenesis have been identified; however, the identities of astrocyte-released proteins that regulate inhibitory synapse formation and function in the brain are still unknown. We identified the chondroitin sulfate proteoglycan Neurocan, NCAN, as a regulator of inhibitory synapse formation. NCAN expression is restricted to the nervous system and is highly expressed and released by astrocytes. In the extracellular matrix, it is cleaved into two fragments, NCAN N-terminal and NCAN C-terminal. We found that the resulting fragments have distinct localization and function in the extracellular matrix. Moreover, our results indicate that NCAN C-terminal domain is necessary and sufficient to induce cortical inhibitory synapse formation *in vitro* and *in vivo*. NCAN does not increase excitatory synapse numbers *in vitro*, underscoring the specificity of this astrocyte-secreted factor. In vivo, we found that Neurocan mutant mice lacking this domain present impaired inhibitory synaptic number and function. Through super-resolution microscopy and in vivo proximity labeling by secreted TurboID, we revealed that the synaptogenic domain of NCAN localizes to somatostatin-positive synapses and regulates their formation. These results show that astrocytes control inhibitory synapse formation through secretion of NCAN and reveal that NCAN C-terminal fragment has distinct functions in the brain in regulating synapse formation. Moreover, our results suggest that through the secretion of multiple signals, astrocytes can differentially regulate excitatory and inhibitory circuits.

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**Topic:** B.09. Glial Mechanisms

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**Title:** Microglial modulation of potentiated dendritic spines visualized with genetically encoded plasticity reporter in mouse organotypic cultures of hippocampus

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**Abstract:** Encoding and storing memories in the central nervous system are related to functional and structural synaptic plasticity. Dendritic spines receive most excitatory synapses in the brain and continuously remodel their shape and number to fulfill the demands of acquiring and evoking memories. In this regard, compelling evidence points to the importance of microglia in spine remodeling, influencing synaptic functionality and plasticity. Microglia regulate dendritic spine density by controlling synapse formation, survival, and pruning, whereas their chronic activation or dysfunction is associated with both excessive phagocytosis of synapses and progression of cognitive deficits.However, it is not known whether and how microglia show a selective preference for potentiated versus non-potentiated spines in its various modulatory activities. So far, synaptic activity mapping of long-term potentiated spines has been limited by the need for an appropriate tool. To this purpose, the genetically encoded SynActive (SA) toolbox, which allows the local translation-dependent expression of fluorescent reporters at synapses undergoing activity-dependent long-term potentiation (LTP), was exploited in organotypic mouse hippocampal slice cultures, to enquire to what extent microglia orchestrate and contributes to the induction/formation, or to the function, of potentiated synapses. Organotypic slices were infected with AAV9 adeno associated viral vectors directing the expression of TdTomato (to label the entire neuron) and of a doxycycline-regulated SynActive-controlled Venus fluorescent protein (to label potentiated dendritic spines). After challenging the organotypic cultures with chemical or electrical LTP we observed: i) an increased microglia density and a higher percentage of microglia with highly branched structures; ii) a larger colocalization of microglia with potentiated spines. Notably, the pharmacological removal of microglia from organotypic slices prevents the detection and expression of the potentiated SA-positive spines. Moreover, by using organotypic slices from fractalkine knockout mice, we found a lower degree of SA-Venus expression, compared to control cultures, suggesting that the alteration of CX3CL1-CX3CR1 signaling interferes with the spine's potentiation. These data show for the first time that spine potentiation is directly reliant on microglia and add new insights on a potential involvement of microglial cells in activity-dependent synaptic remodeling.

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**Nanosymposium**

**NANO84: Alzheimer's Disease: Omics Approaches**

**Location:** WCC 144

**Time:** Wednesday, November 15, 2023, 1:00 PM - 4:00 PM

**Presentation Number:** NANO84.01

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

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**Title:** Hypomorphic a7nAChR leads to neuronal actin cytoskeleton gain of function reinforcing the synapse as a structure

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**Abstract: Background:** Recent advancements in imaging techniques and concerted efforts have led to major strides in deciphering the human brain. Understanding the fundamental differences between the human and pre-human brain is a prerequisite to designing meaningful models. The carrier frequency of 75% in the human population predicts profound translational significance. **Methods:** The physiological role of *CHRFAM7A* in human brain is explored using multiomics approach on 600 post mortem human brain tissue samples (ROSMAP). The emerging pathways and mechanistic hypotheses are tested and validated in an isogenic hiPSC model of *CHRFAM7A* knock-in medial ganglionic eminence progenitors and neurons. Proof of Principle Double blind pharmacogenetic study on the effect of AChEI therapy based on CHRFAM7A carrier status was performed in two paradigms: response to drug initiation and DMT effect. Change in MMSE score from baseline was compared by 2-tailed T-test. Longitudinal analysis of clinical outcome (MMSE) was performed using a fitted general linear model. Model independent variables included age, sex, and medication regimen at the time of the first MMSE, APOE4 carrier status (0, 1 or 2 alleles as categorical variables) and CHRFAM7A genotype. **Results:** *CHRFAM7A* is identified as a modulator of intracellular calcium dynamics and an upstream regulator of Rac1. Rac1 activation re-designs the actin cytoskeleton leading to dynamic actin driven remodeling of membrane protrusion and a switch from filopodia to lamellipodia. The actin cytoskeleton reorganization shifts dendritic spine differentiation from filopodia towards spines with increased head area to stem diameter ratio resulting in increased synapse clustering ("high quality wellcro"). At the same time, CHRFAM7A incorporation into the α7 nAChR pentamer results in a hypomorphic receptor in iPSC derived MGE progenitors with decreased channel open probability. The hypomorphic receptor has diminished response to pharmacological modulation. To assess how CHRFAM7A carrier status affects treatment outcomes we performed double-blind pharmacogenetic analysis of AChEI therapy in two paradigms: response to drug initiation and DMT effect. **Conclusions:** In the presence of CHRFAM7A the hypomorphic α7 nAChR receptor mediates cytoskeletal reinforcement of the synapse in the human brain through $Ca^{2+}$ signaling. The outcome is a more resilient brain in CHRFAM7A carriers (CNV GWAS association) but a more AChEI treatment responsive brain in non-carriers.

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**Title:** Transcriptional Signatures of Tau Pathology in Primary Age-Related Tauopathy and Alzheimer's Disease

**Authors:** G. L. STEIN-O'BRIEN[1], R. PALAGANAS[1], E. M. MEYER[2], J. REDDING-OCHOA[1], O. PLETNIKOVA[3], H. GUO[1], W. R. BELL[4], J. C. TRONCOSO[1], R. L. HUGANIR[1], ***M. MORRIS**[1];
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**Abstract:** Tau pathology is common in neurodegenerative diseases associated with aging. Primary age-related tauopathy (PART) and Alzheimer's disease (AD) are sporadic, age-associated conditions which share a similar structure and anatomic distribution of tau pathology. However, the molecular changes associated with intraneuronal tau pathology in PART and AD and whether the changes are similar in the two diseases is largely unexplored. Using GeoMx spatial transcriptomics, mRNA was quantified in CA1 pyramidal neurons with tau pathology and adjacent neurons without tau pathology in 6 cases of PART and 6 cases of AD, and compared to 4 control cases without pathology. Transcriptional changes were analyzed for differential gene expression and for coordinated patterns of gene expression associated with both disease state and intraneuronal tau pathology. Using these techniques, we identified two novel gene expression signatures and synaptic gene changes associated with intraneuronal tau in PART and AD. The gene expression changes associated with intraneuronal tau pathology were similar in PART and AD. Synaptic gene expression was decreased overall in neurons in AD and PART, however, this decrease was largely driven by neurons lacking intraneuronal tau pathology. Synaptic gene expression in tau-positive neurons in disease was increased compared to tau-negative disease neurons. Genes in the up-regulated expression signature associated with tau were enriched in pathways associated with calcium regulation and synaptic function, specifically in synaptic exocytosis. These gene expression signatures of tau pathology and the synaptic gene changes were confirmed in a published transcriptional dataset of cortical neurons with tau pathology in AD. These novel findings show that transcriptional changes associated with intraneuronal tau pathology are similar in AD and PART, raising the possibility of a mechanistic relationship between tau pathology in the two diseases. Intraneuronal tau pathology was also associated with increased expression of genes associated with synaptic function and calcium regulation compared to tau-negative disease neurons, which may represent a compensatory change. The findings highlight the power of molecular analysis stratified by pathology in neurodegenerative disease and provide novel insight into common molecular pathways associated with intraneuronal tau in PART and AD.

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**Title:** Spatial proteomic signatures of human resilience to human Alzheimer's Disease captured through multiplexed ion beam imaging.

**Authors: *B. CANNON**, C. CAMACHO, M. GOLDSTON, A. SPENCE, M. BOSSE, S. BUKHARI, S. VARRA, D. MRDJEN, K. VIJAYARAGAVAN, M. ANGELO, T. MONTINE, S. BENDALL;
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**Abstract:** Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are characterized by aggregation of misfolded proteins and the progressive loss of neurons and synapses in the brain. However, some individuals show cellular and symptomatic resilience to these diseases despite accumulating a buildup of protein aggregates. In this study, we aimed to identify the local proteomic signatures associated with human neurodegenerative resilience through multiplexed ion beam imaging (MIBI). We analyzed FFPE human brain tissue samples from individuals with Alzheimer's Disease, those who were cognitively healthy and without protein aggregation, and those who were resilient to the development of Alzheimer’s Disease symptoms in the face of existing protein aggregation. By using MIBI we simultaneously measured the expression of 40 proteins in individual cells across multiple brain regions in 33 patients. Using cell segmentation, pixel clustering, and spatial enrichment analysis, we aim to find proteomic signatures associated with resilience that are distinct from those of the disease states. Our dataset will provide insights into the underlying cellular and neighborhood phenotypes of neurodegenerative resilience and may inform the development of novel therapeutic strategies for Alzheimer’s Disease.

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**Title:** Decoding the heterogeneity of Alzheimer's disease at the molecular level: A human post-mortem digital multiplexed gene expression study in sleep-wake modulating lateral hypothalamic area todelineate the molecular signatures of selective vulnerability.

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**Abstract:** Atypical Alzheimer's disease shares similar neuropathological changes with amnestic AD, i.e., accumulation of extracellular amyloid-beta plaques and aggregation of intracellular tau tangles; however, atypical AD presents different symptoms and patterns of brain atrophy. Thus, contrasting amnestic and atypical AD provides a unique framework to investigate factors underlying selective neuronal vulnerability. The lateral hypothalamic area (LHA) is one of the earliest affected areas in AD, and LHA degeneration contributes to sleep and appetite dysfunction. This study investigates the molecular profile of LHA changes in amnestic and atypical AD to identify the molecular signatures of its selective vulnerability to AD subtypes. Using conventional techniques, we extracted RNA from postmortem human LHA of 10 healthy control (HC), 4 atypical AD, and 6 amnestic AD subjects. We used a customized Neuropathology nCounter® (Nanostring) panel for the gene expression study. The Wald statistical test was used to compare the groups, and the genes were considered differentially expressed when log2 fold-change was >=|1|, and the p-value was <0.05. A comparison of amnestic AD and HC demonstrated 41 differentially expressed genes (DEGs), whereas the atypical AD vs. HC comparison demonstrated 65 DEGs. Of those, only the overlap between amnestic and atypical AD was 10-12%. Further, we observed 138 DEGs in atypical AD over amnestic AD. In amnestic AD, gene ontology analysis demonstrated the downregulation of neuropeptide-ligand interaction pathways with simultaneous upregulation of the intestinal immune network IgA pathway. Among the prominent circadian genes, the CLOCK (0.5x, p=0.02), Orexin (HCRT) (-0.4x, p=0.7), and HCRTR2 (-0.8x, p=0.3) genes demonstrated a trend towards a decline, whereas the HCRTR1 (0.4x, p=0.3) gene showed a marginal increase in expression. In contrast, in atypical AD, we observed a significant decline in the CLOCK (0.4x, p=0.04) and HCRTR2 expression (-1.26x, p=0.007). Loss of immune regulation and up-regulation in the GABAergic synapses were also prominent in atypical AD. Our results provide insight into the distinct molecular events in amnestic and atypical AD. Amnestic AD demonstrated a decline in neurotransmitter-receptor interaction with increased intestinal IgA production. In contrast, atypical AD demonstrated a loss of immune regulation and increased synaptic protein expression. The molecular characterization of selectively vulnerable LHA in atypical AD thus provides us a unique opportunity to delineate the mechanisms of selective vulnerability.

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**Title:** A single nucleus transcriptomic profiling of temporal cortex reveals novel AD trait associations confirmed by in situ sequencing

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**Abstract:** Alzheimer’s disease (AD) is a neurodegenerative disorder with complex pathological manifestations and is the leading cause of cognitive decline and dementia in elderly individuals. Given the lack of treatment options, understanding the molecular and cellular changes in AD, it is essential to identify new therapeutic pathways. In this study, we present a comprehensive investigation of cellular heterogeneity from the temporal cortex (TC) region of 40 individuals, comprising healthy donors and individuals with different stages of tau pathology. Using single-nucleus transcriptome analysis of 463,988 nuclei from both gray and white matter of these individuals, we identified cell type-specific subclusters in both neuronal and glial cell types with varying degrees of association with AD pathology. In particular, these associations are present in some layer specific glutamatergic (excitatory) neuronal types, along with other GABAergic (inhibitory) neurons, as well as a few glial cell subtypes. These associations were observed in early as well as late pathological progression. We also performed *in situ* sequencing using CARTANA with 155 genes directly in the tissue of 12 individuals with varying levels of tau pathology. We investigated these genes and their associations with the pathology and were able to replicate key findings that we obtained from our snRNA data analysis. To put these findings in broader context, we also integrated our TC dataset with other published single nucleus RNA-seq studies that profiled other brain regions such as the entorhinal cortex, prefrontal cortex, and superior frontal gyrus. The integrated analysis of 959,237 total nuclei across 9 different studies/regions identified region-specific subpopulations and associations with AD related pathologies in these brain regions. Together, our findings allow us to prioritize specific cell types and pathways for targeted interventions at early and middle stages of pathological spread in AD.

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**Title:** Identification of novel gliovascular interactions in Alzheimer's disease brains and cross-species models

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**Abstract:** Inter-cellular communication within the gliovascular unit (GVU), that comprises astrocytes, pericytes, and endothelia, is critical for the maintenance of the blood-brain-barrier (BBB) properties. The breakdown of BBB in Alzheimer's disease (AD) is well-established, but precise underlying molecular perturbations remain unclear. Additionally, whether GVU molecular alterations observed in AD brains could also be detected in blood from living patients is unknown. Further, although focused snRNAseq studies expanded our understanding about vascular dysfunction, many of the identified molecular perturbations have not been validated in model systems. In this study, we investigated GVU molecules altered in AD brains and prioritized through computational analyses for their conservation in blood and cross-species model systems. We performed snRNAseq of temporal cortex tissue in AD and control brains. We analyzed this data to detect cell specific GVU molecular perturbations and their expected interactions based on computational analyses focusing on vascular and astrocyte clusters, the

major cell types of the GVU of the BBB. To determine whether GVU transcriptional alterations detected in the brain are preserved in the blood, existing blood expression, genetic, and imaging data from two longitudinal antemortem cohorts were analyzed. Using human iPSC-derived pericytes and zebrafish model systems, we evaluated the cross-species conservation of the top GVU alterations detected in AD brains. Brain snRNAseq revealed transcriptional profiles of 6,541 astrocytes and 2,210 vascular cells. The latter formed three distinct vascular clusters characterized as pericytes, endothelia and perivascular fibroblasts. We identified differentially expressed genes and their enriched pathways within these clusters and observed the highest levels of transcriptional changes within pericytes. We prioritized astrocytic ligand - vascular target interaction pairs, which also associate with AD. In living human cohorts, we discovered genetic variants that influence blood expression levels of some of the prioritized GVU genes. Some of the same variants are also associated with brain vascular disease neuroimaging burden. Our ongoing *in vitro* and *in vivo* studies reveal conservation of some of the top prioritized molecular perturbations across species. Our findings prioritized by multiscale, cross-tissue human data revealed GVU perturbations in interacting pericyte and astrocyte molecules, which are conserved across multiple cross-species models. These results nominate new molecular targets and mechanistic insights for BBB disruptions in AD.

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**Title:** An endolysosomal polygenic risk score predicts altered leptomeningeal, neuronal, and microglial gene expression and cell-type specific endolysosome morphology abnormalities

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**Abstract:** Alzheimer’s disease (AD) affects millions of individuals world-wide. While therapies are available that may slow cognitive decline, to-date no cure is available. Genome-wide

association studies have identified multiple loci associated with increased risk of developing AD, many of which include genes associated with functioning of the endolysosomal system. We generated an endolysosomal polygenic risk score (ePRS) to assess the impact of multiple pathway-specific single-nucleotide polymorphisms on AD pathology and gene expression. After genotyping, we stratified individuals by ePRS score and then compared the molecular and cellular phenotypes between individuals in the top (high) and bottom (low) quartiles. We used bulk RNA sequencing (RNAseq) to assess gene expression in leptomeningeal cells taken at autopsy from individuals with high and low ePRS. We also used single-nucleus RNAseq (snRNAseq) on dorsolateral prefrontal cortex tissue to identify gene expression differences between individuals with high and low ePRS who all had high AD pathology. The bulk data demonstrate that ePRS alters gene expression in leptomeningeal cells in interaction with AD pathology, as ePRS had a significant effect on the expression of 480 genes. Our analysis suggests this effect is specific to individuals with no/low AD pathology. Our snRNAseq data showed cell-type specific altered gene expression in neurons and microglia between individuals with high or low ePRS all with AD pathology. These alterations included enrichment of biological pathways associated with the unfolded protein response (neurons) and cytokine regulation (microglia) in high versus low ePRS cases. Immunohistochemistry validated that individuals with high ePRS demonstrate altered endosomal volume in neurons and lysosomal volume in microglia. Together, these data suggest that AD associated with high ePRS may be associated with endolysosomal abnormalities and that targeted therapies that reduce endolysosomal dysfunction may slow progression of AD.

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**Title:** Neurogenesis-dependent cell profile in the hippocampal formation in Alzheimer's disease using spatial transcriptomics

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**Abstract:** Alzheimer's disease (AD) is the most common form of dementia that results in neurodegeneration, particularly in the hippocampal formation (HF) that is critical for episodic memory. Adult neurogenesis occurs in the dentate gyrus (DG) of the hippocampus, plays a role in learning and memory, and is thought to contribute to memory deficits in familial Alzheimer's disease (FAD) mice. Previously, we showed enhanced survival of adult-born neurons (ABNs) in FAD mice restored memory deficits and rescued dendritic spines and transcription profile of ABNs and mature neurons. However, it is not fully known whether neurogenesis modulates the genetic landscape outside the DG and the profile of vulnerable neurons in AD. To address that, we examined the gene profile of cells in the HF of FAD mice and AD patients using spatial transcriptomics (ST). ST data was acquired from *Nestin-CreER$^{T2}$;Bax$^{fl/fl}$* and *Nestin-CreER$^{T2}$;Bax$^{fl/fl}$;5XFAD* mice injected with corn oil (C-NB and C-NBF) or tamoxifen (T-NBF) to enhance ABN survival. We examined differentially expressed genes (DEGs) of 158 genes within the CA1-3, DG, and EC using Fisher's exact test. Ripley's *L* statistic was used to analyze cell clustering within the EC. ST data from healthy aging (HA) and AD patients was used to identify consistent DEGs in the DG between species. We observed that the profile of neurons and glia in the HF was dramatically different between C-NBF and T-NBF and that T-NBF neuron profile had a significant correlation with the C-NB profile relative to C-NBF. Furthermore, in the CA1/3 and EC, we observed significant changes in the excitatory-inhibitory neuron ratio. Interestingly, the largest changes in DEGs were observed across cell types in the EC. In the EC, we observed layer-specific DEGs and increased clustering of microglia and oligodendrocytes in C-NBF, but increased clustering of excitatory and inhibitory neurons in C-NB and T-NBF. Finally, we observed multiple genes that were consistently altered in AD humans and mice. Our data suggests that enhanced neurogenesis in the DG alters the genetic profile in the wider hippocampus. Changes in the excitatory-inhibitory ratio suggest that neurogenesis may have a role in hyperexcitation in AD. Increased neurogenesis greatly alters the genetic profile of the EC relative to other regions, suggesting that changes in ABNs affect the broader hippocampal circuit and may play a role in EC vulnerability in AD. Finally, we found many consistent genes altered between mouse FAD and human AD in the DG. Together, this study provides new insight into the role of neurogenesis regulating the cellular environment of the HF and provides mechanistic evidence for its role in AD pathology.

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**Title:** Transcriptional analysis of new mouse models harboring coding and noncoding human genetic variants associated with late-onset Alzheimer's disease

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**Abstract:** Large-scale genetic studies and meta-analyses have identified dozens of genetic risk variants for late-onset Alzheimer's disease (LOAD). While many candidate loci contain potentially consequential coding variants, others suggest noncoding variation more likely confers risk. Determining the precise genetic mechanisms that contribute to LOAD will enable a deeper understanding of pathogenesis and advanced preclinical models for the testing of targeted therapeutics. We have introduced candidate genetic variants in the *EPHA1*, *BIN1*, *CD2AP*, and *PTPRB* loci into a sensitized mouse model already harboring a humanized amyloid-beta sequence, APOE4, and Trem2.R47H alleles knocked in to a C57BL/6J background. Variants were selected based on predicted function, cross-species conservation, increased risk of LOAD, and allele frequency. The D57N missense variant was chosen in *PTPRB* while promoter variants were modeled in *EPHA1*, *BIN1*, and *CD2AP*. Genome editing with CRISPR-Cas9 was performed and mouse cohorts were aged to four, eight, and 12 months. Homogenized brain hemispheres were assayed from both male and female mice with RNA-seq. Transcriptomic changes were compared to postmortem human brain data to determine the specific disease relevance of each model. All mice were also assessed with a composite frailty measure. Transcriptomic effects from these genetic variants recapitulated a variety of human gene expression patterns observed in LOAD study cohorts. By 12 months of age, *PTPRB*D57N* mice exhibited neuroimmune signatures that correlate with postmortem LOAD cases relative to controls. The *BIN1* promoter variant exhibited both neuroimmune and oligodendrocyte-related changes that correlated with LOAD modules. These changes were more pronounced with age, supporting their role in age-related dementia. We have characterized in vivo signatures of four genetic candidates for LOAD, identifying alterations in specific LOAD-related pathways in each variant on a sensitized genetic background. Combined with our earlier work, these results provide an initial functionalization of 16 LOAD genetic factors and provide animal models for preclinical testing of therapeutics designed to correct specific molecular alterations that contribute to LOAD pathology and progression.

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**Title:** Contribution of age-associated somatic mutations to Alzheimer's disease pathology

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**Abstract:** Somatic mutations normally accumulate in the brain during aging. In Alzheimer's disease (AD), somatic mutations abnormally accumulate in the mitochondrial genome (mtDNA) at early stages of the disease; whether this also happens in nuclear genes is less clear. How these mutations differentially accumulate in brain regions and their cellular components is even less known. In this study, the ultra-accurate Duplex Sequencing (Duplex-Seq) was used to detect

mutations with frequency <1 x $10^{-6}$ in the mtDNA and in a selection of AD-related genes in individuals with sporadic AD. Autopsy brain samples from the UW Precision Neuropathology Core were subdivided into four groups: Control = cognitive unaffected or affected with no dementia, Braak <V and No findings of PART (n=12), CU/CA-ND-PART = cognitively unaffected or affected with no dementia, Braak <V and primary age-related tauopathy (PART) (n=8), CA-ND – High Braak = cognitively affected with no dementia, Braak V-VI (n=9) and AD-High Braak = AD with Braak >III (n=11). Samples from frozen temporal cortex, hippocampus and cerebellum specimens were processed to obtain homogenates and separated them into two fractions: Synaptosomal = containing mainly synaptosomes therefore enriched in synaptic mitochondria and Non-synaptosomal = containing mainly mitochondria from neuronal soma and from other brain cells. The Non-synaptosomal fraction was further processed by a density gradient to separate nuclei from other cell components followed by staining with NeuN antibody and separation of neuronal nuclei by FACS. Duplex-Seq was performed from three resulting isolates: synaptosomal mitochondria, non-synaptosomal mitochondria and NeuN+ nuclei. Duplex-Seq was targeted to the entire mtDNA and in a selection of six nuclear genes (ENO2, PSEN1, PSEN2, APP, APOE, and MAPT). ENO2 was included as a control gene. Consistent with previous studies, somatic mutations in the mtDNA are more common in synaptosomes from brain regions with high neuropathology and in individuals with no dementia. Mutations were more likely to accumulate in specific mtDNA genes such as mt-ND1, mt-CO3 and mt-CYTB, and to cause missense protein changes. In nuclear genes, somatic mutations were more common and clonal in AD-related genes in controls compared to AD cases with high neuropathology. Further analyses are ongoing to determine the predicted pathogenicity of the detected mutations, their impact on protein expression and whether they cluster in specific genomic sequences. Our results support that somatic mutations abnormally accumulate at early stages of AD and that are not not evident at advanced AD stages possibly because of loss of affected neurons.

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**Title:** Microrna regulation of transcriptomics in molecular subtypes of alzheimer’s disease

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**Abstract:** MicroRNAs (miRNAs) as short non-coding RNA molecules with a length of 19 - 25 nucleotides play a critical role in gene transcription and their alterations are associated with various disorders. In recent years, there has been growing interest in studying miRNAs regulation in neurodegenerative diseases such as Alzheimer's disease (AD). miRNAs differentially expressed in the brain between AD and healthy control subjects have been found to be involved in amyloid pathology, tau phosphorylation, inflammation, oxidative stress of nerve

cells, and mitochondrial dysfunction. Our previous work based on large-scale transcriptomic data in multiple brain regions from two AD cohorts systematically identified five molecular subtypes of AD which formed three major classes (namely, the typical, intermediate, and atypical classes), shedding lights on the heterogeneity of AD pathogenesis (Neff et al., 2021). This present study aims to understand the microRNA alterations and regulations of transcriptomics in AD. We conduct a global analysis of the matched miRNA and mRNA sequencing data (309 miRNAs, 20,049 mRNAs) in the prefrontal cortex from 183 control and 315 AD subjects from the Religious Orders Study - Memory and Aging Project (ROSMAP) cohort (Bennett et al., 2018). We perform differential expression (DE) analysis for each AD subtype and control to identify miRNAs associated with AD subtypes and the miRNA-mRNA correlation analysis to identify potential gene targets of AD associated miRNAs in each AD subtype. A hand full of miRNAs were found to be differentially expressed in AD subtypes and many genes were correlated with miRNAs in AD subtypes. In particular, there were over 10 thousand miRNA-mRNA pairs with significant correlations in one typical AD subtype. In addition, our result shows subtype specific miRNA-mRNA correlations, highlighting unique regulatory mechanisms in AD subtypes. miRNAs associated with AD subtypes such as miR-1260 in the typical subtype and miR-431 in the atypical subtype are involved in synaptic signaling and metabolic process, respectively. The miRNAs associated with AD subtypes and the miRNA-mRNA co-expression networks in AD subtypes offer a new avenue to understand the molecular mechanisms of AD and pave a way for developing novel and personalized therapeutics for AD.

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**Title:** Effects of chronic binge ethanol consumption on Alzheimer's disease pathogenesis in 3xTg-AD mice

**Authors: *L. J. SLOAN**[1], P. M. CHILTON[1,2], A. RAO[1], J.-W. ZHANG[1], S. A. MYERS[1], S. REDDY[1], W. E. RODRIGUEZ[1], L. GOBEJISHVILI[1], J. CHARIKER[1], E. ROUCHKA[1], S. GHARE[1,2], C. MCCLAIN[1,3], S. BARVE[1,2];
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**Abstract:** It is becoming increasingly clear that excessive alcohol consumption leads to neurodegeneration. Indeed, recent meta-analyses identify chronic heavy alcohol consumption as a significant risk factor for developing various dementias, including Alzheimer’s disease (AD). However, it is unclear what effect alcohol has on the hallmarks of AD, such as neurological accumulation of β amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) containing hyperphosphorylated Tau proteins. We hypothesize that chronic binge alcohol consumption

accelerates the development of AD neuropathology. To test this hypothesis, we used the triple transgenic (3xTg) mouse model of AD expressing three human proteins in the brain, $APP_{Swe}$, $PS1_{M146V}$, and $tau_{P301L}$, associated with early-onset AD. Female 3xTg mice (n=15) and non-transgenic (nTg) control mice (B6.129; n=13) were given ethanol (EtOH) binges twice/week (4g/kg; n=9/genotype) by oral gavage for four months (6-10 mo) while untreated controls received sham gavages (3xTg n=6; nTg n=4). Statistical analyses were performed using two-way ANOVA (between genotypes) or student's t-test (within genotype). Analyses focused on the hippocampus, a region critical for memory and affected early in AD pathogenesis. In the novel object recognition test (NORT), EtOH binging caused a significant decrease in hippocampal-dependent memory in 3xTg mice compared to untreated 3xTg controls, while nonTg mice were unaffected. RNA-seq data from the mid-brain region containing the hippocampus revealed that binge EtOH treatment significantly affected 8477 genes (4473 up- and 4004 down-regulated) in 3xTg mice, while only 4 genes were significantly affected (2 up- and 2 down-regulated) in nonTg mice. KEGG enrichment analyses found AD pathogenesis was among the top-twenty most affected biological processes by EtOH treatment in 3xTg mice. Within the Aβ pathway, low density lipoprotein receptor-related protein 1 (LRP1; log2FC=1.246), involved with Aβ transport, was significantly up-regulated and neprilysin (log2FC=-0.471), involved in Aβ degradation, was significantly down-regulated. Additionally, examination of targets involved in Tau phosphorylation revealed that EtOH significantly altered expression of GSK3β (log2FC=-0.305) and Akt1 (log2FC=1.014), kinases involved with Tau phosphorylation. Taken together, these data indicate that chronic binge EtOH consumption accelerated AD-associated memory deficits accompanied by significant changes in expression of genes involved in AD pathogenesis in the hippocampus, a region affected early by AD neuropathology.

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**Nanosymposium**

**NANO85: Alzheimer's Disease: Mechanisms, Biomarkers, and Risk Factors**

**Location:** WCC 207A

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:00 PM

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**Title:** Deep subspace learning reveals multiple salient brain subsystems that characterize Alzheimer's disease

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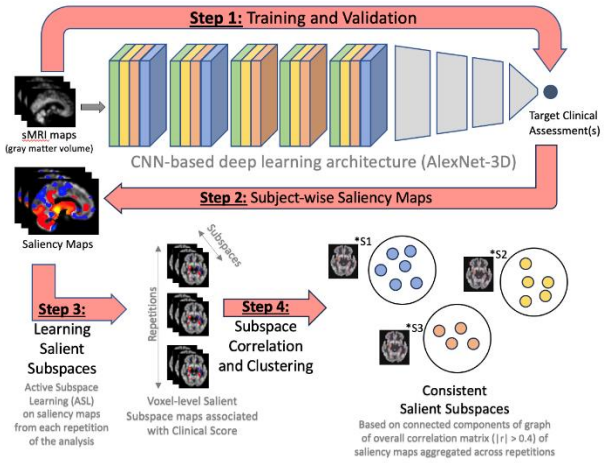
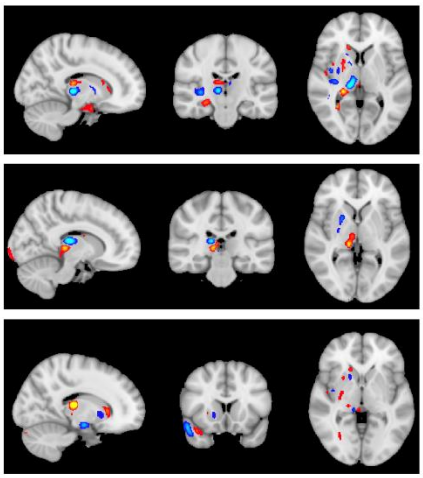
**Abstract:** The brain often undergoes complex changes in multiple sub-systems under the influence of a given disorder. Computational and statistical techniques on structural magnetic resonance imaging (sMRI) data have made it possible to investigate and track these complex changes. However, most approaches work by first reducing the data dimensions either to the level of brain regions or by decomposing the data into low-dimensional representations, followed by associative analysis for brain disorder-related changes. Identifying multiple associated brain subsystems in such scenarios requires manual investigation from an often large set of individual brain areas. We develop a deep learning-based framework which automatically identifies multiple brain subsystems that characterize the changes in cognitive and biological traits related to a given brain disorder or cognitive function. Our approach involves training a deep learning model on sMRI features to predict a target variable (relevant assessment scores or biological measures), followed by saliency analysis to obtain voxel-level importance maps for each subject. Using an active subspace learning approach, these maps are then decomposed and grouped into brain subspaces, which essentially are multiple brain maps, each highlighting a set of brain areas collectively characterizing the changes in the target variable. By using our approach on the ADNI dataset for Alzheimer's disease (AD), we compute multiple brain subsystems associated with AD-related scores (age and MMSE), featuring frontal, temporal, and sub-cortical areas relevant to AD, including the hippocampus, fusiform gyrus, putamen, thalamus, frontal and temporal pole, amygdala etc. In conclusion, our approach successfully uncovers brain subsystems underlying the clinically observed changes in AD via assessments. Developing such approaches is essential for successful biomarker discovery for brain disorders involving complex changes in the brain. In the future, we will be extending this work to include functional MRI and other brain conditions.




(a) Workflow diagram of the overall framework of deep subspace learning



(b) Maps for Top 3 sub-systems that characterize underlying changes for age and MMSE score

**Disclosures: I. Batta:** A. Employment/Salary (full or part-time):; Georgia Institute of Technology, Georgia State 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.; N/A. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); N/A. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. Other; N/A. **A. Abrol:** A. Employment/Salary (full or part-time):; Georgia State University. **V. Calhoun:** A. Employment/Salary (full or part-time):; Georgia Institute of Technology, Georgia State University, Emory 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.; N/A.

**Presentation Number:** NANO85.02

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

**Support:** NIA Grant 1R24AG073138-01

**Title:** Alzheimer's disease-like pathology in a cohort of aged non-human primates (rhesus macaques)

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**Abstract:** Alzheimer's Disease (AD) represents a major public health concern, with more than 5.7 million Americans living with the disease. Despite recent immunotherapeutic advances, no treatment has been able to halt its progression or cure it, underscoring the need for new and improved animal models and biomarkers that may recapitulate key processes underlying AD pathogenesis. Non-human primates (NHPs) are prime candidates, displaying extensive similarity with humans regarding neuronal, endocrine, and immune systems, a highly differentiated prefrontal cortex, and a complex behavioral repertoire, leading us and others to pursue the development of inducible models of AD in rhesus macaques. Significant entry barriers to the use of NHPs in AD research remain, however, including a paucity of information on basal levels of candidate biomarkers, their progression with aging and association with comorbidities, and their relationship with the pathological landmarks that characterize AD. We, therefore, proposed to characterize multiple aspects of AD pathology in a cohort of 36 rhesus macaques aged 20-32 years and correlate our pathological findings with a panel of candidate AD biomarkers and medical histories. Our preliminary results show that, although plaques can be observed in animals between 20-25 years of age, there is an increase in plaques in animals 26 and older. Furthermore, there is substantial variability between animals, with age-matched animals showing multiple-fold differences in plaque load. In addition to amyloid pathology, Tau pathology was also observed in most animals 20 and older, in the form of abnormal phosphorylated Tau (pTau) in axons of the hippocampus, entorhinal cortex, and, to a lesser extent, neocortical areas. Non-axonal Tau pathology was found in a very small number of animals, and late pathology associated-epitopes and neurofibrillary tangles were completely absent. While plaques were not required for axonal Tau pathology to be observed, there is a clear accumulation of pTau+

neurites in dense plaques, suggesting a potential link between the two pathological cascades. Taken together, our results strengthen the rhesus macaque as a model of AD, as both amyloid and tau pathology develop spontaneously in these animals, making them uniquely positioned for AD research. In the next steps, we will perform biomarker assessment in the CSF and plasma of these same animals, including $AB_{40}$ and $AB_{42}$, total and pTau, and NF-L, to determine which biomarkers can be accurately used in rhesus macaques to predict AD pathology. We expect these results to greatly facilitate the use of rhesus macaques in translational research on neurodegenerative diseases.

**Disclosures: G. Diniz:** None. **D. Beckman:** None. **K. Schwartz:** None. **C. Daly:** None. **A.M. Scott:** None. **S. Ott:** None. **D. Griggs:** None. **N.M. Kanaan:** None. **J. Morrison:** None.

**Presentation Number:** NANO85.03

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

**Support:** 5RF1AG071706

**Title:** Positive feedback loop between chronic lysosomal dysfunction and circRNAs in AD mouse models

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**Abstract: Background:** Circular RNAs (circRNAs) and microRNAs (miRNAs) are non-coding RNAs. CircRNAs modulate miRNA levels by sequestration. MiRNAs regulate amyloidogenic pathways, disrupting APP, BACE1, and ADAM10 transcripts. CircRNAs regulate key endolysosomal transcripts. Chronic lysosomal dysfunction (CLD) is linked to Alzheimer's Disease (AD). However, the role circRNAs downstream of CLD and AD is unclear. Here, we tested whether CLD affects the amyloidogenic pathway in 5xFAD mice through circRNAs.**Methods:** Cortical circRNA expression was obtained of 5xFAD, $PPT1^{+/-}$, 5xFAD: $PPT1^{+/-}$ (P5X), $Naglu^{+/-}$, 5xFAD: $Naglu^{+/-}$ (N5X) mice. TruSeq Stranded total RNA was extracted with Ribo-Zero to deplete rRNA and generate stranded-RNA-seq. Libraries were sequenced on a NextSeq 500 platform of Illumina, and analysis was done with the DESeq2 package with an FRD <0.05. *In silico* microRNA, binging was done using circAtlas 2.0 browser. The insoluble Aβ-40 and Aβ-42 were quantified by ELISA in the hippocampal fraction. The Aβ plaque load was quantified in coronal brain sections using immunohistochemistry. **Results:** The P5X and N5X mice exhibit increased amyloid plaque load, hippocampal Aβ-40 and Aβ-42 levels, and reduced lifespan compared to 5xFAD mice. We detected 1286 cicRNAs in all the groups. 38 from P5X and N5X groups, 37 from $PPT1^{+/-}$, and 29 from $Naglu^{+/-}$ passed FRD correction compared with 5xFAD mice. Both linear and circRNAs of 4933406I18Rik, Zfp609, Zfp532, and Nnt changed between $PPT1^{+/-}$ and P5X compared to 5xFAD. The levels of circAdam10, circPan3, circMbtd1, circ4930402H24Rik, circSt6gal2, and circCdc14b were reduced, while circZranb1 and circMyo9a were increased in P5X and N5X compared with 5xFAD. CircNlgn1 levels changed only downstream of $PPT1^{+/-}$. *In silico* analyses show

amyloidogenic-related microRNAs exhibit circRNAs binding sites. MiR-361-3p has binding sites for circZfp609 and circADAM10, and miR-298-3p can bind circNlgn1. Both miR-361-3p and miR-298-3p trap and inhibit BACE1 function and Aβ production, interfering in the amyloidogenic pathway. CircPan3 regulates the autophagy-related miR-421. Experimental evidence shows that overexpression of circPan3 suppresses autophagy through a miR-421/Pink1 pathway. **Conclusions**: CLD ($PPT1^{+/-}$ and $Naglu^{+/-}$) boosted AD pathology and induced differential changes in circRNAs levels affecting amyloidogenic and autophagy pathways. There is a positive feedback loop between CLD and circRNAs in AD mouse models.

**Disclosures: S. Cárdenas Romero:** None. **A. Eteleeb:** None. **N. Nykanen:** None. **M. Nunez:** None. **K. O'Dell:** None. **O. Harari:** None. **J. Cirrito:** None. **M. Sands:** None. **B.A. Benitez:** None.

**Presentation Number:** NANO85.04

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

**Title:** Specificity of Beta-Amyloid Isoforms Detection by Western Blotting

**Authors: *N. SHEN**, X. LIN, H. YANG, Y. HONG, B. BROWN, C. CAO;
Univ. of South Florida, Tampa, FL

**Abstract:** The accumulation of beta-amyloid(Aβ) as senile plaques and eventually lead neuron death in the brain with aging is the critical pathological factor of neurodegenerative diseases like Alzheimer's and Parkinson's disease. Heretofore, numerous studies related to Aβ have been conducted successfully or unsuccessfully. And western blotting was widely used as a common technique to identify the protein of Aβ in these studies. In the long-term study of Alzheimer's diseases, we discovered that as an intrinsically disordered protein, beta-amyloid has its own characteristics when performing western blotting. Beta-amyloid aggregated into different isoforms under different micro-condition. Therefore, it is particularly important to separate and identify the Aβ isoforms by western blotting. However, different antibodies yield different results for the same sample due to epitope variation. We present our findings to encouraging more researcher to join us in exploring qualitative and quantitative methods for Aβ isoforms to further support future studies.

**Disclosures: N. Shen:** None. **X. Lin:** None. **H. Yang:** None. **Y. Hong:** None. **B. Brown:** None. **C. Cao:** None.

**Presentation Number:** NANO85.05

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

**Support:** NIA: R01 AG054048-01

**Title:** Small soluble protein variants as sex specific early biomarkers and therapeutic targets for diagnosing and treating Alzheimer's disease

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**Abstract:** Antibody treatments targeting amyloid beta (Aβ) have recently shown promise as therapeutics for Alzheimer's disease (AD) by slowing the rate of cognitive decline in treated patients. However, cognitive benefit was observed primarily in older male patients, even though females comprise 2/3rds of AD cases and therapeutic benefit has the greatest potential in younger patients. Biomarkers that can predict and diagnose the onset of Alzheimer's disease (AD) are essential for selecting effective therapeutic treatments. We utilized a panel of novel reagents that selectively bind small soluble neurodegenerative disease specific variants of amyloid-beta (Aβ), tau, TDP-43 and α-synuclein to identify blood based biomarkers and potential therapeutic targets for early diagnosis and treatment of AD. We also showed by immunohistochemical analysis of post-mortem AD brain tissue, that key toxic protein variants accumulate in neurons during early stages of AD. We identified distinct differences in protein variant biomarker profiles and brain pathology between male and female AD cases, even during presymptomatic AD stages. In particular female AD cases have different Aβ variants and a stronger dependence on tau variants compared to male AD cases. These results offer a rationale to explain why female AD cases did not respond well compared to male cases in recent therapeutic trials targeting aggregated Aβ. The biomarker panel provides a tool to stratify patients that are likely to respond to a particular therapeutic, including which patients may best benefit from Aβ or tau targeting therapeutics. We provide a rationale for development of precision therapeutics designed to take into account individual differences in toxic protein variants.

**Disclosures: M.R. Sierks:** None. **H.R. Cho:** None. **P. He:** None. **P. Schulz:** None.

**Presentation Number:** NANO85.06

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

**Title:** Evaluation of the plasma Ab42/40 and p-Tau181 in Alzheimer's Disease (AD) using Lumipulse G1200 assays

**Authors: *A. CHENNA**, Y. BADAL, B. YEE, C. PETROPOULOS, J. WINSLOW; LabCorp-Monogram Biosci. Inc, South San Francisco, CA

**Abstract:** Plasma levels of amyloid beta peptide Aβ42 and the Aβ42/Aβ40 ratio are significantly depressed in Alzheimer's disease (AD) patients, whereas phospho-Tau (p-Tau181) protein is significantly elevated. The clinical assessment of plasma Aβ42/Aβ40 ratio and p-Tau181 is a promising approach for early diagnosis, disease progression, and risk stratification of AD. We have evaluated the newly developed blood-based Fujirebio Lumipulse Aβ42/Aβ40 and p-Tau181 assays in AD subjects (n=50) and healthy controls (n= 24), to characterize the discriminatory and correlative relationships for each assay within and between clinical diagnosis groupings. AD sample groups consisting of mild (n=16, MMSE >23-30), moderate (n=14, MMSE=16-22), and severe (n=19, MMSE<16) cognitive impairment, and healthy controls (n=24) were assessed for subgroup measurement differences. Analysis of the median Aβ42/Aβ40 ratio was significantly lower in AD patients ($p<0.0001$), relative to controls. Conversely, the p-Tau181 assay revealed a significant increase in median p-Tau181 (6.7-fold) of the combined AD group ($p<0.0001$, n=50), relative to controls (n=25). All AD sample subgroups had significantly distinguishable Aβ42/Aβ40 ratios ($p=0.0002-<0.0001$) and p-Tau181 levels ($p<0.0001$) relative to the healthy control group, with median p-Tau181 levels fold increases of 1.5, 7.7, and 9.5 for the mild,

moderate and severe AD subgroups relative to the control group. Both Aβ42/Aβ40 ratio and p-Tau181 levels were able to differentiate overall AD compared to controls (AUC=0.82, 0.93 respectively) and between the different AD clinical subgroups (Aβ42/Aβ40 ratio AUC=0.79-0.83) and (p-Tau181 AUC=0.82-0.90). Lumipulse Aβ40/Aβ42 ratio and p-Tau181 assays demonstrated robust analytical performance with the precision of 5-10% CV and clinically differentiated AD plasma from healthy control plasma. Plasma Aβ42/Aβ40 ratio and p-Tau181 biomarkers provide a practical diagnostic opportunity to identify AD patients.

**Disclosures: A. Chenna:** A. Employment/Salary (full or part-time):; LabCorp-Monogram Biosciences. **Y. Badal:** A. Employment/Salary (full or part-time):; LabCorp-Monogram Biosciences. **B. Yee:** A. Employment/Salary (full or part-time):; LabCorp-Monogram Biosciences. **C. Petropoulos:** A. Employment/Salary (full or part-time):; LabCorp-Monogram Biosciences. **J. Winslow:** A. Employment/Salary (full or part-time):; LabCorp-Monogram Biosciences.

**Presentation Number:** NANO85.07

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

**Title:** Unveiling Prospective Alzheimer's Disease Biomarkers via Exosomal Small RNA Signature Decipherment

**Authors: *Y. CHO**[1], H. KIM[1], S. SEO[2], D.-G. JO[1];
[1]Sch. of Pharm., Sungkyunkwan Univ., Suwon, Korea, Republic of; [2]Dept. of Neurol., Samsung Med. Center, Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of

**Abstract:** Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by progressive cognitive decline. The accumulation of amyloid beta (Aβ) in the brain is a notable feature of AD. Apolipoprotein E (APOE), a protein involved in lipid transport and metabolism, has been recognized as the primary genetic risk factor for late-onset AD (LOAD). APOE exists in three isoforms: ApoE2, ApoE3, and ApoE4, with ApoE4 increasing the susceptibility to LOAD and ApoE2 exhibiting a protective effect. Interestingly, individuals carrying the *APOE ε4* allele or displaying Aβ plaques may not always manifest AD symptoms. Exosomes, small extracellular vesicles ranging from 30 to 200 nanometers in diameter, are known to contain proteins, DNA, RNA, including microRNA (miRNA) and long non-coding RNA (lncRNA), and participate in intercellular communication. In this study, we isolated exosomes from healthy individuals and AD patients to investigate the differences in small RNA content among those carrying the *APOE ε4* allele and/or exhibiting Aβ plaques. Exosomes were obtained using a precipitation-based method, and small RNA was subsequently extracted for analysis. Next Generation Sequencing (NGS) was employed to assess the small RNA population. Our NGS analysis revealed significant disparities in small RNA profiles among the examined groups. These findings underscore the potential clinical relevance of exosomes derived from serum. The observed variations in small RNA content suggest the possibility of utilizing exosomal RNA as a biomarker for diagnosing AD, identifying individuals at risk, or monitoring disease progression. Further exploration of this area may enhance our understanding of AD pathology and facilitate the development of novel diagnostic and therapeutic strategies.

**Disclosures: Y. Cho:** None. **H. Kim:** None. **S. Seo:** None. **D. Jo:** None.

**Presentation Number:** NANO85.08

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

**Title:** Proteomic profiling of 54,219 plasma samples reveals peripheral markers of central nervous system disorders

**Authors:** L. HOU[1], A. LI[1], Y. WANG[1], S. LI[1], ***C. D. WHELAN**[3,2];
[1]Janssen, Spring House, PA; [2]Data Sci., Janssen, Cambridge, MA; [3]Data Sci., Johnson and Johnson, Boston, MA

**Abstract:** Blood-based proteomic markers of neurodegeneration, such as plasma p-tau217 and Aβ42/40 ratio, are increasingly implemented in neuroscience research and development. Recent advances in the sensitivity, multiplexing and throughput of proteomics technologies may facilitate the discovery of a broader collection of biomarkers and therapeutic targets for CNS disorders.
In this study, we developed a framework for CNS biomarker and target discovery using antibody-based proteomics, leveraging 2,923 plasma protein analytes measured across 54,219 UK Biobank (UKB) participants using the Olink Explore Proximity Extension Assay. We first conducted cross-sectional association analyses between protein levels and ~1,500 diseases in UKB, as defined by combined ICD codes (PheCODEs). We then performed protein-change trajectory estimations for patients before and after disease onset. Finally, we employed systematic Mendelian randomization (MR-Egger) across ~800 traits using *cis* protein quantitative trait loci identified by the Pharma Proteomics Project (https://doi.org/10.1101/2022.06.17.496443) as instrumental variables for causal inference. Cross-sectional analyses identified 241,501 significant ($p<6x10^{-8}$) associations between 2,389 proteins and 860 illnesses. This included widespread upregulation of inflammatory cytokines in major depressive disorder, broad dysregulation of inflammatory proteins and adhesion molecules in multiple sclerosis, and upregulation of innate immune markers in recurrent epilepsies. Protein-change trajectories revealed that both glial fibrillary acidic protein (GFAP) and neurofilament light-chain (NfL) were elevated at least 5 years before Alzheimer's disease (AD) onset; however, GFAP represented a more specific marker of neurodegeneration vs. NfL, which associated with >200 cardiovascular, metabolic, and respiratory illnesses. MR-Egger revealed dozens of causal relationships between plasma proteins and CNS illnesses, including a negative causal relationship between risk of AD and plasma ApoE ($MR_{Egger}$ beta = -1.1; $MR_{Egger}$ $p=5.7x10^{-232}$). Our findings reemphasize how peripheral proteins may be useful for identification and stratification of patients with debilitating brain illnesses. We underline how proteomic profiling of population biobanks can facilitate discrimination between specific (GFAP) and nonspecific (NfL) markers of brain injury and can accelerate therapeutic target discovery, providing a proof-of-principle for similar large-scale initiatives in cerebrospinal fluid and brain tissue.

**Disclosures: L. Hou:** A. Employment/Salary (full or part-time):; Janssen. **A. Li:** A. Employment/Salary (full or part-time):; Janssen. **Y. Wang:** A. Employment/Salary (full or part-time):; Janssen. **S. Li:** A. Employment/Salary (full or part-time):; Janssen. **C.D. Whelan:** A. Employment/Salary (full or part-time):; Janssen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johnson & Johnson.

**Presentation Number:** NANO85.09

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

**Support:** NIH/NIA Grant 1RF1AG074608-01

**Title:** Unraveling heterogeneity in Vascular Cognitive Impairment and Dementia using monogenic models

**Authors: *J. S. PAULSEN**[1], H. J. BOCKHOLT[2], W. H. ADAMS[3], J. BILLER[3], J. L. ELLIOTT[1], A. CAPRIHAN[4], K. M. JOHNSON[1], L. B. EISENMENGER[1], S. JAYADEV[5], S. SALLOWAY[6], J. GUTIERREZ[7], J. J. MAJERSIK[8], H. L. LUTSEP[9], K. D. ORJUELA[10], D. S. LIEBESKIND[11], S. SESHADRI[12], I. HAJJAR[13], M. D. GESCHWIND[14];
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**Abstract: Goal**: Characterize the presentation and course of biomarkers, neuroimaging, genotypes and phenotypes in presymptomatic or early/moderate symptomatic *NOTCH3* carriers and compare with non-carrier family member controls (NC) in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL). **Background**: CADASIL is the most common monogenic cause of vascular dementia. Persons with CADASIL develop vascular cognitive impairment and dementia (VCID), which can be studied in gene mutation carriers long before clinical presentation. A large cohort in presymptomatic, prodromal and manifest disease stages are investigated to detect changes in biological fluids, neuroimaging, and emerging phenotype of symptomatic VCID. To date, few cross-sectional or longitudinal data describe CADASIL in the USA. *Findings in other countries suggest that specific NOTCH3 mutations predict a more severe course and mortality*. Given findings of variations in CADASIL among global geographic locations, a US cohort is crucial to better understand the clinical progression and identification of potential biomarkers needed to design and interpret clinical trials. **Methods**: A longitudinal cohort of 400 *NOTCH3* carriers and 100 NC from 12 US sites (RF1AG074608) are investigated to find whether VCID subtypes can be illuminated by variations in NOTCH3 mutation. Participants undergo systematic clinical, advanced neuroimaging and blood-based phenotyping. Cognitive function is assessed via pen and paper tests and tablet-based measures from NIH-EXAMINER (a battery emphasizing frontal-executive assessment, often impaired in vascular dementia). Diffusion tensor imaging and newer MRI techniques are examined to clarify underlying mechanism or yield lowest sample size estimates needed for clinical trials. Several outcome assessments, including quality of life, are collected to assess which track progression best. **Results**: Enrollment has begun at all 12 sites. So far, all participants function independently, most often without significant cognitive impairment based on the Montreal Cognitive Assessment. Imaging analyses show MRI abnormalities in CADASIL associated with cognitive decline. **Conclusion**: Findings from our first-in-US multi-site

consortium of the most heritable rare disease for VCID suggest early imaging abnormalities associated with cognitive decline. As data are collected, we will contribute to understanding of vascular dementia. Long-term objectives are to unravel heterogeneity of vascular dementia to clarify contributions to Alzheimer's disease and related dementias as the most common influence in mixed dementias.

**Disclosures: J.S. Paulsen:** None. **H.J. Bockholt:** None. **W.H. Adams:** None. **J. Biller:** None. **J.L. Elliott:** None. **A. Caprihan:** None. **K.M. Johnson:** None. **L.B. Eisenmenger:** None. **S. Jayadev:** None. **S. Salloway:** None. **J. Gutierrez:** None. **J.J. Majersik:** None. **H.L. Lutsep:** None. **K.D. Orjuela:** None. **D.S. Liebeskind:** None. **S. Seshadri:** None. **I. Hajjar:** None. **M.D. Geschwind:** None.

**Nanosymposium**

**NANO86: Parkinson's Disease: Preclinical Models and Therapeutic Strategies**

**Location:** WCC 147A

**Time:** Wednesday, November 15, 2023, 1:00 PM - 4:30 PM

**Presentation Number:** NANO86.01

**Topic:** C.03. Parkinson's Disease

**Title:** Neuroprotective effects of GSK-343 in an in vivo model of MPTP-induced nigrostriatal degeneration

**Authors: *G. CASILI**, D. MANNINO, S. SCUDERI, M. LANZA, A. FILIPPONE, M. CAMPOLO, I. PATERNITI, S. CUZZOCREA, E. ESPOSITO;
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**Abstract:** Parkinson's disease (PD) is characterized by the degeneration of dopaminergic nigrostriatal neurons, which causes disabling motor disorders. Scientific findings support the role of epigenetics mechanism in the development and progression of many neurodegenerative diseases, including PD. In this field, some studies highlighted an upregulation of Enhancer of zeste homolog 2 (EZH2) in the brains of PD patients, indicating the possible pathogenic role of this methyltransferase in PD. The aim of this study was to evaluate the neuroprotective effects of GSK-343, an EZH2 inhibitor, in an in vivo model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic degeneration. Specifically, nigrostriatal degeneration was induced by MPTP intraperitoneal injection. GSK-343 was administered intraperitoneally daily at doses of 1 mg/kg, 5 mg/kg and 10 mg/kg, mice were killed 7 days after MPTP injection. Our results demonstrated that GSK-343 treatment significantly improved behavioral deficits and reduced the alteration of PD hallmarks. Furthermore, GSK-343 administration significantly attenuated the neuroinflammatory state through the modulation of canonical and non-canonical NF-κB/IκBα pathway as well as the cytokines expression and glia activation, also reducing the apoptosis process. In conclusion, the obtained results provide further evidence that epigenetic mechanisms play a pathogenic role in PD demonstrating that the inhibition of EZH2, mediated by GSK-343, could be considered a valuable pharmacological strategy for PD.

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**Presentation Number:** NANO86.02

**Topic:** C.03. Parkinson's Disease

**Support:** R21 AG059391

**Title:** Gene therapy with single domain antibodies reduces α-synuclein pathology in vivo

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**Abstract:** A major challenge for CNS antibody therapies is sufficient CNS entry for efficacy. Single domain antibodies (sdAbs) offer a potential solution. They are much smaller than whole IgGs (15 vs. 150 kDa) and can recognize cryptic epitopes that are inaccessible to larger molecules. Further, sdAbs have great potential as gene therapies. We have generated multiple sdAbs isolated from a llama immunized with human α-synuclein (αsyn). Those with favorable binding profiles were selected for efficacy studies.

Based on their binding profile and cell culture efficacy, two sdAbs, 2D8 and 2D10, were selected for gene therapy. We recently reported on their diagnostic imaging potential, showing their brain entry and binding to intracellular αsyn after an i.v. injection, with their brain signal correlating strongly with αsyn burden (Jiang Y et al Sci Adv May 10, 2023). Female M83 A53T αsyn mice (n=62) received a single i.v. injection of AAV9-2D8-GFP or AAV9-2D10-GFP, or either AAV9-GFP alone or PBS. To test their ability to prevent and reverse αsyn pathology, the mice were treated at 3-4 or 6-8 months of age, and their brains collected 3 months later for analyses. In the younger group, analysed at 6-7 months (n=27), the gene therapy reduced soluble total and phospho-serine 129 (pS129) αsyn by 30-53% in both sdAb groups (n=8-10 per group), compared to controls (n=9). This effect was highly significant (one-way ANOVA: $p<0.0001$ for both. Total αsyn: 2D8 and 2D10, $p<0.0001$; pS129: 2D8 and 2D10, $p<0.001$ (post-hoc Tukey)). However, sarkosyl insoluble αsyn was not reduced in these animals. In contrast, in the older group, analysed at 9-11 months (n=35), the therapy increased soluble total αsyn by 44-45% (one-way ANOVA: $p=0.0018$) in both AAV9-sdAb groups (n=11-14 per group), compared to controls (n=10), and reduced insoluble total and pS129 αsyn only in the 2D10 group by 51-53 % (one-way ANOVA: $p=0.0025$ and 0.0053, respectively. Tukey: $p<0.01$ for both).

Gene therapy sdAb-mediated clearance of soluble but not insoluble αsyn in the younger group likely reflects the limited amount of insoluble αsyn in this cohort. The treatment-induced increase and decrease in soluble vs. insoluble αsyn in the older group presumably relates to continuous clearance of insoluble αsyn, and thereby shift of equilibrium of insoluble to soluble αsyn. Efficacy differences between the age groups likely relate to changes in αsyn pathology over time. These results demonstrate the potential of sdAbs targeting αsyn as gene therapies and show that their peripheral administration is sufficient to significantly reduce αsyn pathology within the brain.

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**Presentation Number:** NANO86.03

**Topic:** C.03. Parkinson's Disease

**Title:** A brain-shuttled antibody targeting alpha synuclein aggregates for the treatment of synucleinopathies

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**Abstract:** Synucleinopathies, which include Parkinson's disease and multiple systems atrophy, are a class of devastating neurodegenerative diseases characterized by the presence of alpha-synuclein (aSyn) rich aggregates in the brains of patients. Passive immunotherapy targeting these aggregates is an attractive disease-modifying strategy. Such an approach must not only demonstrate target selectivity towards aSyn aggregates, but also achieve appropriate brain exposure to have the desired therapeutic effect. Here we present preclinical data supporting ABL301, also known as SAR446159, for treating synucleinopathies. Currently in phase 1 clinical trials, SAR446159 is a bispecific antibody composed of an aSyn-binding IgG and an engineered insulin-like growth factor receptor (IGF1R) binding moiety acting as a shuttle to transport an antibody across blood-brain barrier (BBB). SAR446159 binds tightly to aSyn aggregates and prevents their seeding capacity *in vitro* and *in vivo*. Incubation with SAR446159 prevented aSyn preformed fibrils (PFFs) from inducing synuclein aggregation in primary rat hippocampal neurons. In wild type (WT) mice injected in the striatum with aSyn PFFs, treatment with SAR446159 reduced the spread of aSyn pathology as measured by pSer129 aSyn staining and lowered the severity of motor phenotypes. Additionally, in 9-month-old transgenic mice overexpressing aSyn (mThy1-aSyn, Line 61), repeated treatment with SAR446159 reduced pSer129 aSyn levels in the brain. The potent activity of this antibody is facilitated by both the engineered IGF1R binding moiety, which enhances brain exposure by shuttling the antibody across the BBB, and by its high selectivity for aggregated conformers of aSyn over monomeric aSyn. Moreover, the IGF1R-binding shuttle enabled greater uptake into the endo-lysosomal trafficking pathway of neurons, potentially allowing this antibody to engage aSyn aggregates both intracellularly and extracellularly. These unique properties make SAR446159 a next-generation immunotherapeutic for treating neurodegenerative diseases.

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**Presentation Number:** NANO86.04

**Topic:** C.03. Parkinson's Disease

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**Title:** Knockdown of hdac5 protects midbrain dopaminergic neurons againsta-synuclein-induced neurodegeneration in a rat model of parkinson's disease.

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**Abstract:** Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by midbrain dopaminergic neuron death and intracellular α-synuclein (αSyn) accumulation. Histone deacetylases (HDACs) have been proposed as therapeutic targets for neuroprotection in PD, though which HDAC should be targeted is unclear. Previous work has shown that HDAC5 inhibition protects dopaminergic neurons from 6-hydroxydopamine-induced degeneration *in vitro* and *in vivo*. Whether gene therapy to knockdown HDAC5 can protect these from αSyn-induced degeneration is unclear.In this study, we assessed the therapeutic efficacy of AAV-shHDAC5 in the AAV-αSyn rat model of PD. We first showed an increase in nuclear HDAC5 in tyrosine hydroxylase (TH)-positive neurons in the SN at 24 weeks post unilateral intranigral injection of AAV-αSyn in adult female Sprague-Dawley rats. We also validated that intranigral AAV-shHDAC5 reduced *Hdac5* levels in the SN. We next evaluated the therapeutic efficacy of AAV-shHDAC5. Male and female adult Sprague-Dawley rats received a unilateral intranigral stereotactic injection of a combination of AAV-αSyn with either AAV-shHDAC5 or a scrambled control (AAV-shSCR) vector. At 24 weeks post-surgery brain samples were taken for analysis. We found a significant increase in the numbers of tyrosine hydroxylase (TH)-positive and dopamine transporter (DAT)-positive neurons in SN confirming that AAV-shHDAC5 prevented AAV-αSyn-induced reduction in their number. In agreement with this, densitometry analysis of dopaminergic innervation of the striatum (ST) confirmed neuroprotection of dopaminergic terminals. Furthermore, we evaluated the number of IBA1 positive cells showing a reduced number of IBA1-positive cells in the SN in the AAV-shHDAC5 group. None of the analysed parameters showed a sex specific effect. Collectively these data show a neuroprotective effect of AAV-shHDAC5 in the AAV-αSyn rat model of PD. These data rationalise the further study of HDAC5 knockdown as a potential therapeutic strategy for neuroprotection in PD.

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**Topic:** C.03. Parkinson’s Disease

**Support:** Presidential Postdoctoral Fellowship (021229-00001)
Open Fund Young Investigator Research Grant (OF-YIRG) (MOH-001147)

**Title:** Acidic nanoparticles restore lysosomal function and rescue α-syn-induced neuronal cell death in Parkinson's disease

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**Abstract:** Parkinson's disease (PD) stands as the second most prevalent neurodegenerative disorder worldwide. Although the pathogenic causes of PD are multifaceted, they have been associated with the abnormal build-up of alpha-synuclein (α-syn) in Lewy bodies (LBs), resulting in a gradual decline in the function of dopaminergic neurons. Recent research has highlighted that in cellular and rodent models of PD induced by α-syn overexpression and preformed fibrils (PFFs), the endolysosomal pathway is compromised, although the exact pathogenic mechanism remains unclear. Using transgenic mice overexpressing A30P α-syn (A30PTg), we showed that there is a reduction in lysosomal subunits in young mouse brain tissue lysates, but not in old mouse brain tissue lysates. This suggests that defective lysosomal acidification could be an early driver of neurodegeneration induced by A30P α-syn. To investigate the mechanism, we utilized SH-SY5Y neuronal cells with A30P α-syn overexpression as a PD model, and applied novel pH-responsive acidic nanoparticles (acNPs) which can specifically localize to lysosome and degrade at pH 6 to induce lysosomal acidification. We demonstrated that A30P α-syn overexpression induced lysosomal pH elevation and lysosomal enzyme dysfunction, together with impairments in autophagic and mitochondrial function. In addition, we showed that inhibition of autolysosomal function increased the secretion of α-syn to surrounding cells. Importantly, re-acidification of the impaired lysosomes using acNPs ameliorates defects in autophagic and mitochondrial function, and reduced the release of α-syn to the surrounding media, thereby reducing α-syn induced cell death. Transcriptomics analysis further corroborated the findings by showing a downregulation of pathways regulating lysosomal and mitochondrial function in A30P α-syn overexpressed cells, and application with acNPs modulated this downregulation. We have also applied acNPs in a α-syn PPFs-induced sporadic PD model, and observed that re-acidification of impaired lysosomes with acNPs reduced α-syn spreading via exosomes. In sum, acNPs show great potential as a valuable investigative tool for studying the intricacies of lysosomal acidification mechanisms. Moreover, their use as a potential therapeutic strategy holds promise for addressing Parkinson's disease.

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**Topic:** C.03. Parkinson’s Disease

**Support:** Michael J Fox Foundation Therapeutic Pipeline Program grant with co-funding support from Shake It Up Australia Foundation

**Title:** The CNS-permeable NLRP3 inhibitor RRx-001 is neuroprotective in experimental models of Parkinson's disease

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**Abstract:** Parkinson's disease is the fastest growing neurological disorder globally and there currently are no effective disease-modifying treatments. The neuropathological hallmarks and sequalae of PD includes a complex spectrum of motor and non-motor deficits accompanied by selective dopaminergic degeneration, alpha synuclein accumulation, chronic inflammasome activation, as well as gastrointestinal dysfunction and microbiome dysbiosis. Given the complex multifactorial aetiology of PD, emerging evidence suggests that targeting multiple pathological mechanisms is essential to achieve disease modification. Chronic immune and inflammasome activation, mitochondrial dysfunction and impaired autophagy are well-established mechanisms leading to progressive dopaminergic degeneration and synuclein pathology. We recently confirmed that RRx-001, a Phase 3 small molecule anticancer and chemoprotective agent, is a direct NLRP3 inhibitor that is CNS permeable and exhibits nanomolar potency against inflammasome activation. In this study, we evaluated if RRx-001 could be developed as a novel disease-modifying therapeutic agent for PD. We confirmed that once daily dosing with RRx-001 (10 mg/kg) reduced NLRP3 inflammasome activation markers such as caspase-1 p20 and ASC in the 6-OHDA model of PD. In RRx-001 treated mice, we also found strong activation of the neuroprotective NRF2 pathway in the nigrostriatal system. We also performed in vitro mechanistic studies with RRx-001 in dopaminergic neuronal cells and microglia. We confirmed that RRx-001 inhibits NLRP3 activation in microglia with nanomolar potency and reduced markers of neuroinflammation. In dopaminergic neuronal cells, RRx-001 prevented mitochondrial fragmentation induced by the Parkinsonian neurotoxicant $MPP^+$. RRx-001 also improved markers of mitochondrial function and biogenesis in dopaminergic neuronal cells. Together, our data demonstrates that RRx-001 crosses the blood brain barrier and can mitigate inflammasome activation in microglia and macrophages. Additionally, in neurons, RRx-001 activates neuroprotective NRF2 response pathways to prevent mitochondrial dysfunction and oxidative stress. Our results highlight the neuroprotective properties of RRx-001 as a novel and unique disease-modifying therapeutic agent which can target multiple pathological mechanisms. Given the clinical safety record of RRx-001 in human studies to date, our results suggest that RRx-001 could be an attractive neuroprotective strategy for disease modification of PD.

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**Topic:** C.03. Parkinson's Disease

**Support:** NSERC - Collaborative Research and Development Grants
Mitacs Accelerate

**Title:** Retinal changes detected by ERG and pupillometry in parkinsonian monkeys

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**Abstract:** Diagnosis of Parkinson's disease (PD) is currently made following clinical observation of motor symptoms. By the time these symptoms manifest, around 50% of dopamine neurons are lost in the substantia nigra pars compacta (SNc), limiting the possibility of implementing potential neuroprotective or neurorestorative treatments that could delay the progression of the disease. Non-motor symptoms, such as vision problems, occur much earlier along the progression of the disease. If altered functioning of the retina causes these vision problems, various techniques could be implemented to detect retinal changes, therefore providing early biomarkers for PD. The aim of this project is to determine potential early biomarkers for PD via the retina by using electroretinography (ERG) and pupillometry. In-vivo measurements were performed on 4 non-human primates (NHP), before and after they were rendered parkinsonian by administration of 1-méthyl-4-phényl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that induces degeneration of dopamine neurons. Post-mortem retinal analyses were compared against the retina of 4 control NHPs. ERG results showed reduced a-wave amplitudes with slightly increased b-wave amplitudes in both photopic and scotopic conditions. Pupillometry analysis showed a consistently greater increase in pupil diameter during the post flash period. Post-mortem measurement of retinal layers found a significant thinning of the outer nuclear layer. These results indicate that MPTP caused changes to the retina detectable by ERG and pupillometry and that these changes could be attributed to the observed retinal thinning. As MPTP is a model of PD, these results provide evidence for potential retinal biomarkers that could be used as an earlier or more accurate means of diagnosing PD.

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**Topic:** C.03. Parkinson's Disease

**Support:** MJFF Target Advancement

**Title:** Identification of Synuclein Nitrase, one of a new class of enzymes, and a new target for Parkinson's disease therapeutics

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**Abstract:** Tyrosine nitration can modify the structure, function and/or location of a particular protein and is implicated as a driver of multiple diseases. The prevailing dogma in the oxidative/nitrosative stress field posits that protein nitration is a random chemical reaction but the exquisite selectivity in which proteins are nitrated refutes this assumption. We hypothesized that nitration of alpha-synuclein, a causal, aggregating protein in Parkinson's disease, is catalyzed by an enzyme. Here we show that a previously uncharacterized protein nitrates alpha-synuclein biochemically, in cells and in mice. We call this protein, Synuclein Nitrase. Synuclein Nitrase activity is regulated by another causal Parkinson's disease protein, PARK7/DJ-1. Functionally, we found Synuclein Nitrase impairs neuronal connectivity and propagates pre-formed fibrils (PFF) induced synuclein aggregation in iPSC-dopaminergic neurons. In A53T-synuclein transgenic mice, Synuclein Nitrase knockout reduces paralysis and PFF induced spread of synuclein pathology. Our results suggest inhibiting Synuclein Nitrase could be a promising new Parkinson's disease therapeutic approach. With these data, we have identified a new enzymatic function, catalysis of protein nitration, and begun to characterize these enzymes that we call Nitrases.

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**Topic:** C.03. Parkinson's Disease

**Support:** Michael J Fox Foundation Target Validation Grant
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**Title:** Spleen Tyrosine Kinase (Syk) is a druggable therapeutic target for Parkinson's disease

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**Abstract:** Parkinson’s Disease (PD) manifests as a spectrum of debilitating motor and non-motor deficits for which there are currently no effective treatments beyond symptom management through dopamine replacement therapy. Emerging as a central driver of neuropathology in PD and other neurological conditions, there is growing recognition of the role played by chronic immune and inflammasome activation, triggered by misfolded synuclein aggregates in PD. Indeed, the NLRP3 inflammasome is currently one of the most attractive targets for disease modification in PD, with multiple therapeutic agents in clinical and preclinical development. Previous work from our team, demonstrated extensive NLRP3 inflammasome activation in people with PD. We also demonstrated that pharmacological inhibition of NLRP3 is neuroprotective in multiple disease models. Since kinases and phosphatases orchestrate the assembly and activation of multiprotein inflammasome complexes, we sought to understand the specific kinases responsible for NLRP3 inflammasome activation in PD in our current study. We identified that Spleen Tyrosine Kinase (Syk) was highly activated in central and peripheral immune cells. In primary microglial cells, we found that activation loop phosphorylation of Syk at Tyrosine 525 (pSyk Tyr 525) precedes inflammasome activation and IL1β release triggered by synuclein aggregates. Using the orally-active small molecule Syk inhibitor Entospletinib, we found that once daily dosing could prevent striatal dopamine depletion and dopaminergic degeneration. We also found that Syk inhibition reduced synuclein pathology and spread in the striatum, motor cortex and corpus callosum in a preformed fibril model of synuclein. Our results uncover Syk as new druggable therapeutic target which is highly activated in PD patients. Inhibition of Syk in the CNS could be a novel therapeutic strategy for disease modification in PD by targeting inflammasome driven neuropathology and dopaminergic degeneration.

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**Presentation Number:** NANO86.10

**Topic:** C.03. Parkinson’s Disease

**Title:** Exosome-mediated delivery of Parkin rescues mitochondrial dysfunction in Parkinson's disease models.

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**Abstract:** Parkinson's disease (PD) is the second most common neurodegenerative disorder, caused by a reduction of dopamine release in the midbrain. Parkin (PRKN; also known as PARK2) is an E3 ubiquitin ligase that regulates mitochondrial quality. Mutations in the Parkin gene are associated with early-onset autosomal recessive PD, characterized by mitochondrial dysfunction. Recent studies have suggested the potential therapeutic use of Parkin delivery for the treatment of PD. We have developed to deliver Parkin protein with engineered extracellular vesicles to rescue mitochondrial dysfunction.In our study, we utilized photocleavable proteins, which can be cleaved upon exposure to light of a specific wavelength. By strategically placing the photocleavable protein between Parkin and the exosomal membrane protein, we observed the light-induced release of Parkin into exosomes through cleavage of the photocleavable protein.We evaluated to assess the effectiveness of Parkin exosomes in removing damaged mitochondria and

alleviating oxidative stress, thus rescuing mitochondrial dysfunction in Parkinson's disease models.

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**Topic:** C.03. Parkinson's Disease

**Title:** IPSC-derived midbrain organoids to discover novel biomarkers in Young Onset Parkinson disease (YOPD) model

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**Abstract:** Parkinson disease (PD) is characterized by the progressive loss of dopaminergic neurons with the consequent reduction of dopamine levels in the striatum leading to a range of motor and non-motors symptoms. The mechanisms underlying the degeneration of the nigral-striatal pathway remain unknown. By the time the motor symptoms manifest, at least 30% of dopamine neurons have been lost. Our previous publication using iPSC-derived dopaminergic neurons from sporadic young onset PD (YOPD) patients showed an accumulation of α-synuclein and reduced lysosomal proteins and function. However, the phenotype of dopamine neuron loss has not been established in cell culture models probably due to the amount of time in culture it would take to observe neuron loss. Our aim is to develop a long-term culture model of midbrain organoids which recapitulate the brain physiology and neuron loss in YOPD patient-derived iPSCs.iPSCs were differentiated towards a midbrain fate and kept in culture for 4 months. Cell profile was analyzed by immunostainings during the differentiation period. Staining for floorplate progenitors, neuromelanin, and dopamine neurons showed expression of key markers of the mesencephalon. Moreover, single nuclei RNA-sequencing showing multiple cell types within these midbrain organoids. To assess functionality, dopamine release was analyzed by high-performance liquid chromatography (HPLC), and a multi-electrode array system (MEA) was used to record action potentials generated by the intact organoids.Our results showed that iPSC-derived organoids contain high numbers of mature and functional dopaminergic neurons that can be used as a long-term model for PD. Currently, we are evaluating the potential of recapitulating phenotypes previously observed in a 2D system in this patient-derived organoid model, with the goal of discovering novel characteristics and biomarkers from YOPD neurons.

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**Topic:** C.03. Parkinson's Disease

**Support:** MJFF-020015

**Title:** SCD5-targeting RNAi therapeutics as a potential treatment for Parkinson's Disease

**Authors:** *K. A. HUCKLEBERRY[1], N. KODALI[1], **M. STRICOS**[1], Q. BETHUNE[1], M. KEIBLER[1], L. BRONTESI[2], A. CASTORENO[1], T. NGUYEN[1], L. QIN[1], K. BROWN[1], S. FANNING[2], U. DETTMER[2], L. DANG[1];
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**Abstract:** Parkinson's disease (PD) is a devastating neurodegenerative condition impacting 90,000 new patients in the US alone every year. The pathology of PD is characterized by cytoplasmic inclusions called Lewy bodies, which consist of crowded medley of α-synuclein (αSyn), lipids, and organelles. Dysregulation of αSyn leads to accumulation of di/triglycerides, particularly of oleic acid, and αSyn toxicity is exacerbated by supplementing oleic acid. Meanwhile, inhibition of stearoyl-CoA desaturase (SCD) protects against these effects, reduces oleic acid content, and rescues toxicity in αSyn over-expressing rat neurons and PD mouse models. However, SCD inhibition is toxic to early neuron cultures, suggesting a potential role in development. SCD also plays an important role in the periphery, and SCD1-/- mice develop a number of abnormalities in the skin and eyes. An alternate strategy is targeting the brain-enriched isoform SCD5, thereby bypassing the peripheral on-target toxicities. Alnylam's RNAi therapeutics (RNAiTh) platform targets mRNAs through the RNA-induced Silencing Complex (RISC), which facilitates a catalytic reaction that allows a single siRNA to cleave a large number of target mRNAs. This RNAiTh platform allows us to specifically target SCD5 while sparing SCD1 unlike small molecule inhibitors. In $SNCA^{A53T}$ iPSC-derived neurons these duplexes induced up to 50% reduction in lipid droplet formation. SCD5 is not expressed in mice or rats and only a few other species, such as non-human primates and guinea pigs, express it. Therefore, we utilized guinea pigs as an in vivo model to assess knockdown as well as lipid saturation. We believe that our RNAiTh may provide a unique opportunity to specifically target SCD5 for PD while simultaneously avoiding peripheral on-target toxicity.

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**Presentation Number:** NANO86.13

**Topic:** C.03. Parkinson's Disease

**Support:** NS117968

**Title:** The impact of alpha synuclein overexpression on autophagy activation via a phenotypic TFEB biosensor

**Authors: *E. E. LIAO**, N. NATHAN KOCHEN, A. R. BRAUN, J. N. SACHS;
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**Abstract:** Increasing evidence demonstrates that protein homeostasis and degradation plays a key role in the pathogenesis of neurodegenerative diseases. Dysregulation of the autophagy lysosomal pathway (ALP) has been implicated in synucleinopathies, like Parkinson's disease or dementia with Lewy bodies. Thus, understanding the accumulation of misfolded proteins, like alpha synuclein (aSyn), in these diseases and the ability of the ALP to promote the clearance of these toxic protein aggregates may be a key to increasing neuronal survival. In this study, we investigated whether aSyn overexpression leads to dysfunctional translocation of transcription factor EB (TFEB) into the nucleus. TFEB is the master transcription factor for the CLEAR (Coordinated Lysosomal Expression and Regulation) network. CLEAR-dependent transcription promotes lysosomal biogenesis, autophagy and lysosomal exocytosis. Alpha synuclein has been shown to interact with TFEB and prevent interaction with its canonical partners. In addition to known tool compounds like torin 1, we assessed the ability of previously identified hit compounds to normalize native translocation of TFEB and overcome deficits caused by aSyn. Our phenotypic biosensors provide real time evaluation of autophagy and protein homeostasis and can provide great insight into mechanistic deficiencies of synucleinopathies. Furthermore, these advanced biosensors have potential as a high throughput screening platform to elucidate novel therapeutic interventions.

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**Topic:** C.03. Parkinson's Disease

**Support:** KAIST

**Title:** In-silico paradigm to pinpoint mechanistic subtypes for therapeutics in Parkinson's disease

**Authors:** S. YOO, E. YANG, S. KIM, H. PARK, K.-J. YOON, ***M. L. CHOI**;
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**Abstract:** Parkinson's disease (PD) is the fastest-growing neurological disease and remains progressive and incurable. Treatments are symptomatic, and there has been no disease-modifying drug identified. While most treatments continue to adopt a "generalized method" approach, adjusting drugs according to the unique characteristics of each patient's disease has remained an elusive goal. This lack of personalized treatment hampers the ability to readily identify potential therapeutic efficacy, likely due to the inherent heterogeneity of both patients and the underlying disease mechanisms. Cellular morphology delivers uniquely powerful temporal and spatial

information to profile pathological clues including molecular alteration. However, acquiring accurate features of specific structures by visualizing them remains challenging. It often requires functional characteristics of the application of distinct techniques such as Fluorescence labelling, which has limitations including fluorophore bleaching, toxicity, non-specific signals, and variability. We here introduce a highly easy-access therapeutic screening platform that enables the classification of mechanistic subtypes of PD in human cells using machine learning (ML), which is trained with “intact images”; free of technical contamination (e.g. fluorescent label). The sequential visualization of the developmental trajectory of human stem cells to neurons in the appearance of cellular pathology over time in culture allows a time-resolved sequence of events to be defined, allowing early developmental events to be separated from late events throughout the neuronal differentiation. Our approach establishes a powerful drug screening paradigm that can be used to identify target disease subtypes of a certain drug, thus highly efficient therapeutics based on the molecular underpinning of disease in each patient to maximize the therapeutic benefits.

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**Nanosymposium**

**NANO87: Molecular Mechanisms of Neurodegeneration and Neurotoxicity**

**Location:** WCC 143

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:15 PM

**Presentation Number:** NANO87.01

**Topic:** C.04. Movement Disorders other than Parkinson's Disease

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French National Center for Scientific Research (CNRS
AMMI ‘Association contre les Maladies MItochondriales
Tunisian Ministry of High Education and Scientific Research

**Title:** Mpc2 variants disrupt mitochondrial pyruvate metabolism and cause an early-onset mitochondriopathy

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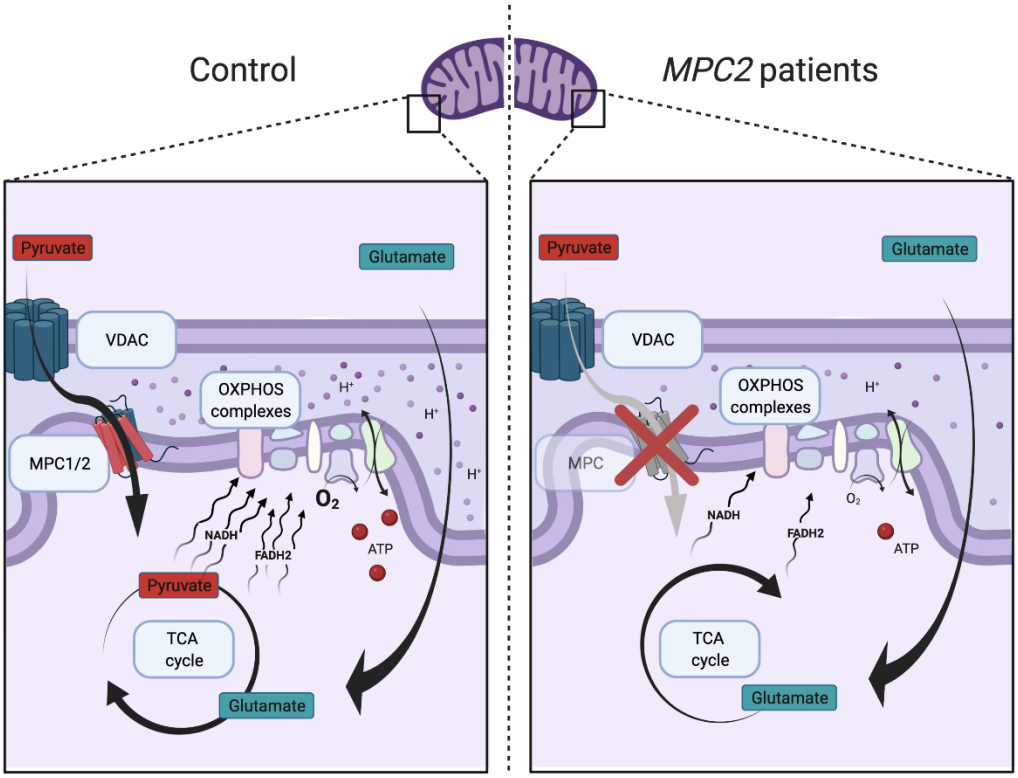
**Abstract:** Pyruvate is an essential metabolite produced by glycolysis in the cytosol and must be transported across the inner mitochondrial membrane into the mitochondrial matrix, where it is oxidized to fuel mitochondrial respiration. Pyruvate import is performed by the mitochondrial pyruvate carrier (MPC), a hetero-oligomeric complex composed by interdependent subunits MPC1 and MPC2. Pathogenic variants in the MPC1 gene disrupt mitochondrial pyruvate uptake and oxidation and cause autosomal-recessive early-onset neurological dysfunction in humans. The present work describes the first pathogenic variants in MPC2 associated with human disease

in four patients from two unrelated families. In the first family, patients presented with antenatal developmental abnormalities and harboured a homozygous c.148T>C (p.Trp50Arg) variant. In the second family, patients that presented with infantile encephalopathy carried a missense c.2T>G (p.Met1?) variant disrupting the initiation codon. Patient-derived skin fibroblasts exhibit decreased pyruvate-driven oxygen consumption rates with normal activities of the pyruvate dehydrogenase complex and mitochondrial respiratory chain and no defects in mitochondrial content or morphology. Re-expression of wild-type MPC2 restored pyruvate-dependent respiration rates in patient-derived fibroblasts. The discovery of pathogenic variants in MPC2 therefore broadens the clinical and genetic landscape associated with inborn errors in pyruvate metabolism.

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**Presentation Number:** NANO87.02

**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Focused Research Award, Center of Excellence in Environmental Toxicology, The University of Pennsylvania, Philadelphia, PA, USA

**Title:** Age-dependent effects of sodium fluoroacetate on mitochondrial respiration and behavior in zebrafish

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**Abstract:** Sodium fluoroacetate (SF, Compound 1080) is a fluorinated pesticide with a toxic mechanism that directly impacts mitochondrial respiration by inhibiting the tricarboxylic acid cycle. Existing supportive care treatments that aim to maintain cardiopulmonary function do not address the mitochondrial impacts of SF. Here, our aim was to investigate how factors of age and sex alter susceptibility to the mitochondrial toxic mechanisms of SF in zebrafish. This study assessed the biomolecular, morphological, physiologic, and behavioral responses to SF (0, 0.1, 0.25, 0.50, 0.75, or 1.0 mM SF) with an acute 24-hour exposure of embryonic (1-2 days post fertilization [dpf]), larval (4-5 dpf), and adult zebrafish (90-220 dpf). Heart rate and oxygen consumption rates of live embryos were assessed at the end of exposure, and exposed embryos raised to the larval stage were assessed for morphology and swimming behaviors. Heart rates, morphology, and behavioral responses to SF were measured in larvae at the end of exposure. Adults were assessed for the behavioral impacts of SF by measuring comparable locomotor effects to those assessed in larvae along with behavior in the novel tank test, an indicator of an anxiety-like response in zebrafish. Oxygen consumption was assessed in embryos, larvae, and adult brain homogenates using O2k respirometry. Exposed embryos showed greater susceptibility to SF-induced reduction in oxygen consumption rate, morphological abnormalities, decreased heart rate, and altered locomotor behaviors, including turn angle, compared to larvae. SF showed dose-dependent effects across most measures, and exposed embryos showed a capacity for greater recovery over time with exposure to lower SF doses. Mitochondrial respiration deficits co-occur with an array of impacts of toxicity, including morphological, heart rate, and behavioral abnormalities in exposed embryos. Adult zebrafish showed sex-differences in the locomotor effects of SF on relative turn angle when assessed in the novel tank test, with females showing greater responsiveness to SF. These results confirm that mitochondrial impairment is an important mechanism underlying the toxic effect of SF and that this can be modeled in zebrafish. The data suggest that vulnerable populations, such as embryos and females, may be more impaired by the toxic mechanism of SF, with increased susceptibility to mitochondrial respiration deficits. Zebrafish are an effective model system for assessing mitochondrial, physiological, and behavioral impacts of toxic substances. This testing system in zebrafish is capable of detecting age- and sex-differences of toxic effects of pesticides such as SF.

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**Presentation Number:** NANO87.03

**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

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**Title:** Primary cilia loss renders pyramidal neurons susceptible to perinatal ketamine-induced dendritic degeneration and learning deficits

**Authors: *N. SARIC**[1], F. SOMAA[1], C. FOSTER SADE[1], N. REDDY[1], L. WANG[1], K. HASHIMOTO-TORII[1], N. ISHIBASHI[1,2];
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**Abstract:** Recent clinical studies have demonstrated a strong link between anesthetic exposure and neurodevelopmental outcomes in children with CHD. Protein-damaging *de novo* gene mutations have been demonstrated to be strong predictors of neurodevelopmental anomalies in congenital heart disease (CHD), and at least 50% of these genes have been found to be associated with primary cilia structure and/or function. Therefore, studying the combined effect of anesthesia and primary cilia gene mutations may shed light on the neurodevelopmental abnormalities observed in CHD children. Using *Emx1cre; Ift88 f/f*, in which primary cilia are lost specifically in cortical excitatory neurons, we administered either vehicle/saline or ketamine at postnatal day 7 (P7). We examined markers of cytoskeletal degeneration at P8 in the medial prefrontal cortex as well as behavioral testing at P30 using the water T-maze to assess their spatial memory performance and cognitive flexibility. Separate cohorts were also tested for gross and fine motor deficits using the accelerated rotarod and pellet reach task, respectively. To assess pyramidal neuron morphology, we used *Thy1-GFPM* transgenic reporter-labeled *Ift88* mutant mice, allowing for tracing and reconstruction of layer V pyramidal neurons. To examine if the observed learning-related deficits were related to altered dendritic spine dynamics we then performed two-photon imaging on *Ift88* cHET+Ket and cKO+Ket *Thy1-GFPM* animals and compared apical spine density and turnover in the motor cortex. We found significant enhancement in immunoreactivity of cytoskeletal degeneration markers in the cKO+Ket mice compared to the other three groups (cHET+PBS, cHET+Ket, and cKO+PBS). The effects of ketamine on dendritic morphology were also specific to this group. During the reversal learning paradigm, *Ift88* knockout mice exposed to ketamine showed a strongly reduced ability to learn compared to other groups, indicative of a cognitive flexibility deficit. This same group demonstrated significant motor deficits, with a reduced rate of motor learning in both gross and fine motor tasks. Ketamine treated cKO mice showed reduced baseline apical spine density as well as diminished spine maturation during motor learning. Our findings indicate that primary cilia deficiency, due to a common genetic predisposition with CHD, might exacerbate the

toxicity of neonatal anesthesia, suggesting a novel therapeutic treatment strategy for the prevention of neurobehavioral abnormalities in this population.

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**Title:** A strategy for normalizing TDP-43 proteinopathy by lowering STAU1 abundance with antisense oligonucleotides (ASOs)

**Authors: *D. SCOLES**, S. PAUL, W. DANSITHONG, K. P. FIGUEROA, M. GANDELMAN, S. PULST;
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**Abstract:** The RNA binding protein STAU1 regulates mRNA neuronal localization and degradation. We identified STAU1 as a protein interacting with ATXN2, that is polyglutamine expanded in spinocerebellar ataxia type 2 (SCA2). We found that STAU1 is overabundant in SCA2 (ATXN2), TDP-43, and C9orf72 mutant patient fibroblasts and mouse models, associated with abnormal autophagic flux. Interestingly, we found that STAU1 directly interacts with the 5'-UTR of *MTOR* enhancing its translation, accounting for autophagy inhibition. The present study is supported by an R61/R33 IGNITE grant to develop ASOs targeting STAU1 as a means for indirectly treating TDP-43 proteinopathy. We screened 118 MOE gapmer ASOs targeting in the *STAU1* coding region in HEK-293 cells. ASOs lowering *STAU1* expression by greater than 45% by quantitative PCR were rescreened in SCA2 patient fibroblasts, and 10 of these were tested for lowering STAU1 abundance *in vivo* in a new BAC-STAU1 mouse model. Among the lead ASOs was ASO45 that targets mouse and human *STAU1* and is valuable for *in vivo* proof-of-concept testing. Here we present new data showing that intracerebroventricular (ICV) injection of ASO45 improves autophagy and neurodegenerative disease molecular phenotypes in multiple mouse models, including mTOR, p62, cleaved caspase 3, NeuN, and ChAT, evaluated by western blotting. The tissues evaluated included cerebella of *Pcp2*-ATXN2-Q127 SCA2 mice, spinal cord of *Thy1*-TDP-43 transgenic mice, and spinal cord of *Prp*-TDP43-Q331K transgenic mice. We have also shown that the autophagy and neurodegenerative disease molecular phenotypes are normalized in C9orf72-BAC(500) transgenic mice, including poly-GA abundance. Targeting *STAU1* may be an effective strategy for treating disorders with STAU1 overabundance and TDP-43 proteinopathy characterized with impaired autophagic flux, including amyotrophic lateral sclerosis (ALS) and limbic-predominant age-related TDP-43 encephalopathy (LATE).

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**Topic:** C.04. Movement Disorders other than Parkinson's Disease

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**Title:** Amyloid like aggregating proteins impair nucleocytoplasmic transport and cause neurodegeneration in vivo

**Authors: *M. D. PADILHA**[1,2], I. RIERA-TUR[2], R. KLEIN[2], I. DUDANOVA[1,2,3];
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**Abstract:** Neurodegenerative diseases are characterized by the gradual deposition of aggregates, consisting of misfolded proteins rich in cross-β-sheet structures, whose presence has been linked to the malfunction of several essential cellular pathways. However, the nature of the toxic agents and the precise mechanisms of action leading to neurodegeneration are still poorly understood. Here, we have generated a doxycycline-regulatable transgenic mouse model expressing an artificial amyloid-like β-sheet protein (β23 protein) in the central nervous system (CNS), to study the effects of aggregation-induced toxic gain-of-function in the absence of loss-of-function phenomena. We found that β23 expression during development led to perinatal lethality. When expression of the β23 protein was suppressed during development and induced at a juvenile age it resulted in the formation of perinuclear aggregates in neurons and in the loss of the specific motor neuron marker, choline acetyltransferase (ChAT), in a subset of spinal cord motor neurons (α-motor neurons). The loss of the ChAT marker was accompanied by a decrease in phasic muscle innervation and neuromuscular junction (NMJ) area, increase in NMJ fragmentation and progressive defects in motor tests. Mechanistically, we detected that expression and perinuclear aggregation of the β23 protein led to the progressive impairment of nucleocytoplasmic transport and caused nuclear accumulation of mRNA, both in primary cultured neurons and *in vivo*. Additionally, we show that β23 expression was associated with a reduction in spinal cord α-motor neurons size and drove a time-dependent decrease of neuronal viability in cultured neurons. Altogether, these results suggest that impairment of the nucleocytoplasmic transport may be one of the mechanisms through which protein aggregates exert their toxic function *in vivo*.

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**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

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**Title:** Omics insight into brain cortex in the mouse model of Cancer-therapy induced cognitive impairment encompassing APOE genotype

**Authors: *H. PANDIT**, L. P. BIRAN, G. REBECK;
Dept. of Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

**Abstract:** **Introduction:** Cancer-therapy Related Cognitive Impairment (CRCI) is observed in cancer survivors after anti-cancer treatments. Although CRCI is well-acknowledged brain toxicity in oncology settings, the mechanism of CRCI is not understood. APOE4 allele is the strongest genetic risk factor for Alzheimer's Disease (AD) and also associated with CRCI. In this study, using a common chemotherapy agent, Doxorubicin, we systematically examined the omics profile of the cerebral cortex in the mouse model of CRCI in human APOE3 and APOE4 backgrounds. **Methods:** Six-month-old female APOE3 and APOE4 mice (Jackson Laboratories) were treated with either 10 mg/kg of Doxorubicin (DOX) or vehicle (control). After 21 days, the brains of these mice were perfused and hemisected, and cortices were isolated from one hemibrain (n=4/genotype/treatment, total n=16 mice). The whole cortex was pulverized to facilitate total protein and total RNA preparations. A Data Independent Acquisition approach was used for proteomics analysis and a poly-A enrichment library approach was used for mRNA sequencing and analysis. **Results:** Principal component analysis (PCA) and correlation hierarchical clustering analysis of RNA expression demonstrated clear separation of APOE3 and APOE4 genotypes. Profound distinction was observed in APOE3 mice between control and DOX-treated groups. In contrast, APOE4 mice did not show separation between control and DOX-treatment. Control APOE4 brains and DOX treated APOE3 brains demonstrated considerable similarity, and shared 210 significant differential expressed genes (DEG) compared to APOE3 controls (Adj P value < 0.05, FDR cutoff= 0.1). Gene-set enrichment analysis (GSEA) of biological pathways (GO:BP) revealed interferon beta, GABAergic, and NLRP3 inflammasome related pathways were upregulated, and ATP regulation, mitochondrial biology and immune response related pathways were downregulated in DOX-E3 and both APOE4 groups, compared to APOE3 control (Adj P value < 0.05, FDR cutoff = 0.1). **Conclusions:** As expected, APOE genotype exhibited a clear effect on cerebral cortex gene expression in control brains. Doxorubicin-induced changes in the APOE3 brain created similarities with the control APOE4 brain, sharing 40% of DEGs. Our findings suggest that doxorubicin induced damages could be partially rescued by activation of protective mechanistic respond in APOE3 cortex environment, whereas in APOE4 brains, these protective responses could be partially abolished due to an intrinsically compromised microenvironment.

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**Presentation Number:** NANO87.07

**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** The pharmacological inhibition of casein kinase 2 induces apoptosis in in vitro and in vivo models of glioblastoma

**Authors: *A. FILIPPONE**[1], M. LANZA[2], G. CASILI[3], M. CAMPOLO[2], I. PATERNITI[1], S. CUZZOCREA[2], E. ESPOSITO[2];
[2]Univ. of Messina, [3]Giovanna Casili, [1]Univ. of Messina, Messina, Italy

**Abstract:** Glioblastoma multiforme (GBM) is the most common and lethal primary malignant cancer of the central nervous system (CNS) of doubtful outcome. It has been reported that casein kinase 2 (CK2), a serine/threonine kinase, is involved in cell growth and survival processes. CK2 acts as an oncogene because of its overexpression in GBM by promoting tumor development and progression via the inhibition of apoptosis. Based on the latest findings, that protein kinase CK2

is a crucial driver of GBM progression, we investigated the effect of CK2 inhibition by CX-4945 in *in vitro* and *in vivo* GBM models. *In vitro*, U87 cells were treated with CX-4945 (5, 10 and 15 μm) for 24 hours. A cytotoxicity assay and western blot analysis were performed after 24 hours. *In vivo*, BALB/c nude mice were inoculated in the right flank with $3 \times 10^6$ U-87 cells. Treatments with CX-4945 (doses of 50 and 75 mg/kg) were administered intraperitoneally every day from week 1 to week 4. We found in *in vitro* study that CK2 inhibition suppressed the proliferation and migration of GBM cells and potentiated the apoptosis pathway. *In vivo*, CK2 inhibition decreased tumor growth and modulated vascular and neuronal growth factors. Also, CK2 inhibition revealed a positive correlation between the neuronal growth factors expression and activation of glial and astrocyte cells in GBM by resolving its pathological features. These novel insights into the molecular signaling of CK2 in GBM demonstrate that CX-4945 may represent a promising approach for future GBM therapy.

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**Topic:** C.07. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NIH RF1NS121992

**Title:** Mechanisms of hippocampal neurotoxicity in a mouse model of prion disease

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**Abstract:** Prion diseases are fatal neurodegenerative disorders caused by prion protein aggregates. The cellular prion protein, $PrP^C$, binds prion aggregates, $PrP^{Sc}$, and also reportedly binds to amyloid beta and alpha-synuclein aggregates. The N-terminus of $PrP^C$ has been implicated in neurotoxicity. To investigate whether and how $PrP^C$ drives neurotoxicity, we generated a knock-in mouse with a G92N substitution in $PrP^C$ (*Prnp*$^{92N}$) that is 100% fatal. This mutation creates an additional glycan at the N-terminus and may disrupt normal protein signaling. By P25-30, *Prnp*$^{92N/92N}$ mice develop neurological signs, including hindlimb clasp and spontaneous seizures. Histologically, late-stage brains show neurodegenerative changes including severe hippocampal pyramidal neuronal necrosis (CA1), neuritic dystrophy, gliosis, and spongiform change. *Prnp*$^{92N/92N}$ brains lack prion protein aggregates or infectivity, thus uncoupling aggregation from PrP-mediated neurotoxicity. To define neurotoxic signaling pathways, we performed phosphoproteomics on whole brain samples at mid-stage disease (P20). *Prnp*$^{92N/92N}$ brains showed higher phosphorylation of GluN2B at S1303, which has been linked with increased excitotoxicity. Furthermore, *Prnp*$^{92N/92N}$ hippocampal neurons have displayed

signs of excitotoxic stress (dendritic beading), which can be rescued by MK801, an NMDA antagonist. *Prnp*$^{92N/92N}$ primary cortical neurons have also demonstrated aberrations in glutamate-mediated calcium signaling, underscoring the role of runaway excitotoxicity in Prnp92N disease pathogenesis. Bulk RNA sequencing of the hippocampus showed the downregulated expression of myelin-related genes, which were also reduced at the protein level by terminal disease. Taken together, these data indicate dysregulated GluN2B-linked glutamatergic signaling and demyelination in the *Prnp*$^{92N/92N}$ mice and suggest that PrP$^{C}$ normally functions to dampen neuronal activity.

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**Presentation Number:** NANO87.09

**Topic:** C.04. Movement Disorders other than Parkinson's Disease

**Support:** Collaborative Center for X-Linked Dystonia-Parkinsonism
Leon Levy Fellowship in Neuroscience
Parekh Center Core Project
MD Anderson Neurodegeneration Consortium

**Title:** A novel mouse model to define the cellular drivers of X-linked Dystonia-Parkinsonism

**Authors: *P. PRAKASH**, W. ZHANG, Y. ZHAO, C. LABORC, Y. ZHU, U. RUFEN-BLANCHETTE, A. MAR, R. BROSH, J. BOEKE, S. LIDDELOW;
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**Abstract:** X-linked Dystonia-Parkinsonism (XDP) is a severe progressive movement disorder that has only been reported in individuals of Filipino origin. It is characterized by dystonic movements and is caused by a mutation in the *TAF1* gene which is located on the X chromosome. *TAF1* encodes TATA-binding protein-associated factor 1—a key protein required for transcription and cell viability. Due to the widespread expression of *TAF1* in various brain cells, identifying the specific cellular drivers of the disease is difficult. To address these challenges, we engineered a humanized model of XDP (*TAF1*$^{PC\text{-}XDP}$), which has a conditional hybrid mouse/human version of the *TAF1* locus. This engineered *Taf1* locus contains exons 1-24 of mouse *Taf1* and exons 25-38 of the human *TAF* gene. A single XDP-causing SVA (SINE-VNTR-Alu) retrotransposon insertion in the intron 32 of the human *TAF1* region. When crossed with cell-type specific Cre-expressing mice, these unique XDP model mice replace exons 25-38 of mouse *Taf1* and replace this with the corresponding SVA-containing human *TAF1* region. This unique mouse can be used to study the *in vivo*development of XDP phenotypes. To evaluate the neuronal contribution to XDP, we first crossed female *Taf1*$^{PC\text{-}XDP}$ (het) with male Nestin$^{Cre}$ mice. Compared to the Cre control mice, the resulting Nestin$^{Cre}$*Taf1*$^{XDP}$ males had a significant reduction in their body weight, and none survived beyond ~60 days. Strikingly, the Nestin$^{Cre}$*Taf1*$^{XDP}$ male mice have drastically reduced brain size, enlarged lateral ventricles, profound striatal and hippocampal pathology. Female Nestin$^{Cre}$*Taf1*$^{XDP}$ (het) mice appeared

grossly normal.We next performed a battery of motor tests to assess the behavioral symptoms exhibited by these XDP animals. Nestin$^{Cre}$*Taf1*$^{XDP}$ male mice exhibit an irregular gait and reduced motor performance in the rotarod test. They also have hind limb reduced grip strength, similar to the human XDP patients. Finally, we asked what are the molecular underpinnings that may describe these severe symptoms exhibited by the XDP mice. Immunohistochemistry revealed dystrophic hippocampal tissue in the Nestin$^{Cre}$*Taf1*$^{XDP}$ male mice while the female mice appeared relatively normal. We also observed a decrease in NeuN$^{+}$ and increase in GFAP$^{+}$ cells in the striatum, indicating the loss of neurons and presence of reactive astrocytes in these brain regions, respectively. Ongoing molecular analysis of these brain regions aims to identify the cell-specific molecular drivers of XPD and explore potential treatment pathways for this devastating disease.

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**Nanosymposium**

**NANO88: Somatosensory Mechanisms**

**Location:** WCC 152A

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:45 PM

**Presentation Number:** NANO88.01

**Topic:** A.08. Development of Neural Systems

**Support:** NIH NINDS Grant NS116168
OHSU OFDIR diversity fellowship

**Title:** Pten signaling drives primary sensory neuron population diversification

**Authors: *A. FERNANDEZ**, K. WRIGHT;
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**Abstract:** Neurotrophin signaling regulates cell survival, differentiation, and population specification in the developing peripheral nervous system (PNS). Downstream of neurotrophin receptors, phosphatase and tensin homolog (*Pten*) modulates intracellular survival and differentiation signaling pathways, and acts as a negative regulator of the *Trk/PI3K/Akt/mTOR* during DRG neurogenesis and neuronal specification. *P*ten has been well studied in the context of neuronal morphogenesis, axon guidance and neurotransmitter receptor function in the central nervous system (CNS). In contrast, our understanding of the role of *Pten* in the PNS is limited to

models of neuropathic pain and nerve injury in adult animals, and it remains unclear how loss of *Pten* affects the development and function of peripheral somatosensory circuits. To address this, we examined sensory neurons within dorsal root ganglia (DRG) in *Pten* hemizygous ($Pten^{het}$) mice. We found altered sensory neuron population diversity in adult $Pten^{het}$ mutants. The number of proprioceptors and TH+ c-low threshold mechanoreceptors (C-LTRMs) increased significantly, while the number of TrkA+ peptidergic nociceptors decreased by 50%. In contrast, the number of IB4+ non-peptidergic nociceptors and Aδ-LTMRs remains constant. Defects in population diversification in $Pten^{het}$ mice are population-specific, as the total number of somatosensory neurons within DRGs remains constant and subsets of mechanosensory and nociceptive populations are unaltered. Moreover, these defects are dependent on diminished phosphatase activity by Pten, and both the Pi3k/mTOR and GSK-3β/β-catenin pathways, downstream of Pten, appear to be dysregulated. Using *Cre* lines to specifically target peripheral neural progenitors ($Sox10^{CreERT2}$) and newly-born primary sensory neurons ($Isl1^{Cre}$) in combination with a conditional allele of *Pten* ($Pten^{Flox}$), we determined that defective population diversity results from altered cell-intrinsic *Pten* signaling during the period of DRG neurogenesis, and these defects persist throughout embryonic development. Altogether, our data unravels a critical role for *Pten* signaling in the cell-intrinsic control of neuronal specification during development of the PNS.

**Disclosures: A. Fernandez:** None. **K. Wright:** None.

**Presentation Number:** NANO88.02

**Topic:** D.01. Somatosensation

**Support:** NIH
CIHR
MCDB, University of Michigan

**Title:** Identification of Specialized Tactual Sensory Apparatus for Mechanical Itch

**Authors: *M. FATIMA**[1], H. LEE[4], H. CHA[2], J. LIU[5], J. DAMBLON[8], F. WANG[9], W. ZHANG[3], R. AJIMAL[6], A. XIONG[6], C. HOR[5], M. CHAI[6], A. ASMAR[6], W. CAI[5], X. ZHOU[6], H. PAN[7], L. HORWITZ[5], H. HU[10], Y. DE KONINCK[11], X. XU[5], B. DUAN[5];
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**Abstract: Identification of Specialized Tactual Sensory Apparatus for Mechanical Itch**Mahar Fatima[1†], Hankyu Lee[1†], Hwayeon Cha[1], Jingyi Liu[1], Jonathan Damblon[2], Feng Wang[2], Wenwen Zhang[3], Ranveer Ajimal[1], Chia Chun Hor[1], Ailin Xiong[1], Xiaowei Zhou[1], Wei Cai[3], Haili Pan[4], Lorraine Horowitz[1], X.Z. Shawn Xu[3], Yves De Koninck[2], Bo Duan[1*1]
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Hairs act as specialized sensory appendage that actively interact, physically and/or molecularly, with the hair associated-myriad of sensory endings to contextualize the environmental cues. Human vellus hairs are the fine hairs present on the skin that are supposed to serve mechanosensory functions, however, the physiological basis of their mechanosensitivity remains unclear. We found a hair type in mice that were homologous to human vellus hairs and termed it as vellus-like hairs (VLHs). Similar to human vellus hairs, VLHs in mice were hypo-pigmented, hypo-medullated, and mechanosensitive. Anatomical mapping of sensory neurons revealed the organization of sensory endings around VLHs consistently conformed to a structured pattern of highly organized longitudinal lanceolate endings. Interestingly, gentle force to mechanically perturb these VLHs evoked itch sensation in mice (VLH-itch). We investigated the VLH-associated sensory neurons and found a distinctive subset of Aβ rapidly adapting-low-threshold mechanoreceptors (RA-LTMRs) co-expressing Toll-like receptor 5 (TLR5) and Calbidin1 (Calb1) mediated the VLH-itch. Our study sheds light on the previously poorly understood somatosensory physiology of these atypical hair types, highlighting the significant role of $TLR5^{+}$/$Calb1^{+}$ Aβ RA-LTMRs in the mediation of VLH-itch**.**

**Disclosures: M. Fatima:** None. **H. Lee:** None. **H. Cha:** None. **J. Liu:** None. **J. Damblon:** None. **F. Wang:** None. **W. Zhang:** None. **R. Ajimal:** None. **A. Xiong:** None. **C. Hor:** None. **M. Chai:** None. **A. Asmar:** None. **W. Cai:** None. **X. Zhou:** None. **H. Pan:** None. **L. Horwitz:** None. **H. Hu:** None. **Y. De Koninck:** None. **X. Xu:** None. **B. Duan:** None.

**Presentation Number:** NANO88.03

**Topic:** D.01. Somatosensation

**Support:** R35NS111643

**Title:** Activity-dependent recomposition of the dorsal horn underlies pruritis during aging

**Authors: *D. ACTON**, S. PIMPINELLA, M. D. GOULDING;
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**Abstract:** Chronic itch, which is characterized by spontaneous scratching and elevated sensitivity to mechanical stimuli (alloknesis), is a debilitating condition that becomes more prevalent during aging. Recently it was shown that mechanosensitive Merkel cells in the skin suppress itch in young healthy mice, presumably by providing excitatory drive to an unidentified population of inhibitory neurons in the spinal cord (Feng et al., 2018). Furthermore, the degradation of Merkel cells during aging was proposed to account for the susceptibility of aged mice to pruritis. A common feature of diverse forms of chronic itch is the simultaneous recruitment of neural pathways in the spinal cord that in healthy animals are dedicated to the transmission of either acute mechanically or chemically evoked itch (Ren et al., 2023). Of particular interest is the mechanical itch pathway, which is gated by NPY acting at Y1 receptors expressed by excitatory neurons (Acton et al., 2019). Here, we show that NPY-Y1 signaling strongly suppresses itch responses in models of both acute and chronic pruritis in young adult (8-

week-old) mice but not in aged (>18-month-old) mice, even though both forms of itch continue to be suppressed by pharmacological activation of Y1 receptors in aged mice. Consistent with this, expression of NPY but not Y1 is reduced in aged mice. Silencing Merkel cells in young adult *Math1$^{CreER}$; Piezo2$^{f/f}$* mice results in the loss of NPY-Y1 gating of itch as well as reduced expression of NPY but not Y1. We report that the reduction of sensory input to the dorsal horn following Merkel cell silencing results in the apoptosis of multiple dorsal horn cell types, including glia and excitatory and inhibitory neurons, with $NPY^+$ neurons displaying far higher levels of apoptosis than $Y1^+$ neurons. These findings indicate that cutaneous mechanosensory input from Merkel cells supports cell survival in the dorsal horn in adult mice, and that the loss of excitatory drive from peripheral mechanoreceptors during aging alters transmission within the mechanical itch pathway in geriatric animals. In addition to itch, the recomposition of the dorsal horn likely has diverse consequences for the processing of touch and pain during aging.

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**Presentation Number:** NANO88.04

**Topic:** D.02. Somatosensation – Pain

**Support:** NIH Grant NS083702
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**Title:** Distinct local and global functions of Aβ low-threshold mechanoreceptors in mechanical pain transmission

**Authors: *M. GAUTAM**[1], A. YAMADA[2], A. YAMADA[2], Q. WU[1], K. KRIDSADA[1], J. LING[2], H. YU[1], P. DONG[1], M. MA[1], J. GU[2], W. LUO[1];
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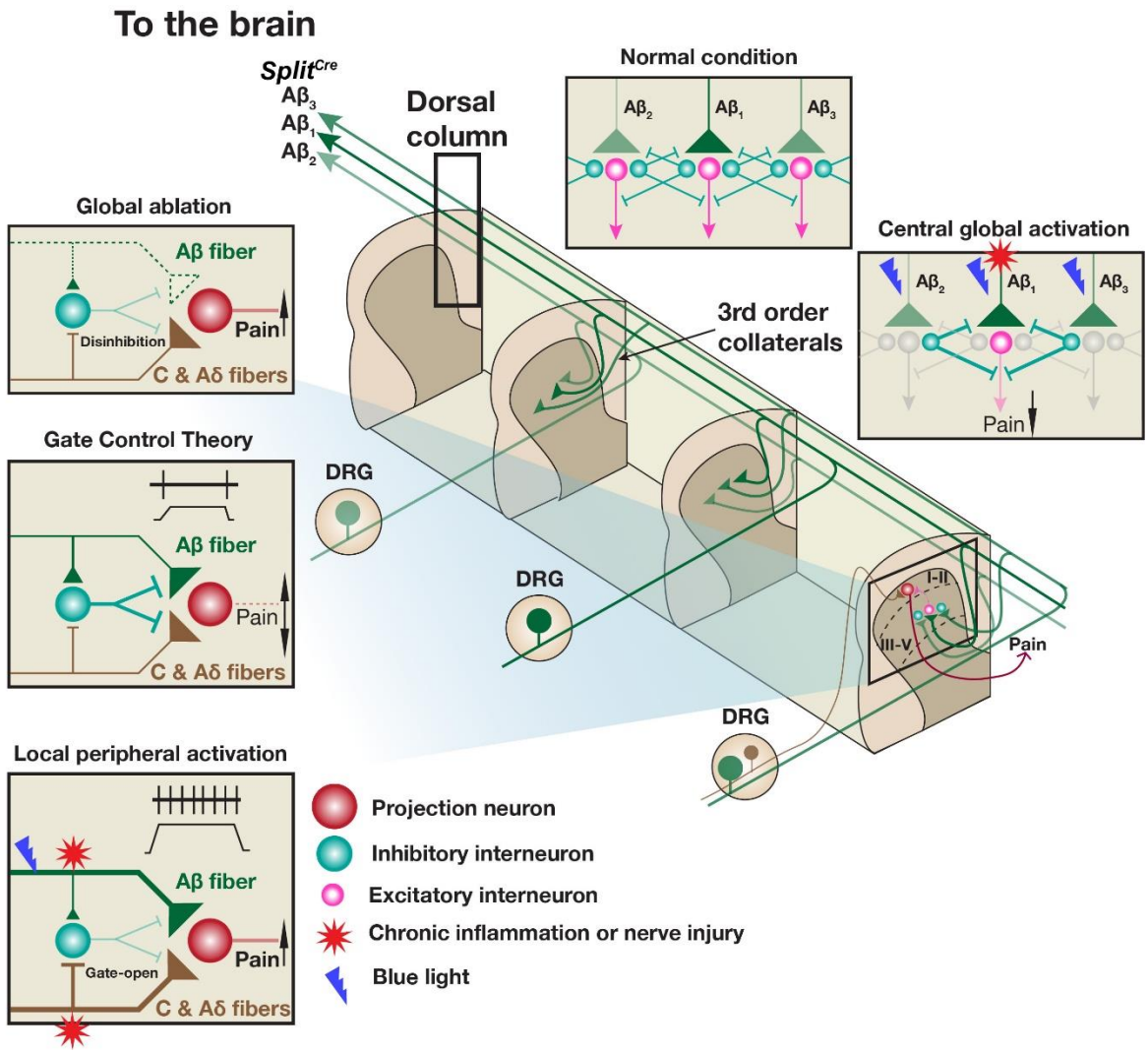
**Abstract:** The roles of A-beta low-threshold mechanoreceptors (LTMRs) in transmitting mechanical hyperalgesia and in alleviating chronic pain have been of great interest but remain contentious. Here we utilized intersectional genetic tools, optogenetics, and high-speed imaging to specifically examine functions of SplitCre-labeled A-beta LTMRs in this regard. Genetic ablation of SplitCre-A-beta LTMRs increased mechanical pain but not thermosensation in both acute and chronic inflammatory pain conditions, indicating their modality-specific role in gating mechanical pain transmission. Local optogenetic activation of SplitCre-A-beta LTMRs triggered nociception after tissue inflammation, whereas their broad activation at the dorsal column still alleviated mechanical hypersensitivity of chronic inflammation. Taking all data into consideration, we propose a new model, in which A-beta LTMRs play distinctive local and global roles in transmitting and alleviating mechanical hyperalgesia of chronic pain, respectively. Our model suggests a new strategy of global activation plus local inhibition of A-beta LTMRs for treating mechanical hyperalgesia.

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**Presentation Number:** NANO88.05

**Topic:** D.03. Somatosensation – Touch

**Support:** NSERC
CIHR

**Title:** Chronic pain mediated changes in the hedonic value of gentle touch in mice

**Authors: *M. ZAIN**, L. BENNETT, H. ZHANG, Q. PAULI, J. CHEUNG, R. BONIN; Pharmaceut. Sci., Univ. of Toronto, Toronto, ON, Canada

**Abstract:** Sensory neurons expressing MrgprB4 detect gentle stroking in mice and their activation is known to be positively reinforcing. This project uses optogenetics and behavioral

techniques to assess whether activation of channelrhodopsin (ChR2) expressing MrgprB4 afferents signal positively valenced tactile information, whether this is altered in chronic pain and whether this is reflected in the downstream circuits recruited. A ceramic ferrule was surgically implanted in the lumbar vertebrae of mice expressing ChR2 in MrgprB4 lineage afferents (MrgprB4-ChR2) to deliver blue light to the central projections of the primary afferents. We used a real-time place preference (RTPP) paradigm with optogenetics to assess the motivational properties of blue light stimulation in implanted male and female MrgprB4-ChR2 mice that had either undergone a spared nerve injury (SNI) or a sham surgery and assessed whether gabapentin administration affected the response. All mice underwent a final stimulation protocol after which the brains, spinal cords and dorsal root ganglions of the mice were dissected out for immunohistochemical analysis. Light stimulation in one arm of the assay increased preference for that arm in the male and female sham surgery mice but not the SNI animals. Preference for blue light could be partially restored in the male SNI mice through treatment with gabapentin. Preliminary results from the immunohistochemistry experiments also show that stimulation successfully induced c-fos expression in the spinal dorsal horn of the stimulated animals and this expression was predominantly found in the superficial dorsal horn of the spinal cord, consistent with the innervation pattern of the MrgprB4+ afferents. Whole brain c-fos results also showed differential recruitment of brain regions following optogenetic activation of MrgprB4 lineage afferents in sham and nerve injured mice.In conclusion, the motivational value associated with gentle touch is plastic and can be abated in models of chronic pain. Future work will continue to elucidate the subtypes of spinal
dorsal horn neurons recruited and the specific brain regions activated in the processing and perception of gentle touch both in chronic pain and in control conditions.

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**Topic:** D.02. Somatosensation – Pain

**Support:** FAPESP grant 2019/26414-2
FAPESP grant 2019/05882-8

**Title:** The DRG serotonin receptor 6 contributes to hypersensitivity

**Authors: *L. KIMURA**[1], W. VIEIRA[3], C. A. PARADA[4], P. DECAEN[5], G. PICOLO[2]; [1]Butantan Inst., São Paulo, Brazil; [2]Butantan Inst., Sao Paulo, Brazil; [3]Dept. of Anat., Univ. de Sao Paulo, São Paulo, Brazil; [4]State Univ. of Campinas - Unicamp, Campinas, Brazil; [5]Pharmacol., Northwestern Univ., Chicago, IL

**Abstract:** Chronic pain affects around 30 % of the world's population— for which there are few nonadditive pharmacological agents available for the patients. We have a limited understanding of the modulation of receptors expression involved in the hypersensitivity acquired over the course of chronic pain development. As it relates to our study, activation of serotonin receptor 6 (5HT6R) in the CNS increases secondary hyperalgesia. While it is postulated that 5HT6R is exclusively expressed in brain cells, such as neurons and astrocytes, nothing is known about the

5HT6R expression in the periphery and its participation in nociception. Thus, we aimed to investigate the role of the 5HT6R of DRG murine cells in terms of excitability, calcium dynamics and hypersensitivity. Because the 5HT6R co-localizes within the primary cilia, we first investigated whether DRG cells would express the primary cilia using confocal and electron microscopy. Even though some DRG cell types produce primary cilia, we find that mature DRG neurons do not. We also observed 5HT6R labeling was not specific to the cilia in fixed DRG cells under endogenous expression. However, 5HT6R was observed in the primary cilia of living cultured DRG cells from transgenic cilia reporter mice (ARL13B-EGFP$^{tg}$) upon overexpression of the receptor. Using current clamp electrophysiology, action potentials frequency of DRG neurons increased after 5HT6R activation (using the 5HT6R agonist EMD 386088), suggesting receptor agonism may have a direct effect on excitability. Looking at calcium response, we incubated WT DRG cells with the agonist and next challenged them with capsaicin. Calcium responses to capsaicin in DRG-TRPV1$^{+}$ cells were potentiated after 5HT6R stimulation. Finally, to assess whether 5HT6R agonism would affect nociceptive behavior, the L5-DRG of rats were injected with EMD 386088 and nociceptive threshold was measured by the electronic von Frey test. Nociceptive threshold decreased after 3 h of injection, suggesting that 5HT6R activation *per se* contributes to hypersensitivity at the DRG level. In conclusion, we showed for the first time the expression of 5HT6R outside the CNS and its participation in pain pathways. The agonism of 5HT6R *in vitro* increases the neuronal firing and calcium response in DRG cultured cells which may explain its hyperalgesic effect *in vivo*.

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**Presentation Number:** NANO88.07

**Topic:** D.02. Somatosensation – Pain

**Support:** HHMI

**Title:** Sars-cov-2 proteases drive airway irritation and inflammation

**Authors: *S. MALI**[1], R. SILVA[2], D. BAUTISTA[3];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]UC Berkeley, Berkeley, CA; [3]UC Berkeley, BERKELEY, CA

**Abstract:** Common symptoms of respiratory viral infections such as COVID-19 include sneezing, coughing, headache, runny nose, and sore throat. Many studies have demonstrated that subsets of somatosensory neurons called nociceptors that innervate the upper airways mediate such symptoms; however, little is known about how these neurons are activated during the course of infection. Our lab has demonstrated that the proteases encoded by the SARS-CoV-2 genome, Papain-like protease (PLpro) and Main protease (Mpro), initially required for viral replication, can be released from infected cells and induce airway irritation and inflammation in mice. Using in vivo and in vitro calcium imaging, we demonstrated that these proteases can activate subsets of airway-innervating TRPV1+ and TRPA1+ nociceptors. Treatment with PLpro or Mpro alone is sufficient to elicit pain and sensitization and the release of inflammatory mediators from nociceptors. Protease activation of nociceptors in concert with other airway-

resident cells drives airway inflammation induced by SARS-CoV-2 infection. Together, these findings have identified a role for viral proteases and nociceptors that drive COVID-19 disease.

**Disclosures: S. Mali:** None. **R. Silva:** None. **D. Bautista:** None.

**Presentation Number:** NANO88.08

**Topic:** F.01. Neuroethology

**Support:** NIH Grant AT011652

**Title:** A neural control circuit for cough-like defensive behaviors in mice

**Authors: *N. GANNOT**, C. PHILLIPS, K. EMERY, P. LI;
Univ. of Michigan, Ann Arbor, Ann Arbor, MI

**Abstract:** Cough-like defensive reflexes, including coughs and expiratory reflexes, are essential defensive respiratory functions triggered by tussive (cough-evoking) stimuli in the airways to protect the body against inhaled substances and invading pathogens. Often, both reflexes occur intermittently during clinically defined episodes of coughing, indicating a likely shared mechanism underlying these two similar cough-like behaviors. Although these behaviors become excessive with severe consequences under pathological conditions, effective anti-tussive medications are lacking due to the limited knowledge of the neural circuit controlling cough. In our lab, we use the mouse model to study the neural pathways for cough-like behaviors, leveraging the genetic and neurogenetic tools available in mice. The nucleus tractus solitarius (NTS) is the first relay nucleus in the brain that receives tussive afferent input from cough receptors in the airways via the vagus nerve. Within the NTS, we identified a sub-population of neurons that express the neuropeptide gene tachykinin 1 (Tac1). These neurons are activated during tussive challenges and play an essential role in cough-like behaviors. Photoactivation of Tac1 neurons is sufficient in inducing cough-like behaviors, while genetic ablation or chemogenetic silencing of these neurons diminishes the coughs and expiratory reflexes induced by tussive agents. Using neuronal tracing and optogenetics, we found that these Tac1 neurons directly innervate and coordinate the medullary regions to control sequential phases of cough-like defensive behaviors. We propose that these NTS neurons are a key component of the central pattern generator for cough-like defensive behaviors in mice, and they coordinate the downstream modular circuits to elicit the sequential motor pattern of forceful expiratory responses.

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**Presentation Number:** NANO88.09

**Topic:** D.02. Somatosensation – Pain

**Support:** Internal Grant Noorda COM

**Title:** Reversible Emerging Pattern of Neurocognitive Changes and Glucose Dysregulation in Chronic Intractable Migraine

**Authors: *K. BILLS**, A. AMEDOLARA, A. PAUL, J. NIELSEN, E. POPE, J. WILLIAMS, S. BAKER, D. SANT, J. KRIAK;
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**Abstract:** Migraine is the most common neurological disorder in the world. It is a multisystemic, multicausal condition characterized by increased neuronal activity in various brain regions including the hypothalamus and trigeminal nerve complex. In this study, we present two emerging, reversible predictive changes in chronic intractable migraine, reactive hypoglycemia and an altered neurocognitive profile. Previous reports have indicated an association between migraine and reduced insulin sensitivity leading to increased average blood glucose levels and conflicting reports have examined neurocognitive changes. In this study, 36 patients with previous diagnosis of chronic intractable migraine for at least 6-months underwent pre- and post-treatment testing of neurocognition (12 domain) and pre- 3-hour glucose tolerance (100g glucose load). Post neurocognitive testing occurred following a minimum of 3 months of chronic migraine resolution. The average blood glucose levels were as follows: post fasting baseline (82 mg/dL +/- 12 mg/dL), 1-hour post glucose ingestion (110 mg/dL +/- 14 mg/dL), 2-hour post glucose ingestion (80.25 mg/dL +/- 14 mg/dL), and 3-hour post glucose ingestion (53 mg/dL +/- 6 mg/dL). The flagged reference glucose range at 3-hours was 65-139 mg/dL. Neurocognitive testing revealed baseline differences in Spatial Awareness and Memory, Episodic Memory, Deductive Reasoning, Grammatical Reasoning, Verbal Short Term Memory and Response Inhibition. Significant improvement after treatment was observed in Spatial Awareness and Memory (p=0.0090), Episodic Memory (p=0.0238), Mental Rotations (p=0.00338), Spatial Planning (p=0.00132), Grammatical Reasoning (p=0.00137), and Response Inhibition (p=8.81E-05). These emerging profiles possess possible predictive power to more rapidly triage patients to effective treatments. Additional mechanistic and clinical studies are needed.

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**Presentation Number:** NANO88.10

**Topic:** D.02. Somatosensation – Pain

**Support:** Rajyoga Meditation Research Foundation (RERF), India

**Title:** Alterations in resting state functional connectivity in central pain processing areas of the brain among Rajyoga meditators, non-meditators and patients with migraine disorder - A cross-sectional study

**Authors: *R. M.G.**[1], R. KV[2];
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**Abstract:** Activation of insular cortex is observed during acute and chronic pain. Altered structural and functional connectivity (FC) in migraine have been reported and studies also report that meditation has a beneficial role in migraine. But till-date, no FC analysis have been studied to compare any possible alterations in brain regions of patients with migraine (PM) vs in Rajyoga meditators (RM) and non-meditators (NM). The aim of the current cross-sectional study

was to identify alterations in resting-state FC in central pain-processing regions among NM, RM and PM. Study participants (n=102) included, age and handedness-matched NM (n=38), regular RM practitioners (n=38), and PM (n=26). NM and PM practicing any mediation methods were excluded from the study. All participants underwent structural and functional resting-state MRI scans after obtaining ethical committee approval. Seed-based FC analysis was done on these scns using CONN software with bilateral insular cortex as seed region. ANOVA was applied for group comparison and significant level is reported at $p<0.05$ corrected for FDR [False discovery rate]. Increased FC for the selected seed region was observed in RM compared to NM in left middle frontal, angular, middle temporal, middle occipital gyri; right precuneus and cuneus gyri. Increased FC was observed in RM vs PM in several pain processing areas including bilateral superior frontal, middle frontal and angular gyri; left superior medial frontal, middle occipital, and middle temporal gyri. No significance was observed when compared for FC between NM and PM. Increased FC in several central pain-processing regions in RM practitioners are involved in emotion, pain and attention control indicating that RM have integrative FC to overcome emotional and cognitive aspects of pain. Thus, Rajyoga meditation practice may be helpful in PM to alleviate migraine pain, although more studies need to be done to use RM as a complementary therapy for migraine.

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**Title:** Voluntary control of mesoscale activity waves in the primary somatosensory and motor cortices

**Authors: *A. A. DOGADOV**, C. PICARD, Z. HAYATOU, D. E. SHULZ, I. FEREZOU, V. EGO-STENGEL, L. ESTEBANEZ;
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**Abstract:** In the cerebral cortex, activity dynamics measured at a mesoscopic (0.1 to 1 mm) scale are characterized by both spontaneous and task-related dynamical waves of synchronized neuronal activity ranging from spreading stationary depolarizations (blobs) to traveling waves that can follow complex trajectories throughout the cortical network. These waves are thought to participate in information processing and propagation, but remain poorly understood. To test whether cortical waves can be actively generated and their trajectory controlled, we have implemented a goal-directed task for head-fixed mice, based on the online processing of wide-field calcium imaging signals. Transgenic mice expressing GCaMP6f in excitatory cortical

neurons (Ai-95 x EMX-Cre) were implanted with a 6-mm diameter optical window covering the left primary somatosensory and motor cortices. Calcium-dependent optical signals were analyzed in real time to track the trajectory of propagating mesoscale waves, and condition the delivery of water rewards to a specific wave trajectory. Using this experimental strategy, we could train mice to voluntarily control the emergence of specific waves of activity in primary somatosensory and motor cortices. Out of 17 mice, 12 increased the number of waves opening reward opportunities, among which 6 increased it by more than 100%, throughout 15 training sessions. Preliminary analyses suggest that mice were also able to coordinate their licking with these waves. Finally, synchronized high-speed videos of the mice behavior reveal that the emergence of these waves was correlated with stereotyped movement of the body part in correspondence with the cortical area where the waves were elicited. Such operant conditioning of large neuronal assemblies is a critical step to assess the potential use of mesoscale activity to control brain-machine interfaces.

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**Nanosymposium**

**NANO89: Depression: Preclinical Models, Human Studies, and Therapeutic Approaches**

**Location:** WCC 152B

**Time:** Wednesday, November 15, 2023, 1:00 PM - 4:00 PM

**Presentation Number:** NANO89.01

**Topic:** G.08. Other Psychiatric Disorders

**Support:** UH3 NS103549

**Title:** Beta Activity In Anterior Cingulate Cortex Mediates Reward Biases In Learning

**Authors: *J. XIAO**[1], J. ADKINSON[1], A. ALLAWALA[3], J. MYERS[1], R. MATHURA[1], V. PIRTLE[1], B. SHOFTY[1], S. MATHEW[1], W. GOODMAN[1], N. POURATIAN[4], K. BIJANKI[1], X. S. PITKOW[2], B. Y. HAYDEN[2], S. A. SHETH[2];
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**Abstract:** Reward is a critical driver of behavior. Its influence permeates our decisions, even when it is ostensibly irrelevant. Understanding how reward biases behavior is important for many reasons, including the fact that diminution in reward biasing is a hallmark of clinical depression. We hypothesized that reward biasing is mediated by anterior cingulate cortex (ACC), a hub region that is associated with the integration of reward and executive control, and with the etiology of depression. To test this hypothesis, we recorded neural activity during a biased judgment task in patients undergoing intracranial monitoring for either epilepsy (N=15) or depression (N=4). In this task, unequal frequency of reward between two correct responses produced a response bias towards the more frequently rewarded stimulus. First, we tested what neural feature represents the outcome value during the feedback period. We found that beta (12-

30 Hz) oscillations in anterior cingulate cortex (ACC) reliably tracked reward receipt ($p < 10^{-4}$). Next, we investigated whether this neural feature is also involved in the value representation of stimulus during the delay period, which is generated by the integration of sensory information and reward history. We found that beta activity in ACC showed a significant difference between the two types of stimuli associated with different reward probability ($p < 0.05$). This suggests that the ACC is engaged in the evaluation of both stimuli and outcomes, potentially representing a common neural mechanism underlying the assessment of reward values. Further, our results revealed that this ACC beta activity predicted stronger choice bias towards the more rewarding stimulus, suggesting its crucial role in translating internal estimate of stimulus value into behavioral preferences. Depression is characterized by an attenuated responsiveness to reward, potentially affecting decision-making processes. Lastly, we assessed neurobehavioral results in a cohort of four patients with treatment-resistant depression undergoing intracranial monitoring as part of a clinical trial. We found that both the behavioral biasing and these beta-specific neural effects were reduced ($p < 10^{-4}$), the impairment of which may contribute to the reduced influence of reward on decision-making observed in depression. In summary, our findings indicate that ACC beta oscillations orchestrate the binding of reward and sensory information to guide adaptive choice. Moreover, these oscillations potentially serve as a biomarker for anhedonia and offer insights into future targeted neuromodulatory interventions.

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**Topic:** G.08. Other Psychiatric Disorders

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**Title:** Identification of personalized therapeutic deep brain stimulation targets for major depressive disorder

**Authors: *K. K. SELLERS**, C. HENDERSON, J. M. FAN, G. S. MAKHOUL, K. WOODWORTH, D. A. ASTUDILLO MAYA, A. N. KHAMBHATI, H. ZAMANIAN, A. M. LEE, R. MARTINEZ, C. K. STARKWEATHER, L. P. SUGRUE, V. R. RAO, H. E. DAWES, K. W. SCANGOS, E. F. CHANG, A. D. KRYSTAL;
UCSF, San Francisco, CA

**Abstract:** Deep brain stimulation (DBS) is a form of neuromodulation in which electrical current is delivered to targeted areas of the brain with the goal of modulating activity. DBS for the treatment of MDD has been very successful in some applications[1,2], but has also failed to replicate in larger studies[3,4]. The Presidio clinical trial (NCT04004169) includes two novel approaches: (a) personalized stimulation target identification across multiple brain regions and (b) closed-loop stimulation based on a symptom biomarker. Here, we present results of

personalized stimulation testing for the first three participants. During this trial stage, participants were implanted bilaterally with 16-channel iEEG leads in the orbitofrontal cortex (OFC), subgenual cingulate (SGC), ventral capsule / ventral striatum (VC/VS), amygdala, and hippocampus. Symptoms were evaluated by self-reported scales (visual analog scales of depression, anxiety, and energy, and Hamilton depression rating scale), patient verbal report, and observer notes. Stimulation was tested across implanted brain regions, at multiple amplitudes and frequencies, and for varying durations. Therapeutic stimulation targets were identified for each participant: right VC/VS for Participant 1[5,6]; right OFC and right SGC for Participant 2; and right SGC and left nucleus accumbens for Participant 3. The identification of different optimal stimulation sites among the bilateral targets may reflect underlying heterogeneity in disease circuitry. We present both our stimulation testing procedures as well as evidence for therapeutic benefit at the identified sites. (1) Mayberg, H. S. *et al.* Deep Brain Stimulation for Treatment-Resistant Depression. *Neuron* 45, 651–660 (2005).(2) Kennedy, S. H. *et al.* Deep Brain Stimulation for Treatment-Resistant Depression: Follow-Up After 3 to 6 Years. *AJP* 168, 502–510 (2011).(3) Holtzheimer, P. E. *et al.* Subcallosal cingulate deep brain stimulation for treatment-resistant depression: a multisite, randomised, sham-controlled trial. *The Lancet Psychiatry* 4, 839–849 (2017).(4) Dougherty, D. D. *et al.* A Randomized Sham-Controlled Trial of Deep Brain Stimulation of the Ventral Capsule/Ventral Striatum for Chronic Treatment-Resistant Depression. *Biological Psychiatry* 78, 240–248 (2015).(5) Scangos, K. W. *et al.* Closed-loop neuromodulation in an individual with treatment-resistant depression. *Nat Med* 27, 1696–1700 (2021).(6) Scangos, K. W., Makhoul, G. S., Sugrue, L. P., Chang, E. F. & Krystal, A. D. State-dependent responses to intracranial brain stimulation in a patient with depression. *Nature Medicine* 27, 229–231 (2021).

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**Topic:** G.08. Other Psychiatric Disorders

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**Title:** Post-stroke depression and related Cognitive deficits are associated with reduced cognitive conflict evoked mid-frontal EEG theta oscillations and can be potentially improved with prefrontal transcranial electrical stimulation

**Authors: *I. BASU**, A. ROSS, O. O. AWOSIKA, F. ROMO-NAVA, B. KISSELA, D. FLECK; Univ. of Cincinnati, Cincinnati, OH

**Abstract:** Post-Stroke Depression (PSD) is the most common neuropsychiatric consequence of ischemic stroke and negatively impacts survival, functional outcome, and quality of life. PSD co-occurs with executive dysfunction resulting in loss of independence in daily functioning. Available psychotherapeutic and pharmacological treatments often fail to alleviate these

comorbidities, hence a better understanding of the brain network changes underlying PSD with executive dysfunction may help to elucidate the associated brain mechanisms and facilitate the development of neuromodulatory interventions. Transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) enhances theta oscillations while improving performance on cognitive control and is a promising intervention for cognitive rehabilitation. Herein, we assessed neural correlates of PSD executive dysfunction and tested if tDCS over the left dorsolateral PFC (dlPFC) can improve cognitive control and associated neural oscillations. We recorded midfrontal scalp EEG from 15 healthy and 9 participants with stroke in the last 2-3 years while they performed a multi-source interference task (MSIT). All participants were administered a MADRS to assess current depressive symptoms. Furthermore, 7 in the stroke cohort performed additional MSIT sessions where they received 10 minutes active and sham tDCS on the left dlPFC in a double-blind, sham controlled crossover study design with a 5 min washout period between sessions. The EEG was pre-processed using EEGLAB to get rid of eye movement and other channel noise artifacts and bandpass filtered to retain 0.5-55 Hz components. A Morlet wavelet decomposition was used to estimate power in theta (4-8 Hz), alpha (8-15 Hz) and gamma (35-50 Hz) frequency bands over a period of 2 seconds following MSIT image presentation. The spectral power was normalized using a baseline period of 0.5 second preceding image onset in the MSIT trials. A generalized linear mixed effects model (GLME) was used to find effect of group (healthy v stroke) on behavior as well as neural oscillations after accounting for task conflict. A GLME was also used to find effects of active tDCS on behavior. Group was a significant predictor of both response time (behavior) and conflict evoked theta power in the frontal channels (F1-Fz, F2-Fz). We also found significant differences in behavior between the active and sham after accounting for cognitive load. Preliminary results indicate that suppressed mid-frontal theta oscillations are a potential neural correlate of post-stroke cognitive dysfunction and tDCS of the left dlPFC might be a promising neuromodulatory therapeutic tool.

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**Support:** Ray and Dagmar Dolby Family Fund

**Title:** Optimizing Discovery of Neural Biomarkers Associated with Naturalistic Mood States

**Authors:** A. N. KHAMBHATI[1], ***N. STAPPER**[2], K. K. SELLERS[1], D. A. ASTUDILLO MAYA[2], J. FAN[3], J. COHEN[2], C. A. HENDERSON[2], V. R. RAO[3], K. W. SCANGOS[2], E. F. CHANG[1], A. D. KRYSTAL[2];
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**Abstract:** Identification of a neural biomarker of a naturalistic mood state is important for elucidating the pathophysiology of mood disorders and developing diagnostics and treatments. Direct neural recordings concordant with self-guided mood surveys can inform biomarker development. Yet, brain activity patterns that represent naturalistic mood must be separated from those that represent behaviors associated with self-guided symptom assessment. The

environmental and interpersonal context during data acquisition may also confound biomarker development in cases where the data are collected outside the naturalistic environment. In this study, we evaluate the impact of survey-taking behaviors and context on biomarker development in three participants of the PRESIDIO clinical trial (NCT04004169), which is investigating closed-loop deep brain stimulation for depression. We collected chronic intracranial electroencephalography (iEEG) while participants reported mood state daily using the Visual Analogue Scale for Depression (VAS-D) and the Hamilton Depression Rating Scale (HAMD-6) for over 300 days. We calculated spectral power in segments of iEEG recordings during each survey report. We compared iEEG and mood ratings collected in the following 3 contexts: 1) in the research lab and triggered manually by the researcher, 2) in the subject's home and triggered manually by the subject, and 3) in the subject's home and triggered automatically in a pre-programmed blinded manner. First, we assessed brain activity dynamics associated with survey taking behavior and found reduced delta and theta (1-8 Hz) power before, elevated alpha activity (8-12 Hz) during, and elevated broadband (15-120 Hz) power after survey completion. We next asked whether these acute dynamics impact our ability to identify a biomarker of mood. We calculated correlations between spectral power and mood rating depression symptoms using a sliding time window before, during, and after survey completion. In the naturalistic environment, biomarkers were consistent between manually triggered and blinded, automatically triggered neural recordings. However, biomarkers developed based on in-laboratory ratings demonstrated a reduction in the correlation between mood and broadband (8-125 Hz) spectral power. We find that biomarkers of mood state remain stable throughout self-guided symptom assessment in the naturalistic environment. However, biomarkers identified using data collected in the research lab environment were discordant. Therefore, environmental context of the data acquisition should be considered as a possible nuisance factor during biomarker development.

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**Presentation Number:** NANO89.05

**Topic:** G.08. Other Psychiatric Disorders

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**Title:** Pre-stimulus cortical excitability as a predictor for cortical response size in the auditory cortex and auditory association areas.

**Authors: *M. M. MOCCHI**[1], E. BARTOLI[2], J. F. MAGNOTTI[8], J. DEGEE[9], B. A. METZGER[3], B. PASCUZZI[1], R. MATHURA[2], J. M. YAU[4], W. K. GOODMAN[5], S. A. SHETH[6], M. J. MCGINLEY[2], K. BIJANKI[7];
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**Abstract:** There is unexpected variability of neural responses which may be explained by global changes in cortical excitability that alter how the brain processes incoming stimuli. Understanding these dynamics is critical to developing biomarkers of cognitive and neural dysfunction in disease. We examine the relationship between cortical excitability and response to auditory stimuli as a means of quantifying this global phenomenon across psychiatric disorders. Intracranial EEG data was collected from 6 patients undergoing epilepsy monitoring. Patients participated in an auditory-oddball paradigm to characterize cortical responses to auditory sensory stimuli. Aperiodic (1/f) slope (cortical excitability), high-gamma power responses, and beta power responses were extracted from 41 channels in the pSTG near the auditory cortex while pupil diameter was also recorded. Pre-stimulus 1/f slope, post-stimulus pupil dilation response, and post-stimulus high-gamma and beta power responses were calculated for all standard and oddball trials before being entered into linear mixed-effects models. Pre-stimulus slope showed a significant interaction effect for predicting the high-gamma ($t(df) = 3.58(6736)$, $p < 0.001$) and beta ($t(df) = 4.507(6688)$, $p < 0.001$) response sizes on a trial-by-trial basis. Furthermore, pupil diameter response size can track high gamma responses ($t(df) = 2.88(1058)$, $p < 0.01$) at the trial level, and shows a similar relationship with beta responses ($t(df) = 1.79(1059)$, $p = 0.07$). These data provide strong evidence that cortical excitability can modulate cortical response sizes in sensory areas, and that these response sizes can be tracked by non-invasive measures such as pupil diameter. Further investigation into other covariates of excitability and salience, such as psychiatric comorbidity and other peripheral nervous system measures of arousal, are necessary for a more comprehensive understanding of the excitability-response variability relationship.

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**Title:** Role of fronto-temporal networks in anxiety and depression during the performance of a cognitive control task

**Authors: *A. SHEKARA**[1], A. ROSS[1], A. C. PAULK[2], A. S. WIDGE[3], S. S. CASH[2], P. K. SHEAR[1], J. P. SHEEHY[1], I. BASU[1];

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**Abstract:** Neuropsychiatric disorders are the number one cause of disability and health-related economic burden in the United States. Established therapies for psychiatric illness primarily focus on diagnostic labels, however, a more robust approach would be to use objective constructs such as brain circuitry underlying functional deficits. Cognitive control is often compromised across mood and anxiety disorders and can be estimated with an interference task where subjects must suppress a natural response to overcome response conflict. Conflict evokes robust electrophysiologic signatures such as theta (4-8 Hz) oscillations in the prefrontal cortex (PFC), and recently published data suggests involvement of the lateral temporal lobe (LTL) in conflict encoding. However, it is unclear how anxiety and depression modulate such circuits/rhythms, and there is little indication about the role of LTL in higher executive function. The objective of this work is to determine the role of fronto-temporal structures modulating cognitive control, and to identify potential differences in these neural signatures between anxious/depressed (A/D) and non-A/D individuals.

We recorded intracranial EEG (iEEG) from fronto-temporal regions of 26 human subjects with intractable epilepsy undergoing invasive monitoring while they performed a multi-source interference task (MSIT). Based on neuropsychological evaluation, we labeled subjects as epileptic controls (SZ) or epileptic with comorbid anxiety/depression (SZ+A/D). We estimated power in theta, alpha (8-15 Hz), beta (15-30 Hz), gamma (30-55 Hz) and high gamma (70-110 Hz) frequency bands. For each frequency band and brain region of interest, we fit a generalized linear mixed effects model (GLME): *Response ~ Conflict + Group + (1|Subject)* where Conflict and Group are binary variables coding the trial conflict and A/D status of participants respectively.

In a subset of our data (n = 16), conflict type was a significant predictor of theta power in dorsolateral PFC (dlPFC) and LTL, theta, alpha, and beta power in dorsomedial PFC (dmPFC), and alpha power in dorsal ACC (dACC). Group was a significant predictor of beta power in dACC. Next, we spectrally decompose evoked response potentials (ERPs) to identify significant time-frequency clusters for subsequent GLMEs assessing group differences in conflict processing. We also explore the orbitofrontal cortex and medial temporal structures to determine their involvement in encoding conflict. Our current results demonstrate roles of both PFC and LTL in cognitive control independent of A/D and motivate further exploration of psychiatric illness on conflict-evoked oscillations.

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**Title:** Prefrontal network engagement by deep brain stimulation in limbic hubs

**Authors: *A. ALLAWALA**[1], K. BIJANKI[2], D. OSWALT[3], R. MATHURA[2], J. ADKINSON[2], V. PIRTLE[2], B. SHOFTY[4], G. BANKS[2], M. ROBINSON[2], M. HARRISON[5], S. MATHEW[2], W. GOODMAN[2], D. BORTON[5], S. A. SHETH[2];
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**Abstract:** Prefrontal circuits in the human brain play an important role in cognitive and affective processing. Neuromodulation therapies delivered to certain key hubs within these circuits are being used with increasing frequency to treat a host of neuropsychiatric disorders. However, the detailed neurophysiological effects of stimulation to these hubs are largely unknown.Here, we performed intracranial recordings across prefrontal networks while delivering electrical stimulation to two well-established white matter hubs involved in cognitive regulation and depression: the subcallosal cingulate (SCC) and ventral capsule/ventral striatum (VC/VS).We demonstrate a shared frontotemporal circuit consisting of the ventromedial PFC, amygdala, and lateral orbitofrontal cortex where gamma oscillations are differentially modulated by stimulation target. Additionally, we found subject-specific responses to stimulation in the dorsal anterior cingulate cortex and demonstrate the capacity for further tuning of neural activity using current-steered stimulation.Our findings indicate a potential neurophysiological mechanism for the dissociable therapeutic effects seen across the SCC and VC/VS DBS targets for psychiatric neuromodulation and our results lay the groundwork for personalized, network-guided neurostimulation therapy.

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**Title:** Low-frequency power in the ventral capsule/ventral striatum and orbitofrontal cortex: a neural biomarker of obsessive-compulsive symptom severity

**Authors: *N. GIRIDHARAN**[1], N. PROVENZA[1], S. RAJESH[1], N. DIAB[1], R. BECHTOLD[3], E. M. DASTIN-VAN RIJN[4], A. ALLAM[1], G. REYES[1], S. REDDY[1], M. AVENDANO-ORTEGA[2], S. MCKAY[2], G. BANKS[1], D. A. BORTON[5], E. STORCH[2], J. A. HERRON[3], W. K. GOODMAN[2], S. A. SHETH[1];
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**Abstract: Introduction:** Deep brain stimulation (DBS) is an underutilized therapy for refractory obsessive-compulsive disorder (OCD) despite high response rates. One barrier to widespread adoption is the challenge in selecting optimal stimulation parameters. An adaptive DBS system that automatically detects OCD-related distress and titrates stimulation accordingly may help increase accessibility of the treatment. However, no robust neural signatures of OCD symptoms exist to serve as an input for the control algorithms in adaptive DBS systems. Our objective was to identify electrophysiological biomarkers of OCD-related distress using longitudinal human intracranial recordings.
**Methods:** We implanted 10 patients with bilateral sensing-capable DBS leads targeting the ventral capsule/ventral striatum (VC/VS) and in 5 participants, additional bilateral electrocorticography electrodes over the orbitofrontal cortex (OFC). VC/VS and OFC leads were connected to investigational bi-directional DBS devices. At each post-operative visit, patients completed a computerized provocation task where they were presented with 120 trials of neutral and provoking images and asked to rate their level of distress from 0 to 10. We recorded local field potentials (LFP) during the task and time-locked this neural data with image presentation and distress ratings.
**Results:** Participants who experienced improvement in OCD symptoms with DBS therapy also reported less distress upon viewing provoking images as they progressed through the study. Decreases in distress ratings over time correlated with reductions in OCD symptom scales ($R^2$=0.318). In the first visit with DBS off, there were significant increases in delta and theta power (1-8 Hz) in the VC/VS and OFC after provoking image presentation compared to neutral images. Over subsequent visits with DBS therapy on, the increase in low-frequency power after provoking image presentation was attenuated.
**Conclusion:** Using chronic intracranial recordings from participants with OCD, we identified potential neural biomarkers of OCD-related distress.

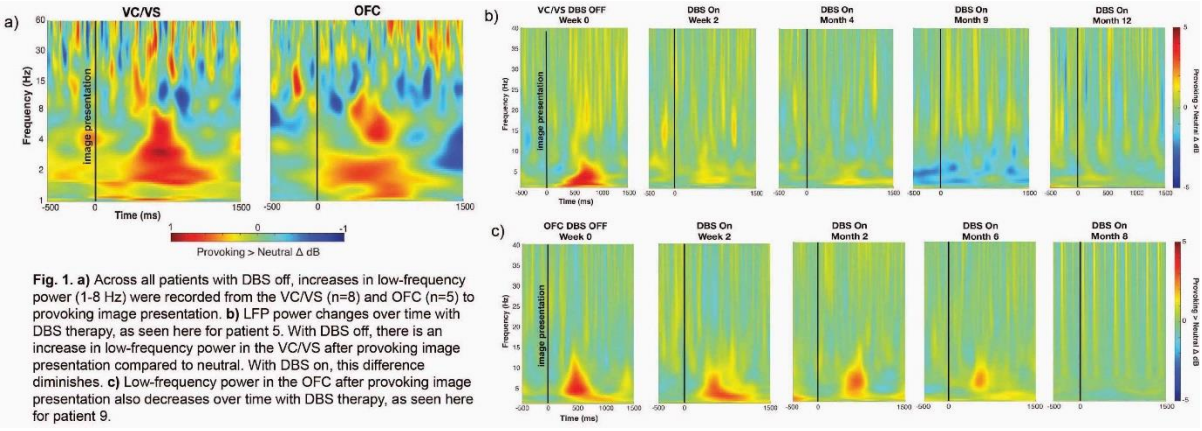


**Fig. 1. a)** Across all patients with DBS off, increases in low-frequency power (1-8 Hz) were recorded from the VC/VS (n=8) and OFC (n=5) to provoking image presentation. **b)** LFP power changes over time with DBS therapy, as seen here for patient 5. With DBS off, there is an increase in low-frequency power in the VC/VS after provoking image presentation compared to neutral. With DBS on, this difference diminishes. **c)** Low-frequency power in the OFC after provoking image presentation also decreases over time with DBS therapy, as seen here for patient 9.

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**Presentation Number:** NANO89.09

**Topic:** G.08. Other Psychiatric Disorders

**Support:** NIH Grant UH3NS103550
Hope for Depression
Devices donated by Medtronic

**Title:** Validation of local field potential marker of subcallosal cingulate deep brain stimulation mediated depression recovery in an independent cohort

**Authors: *S. ALAGAPAN**[1], E. FITOZ[1], M. FIGEE[2], T. NAUVEL[2], K. CHOI[2], M. OBATUSIN[2], S. HEISIG[2], J. CHA[2], A. WATERS[2], R. J. BUTERA, Jr[1], P. RIVA POSSE[3], H. S. MAYBERG[2], C. ROZELL[1];
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**Abstract:** Deep brain stimulation (DBS) of the subcallosal cingulate cortex (SCC) has been demonstrated to be effective in treating patients with treatment-resistant depression (TRD). However, current gold-standard clinical assessments of depression severity are susceptible to extraneous factors unrelated to core depression. Therefore, objective brain-based biomarkers are needed to aid clinical decisions such as stimulation dose adjustment or augmentation with therapy. We have previously reported on a biomarker derived from local field potential (LFP) using a generative causal explainer, an explainable artificial intelligence (xAI) approach. The biomarker tracks transitions in the clinical state of the patients and responds to changes in stimulation dose. In the current study, we aim to validate the biomarker in an independent cohort of TRD patients.

10 participants with TRD were implanted with Summit RC+S (Medtronic, MN, USA), an investigational DBS pulse generator with LFP recording capability. Chronic stimulation was turned on 4 weeks after implantation surgery in the first 5 participants and the day after surgery in the last 5 participants. LFPs were acquired twice daily during the first 6 months of therapeutic stimulation. Spectral features were extracted in 10-second segments and projected through the xAI model to estimate the biomarker. Depression severity was measured using Hamilton Depression Rating Scale (HDRS). We binarized the HDRS into ‘sick’ and ‘stable response’ states and compared them to those derived from the biomarker to verify that the biomarker tracked this critical clinical outcome.

7 out of 10 participants reached treatment response (50% decrease in presurgery baseline HDRS), and 4 out of 10 reached remission (HDRS less than 8). LFP changes in 2 participants were confounded by impedance changes and were excluded from the analysis. The biomarker-derived state tracked the HDRS-derived state with varying accuracy (range: 0.38 - 1.00). The

overall accuracy was 0.71 ± 0.2 (mean, s.d).
In this cohort of participants whose LFP data was not used for training the xAI model, our biomarker was able to track transitions to stable response, suggesting our biomarker generalizes beyond the cohort used for training. However, further analysis is required to identify the factors influencing the variability in performance.

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**Presentation Number:** NANO89.10

**Topic:** G.08. Other Psychiatric Disorders

**Support:** NIH R01-MH127006
UH3-NS103549

**Title:** Brain mechanisms underlying the negative and positive emotion processing biases in treatment-resistant depression

**Authors: *X. FAN**[1], M. MOCCHI[1], B. PASCUZZI[1], J. XIAO[1], B. A. METZGER[2], R. MATHURA[1], C. D. HACKER[3], J. ADKINSON[1], E. BARTOLI[1], S. ELHASSA[1], A. WATROUS[1], Y. ZHANG[1], I. A. DANSTROM[1], W. K. GOODMAN[1], N. POURATIAN[4], S. A. SHETH[1], K. BIJANKI[1];
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**Abstract:** Individuals suffering from depression often display a cognitive bias towards negative information and away from positive information. Although previous studies have revealed increased amygdala response to sad faces and decreased amygdala response to happy faces in major depression disorder, the neural mechanism responsible for the biased emotion processing in depression is not fully understood. Here, we recorded stereotactic electroencephalography (sEEG) signals in amygdala and orbital prefrontal cortex (OFC) from 5 treatment-resistant depression (TRD) patients and 10 epilepsy patients (as control) while they participated in an affective bias task in which happy and sad faces were rated. First, compared with control patients, patients with TRD showed increased amygdala responses to sad faces in the early stage (around 300 ms) and decreased amygdala responses to happy faces in the late stage (around 700 ms). Second, TRD patients showed greater alpha-band activity in OFC as well as greater alpha-phase locking between the amygdala and OFC compared to controls, but only during the late stage of rating happy faces. Finally, direct electrical stimulation of bilateral subcallosal cingulate (SCC) and ventral capsule/ventral striatum (VC/VS) in TRD induced changes especially in the late stage neural responses to happy faces, including increased amygdala response, reduced OFC

alpha-band activity, and reduced alpha-phase locking between the amygdala and OFC. Therefore, our findings suggest that distinct neural mechanisms are responsible for the biased processing of negative and positive emotional information in TRD. The increased activity observed in the amygdala during the early stage of rating sad faces may indicate an overactive bottom-up processing system. While the reduced amygdala response during the late stage of rating happy faces may be attributed to overregulation by OFC through alpha band oscillation.

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**Title:** Associations of EEG-synchronized rTMS treatment of depression with changes in modulated functional connectivity: a concurrent fMRI-EEG-TMS study

**Authors: *H. HE**[1], X. SUN[1], J. DOOSE[2], A. BLANKENSHIP[2], J. MCLNTOSH[1], G. T. SABER[2], J. FALLER[1], Y. LIN[1], J. TEVES[2], S. HUFFMAN[2], S. PANTAZATOS[1], R. I. GOLDMAN[3], M. S. GEORGE[2], T. R. BROWN[2], P. SAJDA[1];
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**Abstract:** Transcranial magnetic stimulation (TMS) is an FDA-approved noninvasive treatment for depression. Studies have shown that TMS might affect both the stimulation site and distant regions, thereby modulating the abnormal network connectivity in depression. Here, we aim to investigate TMS-induced effects on brain networks before and after a six-week EEG-synchronized TMS treatment of depression. We hypothesized that the TMS-modulated functional connectivity (FC) changes might be associated with clinical outcome. This study developed and used an integrated fMRI-EEG-TMS (fET) instrument, where single-pulse TMS was delivered to the left dorsal lateral prefrontal cortex (L-DLPFC). Pre-treatment and post-treatment fET scans were acquired from twenty patients with depression. Patients were randomized into SYNC or UNSYNC group, and only the SYNC group received rTMS pulses (at L-DLPFC) synchronized to the individualized EEG prefrontal alpha phase (random firing in UNSYNC group). To measure TMS-induced effects, we performed general linear modeling and psychophysiological interaction analyses, where estimated beta-weights were summarized based on the Schaefer atlas. Additionally, we measured TMS-induced effects when TMS pulses were delivered at different timing (phase) relative to the EEG prefrontal alpha rhythm. Based on the pre-treatment scan, we labeled each patient's ‘preferred phase’ as the phase bin of TMS trials with the highest induced response at L-DLPFC (in Default-A network). We observed a significant correlation between the pre-treatment evoked response in the left hemisphere Control-

B network and the clinical outcome (percent change in the Hamilton Rating Scale for Depression) only for the SYNC group (Bonferroni corrected $p < 0.05$; UNSYNC: $p > 0.33$). The pre- and post-treatment FC changes (between L-DLPFC in the Default-B network and right hemisphere orbitofrontal cortex in Limbic-B network) modulated by the TMS trials in the preferred phase bins are significantly associated with the clinical outcome only for the SYNC group (corrected $p < 0.01$; UNSYNC: $p > 0.45$). Our results suggest EEG-synchronized rTMS treatment induces FC changes in specific neural circuits that are associated with the clinical outcome only in the synchronized patients. The results may inform future research to temporally optimize and personalize TMS targeting for depression treatment.

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**Topic:** G.08. Other Psychiatric Disorders

**Support:** NIH grant R01-MH128909
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**Title:** High Entropy Deep Brain Stimulation for Treatment Resistant Depression

**Authors: L. S. CHAMAKURA**[1], D. OSWALT[3], E. BARTOLI[1], J. ADKINSON[1], B. HAYDEN[1], S. SHETH[1], X. PITKOW[1], *K. BIJANKI[2];
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**Abstract:** Research shows that deep brain stimulation (DBS) can be useful to treat psychiatric disorders such as depression. A key challenge for such studies is characterizing the individual brain responses to electrical stimulation over the target stimulation space, owing to the combinatorial complexity involved. Here, we propose high entropy stimulation sequences to sample from a wide array of spatiotemporal patterns while adhering to hardware constraints. Our stimulation sequences substantially generalize traditionally parameterized stimuli (amplitude, polarity, frequency, duration, number of pulses) by using marked point processes, a collection of point-like events that are each marked by additional characteristics. We drew samples from a generalized Cox process with time-varying pulse rates and a spectrum of interval distributions from periodic to Poisson to bursty. We defined time-varying pulse characteristics such as pulse amplitude, pulse width, and active stimulation channels according to underlying latent Gaussian processes. Following [1], these latent processes can accommodate flexible spatiotemporal correlations across electrical channels. The proposed approach explores a much larger and more natural portion of the stimulation space than conventional piecewise constant pulse trains. We used the proposed paradigm to stimulate a patient with treatment resistant depression who had implanted DBS electrodes. The stimulation was performed in subcallosal cingulate (SCC) and the ventral capsule/ventral striatum (VCVS) regions of the brain for a duration of 2 hours and the

patient's ongoing brain activity was recorded using intracranial stereo encephalogram (sEEG). We observed that the recorded sEEG exhibits variability in artifacts as a function of the time-varying stimulation parameters. Nevertheless, we show that a linear model of artifacts can account for a large fraction of this variability. We perform artifact correction through template subtraction, where the composite templates were constructed from linear combinations of templates from single-pulse stimulations. Overall, by generating richer and more natural patterns of electrical stimulation, the proposed high entropy stimuli should be useful to efficiently probe the influence of external stimulation on brain states.
[1] Krumin, M. and Shoham, S., 2009. Generation of spike trains with controlled auto-and cross-correlation functions. Neural computation, 21(6), pp.1642-1664.

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**Nanosymposium**

**NANO90: Prefrontal Mechanisms of Executive Function III**

**Location:** WCC 146C

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:30 PM

**Presentation Number:** NANO90.01

**Topic:** H.04. Executive Functions

**Support:** Simons Collaboration on the Global Brain 542993SPI
NSF GRFP 000930410

**Title:** Mechanisms in macaque prefrontal cortex for predicting the motion of occluded objects

**Authors: *N. WATTERS**, J. GABEL, M. JAZAYERI;
MIT, Cambridge, MA

**Abstract:** The primate mind excels at making predictions about physical scenes with moving objects. From crossing a busy street to playing a sport, this capacity is on display at every turn. Cognitive theories have hypothesized that this "intuitive physics" underlies more abstract reasoning such as planning and imagination. However, the neural mechanisms by which the brain predicts the kinematics of moving objects remain largely a mystery. In particular, two competing hypotheses remain unresolved. One hypothesis is online prediction, under which the brain updates the evolving states of occluded moving objects in real-time. Another hypothesis is offline prediction, under which the brain instead tracks elapsed time that can be subsequently combined with a memory of previous states of moving objects to infer their current position. Furthermore, each of these hypotheses could employ either parallel attention to multiple objects or serial attention switching between multiple objects through time.
To resolve these competing hypotheses, we developed a task for non-human primates (NHPs) that requires predicting kinematics of multiple occluded objects as they move in a visual display. We trained two animals on this task and verified that they were able to remember and predict the positions and identities of multiple independently moving occluded objects. Evidence from prior

studies suggests that dorsomedial frontal cortex (DMFC) and frontal eye fields (FEF) are involved in making predictions about dynamic scenes. Accordingly, we conducted simultaneous acute recordings from these areas while the animals performed the task. We recorded thousands of single neurons from two NHPs, both on conditions within the animals' training distribution and throughout generalization conditions to novel dynamics and novel number of objects. We use this neural data to test specific hypotheses about online/offline prediction and attention in the primate brain by developing novel statistical methods for characterizing the geometry of neural dynamics. Furthermore, we validate all of our methods on artificial network models instantiating each hypothesis.

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**Presentation Number:** NANO90.02

**Topic:** H.04. Executive Functions

**Support:** NIH R01MH129492

**Title:** Cognitive Lego: Building Compositional Tasks with Shared Neural Subspaces

**Authors: *S. TAFAZOLI**[1], F. M. BOUCHACOURT[2], A. ARDALAN[2], N. T. MARKOV[2], M. UCHIMURA[2], M. G. MATTAR[3], N. D. DAW[2], T. J. BUSCHMAN[2];
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**Abstract:** Cognition is remarkably flexible, able to rapidly learn new tasks and switch between tasks. Theoretical modeling suggests this flexibility may be due, in part, to the brain's ability to re-use cognitive processes across tasks. To address this, we recorded from frontal, parietal, and temporal cortex while monkeys switched between three compositionally-related categorization tasks. In Task 1, the animals categorized a stimulus based on its shape and indicated their decision with a left/right motor response. In Task 2, the same stimulus was categorized by its color and the animal indicated their decision with an up/down response. In Task 3, the monkeys categorized by color (as in Task 2) but responded along with a left/right response (as in Task 1). In this way, the three tasks could be thought of as compositionally combining sub-tasks to categorize a stimulus according to its shape or color and then responding with either a left/right or up/down action. In line with prior research, task-relevant information was encoded in prefrontal cortex neural activity patterns. Using linear classifiers, we found '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 2 and Task 3 used the same subspace of neural activity to represent the color category of the stimulus, and Task 1 and Task 3 used the same subspace to represent the left/right motor response. In this way, Task 3 could be composed of the sequential engagement of the color categorization subspace (shared with Task 2) and the left/right response subspace (shared with Task 1). The shared representations were dynamically recruited as the monkeys switched between tasks. A classifier was able to decode the animal's belief about which task was in effect on each trial from the neural activity within prefrontal cortex. As the animal discovered the task, we saw an increase in this measure of the animal's internal belief. Furthermore, the strength of the animal's belief on each trial was correlated with

how strongly information was represented in the task's subspaces (i.e., when the animal believed Task 1 was in effect, it used the shape subspace, and when it believed Task 3 was in effect, it used the color subspace). At the same time, the task-irrelevant subspace was suppressed (i.e., color/shape in Task 1/3 were suppressed). Altogether, our results show how the brain can compose a task from the sequential combination of sub—task specific subspaces.

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**Topic:** H.04. Executive Functions

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The Georgia Institute of Technology
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**Title:** Decomposed linear dynamical systems for C. elegans functional connectivity

**Authors: *E. YEZERETS**[1], N. MUDRIK[1], Y. CHEN[2], A. S. CHARLES[1], C. J. ROZELL[3]; [1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Biomed. Engin., [3]Electrical and Computer Engin., Georgia Inst. of Technol., Atlanta, GA

**Abstract:** Recent work has indicated that functional connections between *C. elegans* neurons can be clustered by their role in behavior (Kato 2015, Linderman 2019). Furthermore, they show how data-driven dynamical systems, e.g., recurrent switching linear dynamical systems (rSLDS), can uncover behaviorally-relevant states in an unsupervised way from whole-brain recordings. However, these approaches ascribe a discrete state to the system at each time point, potentially missing dimensions of variability. For example, they cannot flexibly describe the continuous transitions that occur between behaviors nor can they capture simultaneously active states. Using the decomposed linear dynamical systems (dLDS) model (Mudrik 2022), which promote interpretability via sparse dynamics representations and flexibility via continuously varying dynamics coefficients, we demonstrate further insight into *C. elegans* circuit dynamics. We investigated dLDS applied to *C. elegans* $Ca^{2+}$ imaging data from the Zimmer lab (approx. 100 channels, 12 min. during pirouetting behavior under $O_2$ stimulation, n=7). dLDS models the data as an evolving latent state that generates the observed data. The change in the latent state is modeled as a linear dynamical system composed of a linear combination of a dictionary of "core" dynamical systems that can be reused over time. We fit the model parameters (the latent state and the core dynamics) with an expectation-maximization dictionary-learning style procedure that alternates between inferring the latent trajectories (the latent states and the dynamics coefficients dictating which core dynamics are used at what times) and updating the model parameters. We show that while rSLDS outputs unrealistic periods of oscillation between latent state labels in all tested *C. elegans* examples, dLDS smoothly describes these intervals in terms of the dynamics coefficients, which can vary continuously instead of switching on and off. We see substantial inter-individual differences in the learned parameters. However, each individual model not only recapitulates the neural activity, but also reflects meaningful latent

representations for that worm: 1) the dynamics map back to combinations of labeled neurons, 2) the dynamics coefficients can be used to infer the current and next behavioral states, 3) the coefficients reflect nuances in the neural activity due to the experimental protocol ($O_2$ level) or time in the trial, and 4) the coefficients can be clustered to reflect behavior direction (dorsal vs. ventral turning) and speed (crawling vs. slowing). Therefore, we conclude that dLDS can interpretably describe how non-stationary circuits encode behavior.

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**Presentation Number:** NANO90.04

**Topic:** H.04. Executive Functions

**Support:** MOE2016-T2-2- 117
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**Title:** Prefrontal Manifold Geometry Explains Reaction Time Variability

**Authors: *C. LIBEDINSKY**[1], R. HERIKSTAD[2];
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**Abstract:** The stochastic drift-diffusion model proposes that the variability in reaction time is due to randomness during the accumulation of evidence until a decision threshold is reached. However, the neural mechanisms that explain both the randomness and implementation of the decision threshold in the model remain unclear. Here we address these questions using the dynamical systems approach to analyze primate frontal eye field activity and using microstimulation for causal manipulations. We built a mechanistic model in which signals associated with motor plans are bumped out of their attractor state by go-cue signals that emerge around 60 ms after the go cue. The network then travels through a transition subspace towards a movement-initiation subspace that emerges around 35 ms before movement onset and implements the decision threshold. We postulate that the randomness in the neural trajectory, and hence in reaction times, is explained by the amplification of noise during movement preparation by the geometry of the frontal eye field manifold.

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**Presentation Number:** NANO90.05

**Topic:** H.04. Executive Functions

**Support:** NIH R01EB026936
NSF CCF-2217058

**Title:** Neural manifold decoding using low-distortion Riemannian Alignment of Tangent Spaces

**Authors:** D. KOHLI[1], J. NIEUWENHUIS[2], A. CLONINGER[1], ***G. MISHNE**[3], D. NARAIN[2];
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**Abstract:** Since the advent of large-scale high-dimensional extracellular recordings in neuroscience and the widespread adoption of neural population frameworks, there is an increased need to achieve efficient decoding of task-relevant behavioral information from neural dynamics. In various biological fields, high-dimensional datasets often appear to occupy low-dimensional latent geometrical structures that can be approximated as topological manifolds. Manifold learning techniques have enjoyed much success in organizational principles of biological variables and how they relate to each other. Unfortunately, a majority of these techniques introduce distortions while embedding higher-dimensional data into low-dimensional representations, which severely curtails their effectiveness for neuroscience applications. Optimization of these techniques for neural data presents its own challenges since neural data are often noisy, sparse, and nonhomogenous. Based on a novel measure of distortion, we present a new manifold learning technique, Riemannian Alignment of Tangent Spaces (RATS) that is designed to discover low-distortion embeddings of data manifolds in an unsupervised manner. We leverage recurrent neural network models (RNN), which can reliably replicate neural dynamics underlying several behaviors. We use RNNs to construct and control task-based manifolds with known dynamics, intrinsic dimensions, noise, sparsity, and density. Applied to RNN-generated data and neuronal recordings, we demonstrate that RATS yields the lowest distortion embeddings when compared to other manifold learning methods on sensorimotor and navigation data. This leads to improved decoding of behavioral metrics from noisy and sparse task-relevant manifolds. Methods like RATS could help better decipher the elusive links between neural population dynamics and behavior, an issue that remains central to systems neuroscience.

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**Title:** Task flexibility cost arises from interference between relevant and irrelevant information sources

**Authors: *C. XUE**[1], S. MARKMAN[1], R. CHEN[2], L. KRAMER[1], M. COHEN[1];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** In an uncertain and ever-changing world, we make decisions under dynamic task requirements. The flexible switching between tasks comes at a cost, leading to worse task performance and longer response times. We compared task switching behavior in macaque monkeys, human subjects, and artificial neural networks. We observed behavioral and neuronal signs of a flexibility cost in biological agents and an artificial agent trained to reproduce the monkeys' choices, but not in artificial agent trained to produce the correct choices on the same task. The model suggested that the flexibility cost in biological agents results from interference between information related to the two different tasks under task uncertainty. This hypothesis

predicts stronger neuronal and behavioral interference from irrelevant information in conditions where task representation is less certain, which we confirmed with using psychophysics, electrophysiological recordings, and causal experiments. Furthermore, by applying network control theory to the analyses of shared activity of populations of neurons, we confirmed the model prediction of neurons selective for stimulus information and task information in the same brain area. Our findings suggest a neuronal reason for the cost of behavioral flexibility, which illuminates the mechanisms supporting cognitive flexibility in healthy and pathological states can be a basis for interventions to mitigate cognitive impairments. Our work also highlights the potential of a new research paradigm: by comparing artificial networks trained to capture real behavior and optimal behavior, we can generate and test targeted mechanistic hypotheses about the relationships between neurons and cognitively complex behaviors.

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**Title:** Separate neural representations of the time and content of a decision?

**Authors: *N. SO**[1], M. SHADLEN[2];
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**Abstract:** Many decisions involve the accumulation of noisy evidence to a criterion level that both terminates the process and establishes the choice. For difficult perceptual decisions reported by eye movements, the accumulation mimics drift-diffusion and manifests as such in the firing rates of neurons in area LIP and other areas, presumably. The theory and the neural responses explain the stochastic choice and response times of human and nonhuman primates. They do so parsimoniously by 1-dimensional dynamic processes (i.e., scalar functions of time) that link the visual representation of the choice targets to the selection of a saccade. It is natural to wonder whether the coupling of decision formation to the preparation of the saccadic report arises from a principle of neuroscience, or whether it is instead a solution coerced by the design of choice-response tasks. We therefore set out to study perceptual decisions that require distinct actions to terminate the decision process (*When*) and communicate the choice (*What*).
Two rhesus monkeys performed a variant of the random dot motion task. In each trial, the monkeys made two distinct saccadic eye movements corresponding to *When*- and *What*-decisions: first to a choice-neutral target, T0, to report the decision termination, followed ultimately by a final saccade to one of the choice targets to indicate the up or down motion choice.
Simultaneous recordings from >100 neurons in LIP (macaque Neuropixels probes) were used to decode the monkey's ultimate choice (*What*) and decision time (*When*) using activity during motion viewing. The *What* and *When* coding directions (CD) were approximately orthogonal, suggesting a dissociation. However, population activity projected onto the *What*-CD renders a drift-diffusion decision variable (DV) on single trials. The DV is inverted relative to the

expectations in a task where the choice and response time are communicated by the same saccade. During upward motion trials, for instance, neurons representing the up choice-target are suppressed, and neurons representing the down choice-target are excited. Both representations are graded as a function of motion strength and converge to a stereotyped level of activity ~100 ms before the saccade to T0. This implies a termination mechanism applied to the absolute value of either accumulation. This categorical representation (blurring differences in motion strength) is the signal that is passed to other neurons (as in So & Shadlen, 2022) to maintain the decision until its report. In this way, the *When* and *What* of the decision remain coupled via a single drift-diffusion process, and the decision process retains its connection to the saccadic report.

**Disclosures: N. So:** None. **M. Shadlen:** None.

**Presentation Number:** NANO90.08

**Topic:** H.04. Executive Functions

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**Title:** A transient high-dimensional geometry affords stable conjunctive subspaces for efficient cognitive control

**Authors: *A. KIKUMMOTO**[1], K. SHIBATA[2], A. BHANDARI[1], D. BADRE[1];
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**Abstract:** Flexible action selection requires cognitive control mechanisms capable of mapping the same inputs to diverse output actions depending on goals and contexts. How the brain encodes information to enable this capacity remains one of the longstanding and fundamental problems in cognitive neuroscience. From a neural state-space perspective, solving this problem requires a control representation that can disambiguate similar input neural states, making task-critical dimensions separable depending on the context. Moreover, for action selection to be robust and time-invariant, control representations must be stable in time, thereby enabling efficient readout by downstream processing units. Thus, an ideal control representation should leverage geometry and dynamics that maximize the separability and stability of neural trajectories for task computations. Here, using novel EEG decoding methods, we investigated how the geometry and dynamics of control representations constrain flexible action selection in the human brain. Specifically, we tested the hypothesis that encoding a temporally stable conjunctive subspace that integrates stimulus, response, and context (i.e., rule) information in a high-dimensional geometry achieves the separability and stability needed for context-dependent action selection. Human participants performed a task that requires context-dependent action selection based on pre-instructed rules. Participants were cued to respond immediately at varying intervals following stimulus presentation, which forced responses at different states in neural

trajectories. We discovered that in the moments before successful responses, there was a transient expansion of representational dimensionality that separated conjunctive subspaces. Further, we found that the dynamics stabilized in the same time window, and that the timing of entry into this stable and high-dimensional state predicted the quality of response selection on individual trials. These results establish the neural geometry and dynamics the human brain needs for flexible control over behavior.

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**Topic:** H.04. Executive Functions

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**Title:** Neural signatures of cognitive control in high arousal

**Authors: *C. AVANCINI**[1], L. F. CIRIA[1], C. ALAMEDA[1], A. F. PALENCIANO[2], A. CANALES-JOHNSON[3], T. A. BEKINSCHTEIN[3], D. SANABRIA[1];
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**Abstract:** Spontaneous fluctuations in human physical arousal occur naturally throughout the day. These physiological changes unfold in a nonlinear manner and become severe during extreme states such as sleep or intense physical exertion, modulating cognition and the processing of exogenous and endogenous stimuli. Cognitive control has been shown to be robust even under strained states of arousal; however, little is known about the neural mechanisms underlying this robustness during high arousal. We predicted that preserved behavioural measures of cognitive control under high arousal would be accompanied by changes in its typical neural correlates.In an electroencephalography (EEG) study, 39 expert cyclists were presented with an auditory stimulus-response conflict task while cycling on a stationary bike. Prior to testing, individuals' maximum power output, oxygen consumption and heart rate had been measured to individually adjust and monitor their effort during the subsequent EEG sessions. Using a repeated-measures design, participants performed two experimental sessions on different days: one at low intensity (average heart rate of 54.3% of their maximum, SD = 6.4) and one at high intensity (average heart rate at 85.7% of their maximum, SD = 4.2). Participants had to respond to the content of the auditory stimuli (the words "left" and "right"), while ignoring the task-irrelevant spatial location (left or right). Stimulus content and spatial location could be either congruent or incongruent. The differential response to congruent and incongruent trials allowed us to quantify the conflict effect (CE), which serves as a measure of cognitive control.Consistent with our predictions, we found no significant difference in the behavioural CE between the two exercise conditions. However, midfrontal-theta power changes (a typical neural measure of cognitive control) showed an interaction between congruency and exercise condition.

Specifically, the CE was no longer reliable at high-intensity exercise. Similarly, time-frequency multivariate decoding failed to decode stimulus conflict, while other features such as stimulus content were decodable above chance levels. Whole-brain connectivity measures (weighted Symbolic Mutual Information) revealed no difference in the CE between intensity levels. These results demonstrate the robustness of the human cognitive control system even under strained high arousal states. We propose that the dissociation between behavioural and neural measures could indicate the activation of neural compensatory mechanisms as a response to physiological pressure.

**Disclosures: C. Avancini:** None. **L.F. Ciria:** None. **C. Alameda:** None. **A.F. Palenciano:** None. **A. Canales-Johnson:** None. **T.A. Bekinschtein:** None. **D. Sanabria:** None.

**Presentation Number:** NANO90.10

**Topic:** H.04. Executive Functions

**Support:** NIH Grant MH064498

**Title:** Representation of context and priority in working memory (in silico and in vivo)

**Authors: *Q. WAN**[1], A. ARDALAN[2], B. R. POSTLE[1];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

**Abstract:** How does the brain keep information in a readily accessible state while preventing it from interfering with ongoing behavior? A past fMRI study, using a double serial retrocuing (DSR) task, suggested that the neural representation of the stimulus undergoes a transformation as a function of priority (Yu, Teng & Postle, 2020). The task begins with the presentation of two samples, one after the other, followed by a retrocue (cue1) designating one the prioritized memory item (PMI) that will be tested at recall1. The uncued item (unprioritized memory item; UMI) can't be forgotten, however, because with $p = .5$ cue2 might designate it for recall2. To gain further mechanistic insights into how prioritization in working memory might be implemented computationally, we trained recurrent neural networks (RNNs) to perform a DSR task analogous to Yu, Teng and Postle (2020). To visualize the representational dynamics of stimulus information during the task, we conducted a principal component analysis (PCA) on the activity in the hidden layer of the RNN. We observed that the two stimuli are represented in quasi-orthogonal dimensions throughout the task, which serves to individuate the stimuli as a function of the temporal order in which they were presented. (A similar phenomenon was shown in RNNs trained on a 2-back working memory task.) This "context code" was confirmed when a multiclass linear support vector machine (SVM) classifier trained on items when presented in one ordinal position (e.g., "1$^{st}$") yielded only chance-level decoding accuracy when tested on these same items when they had been presented in the other (i.e., "2$^{nd}$"). The same cross-condition decoding approach showed evidence for concurrent priority coding (e.g., SVMs trained on PMI could not decode UMI). Armed with these observations, we applied the same logic of cross-condition decoding to the fMRI data from Yu, Teng and Postle (2020), in which subjects recalled the orientation of gratings presented serially, and at various locations. We found that, in early visual cortex, stimulus location was represented in a priority code, but not a context code (i.e. cross-condition decoding by context was successful). In FEF, in contrast, it was represented

in both a context code and a priority code. This regional heterogeneity of coding schemes may reflect difference in functional roles. For FEF, tracking context and behavioral priority are both necessary functions of a priority map. For representational cortex, in contrast, it may be that only representational transformations are needed to prevent interference at behavioral readout.

**Disclosures: Q. Wan:** None. **A. Ardalan:** None. **B.R. Postle:** None.

**Nanosymposium**

**NANO91: Synaptic and Cellular Mechanisms of Autism and Behaviors**

**Location:** WCC 150

**Time:** Wednesday, November 15, 2023, 1:00 PM - 2:45 PM

**Presentation Number:** NANO91.01

**Topic:** A.07. Developmental Disorders

**Support:** Weston Foundation Grant

**Title:** Effect of Feceal derived metabolites from Autistic individuals on zebrafish neural development

**Authors: *T. VAN RAAY**, V. REA, T. BALL;
Univ. of Guelph, Guelph, ON, Canada

**Abstract:** The microbiome has been implicated in autism due to the frequent comorbidity of gastrointestinal symptoms in idiopathic ASD, and correlations between gut microbiota metabolites and mRNA processing of the host. There are presently many difficulties investigating the role of microbiota on the host due to the complexity in microbiome taxonomy and variation between individual host microbiomes. In contrast, microbiome metabolites represent the net chemical output of the microbiome, and consequently, may be more conserved in biochemical function. Here, we use 2-day old zebrafish neurodevelopment as a proxy for evaluating the contribution of gut microbe metabolites derived from ASD versus neurotypical (NT) children on neural gene expression, mRNA processing, and sensory system patterning. Germ-free zebrafish were treated with different metabolite samples derived from a collection of age and gender matched NT and ASD children. We identified 275 genes with differential gene expression and 211 genes with differential exon use across treatments between NT and ASD but also differences between two ASD subgroups. Gene ontology analysis strongly suggests a link to RNA binding, including a group of 13 ribosomal protein genes that were upregulated in one of the two ASD subgroups. Investigation at a cellular level demonstrated perturbations in peripheral nervous system including terminal neuromasts of the posterior lateral line and peripheral nervous system ganglia. Overall, this data provides evidence that germ-free zebrafish are a useful model for understanding the impact and potential mechanism of human gut-derived metabolites on neural development.

**Disclosures: T. Van Raay:** None. **V. Rea:** None. **T. Ball:** None.

**Presentation Number:** NANO91.02

**Topic:** A.07. Developmental Disorders

**Support:** NICHD P50 HD093079

**Title:** Modeling brain overgrowth in autism using human pluripotent stem cells

**Authors: S. CHEN**[1], J. RAMESH[1], M. MAMUN[1], *S. CHETTY[2];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA

**Abstract:** Approximately 15-20% of individuals with Autism Spectrum Disorder (ASD) have disproportionate megalencephaly (ASD-DM), with disproportionate enlargement in both gray and white matter volume. Individuals with ASD-DM have more severe behavioral and cognitive problems and are less responsive to standard therapeutic interventions, leading to very poor prognoses relative to individuals with ASD and normal head circumferences. Increases in brain size often precede clinical symptoms, suggesting that understanding the underlying mechanisms regulating brain overgrowth could provide a window of opportunity for intervention or mitigation of symptoms. Here, we generated ~40 human iPSC lines from cohorts of children (2-4 years old) with complete clinical and phenotypic data, including A) ASD subjects with disproportionate megalencephaly, ASD-DM; B) ASD subjects with normal sized brains, ASD-N; C) Typically developing (TD) subjects with disproportionate megalencephaly, TD-DM; and and D) TD subjects with normal sized brains, TD-N. We differentiated each of the iPSC lines into neuroglial cells and investigated changes at the molecular and cellular levels contributing to brain overgrowth. In the differentiated neural and glial progenitor cells, we observe increased proliferation and suppressed phagocytosis by microglia and macrophages in ASD-DM. RNA-sequencing of the differentiated progenitor cells reveals important signaling mechanisms related to the neuroimmune system in regulating cellular phagocytosis. In prior work, we have demonstrated that CD47 (a ‘don’t eat me’ signal) is overexpressed in both NPCs and OPCs in 16p11.2 deletion carriers with macrocephaly contributing to reduced phagocytosis in vitro and in vivo. Treatment of 16p11.2 deletion NPCs and OPCs with an anti-CD47 antibody to block CD47 restores phagocytosis to control levels in cellular and mouse models. Here, we show that similar neuroimmune mechanisms commonly implicated in cancer regulate cellular homeostasis in idiopathic forms of autism. Furthermore, we highlight new forms of therapy for selected autistic individuals with brain overgrowth early in the disease.

**Disclosures: S. Chetty:** None.

**Presentation Number:** NANO91.03

**Topic:** A.07. Developmental Disorders

**Support:** NIH/NICHD R01 HD099162
NIH/NICHD F31 HD110206

**Title:** The role of the E3 ubiquitin ligase UBE3B in synaptic function and pathology

**Authors: *S. VASHISTH**[1], A. SHEDD[2], K. KAUR[3], K. M. HUBER[5], M. CHAHROUR[4];
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Southwestern Med. Ctr., Dallas, TX; [5]Dept. of Neuroscience, Peter O'Donnell Jr. Brain Inst., UT Southwestern Med. Ctr. at Dallas, Dallas, TX

**Abstract:** Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired communication, abnormal social behaviors, and restricted and repetitive behaviors. Pathogenic mutations in UBE3B result in neurodevelopmental disease, including intellectual disability, lack of speech, and ASD. UBE3B is an E3 ubiquitin ligase that tags substrate proteins with ubiquitin, marking them for proteasomal degradation. The ubiquitin-proteasome system (UPS) is known to regulate several signaling pathways critical for neurodevelopment, including neurogenesis and synaptogenesis, and mutations in various UPS genes have been identified in ASD and related neurodevelopmental disorders. To investigate the function of UBE3B and how its disruption gives rise to neurodevelopmental abnormalities, we generated a central nervous system (CNS)-specific conditional Ube3b knockout ($cKO^{nestin}$) mouse model and evaluated the resulting neurobehavioral phenotypes. We found that loss of UBE3B from the CNS results in significant deficits in ultrasonic vocalizations, social interaction, learning, and memory. Golgi-Cox staining and immunofluorescence showed significantly reduced dendritic complexity, length, and spine density of cortical neurons from $cKO^{nestin}$ mice, as well as fewer GluA1 puncta throughout the cortex. Further, $cKO^{nestin}$ mice demonstrate fewer spontaneous UP states, or periods of persistent network activity, at a reduced frequency in the cortex. To identify neuronal UBE3B substrates, we used stable isotope labeling by amino acids in cell culture (SILAC) of neural stem cells from wild type (WT) and constitutive Ube3b knockout (KO) mice followed by quantitative mass spectrometry. We filtered the data for candidate direct substrates that exhibited increased protein level and decreased ubiquitination in KO compared to WT cells. Ontology analyses indicated that many of the identified candidates (~41%) are involved in synaptic development and function, suggesting dysfunction in synaptic proteostasis following the loss of UBE3B. Our findings identify a role for UBE3B in regulating social behavior, learning, memory, and neuronal morphogenesis by fine-tuning the synaptic proteome, and suggest that it may be involved in the synaptic function underlying these behaviors. Ongoing and future studies will further investigate the specific neuronal substrates of UBE3B and the molecular pathways it regulates.

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**Presentation Number:** NANO91.04

**Topic:** A.07. Developmental Disorders

**Support:** RGC GRF 17620520
RGC RFS 2021-7H05

**Title:** Spectro-temporal dynamics and spatial characteristics of disrupted emotion-cognition interplay in the autistic brain

**Authors: *M. ZHANG**, X. WANG, H. K. LEE, S. X. TONG;
The Univ. of Hong Kong, Hong Kong, China

**Abstract:** Autistic individuals experience difficulties in perceiving and utilizing socio-emotional information to regulate their behaviors according to situational contexts. Despite accumulating

evidence indicating widespread neural anomalies in emotional and cognitive processing in autism, the specific neural mechanisms underlying the disrupted interplay between these two domains remain elusive. To address this issue, we used an emotional Go/Nogo task, where autistic ($n$ = 25, mean age = 11.01 ± .78 years, 2 girls) and non-autistic ($n$ = 25, mean age = 10.99 ± .73 years, 2 girls) children were instructed to respond to one particular facial expression (i.e., happy, angry, surprised or neutral; Go trials) and withhold responses to the other three expressions (Nogo trials), while measuring their electroencephalography (EEG). We applied generalized eigendecomposition (GED; a hypothesis-driven source separation technique) and time-frequency analysis to capture both the spatial characteristics and spectro-temporal dynamics of emotion-cognition interplay. A significant Emotion × Task interaction was observed in delta (1.5-3.5 Hz, 0-170 ms; $F_{3, 144}$ = 3.00, $p$ = .032) and alpha (8.5-13.5 Hz, 300-650 ms; $F_{3, 144}$ = 2.66, $p$ = .050) power over a frontocentral site in two separable time-frequency windows. In particular, when cued with happy faces, non-autistic children showed greater delta ($t_{24}$ = 2.25, $p$ = .034) and alpha ($t_{24}$ = 3.18, $p$ = .004) desynchronization in Nogo compared to Go trials, whereas such an effect was absent in autistic children ($p$s ≥ .100). This suggests a sluggish retrieval of social relevance (early delta) and attenuated attentional modulation of cognitive resources (alpha) in autistic children when they encounter conflicts between emotional information and task demands (e.g., happy faces signaling inhibition). Moreover, we found a significant Emotion × Group modulation on the parietooccipital beta power (24.5-29.5 Hz) in a later time window (400-750 ms; $F_{3, 144}$ = 2.96, $p$ = .033). Relative to the processing of neutral expression, the beta desynchronization to angry faces (irrespective of task demands) was reduced in autistic ($t_{24}$ = -2.12, $p$ = .045) but not in non-autistic children ($t_{24}$ = -.006, $p$ = .995). This could be attributed to diminished awareness of and/or fast disengagement from angry faces in autistic children after response selection. These findings suggest that compared to their neurotypical peers, autistic children are less sensitive to emotional valence and deficient in employing emotional cues to guide their cognition and behavior, and thus possess difficulties navigating the social world.

**Disclosures: M. Zhang:** None. **X. Wang:** None. **H.K. Lee:** None. **S.X. Tong:** None.

**Presentation Number:** NANO91.05

**Topic:** A.07. Developmental Disorders

**Support:** (NSERC) Discovery grants
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(CIHR) Project Scheme grant
(SSHRC) Insight grant

**Title:** Brainstem development of autistic children measured by the auditory brainstem response

**Authors: *A. A. SEIF**[1], M. RAJAB[1], R. GUERVILLE[1], K. SCHAAF[1], R. A. STEVENSON[2], S. SCHMID[1];
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**Abstract:** *Introduction:* Autistic children often display sensory sensitivities, sensory seeking/avoidance behaviors, and a range of other issues related to auditory sensory processing

disruptions. It has been shown that autistic individuals present abnormal cortex activation when processing acoustic stimuli and it is proposed that the brainstem is involved in that process. We have hypothesized that autistic children display delays in auditory brainstem maturation, which has been structurally observed by neuroimaging and post-mortem analysis. Functionally, this delay could be measured through the auditory brainstem responses (ABR), an auditory evoked potential recorded through electrodes on the scalp.
*Objective:* To investigate brainstem development of autistic children indexed via ABR.
*Methods:* Autistic (n=12; data collection ongoing) and non-autistic (n=12) children aged 8-13years completed a hearing evaluation and an ABR recording session. The hearing evaluation consisted of a visual inspection using an otoscope, tympanometry (226Hz probe), audiogram (250-8kHz), and distortion product otoacoustic emission evaluation (2kHz-8KHz). The ABR paradigm consisted of a slow click-rate (19.1clicks/second) and a fast click-rate (59.1clicks/second), and acoustic stimuli at 80dBnHL intensity across both ears. Participants listened passively while watching a silent video.
We conducted three-way, mixed-model, repeated-measure ANOVAs with within-subject factors of ear and stimulus click-rate, and a between-subject factor of diagnostic group. Absolute latencies of peaks I, II, III and V, and the inter-peak latencies (IPL) of peaks I-III and I-V were evaluated. In addition to the amplitude of peaks I and V, and the peak V to peak I ratio.
*Results:* Eight participants failed screening and were excluded. The overall ABR waveform showed a trend towards prolongation in the autistic relative to the non-autistic group. Main effects of group were observed for peak III ($F_{(1,12)}$ =5.806, p=.033) and IPL of peaks I-III ($F_{(1,12)}$=4.783, p=.049). No significant interactions were observed.
*Conclusion:* Autistic children showed a prolonged ABR waveform with increased latency of peak III and IPL I-III, which is indicative of reduced speed of neural conduction. The prolonged ABR waveforms of autistic children observed here resemble those of younger non-autistic children, supporting the hypothesis of a delay in auditory brainstem development.

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**Presentation Number:** NANO91.06

**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** R01DC018650
R00DC015014

**Title:** Revealing abrupt transitions from goal-directed to habitual behavior

**Authors: *S. MOORE CORONA**, Z. WANG, Z. ZHU, R. SUN, A. LEE, A. CHARLES, K. V. KUCHIBHOTLA;
Johns Hopkins Univ., Baltimore, MD

**Abstract:** A fundamental tenet of animal behavior is that decision-making involves multiple 'controllers.' Initially, behavior is goal-directed, driven by desired outcomes, shifting later to habitual control, where cues trigger actions independent of motivational state. Clark Hull's question from 1943 still resonates today: "Is this transition [to habit] abrupt, or is it gradual and progressive?" Despite a century-long belief in gradual transitions, this question remains

unanswered as current methods cannot disambiguate goal-directed versus habitual control in real-time. Motivation is the basis of goal-directed behaviors, while not the main driver of habitual performance, and thus we sought to study habit expression *en passant*, in individual mice. To do so, we introduce a novel 'volitional engagement' approach, motivating animals by palatability rather than biological need. Offering less palatable water in the home cage reduced motivation to 'work' for plain water in an auditory discrimination task when compared to water-restricted animals. Using quantitative behavior and computational modeling, we found that palatability-driven animals learned to discriminate as quickly as water-restricted animals but exhibited state-like fluctuations when responding to the reward-predicting cue—reflecting goal-directed behavior. These fluctuations spontaneously and abruptly ceased after thousands of trials, with animals now always responding to the reward-predicting cue. In line with habitual control, post-transition behavior displayed motor automaticity, decreased error sensitivity (assessed via pupillary responses), and insensitivity to outcome devaluation. Surprisingly, some animals reverted to goal-directed behavior after several sessions showing habitual behavior, suggesting that transitions to habitual decision-making are not permanent. Bilateral lesions of the habit-related dorsolateral striatum (DLS) blocked transitions to habitual behavior. Preliminary recordings taken simultaneously in the dorsomedial striatum (DMS) and DLS are used to understand the interplay between these two areas around the transition point. Thus, 'volitional engagement' reveals spontaneous and abrupt transitions from goal-directed to habitual behavior, suggesting the involvement of a higher-level process that arbitrates between the two. Understanding the exact time course of habit formation opens new avenues to further explore its neural correlates, develop predictive models of habitual behavior and implement timed interventional strategies to manipulate it.

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**Presentation Number:** NANO91.07

**Topic:** G.02. Reward and Appetitive Learning and Memory

**Support:** Intramural Research Grant

**Title:** Two groups of midbrain dopamine neurons have opposing and complementary functions in regulating behavior

**Authors: *G. COSTELLO**, O. HIKOSAKA;
Natl. Eye Institute/NIH, Bethesda, MD

**Abstract:** Midbrain Dopamine (DA) is known to have multiple functions. It plays a critical role in reward-related learning, and the reward prediction hypothesis (RPE). Recently, an alternative saliency hypothetical framework has proposed that DA activity reflects the relative salience of stimuli independent of their reward value. Favoring this hypothesis, our lab and others have also observed increased phasic activity in dopamine neurons to non-rewarding or even aversive stimuli. In this study we aimed to examine whether aversion, movement and reward encoding co-occurs in the same neurons or are separately encoded by different groups of dopamine neurons and how these fit within the current reward prediction error framework or salience framework. To solve this, we further characterized the functional specialization of dopamine cells within the

dorsolateral and ventromedial Substantia Nigra pars compacta (SNc) by screening individual neurons with a broad spectrum of behavioral tasks. We found two subtypes of DA neurons 1) localized in the dorsolateral SNc with closely resembling DA salience and long-term memory. These neurons also showed pre-saccadic activity and spatial selectivity. 2) DA neurons localized in the ventromedial SNc more closely resembled DA value responses and short-term memory. They had post-saccadic activity and no spatial selectivity as well as showed modulation based on RPE. This study reveals insights into the precise relationship between dopamine activity, object selection and the movement initiation. Taken together, these results suggest that these two groups of DA neurons, as well as two parallel circuits in the basal ganglia, have opposing functions in regulating behavior but work simultaneously in concert and compete at a global scale allowing animals to adapt in different environments.

**Disclosures: G. Costello:** None. **O. Hikosaka:** None.

**Nanosymposium**

**NANO92: Computational Tools for Physiological Data Analysis**

**Location:** WCC 147B

**Time:** Wednesday, November 15, 2023, 1:00 PM - 3:00 PM

**Presentation Number:** NANO92.01

**Topic:** I.06. Computation, Modeling, and Simulation

**Title:** Latent quotient space for extreme points neighborhood applied over discrete signal time series of MEG recordings

**Authors: *A. KATZ**;
Mathematics, Bar Ilan Univ., Ramat Gan, Israel

**Abstract:** Several studies have reported methods for signal similarity measurement; however, a similarity (or distance function) that concludes time and space features in data remains to be accomplished. In the research reported here, we developed a new metric that relates to the properties of time and space together with great precision. The proposed application of the newly defined metric shows that its latent geometric characteristics may substantially affect the similarity between signals in the absence of differences measuredby leading existing metrics. Latent geometric characteristics are expected to be an important analytic feature of MEG recordings, known for their high spatial and temporal resolution, which can benoticed in a cross-area projection of brain activity.

**Disclosures: A. Katz:** None.

**Presentation Number:** NANO92.02

**Topic:** I.06. Computation, Modeling, and Simulation

**Support:** NIH SPARC OT2 OD025340
NIH SPARC 75N98022C00018

**Title:** Decoding the distribution of conduction speeds from neural recordings in rat cervical vagus nerve in silico and in vivo

**Authors: *E. PEÑA**, W. M. GRILL, N. A. PELOT;
Biomed. Engin., Duke Univ., Durham, NC

**Abstract:** Nerves transmit a range of information about homeostasis and organ function that can serve as feedback for closed-loop therapies to treat hypertension, diabetes, and inflammatory diseases. Nerve fibers conduct action potentials at different speeds depending on their diameter and myelination; reconstructing the distribution of these speeds (conduction velocity distribution; CVD) using neural recordings could facilitate decoding of clinically relevant physiological information. Previous studies developed optimization approaches for CVD reconstruction, but there is limited information quantifying the accuracy of reconstruction methods against ground truth or comparing performance across methods, thus limiting the reliability of CVD reconstructions. We compared accuracy of published methods in reconstructing CVDs from compound nerve action potential (CNAP) recordings from rat cervical vagus nerve that were either recorded in vivo or simulated in a computational model. We recorded CNAPs in vivo and we collected nerve samples to quantify nerve-specific fiber diameter distributions from histology. We simulated CNAPs using a biophysically-based framework for modeling nerve recordings, which incorporated the nerve-specific fiber diameters (and associated CVD), nerve morphology, and cuff geometry. The optimization problem formulation and parameters used greatly affected accuracy of the CVD reconstructions. The simplest and most widely used approach—unconstrained, non-regularized least squares—produced biologically infeasible features (e.g., negative counts of some fiber diameters) at certain discretization values of conduction speeds. Constrained and regularized approaches were less sensitive to discretization value. The nonnegative-constrained and smoothness-regularized approach with 100 bins applied on the simulated CNAP produced a CVD that matched the nerve-specific CVD shape, fiber counts, and median and mode of conduction speeds. The same approach on in vivo CNAP data produced a CVD with similar shape, fiber counts, and median of conduction speeds, but the mode of conduction speeds was 74% faster due to the in vivo CNAP having slightly shorter latency than the simulated CNAP. Our comparisons of methods for reconstructing CVD identified nonnegative-constrained smoothness-regularized least squares as the most robust among published methods. Our work highlights the potential for biophysically-based CNAP modeling to extract information from peripheral nerves that can inform closed-loop bioelectronic therapies.

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**Title:** Efficient Sampling-Based Bayesian Active Learning for synaptic characterization

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**Abstract:** Optimizing the parameters of an experiment is a critical problem in neuroscience. Experimental design usually involves setting a plethora of parameters: which stimulations to perform, how to determine measurement points, etc. These parameters will be critical to the output of an experiment. However, most neuroscience experiments still rely on fixed, non-adaptive designs, which may not yield sufficient information about the studied system. Consequently, experiments often require more observations to reach a certain result, which increases their cost and need for subjects.
An efficient framework to alleviate this issue is called Bayesian Active Learning (BAL). BAL selects experimental parameters to optimize a given output (for instance, to reduce the uncertainty of inferred parameters, or to increase the information gained per observation). But the applicability of theoretical BAL methods to actual experiments is limited as it requires performing high-dimensional optimizations in real time. Current methods are either too time consuming for sequential experiments, or only applicable to specific models.
Here, we developed a method called Efficient Sampling-Based Bayesian Active Learning (ESB-BAL). After each new observation, ESB-BAL computes on-the-fly the next experimental input to maximize the mutual information between the output of the experiment and the parameters to be estimated. The experimental design is thus continuously optimized in a closed-loop manner. To achieve this, we implemented three technical innovations: the use of particle filtering for representing the knowledge from previous observations, an efficient GPU implementation for parallel particles propagation, and mean-field approximations to estimate the current state of the system.
To validate it, we apply ESB-BAL to the problem of estimating the parameters of a synapse (e.g., the number of vesicles, or the time constant of depression) from the postsynaptic responses to evoked presynaptic action potentials: the accuracy of these estimated parameters will greatly depend on the chosen stimulation times. After each new observation, the optimal next stimulation time is computed on a millisecond scale. Using synthetic data and synaptic whole-cell patch-clamp recordings, we show that our method can significantly reduce the uncertainty of inferred parameters. We also demonstrate that ESB-BAL can be used to optimize the rate of information gained by unit of time, and to optimize batches of future experimental inputs. Overall, ESB-BAL is fast enough to be applicable to different experimental settings and is paving the way towards adaptive experimental designs in neuroscience.

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**Title:** Evaluation of time-delayed functional connectivity with whole-brain simulations

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**Abstract:** As electroencephalography (EEG) lacks a ground-truth, simulation studies are used to evaluate methods such as functional connectivity (FC) prior to applying them to real data. FC refers to the statistical dependencies among the activity of distinct brain regions and is widely used to investigate brain function. EEG-based simulation methods are usually based on an underlying model for brain activity at different regions (or sources) which is projected to the channel level via a lead field. While the lead field incorporates the effect of the volume conduction through linear mixing of the simulated sources, the simulated source activity should mimic other aspects of EEG data such as distinct frequency peaks of the power spectral density, the 1/f noise or time-delayed FC. In this work, we propose to use whole-brain simulations of EEG activity to serve as ground-truth for the evaluation of EEG-based FC measures. More specifically, the Jansen-Rit model [Jansen & Rit, 1995] was considered to model the local brain activity at the individual sources. Neural models were placed at the centers of 68 brain regions based on the Desikan-Killiany atlas [Desikan et al., 2006] and were interconnected based on the structural connectome and coupling functions (global model) to simulate whole-brain EEG at the source level [Sanz Leon et al., 2013]. In our study, the structural data from eight healthy adult participants from the LEMON dataset [Babayan et al., 2019] were used to derive structural connectomes from diffusion weighted imaging (DWI). The source-level simulated neural data were then projected into the EEG channels through individual lead fields for each participant. Through controlled simulations, we found sets of parameters that can mimic the time-delayed functional connectivity aspects of EEG data such as imaginary coherency [Nolte et al., 2004] and time-reversed Granger causality [Haufe et al., 2013]. Furthermore, we provide a pipeline for the evaluation of existing measures that can be extended to newly developed EEG-based FC measures in the future. Therefore, our simulation approach brings the community one step closer to the realistic evaluation of EEG-based functional connectivity prior to application to real data.

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**Title:** Monitoring tonic concentrations of dopamine and serotonin after drug of abuse administration using voltammetry and deep learning

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**Abstract: Background and Aims** Substance use disorder (SUD) is a prevalent problem globally. It is thought that drugs of abuse (DoA) increase signaling of several neurotransmitters, including dopamine (DA) and serotonin (5-HT), and that this signaling underlies their addictive potential. Voltammetry offers the ability to track tonic neurotransmitter concentrations with high spatiotemporal resolution. However, voltammetry struggles to discriminate between

electrochemically similar neurotransmitters. To overcome this issue, we have developed a deep learning algorithm (DiscrimNet) to resolve individual concentrations of DA and 5-HT. We then utilized multiple cyclic square-wave voltammetry (M-CSWV) in combination with DiscrimNet to track individual tonic neurotransmitter concentrations with high spatiotemporal resolution (~10s/scan) during DoA (cocaine and oxycodone) action and ventral tegmental area (VTA) deep brain stimulation (DBS). **Methods** A carbon fiber microelectrode was stereotactically implanted into the nucleus accumbens (NAc) of urethane-anesthetized Sprague-Dawley rats. After one hour of baseline neurochemical recording was obtained using M-CSWV, cocaine or oxycodone was administered (i.v.). 30 minutes after drug administration, VTA DBS (90Hz, biphasic 200μs pulse-width, 0.2mA) was delivered continuously for 30 minutes, and the resulting effects on tonic neurotransmitter levels were monitored. To capture the entire time course of DoA action, 3 hours of tonic measurements were performed. DiscrimNet was used to isolate individual concentrations of DA and 5-HT from the total neurotransmitter signal captured by M-CSWV. **Results** Tonic extracellular DA and 5-HT concentrations were increased from baseline by cocaine (DA: +48.3±7.1nM, 5-HT: +19.0±4.1nM) and oxycodone (DA: +95.2±14nM, 5-HT: +47.2±7.4nM). VTA DBS abolished the DoA-elicited DA increase and did not affect 5-HT concentrations. **Conclusions** DiscrimNet and M-CSWV were able to capture and monitor dopamine and serotonin concentrations separately during DoA exposure with high spatiotemporal resolution. VTA DBS was shown to reverse the acute dopamine increase resulting from DoA exposure. These results suggest the exciting possibility that DBS can modulate the addictive potential of drugs of abuse and may perhaps be a treatment for SUD.

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**Title:** Signal unmixing and spatiotemporal pattern extraction algorithms for the analysis of fluorescence voltage imaging recordings of neural population activity

**Authors: *V. KRUZHILIN**[1], R. T. CHRAPKIEWICZ[2], S. HAZIZA[2], Y. ZHANG[2,3], M. J. SCHNITZER[4,5,3];
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**Abstract:** Neocortical dynamics can organize into waves and oscillations that have been implicated in sensory processing, attention, motor control, and various brain disorders. Certain oscillations may facilitate brain area interactions, such as memory consolidation. Toward understanding these dynamics, recent methods for voltage imaging now allow recordings of the collective activity patterns of specific neuron types. Accompanying this experimental approach, there are two important data analysis challenges: (1) The small dynamic range of fluorescence voltage signals implies that noise and optical artifacts must be removed from the optical voltage traces; (2) The spatiotemporal patterns of propagating voltage oscillations must be quantitatively characterized. We present methods to address each of these challenges.

First, we created a frequency-dependent filtering approach that separates signals reflecting factors other than neural voltage dynamics (e.g., hemodynamics, brain motion, or electronic noise) from neural voltage signals without introducing further noise. Our unmixing method accounts for spatially varying spectral amplitudes and phase delays between the voltage and reference fluorescence channels in optical recordings. With this method, we observed cortical voltage waves with amplitudes as weak as ~0.1% changes in fluorescence intensity at temporal frequencies up to ~80 Hz during visual stimulus presentation on a single trial level. We also applied our method to fiber photometry recordings from hippocampus and found that unmixing improved the coherence of cell-type-specific fluorescence voltage traces with concurrently acquired local field potential recordings.
Second, to extract the spatiotemporal structure of cortical voltage dynamics, we used an autoregressive method that fits a linear dynamical system model to the observed activity. Low-rank representations allowed us to compare voltage activity across different brain states and animals. When applied to data from anesthetized mice, our analysis revealed stereotypical spatiotemporal patterns of propagating delta-band (0.5-3 Hz) activity. In awake mice, we consistently found spontaneous alpha-like waves (5-10 Hz). Finally, we analyzed activity changes during transitions between anesthetized and awake brain states, which revealed signature shifts in the frequencies and propagation directions of the dominant cortical voltage wave patterns.
Overall, our analysis methods constitute essential new tools to help neuroscientists achieve empirically grounded insights into the characteristic dynamics of neocortical activity, as captured by voltage imaging.

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**Title:** A machine learning approach to identifying brain-wide connectivity patterns resulting from selection on social behavior in the Russian farm-fox experiment

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**Abstract:** Understanding the underlying neural mechanisms that govern behavior is a major goal of neuroscience. We contribute to this aim by using machine learning (ML) to identify the differences between DTI connectivity matrices, acquired with a multishell diffusion sequence on a Bruker 9.4T MRI at a resolution of 300 cubic microns, of 30 male foxes from the Russian farm-fox experiment. The ongoing 64 year-old farm-fox experiment is a uniquely rigorous study which parallels wolf-to-dog domesticity via the behavioral selection of foxes towards three lineages: tame, aggressive, and control. Previous neuromorphological research of these 30 foxes

has identified gray matter alterations in prefrontal and limbic regions. Our work now extends this neuroscientific analysis of domestication using an ensemble of ML algorithms to (1) quantify the degree of difference between the DTI connectivity matrices across the three fox lineages, and (2) to specifically identify a small subset of voxels which allow an algorithm to classify the DTI matrices with novel accuracy. Our approach represents a data-driven analysis of all voxel-to-voxel connections across individual whole-brain connectomes, an analysis goal that has historically proven challenging in neuroimaging connectivity research. We achieve this using data augmentation to enhance the small dataset, Recursive Feature Elimination to identify key voxels, supervised ML to classify the DTI matrices with unprecedented accuracy, and unsupervised ML for dimensionality reduction to visualize the results. We postulate that our algorithm's ability to distinguish between DTI matrices of the lineages with high accuracy using just a minute fraction of the provided voxels can substantiate neuromorphological evidence of the brain regions in which domestic behavior is manifested. Results are further discussed in relation to the neural mechanisms of social behavior in other species.

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**Title:** Discovering neural policies to drive animal behavior using deep reinforcement learning

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**Abstract:** Deep reinforcement learning has been successful in a variety of domains but has not yet been directly used to learn biological tasks by interacting with a living nervous system. As proof-of-principle, we show how to create such a hybrid system trained on a target-finding task. Using optogenetics, we interfaced the nervous system of the nematode *Caenorhabditis elegans* with a deep reinforcement learning (RL) agent. Agents adapted to strikingly different sites of neural integration and learned site-specific activations to guide animals toward a target. This adaptation to the site of integration was reflected in the different policies learned by the agents and demonstrated the agents' ability to use information in a neural circuit-specific fashion without any prior knowledge about neural functions. Six different optogenetic lines were evaluated (n=10 for each experimental condition) and for five of these, agents succeeded in driving animals to targets compared to controls ($p<.01$ for the three best-performing lines). Further, by exploiting the animal's innate sensory abilities, RL agents could drive effective behaviors in a novel environment that they had not previously seen. For instance, in food search tasks with obstacles, agents improved animals' abilities to find food from 0 of 20 trials to 11 of 20 trials ($p<.001$) for the best-performing line. Thus, the animal-RL agent hybrid achieved cooperative computation rather than the agent acting as a controller for a soft robot. Our system demonstrates that deep RL is both a viable tool for learning neural circuit dynamics that can

produce goal-directed behavior without prior knowledge and for improving biologically relevant behavior in a flexible and robust way.

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**Topic:** I.06. Computation, Modeling, and Simulation

**Title:** A signal processing method applied to the electroencephalogram as inspired by the concept of striatal beat frequency is used to form a basis from which to separate frequency specific beat patterns is developed through the exploitation of single periods of single frequencies and correlation coefficient

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**Abstract:** This paper proposes a method for time-domain analysis of an electroencephalogram (EEG) using the theory of interval timing and Striatal Beat Frequency (SBF) first developed by Matell & Meck back in the year 2000. We first form a bank of single-period single-frequency (SPSF) sinusoids where the number of points making up each SPSF sinusoid is commensurate with the EEG device sampling rate. Then we choose a common starting reference point in the EEG where we center each individual SPSF and record each separate correlation coefficient (CC) in a column of a matrix where each row corresponds to a separate SPSF. As the bank of SPSFs is moved to the next point in time in the EEG another set of CCs is calculated and recorded in the next column of the matrix. The process is continued until the end of the EEG. In this way we avoid the inevitable drawback of non-periodic chopping within a windowed DFT and that of non-normalized outputs and frequency spreading of the Geophysics-derived Wavelet constructs. We may think of this process as a slow Fourier transform that is organized and normalized at each instance. From this matrix of CCs we can now place intuitive thresholds in order to visualize the on-off pattern of our modeled SBFs across each individual row in time and may act as a common standardized basis that is easily reproducible because of its bare-bones construction and more importantly, current algorithms will no longer be constrained the usual (delta, theta, beta, and gamma bands) bands or to a particular wavelet shape, and in the case of estimating phase locking values the SPSF approach may mitigate the problems associated with applying the Hilbert transform function inside a band-passed filtered window of EEG. Using epileptic seizure data, sampled at 256 Hz, from the ChB MIT scalp EEG database and Temple University Seizure Detection Corpus we compared the outputs from the DFT, Wavelet, and SPSF methods and found that the SPSF method identified low amplitude frequency components that were overlooked by the Wavelet method and it outperformed the DFT by clearly identifying on-off sequences of specific rows of frequency components, in particular at the 2, 7, and 15 Hz rows for this particular patient, whereas the DFT was smeared across the spectral output. There was evidence that the SPSF could draw attention to early-onset detection of non-convulsive status epilepticus (NCSE). We will show additional results of the SPSF method from an EEG recording from this 63-year-old male author at a sampling rate of 19.2 KHz which is free from 60 Hz contamination.

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