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Nanosymposium

NANO01: Ligand:Gated Receptors and Ion Channels: Structure, Function and Regulation

Location: MCP Room N227

Time: Saturday, October 5, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO01.01

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

Support: VR Grant 2019-02433
VR Grant 2021-05806

Title: Potentiation of ligand-gated ion channels by stimulant derivatives via a vestibular binding site

Authors: E. KARLSSON[1], N. HALOI[2], M. DELARUE[3], ***R. J. HOWARD**[1,4], E. LINDAHL[1,4]; [1]Biochem. & Biophysics, Stockholm Univ., Solna, Sweden; [2]Applied Physics, KTH Royal Inst. of Technol., Solna, Sweden; [3]Unite Dynamique Structurale des Macromolecules, Pasteur Inst., Paris, France; [4]Applied Physics, KTH Royal Institute of Technology, Solna, Sweden

Abstract: The superfamily of pentameric ligand-gated ion channels includes eukaryotic and prokaryotic receptors for GABA, glycine, acetylcholine, serotonin, glutamate, and environmental factors such as pH and metal ions. Allosteric modulation in this family is critical to the action of neurotransmitters and many psychoactive drugs. However, details of their modulatory mechanisms remain unclear, especially beyond the orthosteric neurotransmitter-binding sites. Here, we show that both the mammalian serotonin-3A receptor and the homologous bacterial protein sTeLIC are functionally enhanced by psychostimulant derivatives. Analysis of electron cryomicroscopy structures, enhanced-sampling molecular dynamics simulations, and electrophysiological recordings from engineered mutants supports a modulatory site distal to the neurotransmitter interface. By binding in an intrasubunit pocket facing the extracellular vestibule, these compounds facilitate intersubunit interactions associated with channel activation. This work supports a detailed structure-function mechanism for receptor potentiation via a noncanonical site, with direct implications for understanding and modifying psychiatric and gastrointestinal drug effects.

Disclosures: E. Karlsson: None. **N. haloi:** None. **M. Delarue:** None. **R.J. Howard:** None. **E. Lindahl:** None.

Presentation Number: NANO01.02

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

Support: EMBO ALTF 542-2021
Swedish Research Council 2019-02433
Swedish Research Council 2021-05806
Stockholm University FV-5.1.2-0523-19
Swedish e-Science Research Center

Title: Structural basis for activation and potentiation of human a5b3 GABAA receptors

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Abstract: General anesthetics like etomidate mediate amnesic as well as sedative effects through different populations of gamma amino butyric acid (GABA) receptors in the central nervous system. The amnesic effects have largely been attributed to α5-subunit-containing receptors in the hippocampus, indicating a clear role in learning and memory for these receptors. The α5 subunit is thought to primarily coassemble with β3 and, in some cases, γ2 subunits, generating a variety of receptor subtypes with differential functional and pharmacological properties. However, the stoichiometry, structure, and gating mechanisms of these different subpopulations are not well understood. Here we report structures of human α5β3 GABAA receptors with various modulators, assembled in two stoichiometries. Our electron cryomicroscopy (cryo-EM) structures, combined with electrophysiology in Xenopus oocytes, support a primary assembly of 2:3 α:β subunits, though a minority population of 1:4 α:β indicates multiple assemblies are possible. Differential glycosylation of the α5 and β3 subunits enabled reconstruction of the heteromeric complex even in the absence of added fiducials. In the resting state of the receptor, Zn2+ binds to histidine residues at the M2-17′ position in the β3 subunit, blocking ion passage. In the activated state, GABA binding to the orthosteric site is associated with global rearrangements propagating to unbinding of the 17′ Zn2+ atoms and opening of the 9′ hydrophobic gate. Unlike the α1β3 receptor where GABA is effectively a partial agonist, saturating GABA binding to α5β3 appears to drive activation of nearly all receptors, resulting in a single desensitized state observed under the cryo-EM conditions. In agreement with this high GABA efficacy, the GABA-bound structure is virtually unaffected by further addition of the anesthetic etomidate at the β-α transmembrane domain interface. Taken together, these structures offer newly detailed models for gating and modulation of a GABAA-receptor subtype critical to learning and memory, including prospective templates for structure-based drug discovery.

Disclosures: J. Cowgill: None. **C. Fan:** None. **R.J. Howard:** None. **E.R. Lindahl:** None.

Presentation Number: NANO01.03

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

Support: NIH Grant F32GM142233
NIH Grant R35GM134896
NIH Grant R35GM134896-2S1
BBSRC BB/S001247/1
UKRI-BBSRC BB/M011224/1

Title: Mechanistic studies of canonical and non-canonical activation of a heteromeric glycine receptor.

Authors: *E. GIBBS[1], P. BIGGIN[2], D. SEIFERTH[2], B. FEDDERSEN[2];
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Abstract: Glycine receptors (GlyR) are chloride-conducting pentameric ligand-gated ion channels (pLGICs) that provide inhibitory input within the spinal cord. Their proper function is essential for muscle relaxation and also plays an important role in modulating responses to auditory, visual and noxious stimuli. Glycinergic signaling is an interesting pathway for pain therapeutics due to its limited influence on higher brain structures. However, targeting GlyR requires a better understanding of synaptic GlyR architecture and the molecular mechanisms underlying channel activation. Notably, while most mechanistic work on GlyR has been done with homomeric channels, synaptic GlyR is necessarily heteromeric as homologous α and β subunits play distinct and necessary roles in channel function and synaptic localization. Here, we present single particle cryo-EM structures of zebrafish α1/β heteromeric GlyR in the presence of a canonical agonist, glycine, a canonical antagonist, strychnine and a positive allosteric modulator/non-canonical agonist ivermectin. We observe a 4α:1β channel stoichiometry, consistent with other recent studies. Canonical agonists bind in subunit interfaces in extracellular domain of GlyR and like other pLGICs cause a global symmetric twist of both the extracellular and transmembrane domains. Canonical antagonists induce similar movements but in the opposite direction. Global changes are consistent with past work done with homomeric receptors, but differences in subunit side chains near the channel pore rationalize known functional differences between homomeric and heteromeric channels. Canonical ligand binding between different subunit interfaces is near symmetric as the binding pocket residues are mostly conserved. By contrast, ivermectin binds at the extracellular leaflet between subunits in a pocket that is not conserved between GlyR subunits. Binding is observed to differ at subunit interfaces that involve the sole β subunit and asymmetric effects on the channel are observed. Together these results highlight the relationship between the structure and function of GlyR. This information is valuable not only to molecular studies but also more broadly as technological advances bridge the gap between molecular and cellular research.

Disclosures: E. Gibbs: None. **P. Biggin:** None.

Presentation Number: NANO01.04

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

Support: NIH NINDS NS077114

Title: Effects of chaperones RIC-3 and NACHO on the functional surface expression of nACh receptor subtype α7

Authors: *H. Q. DO[1], I. KIM CAVDAR[1], N. SARAYLI BELIRGEN[1], P. N. GROZDANOV[2], R. RAMANI[1], J. THERIOT[1], M. JANSEN[1];
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Abstract: Neuronal nicotinic acetylcholine receptor subtype α7 (α7 nAChR) is widely expressed throughout the central nervous system, including in hippocampal interneurons and astrocytes, which regulate information processing, learning, and memory formation. The α7 nAChR is a member of the pentameric ligand-gated ion channel superfamily and highly permeable to calcium. This leads to its extensive involvement in multiple signaling pathways, including Janus kinase 2 (JAK2), phosphatidylinositol 3-kinase (PI3K) or PI3K/AKT, and ERK-MAP kinase. The α7 nAChR, therefore, is implicated in both neuroprotection and neurotoxicity in

Alzheimer's disease, schizophrenia, and autism spectrum disorders. The dual roles of α7 nAChR in neuroprotection and neurotoxicity may result from the unbalanced regulation of its functional surface expression by scaffold proteins (PICK1), regulatory proteins (Ly6h), and chaperones (RIC-3, NACHO). PICK1 and Ly6h negatively regulate functional expression, while chaperones RIC-3 and NACHO positively regulate this expression. The interactions of α7 nAChR with PICK1, Ly6h, RIC-3, and NACHO and their modulation of the functional expression of α7 nAChR have been explored in the last two decades. However, the underlying mechanisms governing the functional expression of α7 nAChR through these interacting partners, as a whole, are not fully understood. Therefore, there are no effective therapeutics to control the neuroprotective and neurotoxic functions of α7 nAChR. Here, we explore the functional surface expression of α7 nAChR under the regulation of the chaperone proteins RIC-3 and NACHO. Using AlphaFold, mutagenesis, pull-down assays, and electrophysiology, we reveal the sequence determinants in the interaction of RIC-3 and α7 nAChR, which affect the functional expression of α7 nAChR. We also characterize the current properties of α7 nAChR under the effects of RIC-3, NACHO, and α7 nAChR modulators. Our findings suggest that RIC-3 and NACHO independently regulate the assembly of α7 nAChR via distinct interaction mechanisms. Our work provides insights into the modulation of α7 nAChR assembly and sets the stage for additional studies.

Disclosures: H.Q. Do: A. Employment/Salary (full or part-time):; Texas Tech University HSC. **I. Kim Cavdar:** None. **N. Sarayli Belirgen:** A. Employment/Salary (full or part-time):; Texas Tech University HSC. **P.N. Grozdanov:** None. **R. Ramani:** None. **J. Theriot:** None. **M. Jansen:** A. Employment/Salary (full or part-time):; Texas Tech University HSC.

Presentation Number: NANO01.05

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

Title: Magnetogenetic silencing of neuronal activity

Authors: *C. PARK[1], Y. LEE[1], M. KWAK[1], L. JAE HYUN[1], C. LEE[2], J. CHEON[1];
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Abstract: The reversible silencing of neuronal activity with spatiotemporal precision is a potent approach for unraveling the functional roles of specific brain regions, circuits, and cells. Magnetism possesses the unique capability to penetrate deeper into tissues, offering unprecedented potential for wireless and remote deep brain stimulation. In this study, we introduce a novel magneto-mechanical-genetic toolbox for neuronal silencing utilizing the mechanosensitive anion channel FLYC1. Our approach leverages nanoscale magnetic force actuators (m-Torquers), which apply torque force under uniform, rotating magnetic fields to trigger the mechanical gating of FLYC1 ion channels and induce chloride ion influxes. The magneto-mechanical activation of FLYC1 effectively hyperpolarizes neurons and inhibits action potential firing, as confirmed by optical and electrophysiological recordings. Furthermore, FLYC1 was genetically engineered to have mutations for the increased mechanosensitivity and chloride permeability, leading to stronger neuronal inhibition. Our study demonstrates, for the first time, that magnetism can be used to silence neuronal activity of freely moving animals in wireless and spatiotemporal manner.

Disclosures: C. Park: None. **Y. Lee:** None. **M. Kwak:** None. **L. Jae hyun:** None. **C. Lee:** None. **J. Cheon:** None.

Presentation Number: NANO01.06

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

Support: NIH Grant DA04735
NIH Grant NS130831

Title: Structural investigations of GABA-A autoimmune encephalitis

Authors: *C. M. NOVIELLO[1], R. E. HIBBS[2], J. KREYE[3], J. TENG[2];
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Abstract: Autoantibodies targeting GABAA receptors can cause encephalitis, seizures, and severe behavioral abnormalities. Here we explored mechanisms of autoantibody attack on GABA-A receptors via cryo-electron microscopy. These antibodies induced severe encephalitis by directly inhibiting GABAA function, resulting in nervous-system hyperexcitability. We further explore mechanisms of autoantibody interference with GABAA receptors by structural comparisons, and we identify key residues in these antibodies involved in specificity and affinity. Finally, we confirm structure-based hypotheses for functional effects using electrophysiology. Together these studies identify mechanisms of direct functional antagonism of neurotransmission underlying autoimmune encephalitis in humans.

Disclosures: C.M. Noviello: None. **R.E. Hibbs:** None. **J. Kreye:** None. **J. Teng:** None.

Presentation Number: NANO01.07

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

Support: NIH DA047325
24PRE1189840

Title: Structural insights into $GABA_A$ receptor potentiation by Quaalude.

Authors: *W. CHOJNACKA[1], R. E. HIBBS[2];
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Abstract: Methaqualone, a quinazolinone marketed commercially as Quaalude, is a central nervous system depressant that was used clinically as an anxiolytic and a sedative-hypnotic, then became a notorious recreational drug in the 1960s-80s. Due to its high abuse potential, methaqualone was outlawed for medical use, but remains a drug of abuse around the world. Methaqualone principally acts upon GABAA receptors, which are the major inhibitory neurotransmitter receptors in the central nervous system. Its restricted status and limited accessibility have led to methaqualone's pharmacology being understudied. Here we use cryo-EM to localize the GABAA receptor binding sites of methaqualone and its more potent derivative, PPTQ, to the same intersubunit transmembrane sites shared by the general anesthetics

propofol and etomidate. We observe these ligands inserting more deeply into subunit interfaces than previously-characterized modulators. We elucidate the mechanism of action of quinazolinones; where their binding causes widening of the extracellular half of the ion-conducting pore, establishing a trend among positive allosteric modulators in destabilizing the hydrophobic activation gate in the pore as a mechanism for potentiation.

Disclosures: W. Chojnacka: None. **R.E. Hibbs:** None.

Nanosymposium

NANO02: Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Room S404

Time: Saturday, October 5, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO02.01

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

Support: PRIN 2022ZYLB7B

Title: A vicious circle among interleukin-1β, amyloid-β and Tau underlies synaptic and memory deficits in mouse models of Alzheimer's disease infected with Herpes simplex virus type-1

Authors: *D. D. LI PUMA[1,2], R. PIACENTINI[1,2], B. BANDIERA[1], G. PULIATTI[1], G. BONI[1], G. DE CHIARA[3], A. T. PALAMARA[4,5], C. GRASSI[1,2];
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Abstract: We recently demonstrated that spreading of Herpes Simplex virus type 1 (HSV-1) infection to the CNS, induced by thermal stress (TS), triggers the accumulation of Alzheimer's disease (AD) molecular hallmarks and the development of an AD-like phenotype in wild-type (WT) C57BL/6 mice. Specifically, infected mice undergone 2TS exhibited synaptic and memory deficits along with increased levels of interleukin-1β (IL-1β) and a slight accumulation of Amyloid-β (Aβ) and Tau proteins. Here we investigated the cross-talk among IL-1β, Aβ and Tau in synaptic and memory impairment observed in infected mice. The causal role of IL-1β was assessed by treating WT mice with the IL-1 blocker, anakinra, that fully rescued all structural and functional indices of neurodegeneration. HSV-1-infected $APP^{-/-}$ and $Tau^{-/-}$ mice undergone 2TS exhibited IL-1β mRNA levels that were higher than in mock-infected transgenic mice (1.6- and 1.4-fold induction, respectively, $p<0.05$) but significantly lower than in WT mice (-32% and -43% in HSV-1-infected $Tau^{-/-}$ and $APP^{-/-}$ mice, respectively, $p<0.05$). Accordingly, transgenic mice showed milder synaptic deficits than WT mice: long-term potentiation at the hippocampal CA3-CA1 synapses in HSV-1- vs mock-infected mice was 73.7±6.7% vs 98.6±10.1% in $Tau^{-/-}$ mice, 69.5±5.6% vs 94.6±6.4% in $APP^{-/-}$ mice and 47.3±6.4% vs 98.0±12.4% in WT mice. Further investigations revealed that the decreased levels of IL-1β observed in HSV-1-infected

transgenic mice were associated with a lower activation of microglia. In particular, the upregulation of M1 phenotype marker CD86 in $Tau^{-/-}$ and $APP^{-/-}$ mice (+63% and +64% vs mock-infected mice, respectively; $p<0.05$) was significantly lower (-45% for both mouse strains) than that observed in HSV-1-infected wild-type mice (+197%). Collectively, our findings suggest that IL-1β-mediated neuroinflammation is a major determinant of the synaptic dysfunction observed at early stages of our mouse model of sporadic AD. The lower levels of IL-1β resulting from altered microglial activation, and the milder synaptic deficits we observed in $Tau^{-/-}$ and $APP^{-/-}$ mice indicate the critical role of the vicious circle established among IL-1β, Aβ and Tau in the pathophysiology of synaptic dysfunction in AD.

Disclosures: D.D. Li Puma: None. **R. Piacentini:** None. **B. Bandiera:** None. **G. Puliatti:** None. **G. Boni:** None. **G. De Chiara:** None. **A.T. Palamara:** None. **C. Grassi:** None.

Presentation Number: NANO02.02

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

Support: PRIN 2022ZYLB7B

Title: Herpes simplex virus-1 infection induces complement proteins upregulation in brain cells: possible role in synaptic damage

Authors: *M. MITEVA[1,2], V. PROTTO[3], F. ZANZI[1], R. PIACENTINI[4,5], C. RIPOLI[4,5], M. MARCOCCI[6], C. GRASSI[4,5], A. PALAMARA[3,2], G. DE CHIARA[1];
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Abstract: Several pieces of evidence suggest that recurrent herpes simplex virus-1 (HSV-1) infection reaching the brain is one of the risk factors for Alzheimer's disease (AD). Numerous studies suggest that abnormal upregulation of the complement cascade, a key component of the innate immune system, is involved in the pathogenesis of AD, also concurring to synaptic elimination in the brain. Hence, we investigated if HSV-1 triggers complement cascade activation in brain cells likely leading to synaptic loss through microglia phagocytosis. We exploited murine primary neuronal cultures co-cultured or not with BV2 microglial cells (ratio 1:5 = microglia: neurons) as in vitro models of infection, and analyzed cells 24 h after HSV-1 or mock infection with different techniques. We first checked the effect of HSV-1 infection on the intracellular expression of C1q, C3 and C4 in the primary neuron/glia co-cultures. Real-time PCR and western blotting (WB) analyses of cell lysates revealed that HSV-1 infection caused a significant increase of complement proteins at both mRNA and protein levels as well as their release in the supernatant of infected cells. Interestingly, we found that C1q and C4 localized at the synaptic level upon HSV-1 infection, suggesting that they may take part in HSV-1-driven synaptic damage. To explore this possibility, we infected cells in the presence or absence of agents able to inhibit complement activation and monitored the expression levels of pre-and post-synaptic markers, such as PSD-95 and synaptophysin. WB analyses revealed that HSV-1 infection significantly downregulated both the synaptic proteins in untreated cultures, whereas a

partial rescue was observed when the infection was performed in the presence of complement inhibitors. We then performed an engulfment assay and confocal immunofluorescence (IF) analyses to study microglial phagocytosis of synaptic material, investigating possible interaction between CD68 (microglial lysosome marker) and synaptic proteins within HSV-1 infection in complement inhibitor-treated and untreated co-cultures. Results from these experiments indicate that HSV-1 infection triggers increased microglial phagocytosis of synaptic materials, which was partially prevented when the complement cascade is inhibited. Overall our data indicate that HSV-1 infection triggers aberrant complement activation and microglial engulfment of complement-tagged synapses in primary brain cultures, further supporting the role of HSV-1 in neurodegeneration.

Disclosures: M. Miteva: None. **V. Protto:** None. **F. Zanzi:** None. **R. Piacentini:** None. **C. Ripoli:** None. **M. Marcocci:** None. **C. Grassi:** None. **A. Palamara:** None. **G. De Chiara:** None.

Presentation Number: NANO02.03

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

Support: Telethon foundation TCP11015 to L.F.
Alzheimer Association AARG 17-505136 to L.F.
Canadian Institutes of Health Research PJT-173497 to P.E.F.
National Institute of Health NINDS R01NS110024 to O.A.

Title: Differential effect of SUMO1 and SUMO2 conjugation on Tau-mediated synaptic toxicity in the PS19 mouse model of Frontotemporal dementia.

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Abstract: Hyperphosphorylated tau and insoluble tau aggregates represent the main histopathological hallmarks of tauopathies. Although phosphorylation has been mainly studied for its role in Tau function, other post-translational modifications seem to have a central impact in Tau physiology and pathology. Among them is SUMOylation, which is involved in several neurodegenerative diseases, including tauopathies like Alzheimer's disease (AD). With respect to the CNS, SUMOylation refers to the binding of one of the three SUMO paralogs (SUMO1-3) to the target protein. SUMO2/3 are almost identical in sequence homology and functionality, while SUMO1 differs significantly in sequence and has distinct functionality in comparison to SUMO2/3. Previous studies have shown that both SUMO1 and SUMO2 could bind to Tau. Importantly, SUMO2 conjugation provides resistance to stress conditions and it is required for cognitive processes, while SUMO1 conjugation impairs neuronal plasticity and memory

formation. In the present study, we employ the transgenic mouse model PS19 that expresses the P301S mutation in order to study the effects of SUMOylation in primary tauopathies, such as Frontotemporal Dementia (FTD). Specifically, we examined the effect of overexpressing either SUMO1 or SUMO2 in PS19 mice on plasticity and memory formation by employing hippocampus-dependent behavioral tasks, electrophysiological recordings, and biochemical analysis of plasticity markers. Analysis of global SUMO1 and SUMO2 conjugation in PS19 mice showed augmentation of SUMO1 binding and reduction of SUMO2 binding to target proteins in comparison to age-matched control mice. In addition, overexpression of SUMO1 in PS19 mice at the age of 4-5 months, when PS19 mice do not still exhibit plasticity or memory impairments, caused an earlier onset of defects in basal synaptic transmission, long-term potentiation (LTP), as well as spatial and reference memory. Conversely, overexpression of SUMO2 in 8-9 months old PS19 mice, rescued impairments of LTP and memory formation, encountered in PS19 mice at this age, suggesting a neuroprotective effect of SUMO2 after the manifestation of synaptic and cognitive decline in PS19 mice. Finally, the levels of several plasticity markers, such as GluA2, synapsin, alpha-synuclein and SynGAP were restored in PS19-SUMO2 mice. Collectively, these results confirm the distinguished role of SUMO1 and SUMO2 conjugation in an animal model of FTD, as it was shown before in AD animal models. Importantly, we suggest that SUMO2 overexpression could ameliorate plasticity and memory deficits caused by pathological tau, opening new therapeutic revenues in tauopathies.

Disclosures: E.K. Argyrousi: None. **F. Orsini:** None. **P.E. Fraser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual Properties on Biologics Enhancing SUMO2 Conjugation. **L. Fioriti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual Properties on Biologics Enhancing SUMO2 Conjugation. **O. Arancio:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual Properties on Biologics Enhancing SUMO2 Conjugation, Founding Member of Neurokine Therapeutics. F. Consulting Fees (e.g., advisory boards); Advisory Board of Appia Pharmaceutical.

Presentation Number: NANO02.04

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

Support: Italian Ministry of Research PRIN 2020AMLXHH_002
Italian Ministry of Health (Oasi Research Institute IRCCS) Ricerca Corrente

Title: The lack of Amyloid-beta production and function prevents the beneficial effects of phosphodiesterase inhibitors on hippocampal synaptic plasticity and memory

Authors: *M. TROPEA[1], V. VACANTI[1], R. TROVATO[1], D. PUZZO[1,2];
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Abstract: Phosphodiesterases (PDEs), the enzymes responsible for degrading cyclic nucleotides (CNs), have been studied as potential drug targets in neurodegenerative disorders like Alzheimer's disease (AD). Extensive preclinical research has demonstrated that PDE inhibitors

(PDE-Is) can counteract the detrimental effects of Aβ and tau on synaptic plasticity and memory. However clinical trials have not yet met expectations. In light of this, our study aimed to determine whether amyloid-β (Aβ) production and function might interfere with the beneficial effects of PDE-Is on hippocampal synaptic plasticity and memory.
We performed in vitro electrophysiological recordings on hippocampal slices to examine how the absence of Aβ in APP knockout (KO) mice and the lack of its physiological receptor, i.e., α7 nicotinic acetylcholine receptor, in α7KO mice impact CN-mediated synaptic plasticity. Additionally, we assessed the effects of PDE-I treatment on memory performances in these models.
We found that treatment with the selective PDE4-I roflumilast or the PDE5-I vardenafil, increasing cAMP or cGMP levels respectively, failed to enhance hippocampal long-term potentiation (LTP) and memory in young APPKO and α7KO mice compared to WT controls. Furthermore, chronic treatment with roflumilast or vardenafil did not rescue the age-dependent cognitive phenotype in APPKO and α7KO, the latter having previously been shown to develop an AD-like cognitive phenotype.
Our results suggest that Aβ production and function are needed for CN-mediated synaptic plasticity and memory, and that the lack or malfunction of Aβ prevents PDE-Is from rescuing cognitive dysfunction, providing new insights into the pathophysiology and treatment of AD.

Disclosures: M. Tropea: None. **V. Vacanti:** None. **R. Trovato:** None. **D. Puzzo:** None.

Presentation Number: NANO02.05

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

Support: NIH Grant F31AG079620
NIH Grant R21AG072811

Title: Dopamine neuron hyperexcitability in a mouse model of Alzheimer's disease

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Abstract: Alzheimer’s disease (AD) patients suffer from severe comorbidities including depression which could arise from dysfunctional dopamine release from subcortical areas. Tonic dopamine release, driven by spontaneous firing of ventral midbrain dopamine neurons, is essential for modulating classic dopamine-dependent mechanisms and learning and memory. Here we show that in the 3xTg-AD mouse model of AD, mice display normal locomotion, but age dependently fail to acquire an operant conditioning task, suggesting ventral tegmental area (VTA) dysfunction. Using patch-clamp electrophysiology in brain slices, we found that VTA dopamine neurons display heighted spontaneous firing rates, decreased firing fidelity, and an overall depolarization of the inter-spike interval. Using specific pharmacology and patch clamp electrophysiology paired with single cell RNAseq (Patch-seq), we uncovered evidence that overactive casein kinase 2 (CK2) may decrease the calcium sensitivity of small conductance calcium activated potassium (SK) channels. Altered firing rates and SK currents were restored in 3xTg-AD mice by a CK2 inhibitor, administered either *in vivo* or *ex vivo*. CK2 is known to be activity dependent, thus we next hypothesized that altered synaptic excitation or inhibition may

be responsible for CK2 dysregulation. In support, we found that VTA dopamine neurons from young 3xTg-AD mice exhibit less frequent mIPSCs with no apparent change in presynaptic release probability. A robust dendritic retraction suggested a potential postsynaptic locus for decreased inhibition. In contrast, mEPSC frequency and amplitude are increased at early ages, indicating an early alteration to the excitation-inhibition relationship. Evoked AMPA receptor-mediated currents displayed a decrease in paired-pulse ratio and an increase in glutamate release, suggesting a pre-synaptic locus for increased synaptic excitation. These results suggest homeostatic shifts in the synaptic balance may promote an intrinsic, activity dependent, positive feedback loop resulting in VTA dopamine hyperexcitability in Alzheimer's disease.

Disclosures: H.E. Blankenship: None. **K.A. Carter:** None. **M.J. Beckstead:** None.

Presentation Number: NANO02.06

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

Support: AARF-21-721588

Title: The Epigenetic Landscape of Memory: Targeting Histone Acetylation for Alzheimer's Disease Therapeutics

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Abstract: Memory is influenced by epigenetics, which regulates gene expression without altering DNA sequences, primarily through modifications to DNA or histone proteins, impacting gene accessibility and transcription. Histone acetyltransferases (HATs) such as CBP, p300, PCAF, Tip60, and Src3 are key players in memory formation, adding acetyl groups to histone proteins. These enzymes play pivotal roles in facilitating synaptic plasticity and long-term memory consolidation through their histone acetylation activity. The hypothesis underlying these studies is that histone acetylation is disrupted in neurodegenerative diseases such as Alzheimer's disease (AD) and related dementias, contributing to memory impairments and synaptic dysfunction. To address this hypothesis, we performed a multidisciplinary study in which we combined medicinal chemistry techniques with biochemical, electrophysiologic and behavioral methods. Measurement of HAT expression revealed diminished CBP, p300, PCAF, Tip60, and Src-3 levels in postmortem AD brains compared to age-matched controls. Additionally, consistent with these findings, acetylation of H3K4, H3K9, and H3K14 residues crucial for memory formation was decreased in AD brains compared to controls. Inducing memory formation in wild-type mice, using fear conditioning, resulted in increased histone acetylation at lysine residues H3K4, H3K9, and H3K14 as well as increased expression of memory-associated genes, including CREB, pCREB, Arc, c-Fos, and BDNF. However, in 14-month-old hTau/Mapt-KO mice, a tau oligomer elevation model of AD, we did not detect elevated histone acetylation or expression of memory-related genes after memory induction. Most importantly, upregulation of histone acetylation including AD-related lysine residues H3K4, H3K9 and H3K14 through compounds OA57 and OA8, two structurally unrelated small molecules that increase activity of

memory-related HATs, CBP, p300 and PCAF, rescued the memory and synaptic plasticity defects in hTau/Mapt-KO mice and APP/PS1 mice, an amyloid-deposition model of AD. These findings highlight a downregulation of epigenetic mechanisms linked with histone acetylation and suggest that HAT activators offer a potential therapeutic tool for mitigating cognitive decline in AD and related dementias.

Disclosures: E. Calcagno: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property on the development of small molecules that activate histone acetyltransferases. **E. Zuccarello:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property on the development of small molecules that activate histone acetyltransferases. **A. Staniszewski:** None. **H. Zhang:** None. **E.K. Argyrousi:** None. **S. Deng:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property on the development of small molecules that activate histone acetyltransferases. **D.W. Landry:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property on the development of small molecules that activate histone acetyltransferases. **J. Fiorito:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property on the development of small molecules that activate histone acetyltransferases. **O. Arancio:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founding Member of Neurokine Therapeutics, Intellectual property on the development of small molecules that activate histone acetyltransferases.

Presentation Number: NANO02.07

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

Support: R35NS116879

Title: Amyloid-beta oligomer induced fragmentation of synaptic nanostructure.

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Abstract: Amyloid Beta Oligomer (Aßo)-Induced Fragmentation of Synaptic Nanostructure.
*Ramsay, H.J., Kennedy, M.J.
Several synaptic proteins organize nanostructurally as sub-synaptic domains (SSDs). Experimental evidence that postsynaptic SSDs co-register with presynaptic neurotransmitter release sites, support the notion that synaptic nanostructure contributes to synaptic transmission. Despite this progress, it remains unclear whether synaptic perturbations associated with brain disease impact the delicate nano-positioning of these receptors, and if so, what implications this holds for synaptic health. Alzheimer’s Disease (AD) is associated with synaptic accumulation of the Amyloid-Beta (Aß) protein, and soluble Aß oligomers (Aßo) rapidly, and potently impact synaptic function. Here, DIV17 dissociated hippocampal neurons were treated for 15 minutes with 500 nM Aßo. Coverslips were then immunolabeled, and imaged using 2D-STED

microscopy, and an ImageJ segmentation analysis. Comparing dendritic spines bound by Aßo to those from untreated cultures, we observed that Aßo-bound spines were increased in their number of PSD95 and GluA1/GluA2-containing AMPAR SSDs, with AMPAR SSDs smaller than those at control synapses. Additionally, this Aßo-induced nanostructural "fragmentation" was accompanied by an increase in the summed synaptic intensities of PSD95 and GluA1 SSDs, suggesting that Aßo also leads to the incorporation of new protein at the synapse, in line with previous research. Lastly, by instead GluA1-immunolabeling prior to our Aßo application, we were able to prevent the fragmentation of GluA1-containing AMPAR populations at the synapse, which enables us to further decipher what role this disruption plays in known AD pathology. Experimental model / rigor statement: In this confirmatory work, cultured neurons from male/female P0-P1 Sprague-Dawley rat pups were pooled and used for all experiments. Biological sex differences were not assessed. We ensured scientific rigor using appropriate controls, biological/technical replicates with sufficient sample size, and blinding when possible.

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

Presentation Number: NANO02.08

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

Support: RF1 AG077103
R01 AG065628
R21 AG083638
RFI AZ170142

Title: Shared pathogenic features of neurological disorders contributing to enhanced risk for Alzheimer's disease.

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Abstract: The symptoms, histological features and major risk factors for Alzheimer's disease are deeply characterized and include cognitive decline, amyloid and tau aggregates, neuroinflammation and neurodegeneration. And despite decades of research devoted to this disease, remarkably little is understood regarding underlying pathophysiological mechanisms that initiate and drive AD. However, with recent technology that expands the interrogation of clinically-based models, such as transcriptomics, human iPSC-derived brain cells from patient populations, and gene editing approaches, a consensus is forming around core pathways that play a pivotal role in triggering AD. An additional strategy to identify AD drivers is to examine common physiological and genetic changes in conditions that greatly increase AD risk. Here, we selected traumatic brain injury (TBI; one of strongest environmental risk factors for AD) and

Down syndrome (DS; the greatest genetic risk factor for AD) to identify early mechanistic features shared with AD. By integrating human and mouse single cell transcriptomics and iPSC-derived neurons, we are synthesizing a genetic and pathophysiological model for early drivers of AD. Consistent with their overlap in differentially expressed gene pathways, key lines of convergence among TBI, DS, and AD point to an early and upstream dysregulation of intracellular Ca2+ homeostasis, mitochondrial dysfunction, synaptic deficits, and endo-lysosomal deficits. Using a series of electrophysiological assays, live cell imaging of targeted biosensors, and immunoassays in human neurons derived from these patients and/or respective mouse models, we probed each of these core pathogenic hubs and compared across conditions to identify shared upstream mechanisms. We found that dysregulated Ca2+ release from the intracellular ryanodine receptor was a common essential component, and triggered pathogenic cascades driving amyloid and tau accumulation, as well as synaptic signaling alterations leading to plasticity deficits. Likewise, TBI and DS brains and neurons displayed amyloid and tau expression similar to AD. The excess intracellular Ca2+ underlies tau accumulation via impaired lysosome activity, as well as mitochondrial dysfunction and superoxide production, and synaptic defects associated with impaired memory encoding. In sum, there is increasing compelling evidence from human and animal models, from the gene-level to the brain network level, that alterations in Ca2+ handling and mitochondrial function are potent initiators of AD, and may serve as underlying mechanisms for converting TBI and DS to AD.

Disclosures: G. Stutzmann: None. **S.D. Ginsberg:** None. **R.A. Marr:** None. **M.J. Alldred:** None. **N.M. Barrington:** None. **E.K. Webber:** None. **R. Ramachandra:** None. **D.F. Steinbrenner:** None. **S.H. Mustaly:** None.

Presentation Number: NANO02.09

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

Support: NIH Grant RF1AG033570

Title: The Role of PICALM in Neuronal Vulnerability

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Abstract: Alzheimer’s disease (AD) is the most common cause of dementia worldwide. AD is characterized by brain aggregates of β-amyloid (Aβ) and neurofibrillary tangles. Clinically it is characterized by progressive memory loss and cognitive deficits. The familial forms of AD are cause by mutations in amyloid precursor protein (APP) and presenilin 1 and 2. However, what causes the late onset (LOAD) form is unknown. Aging is the greatest risk factor of LOAD. Several genome-wide association studies (GWAS) have PICALM, the gene encoding for Phosphatidylinositol Binding Clathrin Assembly Protein, as a genetic risk factor for LOAD. However, how PICALM polymorphism increases the risk for AD is yet to be fully understood. My preliminary studies show that levels of PICALM in the hippocampus of adult mice are significantly reduced with age, while its cleavage products are increased, suggesting that PICALM stability in the brain may be compromised with age. Further, PICALM was expressed in neurons derived from human induced pluripotent stem cells (iPSC), and its expression

appeared to be stage-specific. Knocking out PICALM in iPSC- derived neural progenitor cells, precursors and neurons reduced expression levels of β-III-tubulin and neurofilament in these cells, suggesting that PICALM regulates neuronal maturation. Importantly, dendritic arborization of PICALM KO neurons was compromised, suggesting that PICALM plays a role in neuronal morphology. Additionally, the ratio between the mature and immature form of β-Amyloid precursor protein (β-APP) was altered in PICALM KO cells throughout neurogenesis stages, suggesting that PICALM regulates β-APP maturation. Together, these experiments suggest that PICALM regulated neuronal differentiation and maturation. Future experiments will examine its role in neuronal plasticity.

Disclosures: L. Aponte Cofresi: None. **T. Stephen:** None. **K. Rakowiecki:** None. **O. Lazarov:** None.

Nanosymposium

NANO03: Mechanisms of Neuroprotection: Therapy Development

Location: MCP Room S103

Time: Saturday, October 5, 2024, 1:00 PM - 4:30 PM

Presentation Number: NANO03.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01AG071512
NIH 1R21AG073684

Title: Mechanisms of neuroprotection and resilience in aging and neurodegeneration

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Abstract: Besides its essential role as a building block for protein synthesis, cysteine is utilized to generate numerous sulfur-containing molecules including the antioxidant glutathione and coenzyme A. We previously showed that the metabolism of cysteine is dysregulated in Huntington's disease (HD), a neurodegenerative disorder triggered by the expansion of polyglutamine repeats in the protein huntingtin as well as aging. In this recent study, we show that cysteine metabolism is compromised at multiple levels in HD, both transcriptional and post-translational. Accordingly, restoring cysteine homeostasis may be beneficial in HD.

Disclosures: B. Paul: None.

Presentation Number: NANO03.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AG071512
R21AG073684-01
Johns Hopkins Catalyst Award
19PABH134580006

Title: Limiting transsulfuration pathway accelerates Alzheimer's-like neurodegeneration

Authors: *S. CHAKRABORTY[1], S. J. TRIPATHI[1], E. F. VAZQUEZ-ROSA[2], K. CHAUBEY[2], S. BARKER[3], E. MILLER[2], H. FUJIOKA[4], S. VAZQUEZ[1], A. A. PIEPER[5,6,7,8,9], B. D. PAUL[1,10,11,12];
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Abstract: The reverse transsulfuration pathway is critical for the production of gasotransmitter hydrogen sulfide (H_2S). In the brain, H_2S is essential to maintain the redox state, cerebral blood flow and plasticity. Cystathionine gamma-lyase is a crucial neuronal enzyme that generates cysteine and H_2S for neurons. Notably, H_2S modulates the activity of several proteins via sulfhydration, a post-translational modification that occurs on reactive cysteine residues. Sulfhydration is critically implicated in numerous neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and traumatic brain injury. Here, we demonstrate that abrogation of the transsulfuration pathway via depletion of cystathionine gamma-lyase leads to redox imbalance, changes in sulfhydration, and accelerates neurodegeneration through anomalous neuroplastic alterations leading to cognitive deficits. We speculate that pharmacotherapies targeted to normalize the reverse transsulfuration pathway represent a new broad approach to neuroprotection in neurodegenerative disorders.

Disclosures: S. Chakraborty: None. **S.J. Tripathi:** None. **E.F. Vazquez-Rosa:** None. **K. Chaubey:** None. **S. Barker:** None. **E. Miller:** None. **H. Fujioka:** None. **S. Vazquez:** None. **A.A. Pieper:** None. **B.D. Paul:** None.

Presentation Number: NANO03.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Intranasal insulin administration reduces oxidative stress induced damage and improves mitochondrial bioenergetics in Alzheimer disease.

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Abstract: Brain insulin resistance (bIR) heavily impacts on the core pathological processes of aging and Alzheimer disease (AD) since insulin regulates brain metabolism and cognitive functions. A close link among bIR, oxidative stress (OS) and mitochondrial defects exists, that

contributes to brain dysfunctions observed in AD. Intriguingly, several studies suggest that intranasal insulin treatment (INI) enhances cognitive performance and reduced AD neuropathology both in humans and murine models of AD. We focused on the interplay between OS and bIR, by testing the hypothesis that rescuing brain insulin signaling activation by INI results in improved mitochondrial functions and reduced OS-induced damage to proteins in a mouse model of AD (3xTg-AD).
Methods. 12-month-old 3×Tg-AD and wild-type (non-Tg) mice were treated with INI (2 UI) or vehicle (saline) every other day for 2 months. Insulin signaling pathway and OS marker levels, i.e., PC, 4-HNE and 3-NT were evaluated in the frontal cortex. Then, due to the link between bIR and nitrosative stress, a redox proteomics approach was used to identify specific protein targets of 3-NT modifications. Mitochondrial functions were evaluated by measuring mitochondrial complexes (OXPHOS) and activities in all experimental groups. *Results.* INI administration improved insulin signaling and reduced OS levels in 3xTg-AD mice. In particular, a consistent reduction of 3-NT levels was observed. Redox proteomics allowed to identify several proteins with reduced 3-NT modifications, that belong to key pathways, such as protein degradation and energy metabolism, known to be involved in the progression of AD. Remarkably, reduced 3-NT levels on mitochondrial proteins were responsible for an improvement of mitochondrial activity and brain energy metabolism. *Conclusions.* We propose that INI represents a promising approach to reduce OS-induced damage to proteins and restore mitochondrial bioenergetics in AD brain.

Disclosures: A. Tramutola: None.

Presentation Number: NANO03.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Differential action of PTPN6 on amyloid-associated pathology and white matter damage in a mouse model of Alzheimer's disease

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Abstract: Neuroinflammation is one of the hallmarks of Alzheimer's disease and a majority of AD risk genes are expressed in microglia. The cytoplasmic protein tyrosine kinase PTPN6 interacts with ITIM (immunoreceptor tyrosine-based inhibitory motif) of multiple AD risk gene products including Siglec-11, LILRB2, PILRA, and CD33 and it is also known as a negative regulator of DAP12/TREM2 signaling. To understand the role of ITIM receptor signaling and PTPN6 regulation in AD pathology, we examined effect of Ptpn6 deficiency in TauPS2APP mouse model. Conditional knockout (cKO) of Ptpn6 in microglia augmented microglia engagement around plaques and protected neurites from plaque-associated neurite damages. But Ptpn6 cKO exacerbated neurodegeneration in white matter areas. Interestingly, heterozygous deletion of Ptpn6 retained beneficial effects on neurites around plaques without aggravating white matter damage. scRNA-seq revealed elevation of Lgals3^{high} DAM states by Ptpn6 cKO, but not by heterozygous deletion of Ptpn6. Lgals3^{high} microglia are in close proximity to Gfap^{high} cells as well as Serpina3n^{high} cells compared to Lgals3^{low} microglia in the brain. Current results indicate that different degree of inhibition of PTPN6-associated pathways may lead to different

microglial states that impact the pathology of surrounding cellular context, and the milieu also closely influence microglial states.

Disclosures: S. Lee: A. Employment/Salary (full or part-time):; Full time employee of Genentech.

Presentation Number: NANO03.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS116914

Title: Bridging inflammation and neurogenesis: the dual impact of Interleukin-1 on adult hippocampal neurogenesis

Authors: *M. SMIRNOVA[1,2], M. C. MONET[1,2], D. P. NEMETH[3], H. VAN PRAAG[1,4], N. QUAN[1,4];
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Abstract: The process of adult hippocampal neurogenesis (AHN) represents a dynamic interplay between neural stem cells (NSCs) and their microenvironment within the dentate gyrus (DG) of the hippocampus. AHN is essential for learning and memory; however, its regulation can be influenced by factors such as interleukin-1 (IL-1), a proinflammatory cytokine implicated in neuroinflammatory processes in neurodegenerative diseases. IL-1 signals through its receptor, interleukin 1 receptor type 1 (IL-1R1). Utilizing transgenic mouse models allowing for cell type-specific expression of IL-1R1, we are able to analyze the role of IL-1 in modulating AHN. We established a minimum dose of IL-1, delivered stereotaxically via an adeno virus to induce NSC proliferation, the first phase of AHN. Intriguingly, we discovered that low-dose IL-1 promotes NSC proliferation in the DG. Conversely, high doses of IL-1 lead to a bilateral suppression of NSC proliferation, accompanied by the induction of neuroinflammation. The detrimental effects of high-dose IL-1 signaling on AHN potentially simulate the neuroinflammatory conditions observed in neurodegenerative diseases. Additionally, we observed that when IL-1R1 is selectively expressed on astrocytes, not other cell types, the low dose IL-1-stimulated NSC proliferation was replicated. Astrocytes, known for their supportive roles in neuronal function and plasticity, appear to play a critical role in mediating the pro-neurogenic effects of IL-1. Hence, IL-1 functions as a double-edged sword in AHN depending on its concentration and cellular target. To gain deeper insights into the mechanisms underlying IL-1's effects on AHN, we will use retroviral tagging of proliferating NSCs allowing for the tracking of their fate and morphological changes during differentiation into mature neurons. Furthermore, we designed a virus capable of visualizing IL-1 ligand expression, which offers a novel tool to investigate the cellular interactions underlying IL-1's effects on AHN. By identifying the specific cell types targeted by IL-1 and elucidating the signaling pathways involved, we can gain a more comprehensive understanding of IL-1-mediated regulation of AHN. Overall, this research will establish a dose-dependent and cell type-specific response of IL-1 signaling on AHN. By

deciphering how IL-1 influences AHN, this work has the potential to uncover novel therapeutic targets for mitigating the neuroinflammatory phenotype associated with neurodegeneration.

Disclosures: M. Smirnova: None. **M.C. Monet:** None. **D.P. Nemeth:** None. **H. van Praag:** None. **N. Quan:** None.

Presentation Number: NANO03.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS124123

Title: TNFR2 activation attenuates behavioral dysfunction in a mouse model for multiple sclerosis

Authors: *K. L. NGUYEN[1], T. MARTYNYUK[2], S. GUPTA[3], S. ARNAB[4], V. BRACCHI-RICARD[5], R. FISCHER[6], J. R. BETHEA[7];
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Abstract: **Background & Aims:** Chronic pain (CP) is among the leading causes of disability in adults and is estimated to affect approximately 25% of the world population with greater prevalence among females. Currently available analgesic drugs provide minimal relief from CP, in part, due to poorly understood underlying mechanisms. Tumor necrosis factor (TNF), a pleiotropic cytokine, has been shown by our lab as well as other to be critical mediator in both physiological and pathological processes crucial to pain development. However, anti-TNF therapies are often ineffective, and patients experience robust side effects due to inhibition of both pathological (TNF receptor 1; TNFR1) and neuroprotective (TNF receptor 2; TNFR2) pathways. **Methods:** We have previously demonstrated that inhibition of soluble TNF/TNFR1 is ineffective in reducing mechanical hypersensitivity in females following peripheral nerve injury (chronic constriction injury; CCI) or experimental autoimmune encephalomyelitis (EAE; mouse model of multiple sclerosis). Using a murine-specific selective TNFR2 agonist, we wanted to first determine if CP attenuated following EAE. Subsequently, we wanted to explore changes to dendritic cell populations, which control T-cell tolerance and activation of pathogenic response in autoimmunity, and if TNFR2 activation contributes to changes in dendritic cell presence. **Results:** We found robust attenuation of mechanical allodynia in both females and males; however, spontaneous pain is mitigated primarily in females. At acute motor disease, both onset and severity of EAE motor symptoms appear to be delayed and reduced in females. In males, motor symptoms are delayed, and the penetrance of motor disease is reduced. In addition, increased presence of dendritic cells (CD11b+/CD11c+) was sexually dimorphic in the brain and spinal cord during chronic EAE following TNFR2 activation. **Conclusions:** Together, these data demonstrate that TNFR2 agonism affectively attenuates female and male evoked pain with greater efficacy in females for reducing spontaneous pain in EAE. In addition, TNFR2 agonism is sexually dimorphic in mitigating EAE motor deficits along with underlying changes in CNS

presence of dendritic cells. Overall, TNFR2 activation holds therapeutic potential to broadly treat CP.

Disclosures: K.L. Nguyen: A. Employment/Salary (full or part-time):; BioNTech/resano. **T. Martynyuk:** None. **S. Gupta:** None. **S. Arnab:** None. **V. Bracchi-Ricard:** None. **R. Fischer:** A. Employment/Salary (full or part-time):; BioNTech/resano. **J.R. Bethea:** 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.; BioNTech/resano. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BioNTech/resano.

Presentation Number: NANO03.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS: R01NS124123

Title: Antagonistic role of male sex-hormone and male sex-chromosome complement on the therapeutic efficacy of TNFR2 activation in a model of Multiple Sclerosis.

Authors: *S. GUPTA[1], K. A. SWANSON[2], R. FISCHER[3], J. R. BETHEA[4];
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Abstract: Transmembrane TNF/TNFR2 signaling has recently gained importance as a potential therapeutic for neuroinflammatory disorders, including Multiple Sclerosis (MS). Previous data from our lab has demonstrated the therapeutic role of TNFR2 activation in alleviating chronic neuropathic pain (CNP) in males and females but has also revealed the sex-specific efficacy of TNFR2 activation in alleviating motor deficits only in female EAE (a rodent model for MS) mice, at a chronic timepoint. **The current study was hence designed to investigate the male sex-specific characteristics which limited the motor recovery in EAE males upon TNFR2 activation**. We utilized sophisticated pharmacological approaches along with transgenic mice to interrogate the role of male sex-hormone and male sex-chromosome complement on the limited therapeutic efficacy of motor recovery in EAE males. Using four core genotype mice, XX^{sry+} males and XY males were immunized with EAE and were treated with a novel TNFR2 agonist. Motor deficits were analyzed daily. EAE XX^{sry+} males had a significant decrease in motor disease severity as compared to EAE XY males upon TNFR2 activation. Moreover, TNFR2 activation reduced the motor disease incidence in EAE XX^{sry+} males 3.5-fold as compared to EAE XY males. Concluding that it is the XY sex-chromosome complement which decreases the efficacy of TNFR2 activation in alleviating motor deficits in EAE males. To investigate the role of male sex-hormones, adult males were gonadectomized and were either exogenously provided with continuous release testosterone or placebo pellets 10 days before EAE immunization. Upon TNFR2 activation, gonadectomized males with testosterone replacement had a delayed motor disease onset and decreased motor disease severity as compared to the gonadectomized males without testosterone replacement. This indicated the protective role of testosterone and concluded **testosterone is not limiting the therapeutic effects of TNFR2 activation for motor recovery.** However, we surprisingly found that TNFR2 activation does not alleviate CNP in gonadectomized males, even with testosterone replacement, suggesting **a gonad-dependent but**

testosterone-independent effect of TNFR2 activation on pain alleviation in EAE males. This was also confirmed by pharmacological studies using flutamide (anti-androgens) in gonadally intact male mice, where TNFR2 activation still relieved CNP. This study is the first to investigate the chronic role of male sex-dependent characteristics involved in attenuation of CNP and motor deficits (the two predominant symptoms of MS) in EAE upon TNFR2 activation.

Disclosures: S. Gupta: None. **K.A. Swanson:** None. **R. Fischer:** A. Employment/Salary (full or part-time):; BioNTech/resano. **J.R. Bethea:** 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.; BioNTech/resano. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BioNTech/resano.

Presentation Number: NANO03.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 DC020528
NIH R01 AG 029493
DOD MS 220167

Title: Astrocyte FAK inhibition female-specifically alleviates experimental autoimmune encephalomyelitis, possibly through vitronectin-FAK-IL6 signaling

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Abstract: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) that leads to demyelination and axonal injury. Astrocytes are major glial cells in the central nervous system (CNS) and affect myelin maintenance and remodeling. In a mouse experimental autoimmune encephalomyelitis (EAE) model, we found that inducible Cre-*lox* knockout of focal adhesion kinase (FAK) in astrocytes delayed the clinical onset of EAE symptoms and substantially reduced the peak disease severity and chronic disease activity in female but not male mice. Knockout of FAK in astrocytes also reduced demyelination and axonal injury in females. Importantly, systemic treatment with a small-molecule FAK inhibitor, FAK14, had the same effect in female wildtype but was not efficacious in astrocytic FAK knockout mice or males. These data suggest that FAK in female astrocytes exacerbates EAE lesions and that FAK14 acts by inhibiting astrocyte FAK. We further investigated the upstream and downstream signaling involved in the astrocyte FAK effect on EAE. Plasma proteins, including vitronectin, leak into the injured CNS and contribute to MS pathogenesis by activating microglia. Our previous in vitro study in a cell model of astrocytes (astroglioma C6 cells) showed that vitronectin is the only protein among several tested extracellular matrix molecules that activates FAK and stimulates IL-6. Sustained IL-6 expression in astrocytes is restricted to EAE demyelination areas, and knockout of IL-6 in astrocytes delays EAE onset in female mice only, suggesting a female-specific role of astrocytic IL-6 in EAE. Here, we found that knockout of vitronectin mitigated EAE in female but not male mice. Further, IL-6 was upregulated in the spinal cord of astrocyte FAK knockout EAE mice and FAK14-treated wildtype females. These

data suggest that the female-specific role of astrocyte FAK in EAE might be through VTN-FAK-IL6 signaling. Together, this study reveals that inhibition of FAK improves neurological and histological outcomes during the acute and chronic phases of MS and points to opportunities for the rational development of FAK inhibitors, such as FAK14, as a novel MS therapy.

Disclosures: J. Bullen: None. **D. Cox:** None. **K. Taylor-Cox:** None. **T. Hagg:** None. **C. Jia:** None.

Presentation Number: NANO03.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Alzheimer's Association 2019-AARG-643091

Title: Sex-associated differences in brain insulin signaling and energy metabolism uncover early pathological alterations driving neurodegeneration

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Abstract: Background. Biological sex influences Alzheimer's disease (AD) development, particularly concerning brain insulin signaling (bIS) and energy metabolism defects. Biliverdin reductase-A (BVR-A) plays a crucial role in IS, and its downregulation leads to insulin resistance. However, the sex-related differences in AD neuropathology and underlying mechanisms remain unclear. We aimed to identify early changes of bIS in males and females, shedding light on pathological signs preceding overt brain insulin resistance and AD neuropathology.**Methods.** C57BL/6J mice (WT and BVR-A knock-out, M and F, n=10/sex/group) received a chow or a high-fat diet (HFD, 60% kcal by fat) for 1 or 8 weeks. Peripheral metabolic measurements (fasting glucose, insulin, and OGTT) and cognitive tests (NOR and Y-maze) were performed. bIS activation (basal and intranasal insulin-induced), oxidative stress marker levels, mitochondrial activity, and AD neuropathology markers were evaluated in the hippocampus and cortex. Correlations were performed with peripheral metabolic measurements (fasting glucose, insulin, and OGTT) and cognitive tasks (spatial memory). **Results.** Male mice exhibit greater alterations of bIS and mitochondrial functions compared to females. A consistent accumulation of APP-C99 (precursor of beta-amyloid peptide) occurs in male mice, but not in female. No changes for oxidative stress markers (PC and 4-HNE) were observed. These alterations start in the cortex and progress in the hippocampus, leading to an impairment in associated memory functions. Despite mild alterations, female mice show worse cognitve outcomes following HFD, suggesting heightened susceptibility. PCA analysis shows that mice are separated along the PC1 based on the diet received (SD vs HFD), while they are separated on PC2 based on sex (M vs F). Variables that drive diet-mediated effects include the IRS1AKT/mTOR axis along with mitochondrial OXPHOS complexes, while PTEN and p70S6K are responsible for sex-associated differences. Both in male and female mice, these changes are linked to the loss of BVR-A, a connection confirmed by data from knockout mice. **Conclusions.** This study highlights the importance of identifying sex-related differences as well as shared features in the progression of bIS alterations in AD. Loss of BVR-A is a common event, although sex-, temporal- and regional-associated differences are observed. These observations

warrant further investigations, but provide solid bases to support personalized approaches for AD prevention.

Disclosures: E. Barone: None.

Presentation Number: NANO03.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: College of Medicine
John G Kulhavi Professorship
Neuroscience Program
E. Malcolm Field
Gary Leo Dunbar

Title: Delivery of curcumin using G4 PAMAM dendrimers in GFAP-IL6 mouse model of chronic neuroinflammation

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Abstract: Neuroinflammation is the innate immune response involving the recruitment of immune cells and nutrients in neural tissue of direct tissue injury. The underlying physiological mechanism of the inflammatory response in the central nervous system involves activating microglia and astrocytes after pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPS) are detected. Chronic neuroinflammation, which impacts neurons and leads to neuronal cell death, resulting in varying degrees of impaired cognitive function, is associated with the progression of various neurodegenerative disorders, including Alzheimer's, Huntington's, and Parkinson's disease. GFAP-IL6 mice are a model of chronic neuroinflammation and subsequent neurodegeneration. This is created by upregulating the expression of the pro-inflammatory cytokine IL-6 gene in astrocytes. GFAP-IL6 mice show significantly increased microglia and astrocytes in the hippocampus and cerebellum. Previous studies have identified curcumin as a promising treatment for neuroinflammation. Curcumin is a natural cytokine suppressive anti-inflammatory drug (CSAIDs) derived from the spice turmeric, which targets the pro-inflammatory signaling pathways CP1 and NFKB to inhibit the expression of cytokines like COX-2, iNOS, TNF- α, IL-1, -2, -6, -8, and -12. However, the efficacy of this treatment is hindered by its low solubility and bioavailability. One method to increase the treatment efficacy is to deliver curcumin using poly-amido(amine) (PAMAM) dendrimers. Generation 4 (G4) 70/30 PAMAM dendrimers contain hydroxyl and amine surface functional groups at a 70:30 ratio. In this study, we used G4 70/30-cystamine core PAMAM dendrimer-encapsulated curcumin (D-Curc) for intracranial administration into the hippocampus and cerebellum of the GFAP-IL 6 mouse model. The efficacy of D-Curc was measured by testing motoric function via the accelerated rotarod (accelerod). We performed Western blot analysis to confirm GFAP and IL-6 expression. We observed a significant genotype effect in the accelerod

($p<0.001$) as well as in GFAP expression in the hippocampus ($p=0.005$), GFAP expression in the cerebellum ($p<0.001$), IL-6 expression in the hippocampus ($p=0.021$), and IL-6 expression in the cerebellum ($p<0.001$). We also found a trend toward reduction of GFAP expression within the hippocampus of curcumin-dendrimer complex treated mice. Additionally, we observed an intermediate effect in the accelerod test in mice treated with curcumin-dendrimer complex.

Disclosures: S. Schwind: None. **A. Poudel:** None. **B. Srinageshwar:** None. **A. Uprety:** None. **O. Smith:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Presentation Number: NANO03.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: AHA/Allen Initiative in Brain Health and Cognitive Impairment 19PABH134580006
Valour Foundation
NIH/NIA F99/K00 1F99AG083111-01
the Weill Cornell Autism Research Program and National Institute of Health DA029122, DA053261, and DA054368
Rebecca E. Barchas, MD, Professor in Translational Psychiatry of Case Western Reserve University and as the Morley-Mather Chair in Neuropsychiatry of University Hospitals of Cleveland Medical Center

Title: Loss of brain Cav1.2 leads to blood brain barrier deterioration and neuroinflammation.

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Abstract: Voltage-gated calcium channel Cav1.2 is encoded by the CACNA1C gene that has been frequently implicated in neuropsychiatric diseases, such as bipolar disorder, schizophrenia, and autism spectrum disorder. Previous research has reported that loss of Cav1.2 in the forebrain results in cognitive impairment and anxiety-like behavior in mice associated with impaired survival of young hippocampal neurons. It has also recently been reported that Cacna1c genetic variants are associated with Alzheimer's disease as well. However, the mechanism of how altered Cav1.2 contributes to such as wide spectrum of neuropsychiatric disease has remained unclear. To specifically examine the function of Cav1.2 in the brain, we generated brain-specific Cacna1c knock out (KO) mice using the Nestin promoter driven Cre recombinase. Interestingly, brain *Cacna1c*KO mice displayed both structural and functional damage to the blood-brain barrier (BBB), as evidenced by astrocytic endfeet swelling around the blood vessels and severe peripheral molecule IgG extravasation. In multiple neurodegenerative conditions, the BBB has been shown to deteriorate and lead to neuroinflammation that is associated with neurodegeneration and resulting neuropsychiatric dysfunction. In brain-specific Cacna1c KO mice, we also observed neuroinflammation driven by astrocytes, increased oxidative stress, impaired neurogenesis, and neurodegeneration. Additionally, brain chemokine levels were

significantly increased, suggesting that immune cell infiltration might play a role in BBB deterioration in this model. Taken together, our data indicate a previously unrecognized role for brain Cav1.2 in maintaining BBB integrity and brain homeostasis.

Disclosures: Y. Koh: None. **M.F. Noterman-Soulinthavong:** None. **B.D. Paul:** None. **A.M. Rajadhyaksha:** None. **E.B. Taylor:** None. **A.A. Pieper:** None.

Presentation Number: NANO03.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: F30AG076183
T32NS077888
T32GM007250

Title: Acutely blocking aberrant mitochondrial fission prevents chronic neurodegeneration following traumatic brain injury

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Abstract: Progression of traumatic brain injury (TBI) into a chronic neurodegenerative disorder is a poorly understood pathology and major worldwide health problem. TBI patients can suffer for many decades with problems such as chronic headaches, nonspecific visual difficulties, irritability and depression, memory decline, and increased chance of developing earlier and more severe forms of aging-related neurodegenerative disease. Currently, there are no protective treatments for TBI patients that prevent the transition of acute TBI into a chronic condition. Here, we report that mitochondrial fission and fragmentation in the brain is acutely elevated after TBI in mice, triggering chronic neurodegeneration that persists 17 months later. We demonstrate that this acutely elevated mitochondrial fission is driven by increased Fis1 expression, which we also show is elevated in human TBI brain. Pathological mitochondrial fission can be pharmacologically inhibited using the small peptide, P110, which blocks the interaction between Fis1 and its binding partner, Drp1. We show that treating mice with P110 in the two-week period following TBI is sufficient to prevent chronically impaired mitochondrial bioenergetics, oxidative damage, microglial lipid droplet formation, blood-brain barrier deterioration, neurodegeneration, and cognitive impairment. Delaying this same treatment until 8 months after injury, however, is not neuroprotective. Thus, we identify a time-sensitive treatment of mitochondrial dysfunction in the acute phase of injury that is critical for preventing progression of TBI into a chronic neurodegenerative disease.

Disclosures: P. Sridharan: None. **Y. Koh:** None. **E. Miller:** None. **S.J. Tripathi:** None. **T. Kee:** None. **K. Chaubey:** None. **E.F. Vazquez-Rosa:** None. **S. Barker:** None. **D.E. Kang:** None. **J. Woo:** None. **B.D. Paul:** None. **X. Qi:** None. **A.A. Pieper:** None.

Presentation Number: NANO03.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CJD Foundation

Title: Adam8 is a major alpha-cleavage enzyme for cellular prion protein in CNS

Authors: J. ZHANG[1], C. WONG[1], K. KOO[1], S. JIANG[1], M. V. CAMACHO[1], J. LIANG[3], ***Q. KONG**[2,1];
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Abstract: The cellular prion protein (PrP^C) is a widely expressed cell surface glycoprotein implicated in many physiological processes (such as neuronal survival, stress protection and peripheral myelin maintenance) and the pathogenesis of several neurodegenerative disorders (such as prion diseases and Alzheimer's disease). PrP^C undergoes three types of cleavages, of which the a-cleavage occurs at the conserved hydrophobic central region of PrP, resulting in the membrane-attached C-terminal C1 fragment and the N-terminal N1 peptide released to the extracellular space. α-cleavage is the most beneficial PrP^C cleavage and of great therapeutic potential, because it not only produces the neuroprotective N1 fragment and the prion-inhibiting C1 fragment but also lowers the level of cell surface full-length PrP^C (FL-PrP) that is the essential substrate for prion replication as well as a key receptor for cytotoxic oligomers of PrP, amyloid β, tau, and α-synuclein. We have previously reported that ADAM8, a metalloprotease, is the primary PrP α-cleavage enzyme (termed αPrPase or PrP α-secretase) in skeletal muscle tissue and muscular cells. However, the identity of αPrPase(s) in the CNS is still undefined, hindering our understanding of the physiological functions of PrP^C in CNS and development of effective therapeutics against relevant brain diseases.
Here we show that ADAM8 is also a major αPrPase in the brain and neuronal cells. First, in primary neurons isolated from ADAM8 knockout (A8KO) mice, the PrP α-cleavage activity is >60% lower than that in primary neurons from wild type mice. Second, in brain tissues, the PrP α-cleavage activity in ADAM8 knockout mice is only ~70% of that of wild type mice. Third, overexpression of ADAM8 from a plasmid or rAAV vector in M17T cells (modified from the M17 human neuroblastoma cell line to overexpress human PrP) led to very significant increases in PrP α-cleavage activity and substantial decreases in FL-PrP and total PrP levels. Our preliminary data in mice indicate that treatment with rAAV-ADAM8 also led to similar effect in the brain, further supporting a significant role for ADAM8 in PrP α-cleavage in the brain and a high potential for rAAV-ADAM8 in treatment of prion disease, Alzheimer's disease and other neurodegenerative diseases where PrP plays a critical role in pathogenesis.

Disclosures: J. Zhang: None. **C. Wong:** None. **K. Koo:** None. **S. Jiang:** None. **M.V. Camacho:** None. **J. Liang:** None. **Q. Kong:** None.

Presentation Number: NANO03.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Cornerstone grant, Histochemical Society
Graduate Council Research Funding award, Southern Illinois University

Title: Cannabidiol (CBD) attenuates Doxorubicin-induced cognitive impairment through modulation of inflammatory mediators

Authors: *B. POUDEL;
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Abstract: Post-treatment side effects of chemotherapy can include cognitive deficits commonly known as Chemo-brain. Treatment with Doxorubicin (DOX), one of the most widely used chemotherapeutic drugs in the treatment of cancer, can induce several neurological disorders like depression, anxiety, and impaired cognitive function. Cannabidiol (CBD) is a non-psychoactive component of *Cannabis sativa* that has been identified as a possible therapeutic agent against many neurodegenerative disorders including traumatic brain injury, spinal cord injury, Tau protein-induced neurodegeneration, and neuropathic pain. Therefore, this study aimed to assess whether oral CBD administration could prevent DOX-induced Chemo-brain and its underlying mechanisms. Female Long Evans Hooded rats received intraperitoneal injections of DOX (6mg/kg) or vehicle (0.9% saline), once a week for four weeks, followed by oral administration of CBD (10mg/kg) three times a week for the same period. CBD was significantly protective against DOX-induced cognitive impairment as measured by several tests of anxiety and depression-like behaviors. Furthermore, CBD restored DOX-induced inflammatory insults by regulating gene expression of biomarkers of neuroinflammation in the hippocampus and prefrontal cortex. In conclusion, our findings suggest that CBD offered neuroprotection against DOX-induced cognitive impairment which may be explained at least partly by its anti-inflammatory effects. This provides insights into a possible mechanism by which CBD could alleviate DOX-induced cognitive dysfunction.

Disclosures: B. Poudel: None.

Nanosymposium

NANO04: Somatosensory Restoration Through Neuroprosthetics

Location: MCP Room N426

Time: Saturday, October 5, 2024, 1:00 PM - 4:30 PM

Presentation Number: NANO04.01

Topic: D.02. Somatosensation – Touch

Support: ERC Consolidator grant 772242 ARTTOUCH
Swedish Research Council grant 62X-3548
Sahlgrenska University Hospital grant 3161

Title: Precise sensory feedback from electrically stimulating single mechanoreceptive afferents in the human hand

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Abstract: The domain of neuroprosthetics is expanding rapidly, including the development of more user-friendly prostheses, restoring motor control, and enhancing sensory feedback. We explored the limits of sensory perception and discrimination from stimulating single mechanoreceptors in the glabrous skin of the human hand. We used microneurography to make axonal recordings from single mechanoreceptive afferents in the median nerve in healthy humans. All experiments were approved by a local ethics committee and conformed to the Declaration of Helsinki, including obtaining written, informed consent. Once a single low threshold mechanoreceptive unit was identified, we stimulated it with low current (up to 8 µA) short square wave pulses. When there was a match between the afferent recorded and the one stimulated, we continued to test different trains of electrical stimulation. We explored to what extent varying the stimulation frequency would elicit perceivable differences in sensation intensity. We conducted experiments in 71 healthy humans, where a microelectrode was inserted into the median nerve at the wrist, in order to record from and stimulate fast-adapting type I (FA-I), fast-adapting type II (FA-II), slowly-adapting type I (SA-I), and slowly-adapting type II (SA-II) afferents. The characteristics of the sensations were noted, including the size, border, and shape of the perceptual field and the quality of the sensation evoked. In intensity discrimination tests, a two forced-choice staircase procedure was used to determine the psychophysical discrimination threshold, where a reference stimulus of 30, 60, or 120 Hz was compared to a test stimulus of higher frequency, starting at 100% higher and decreasing until a threshold was found for detecting a difference between the two stimuli. A total of 250 mechanoreceptive afferents were stimulated, consisting of 125 FA-Is, 90 SA-Is, 14 FA-IIs, 21 SA-IIs. The FA-Is typically produced a small point of vibration or tingle sensation, SA-Is a very small point of pressure or pulling sensation, FA-IIs a slightly larger area of pure vibration, and SA-IIs a larger area of natural pulling/pushing sensation. Due to the larger numbers of type I afferents tested, we conducted the frequency discrimination analyses on these for 96 trials, where participants could reliably differentiate between frequencies with a 25% difference in both FA-I and SA-I units. These results show how even individual low threshold mechanoreceptors can reliably signal discriminative tactile information, which can be applied in neuroprosthetics to provide finer feedback, such as required in texture perception.

Disclosures: R. Ackerley: None. **H.B. Wasling:** None. **R.H. Watkins:** None. **M. Dione:** None. **J. Wessberg:** None.

Presentation Number: NANO04.02

Topic: D.02. Somatosensation – Touch

Support: Swiss National Science Foundation
European Union's Horizon 2020 research and innovation program under Marie Skłodowska-Curie grant 754354

Horizon Europe Research & Innovation Programme under grant 101092612

Title: Simultaneous encoding of multiple haptic modalities via transcutaneous electrical nerve stimulation

Authors: F. LEONG[1], J. MUHEIM[2], A. XU[3], C. BERNARDONI[4], O. RUGGABER[2], M. R. BRICQ[3], ***S. SHOKUR**[6,3], S. MICERA[5];
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Abstract: Haptic feedback plays a critical role in the manipulation of objects, providing essential sensory information and guiding motor control. Transcutaneous Electrical Nerve Stimulation (TENS) is a non-invasive stimulation method that allows the elicitation of various sensory feedback. Due to its potential in integration with prosthetic devices, TENS has been used extensively in the sphere of sensory feedback research for the encoding of different haptics modalities. However, most studies have focused on providing individual modalities. This study seeks to synthesize prior efforts by simultaneously encoding multiple modalities. We propose utilizing orthogonal stimulation parameter spaces to concurrently encode different haptic feedback modalities such as pressure, stiffness, texture, and motion.We ran a series of experiments using up to 4 TENS bipolar channels placed on the forearm of healthy participants. Our experiments demonstrated promising results: participants could discriminate between two directions of motion and distinguish three levels of object stiffness. Each modality was independently validated using its respective encoding strategy within the orthogonal stimulation parameter spaces. This strategy was designed to minimize the interference between different haptic modalities, thereby potentiating future multimodal solutions. To encode the directionality of motion, we tested different electrode placement arrangements; the best arrangement resulted in more than 95% accuracy in detection rate. For encoding the objects' stiffness, we varied the rate of change of stimulation current amplitude. Additionally, we tested different hand closure speeds (0.70 - 1.17 rad/s) to investigate possible interference with stiffness detection. Participants' accuracy was above chance level in all cases, with best detection at a slow speed (73%) and decreasing detection at a higher one (54%). Subsequently, we present results from experiments where multiple haptic modalities were encoded simultaneously. In our view, TENS has the potential to significantly enhance the sensory experience for prosthetic users. By building upon previous research that refined individual modalities, we propose that it is time to shift our focus towards the simultaneous encoding of multiple haptic modalities. This could open up new possibilities in the design and development of prosthetic devices, leading to richer user experiences.

Disclosures: F. Leong: None. **J. Muheim:** None. **A. Xu:** None. **C. Bernardoni:** None. **O. Ruggaber:** None. **M.R. Bricq:** None. **S. Shokur:** None. **S. Micera:** None.

Presentation Number: NANO04.03

Topic: D.02. Somatosensation – Touch

Support: MITI Grant CNRS

Title: Characterization of touch perception on the torso for application in prosthetics after breast cancer

Authors: *S. FARESSE, R. ACKERLEY;
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Abstract: Many studies have investigated touch on the hands, but far fewer have looked at touch over the rest of the human body. The skin over the whole body contributes to our sense of self and interactions we have with our environment. Presently, we investigate somatosensory perception on the human torso, focusing on the breast, and comparing this to the forearm and glabrous hand skin. Our goal is to better understand the fundamental mechanisms in somatosensory perception, to apply the insights to reproduce realistic sensations in breast prosthetics after mastectomy. We investigated affective touch, as well as tactile and thermal perception, on the palm, forearm, torso under the breast, nipple-areola complex, and upper breast. The study was approved by an ethical committee and conformed with the Declaration of Helsinki. Participants gave written informed consent. For affective touch, we used a standard skin stroking approach with a rotary tactile stimulator (Dancer Design) and a soft brush. We applied five stroking velocities per skin site (0.3, 1, 3, 10, 30 cm/s), repeated three times, at a force of 0.4 N. Participants rated tactile pleasantness, from very pleasant to very unpleasant, and intensity, from very weak to very intense, on a visual analog scale. We measured warm and cool detection thresholds with a thermode (QSTlab) at the same skin sites. Results show maximum pleasantness for the intermediate stroking velocity (3 cm/s) at all skin sites, with an inverted-U shape curve, although the nipple skin site was rated as overall less pleasant than other sites. We observed a positive linear relationship between tactile intensity and stroking velocity for all sites. For warm perception, the threshold was lower (higher sensitivity) for the palm (5.5^{o}C above skin temperature), as compared to the other sites (all around 7.5^{o}C). For cool perception, all the skin sites had a similar threshold of around 2^{o}C below skin temperature. Cool detection thresholds were closer to skin temperature than the warm thresholds, showing a higher sensitivity to cool for all sites, although we found increased variability in temperature detection threshold on the nipple-areola complex. It appears that pleasantness in nipple-areola complex is different to other zones, and it does not resemble the glabrous or hairy skin perception, indicating a different type of skin innervation. We relate these findings to the underlying neurophysiology of the skin and identify how somatosensation can be dependent on the body area stimulated, requiring a consideration of this throughout all prosthetics, but especially for the breast, which seems to be different to other skin.

Disclosures: S. Faresse: None. **R. Ackerley:** None.

Presentation Number: NANO04.04

Topic: D.02. Somatosensation – Touch

Support: Horizon Europe Research & Innovation Programme under grant 101092612 (Social and hUman ceNtered XR - SUN project)

Title: Investigation of individual differences explaining the presence/absence of thermal phantom maps in upper-limb amputees

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Abstract: In the effort to restore the natural somatosensory palette for upper limb amputees, the focus has long been centered on providing tactile cues. Lately, additional attention has been directed at targeting another fundamental haptic modality: thermosensation. We recently reported the presence of thermal phantom maps — locations on the residual arm that elicit temperature sensations perceived on the missing hand when stimulated with cold or warm objects. By targeting these skin areas with a prosthetic device, participants regained homologous thermoception. We found that such thermal phantom maps were naturally present in a majority of transradial amputees that we tested (17/27) (Iberite et al., 2023). While these results were encouraging, they also raised a fundamental question: what defines the presence/absence of phantom sensation? An answer to this question could also help us find new strategies to enlarge the spectrum of amputee recipients of thermal feedback.
We found no demographic or amputation-related aspects explaining the presence/absence of phantom thermal sensation. Indeed, neither the length of the residuum, participants' baseline temperature on the residuum, the age at amputation, nor the time since amputation were related to the absence/presence of thermal phantom maps (n=27, test name, $p>0.1$ for all tests). Instead, we hypothesize that the chaotic postoperative neural regrowth is the main underlying mechanism. In fact, the 3D analysis of the spatial distribution of thermal phantom maps (tested in 10 amputees) showed clusters of locations grouped according to the natural innervation of the radial, median, and ulnar nerves.
We propose two new directions to provide temperature sensations to the non-respondent participants: personalization of the thermode size and thermal remapping. The personalization of the thermode size could allow for more precise targeting of the reinnervated spots and finding new thermal phantom locations. Second, thermal remapping on an intact skin area could allow a non-homologous yet efficient approach to providing amputees with discriminative thermal capabilities. Tests with 5 non-responding amputee participants using this approach showed that participants' thermal discrimination accuracy was similar to the ones of their intact hand (63% accuracy for discriminating copper, glass, and plastic on the residuum skin and 64% accuracy for direct contact with the intact index). Whether it is by targeting thermal phantom spots or bypassing the afferents originally innervating the hand, we argue that current technology could potentially restore thermal perception in almost all upper limb amputees.

Disclosures: J. Muheim: 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.; Horizon Europe Research & Innovation Programme under Grant agreement N. 101092612 (Social and hUman ceNtered XR - SUN project). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coinventor of a thermal sensing device and sensory feedback system and method using said thermal sensing device (application number EP22207038.5). **F. Iberite:** A. Employment/Salary (full or part-time):; Scuola Superiore Sant'Anna. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); Coinventors of a thermal sensing device and sensory feedback system and method using said thermal sensing device (application number EP22207038.5). **S. Micera:** A. Employment/Salary (full or part-time):; EPFL, Scuola Superiore Sant'Anna. 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.; Horizon Europe Research & Innovation Programme under grant 101092612 (Social and hUman ceNtered XR - SUN project). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); coinventors of a thermal sensing device and sensory feedback system and method using said thermal sensing device (application number EP22207038.5). **S. Shokur:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coinventor of a thermal sensing device and sensory feedback system and method using said thermal sensing device (application number EP22207038.5).

Presentation Number: NANO04.05

Topic: D.02. Somatosensation – Touch

Support: Department of Veterans Affairs RR&D Service Grant RX003699

Title: Examining the temporal discrimination threshold of sensation produced by peripheral nerve stimulation in persons with upper limb loss

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Abstract: When using our hands to explore complex objects and environments, detecting the order, temporal overlap, and temporal gap between different somatosensory stimuli is crucial for effective object acquisition, discrimination, and grasp control. This study investigates the temporal processing of tactile stimuli using the somatosensory temporal discrimination threshold (TDT), which describes the shortest temporal gap between two sequential stimuli required for them to be perceived as distinct. While prior studies have examined TDT for electro-cutaneous stimulation, the TDT for sensations evoked through extra-neural peripheral nerve stimulation (PNS) remains unexplored. As the application areas of PNS broaden across clinical populations, it is important to understand the temporal properties of evoked sensations to help design stimulation strategies which more closely reproduce natural temporal properties of touch. This study was conducted in two individuals with unilateral upper extremity amputation who were implanted with 16-contact composite flat interface nerve electrodes (C-FINEs) around median, ulnar, and/or radial nerves in their residuum. We determined TDT with an ascending staircase method consistent with standard clinical methods. Across eight sessions we assessed two C-FINE contacts in each of two participants. In each session, the PNS pulse train duration or intensity pseudo-randomly varied across three values, which were selected to correspond to levels likely to occur during the daily use of a neuro-prosthesis. For each contact, 15 trials per intensity level and 10 trials per duration condition were collected. The range of PNS parameters, from threshold to the participant's self-selected maximum level, were established in an initial mapping period within each session. The mean TDT across all trials was 37.2±13.8 ms,

consistent with previously reported values for electro-cutaneous stimulation (20-90 ms). For all contacts tested across both participants, the TDT was significantly lower at mid and high PNS intensities compared to low intensities (Kruskal-Wallis test, $p < 0.01$). Over the range of PNS pulse train durations tested (100-1000 ms), no significant effect on TDT was observed (Kruskal-Wallis, $p > 0.05$). This study provides insight into how variations in common PNS parameters affect a user's ability to rapidly detect inter-stimulus timing and dynamics across stimuli. Future work can leverage these findings to more closely mimic the temporal detection properties of normal sensation to improve the performance of closed loop sensory neuro-prostheses.

Disclosures: J. Wright: None. **H. Charkhkar:** None. **D.J. Tyler:** A. Employment/Salary (full or part-time):; Afference. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Barologics, Stimulation Patents. **E.L. Graczyk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stimulation Technology Patents.

Presentation Number: NANO04.06

Topic: D.02. Somatosensation – Touch

Support: JHUAPL Internal Research Support

Title: Enabling rapid thermal perceptions using thin-film thermoelectric cooling technology

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Abstract: After amputation, touch feedback to the missing limb can help improve function for prosthesis user. Noninvasive stimulation techniques have been demonstrated for exciting peripheral nerve pathways to elicit sensations of pressure, vibration, texture, tingling, pain, and most recently temperature. In this study, we used a thin-film thermoelectric cooling device to rapidly provide thermal stimulation at speeds up to 6 °C/s and evoke sensations of cooling in the phantom hand of individuals with arm amputation. The goals of the study were both to quantify tactile experiences of receiving targeted thermal, mechanical, and electrical stimulation and to demonstrate the benefit of fast-acting thermoelectric cooling devices to enable real-time thermal feedback to enhance sensorimotor tasks. We quantified the regions of phantom hand activation and the perceived modality of sensation as a result of noninvasive thermal, electrical, and mechanical stimulation to targeted regions of the residual arm in five participants with upper limb amputation. In all participants, stimulation sites were found that resulted in touch sensations in the missing hand for all stimulation modalities. Thermal stimulation was delivered between 11-16 °C. Electrical stimulation was delivered using targeted transcutaneous electrical nerve stimulation (tTENS) with amplitudes up to 4 mA at 4 Hz. Mechanical stimulation was delivered using a 1 cm plastic probe. The experiments were approved by the Johns Hopkins Medicine Institutional Review Boards. We found that sensations of cooling or pressure could be induced in the phantom hand of all participants using all three stimulation modalities, and stimulation sites remained stable over multiple years. Thermal sensations were perceived up to 63% faster when

using the thin-film thermoelectric cooling device compared to conventional thermal stimulating technologies. During a sensorimotor task in which two participants with amputation and two additional participants without amputation controlled a virtual or physical prosthesis to grasp and identify objects based on temperature, the time spent touching each object was as little as 43% less and participants were up to 2.8 times more accurate at identifying objects when using the thin-film thermoelectric device. In this study, we demonstrated that rapid thermal feedback ultimately improved sensorimotor task performance when incorporated into a prosthesis. This work has implications for enabling future prosthetic limbs to provide more complex sensations of touch and further enhance sensorimotor function after amputation without the need for additional invasive surgical procedures.

Disclosures: L. Osborn: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder for US Patent 11,532,778, Royalties from US Patent 11,532,778. **C. Moran:** None. **B. Christie:** None. **M. Pelos:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder for US Patent 11,532,778, Royalties from US Patent 11,532,778. **R. Venkatasubramanian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder for US Patent 11,532,778, Royalties from US Patent 11,532,778. **M.S. Fifer:** None. **R. Armiger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder for US Patent 11,532,778, Royalties from US Patent 11,532,778.

Presentation Number: NANO04.07

Topic: D.02. Somatosensation – Touch

Support: Marie Skłodowska-Curie Individual Fellowship

Title: The contribution of visuo-thermal signals to the sense of body ownership: Behavioural and neural evidence

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Abstract: Introduction: The feeling that the body belongs to oneself (i.e., sense of body ownership) is the result of sophisticated processes of multisensory integration, whereby exteroceptive, proprioceptive, and interoceptive signals are continuously combined. Illusions of body ownership, such as the rubber hand illusion (RHI), can provide some insight into the interplay between vision, proprioception, and touch. The RHI experience can be modulated by manipulating the characteristics of the tactile input, for example in terms of velocity and softness (e.g., Crucianelli et al., 2018). However, the contributing role of thermal signals to the sense of body ownership and related neural mechanism remains unexplored.**Methods:** Across one behavioral and one fMRI experiments, we investigated the role of thermal interoceptive congruency to the sense of body ownership by means of a RHI, which takes place under temporal and spatial congruency rules. In Experiment 1, we manipulated the temperature of the

felt (on the real hand) and seen (on the rubber hand) touch and measured the subjectively experienced illusion, the shift in perceived hand location as an objective index of the illusion, and the perceived temperature of touch. In Experiment 2, we investigated which brain areas are related to such visuo-thermal integration effects. **Results:** We found that thermal incongruencies between the thermosensory and visual stimuli reduced the RHI both in terms of subjective experience and proprioceptive drift and gives rise to a visuo-thermal illusion effect towards the seen touch, but only when the rubber hand was placed in a plausible position (Crucianelli & Ehrsson, 2022). The results of the fMRI experiment show that the superior temporal gyrus might play a crucial role in the integration of visuo-thermal signals in the context of body ownership. **Conclusions**: Our results suggest that thermosensation contribute to the sense of body ownership, by a mechanism of dynamic integration of visual and thermosensory signals, and further highlight the importance of skin-mediated interoceptive signals to the awareness of ourselves as embodied beings (Crucianelli & Ehrsson, 2023).

Disclosures: L. Crucianelli: None. **R. Ohata:** None. **D. Radziun:** None. **H. Ehrsson:** None.

Presentation Number: NANO04.08

Topic: D.02. Somatosensation – Touch

Support: UH3NS107714
R35 NS122333
University of Chicago (Chicago Postdoctoral Fellowship)

Title: Feeling without touching: cortical and perceptual hand representations explored using intracortical microstimulation

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Abstract: By measuring tactile perception, we learn not only about the organization of the somatic sensory system, but also about the formation and maintenance of coherent mental representations of the body. Extensive evidence demonstrated large and highly-systematic perceptual distortions of tactile space. Traditional concepts of somatosensation have been shaped by the principles of somatotopic and hierarchical organization of the primary somatosensory cortex (S1). However, emerging psychophysical phenomena have been studied mostly with natural touch only that undergoes extensive processing along the tactile system, and it is unclear at which stages these phenomena arise. Intracortical microstimulation (ICMS) of S1 allows to directly evoke vivid touch sensations on the body, the properties of which can by systematically manipulated by varying the parameters of stimulation. In this work, we use ICMS of human S1 in three implanted participants to define cortical-body maps of the human hand and then to link theme with the mental-body maps. Firstly, we identified the spatial acuity of ICMS-based artificial touch and its reliability compared to natural mechanical touch. This neuroprosthetic tool allows us to explore the cortical representations of the human hand in an unprecedently granular

way. Therefore, we adopted ICMS of S1 to evoke percepts on the contralateral hand (i.e., projected fields - PFs) thanks to the direct activation of cortical neurons projecting on specific patch of skin. After marking these specific points on the participants skin, we also stimulated them through mechanical touch. Then, we directly compared distances of these points on skin and cortex (using the inter-electrode distances) generating detailed cortical-body maps. We directly observed somatotopic and magnification factors typical of the somatosensory homunculus. Collecting not only experimentally-measured distances, but also the perceived distances between two points, we directly defined the relationship between cortical-body and mental-body spaces. Our findings showed the occurrence of perceptual biases both in skin and cortical stimulation, rejecting the hypothesis of a confounding effects of peripheral processing on mental-body representations. Finally, in the context of bionics, our study sheds light on the needs of current neurotechnology, defining new implant design for sensory restoration together with novel robotic hand sensorization.

Disclosures: G. Valle: None. **G. Risso:** None. **T. Hobbs:** None. **S.J. Bensmaia:** None. **R.A. Gaunt:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Blackrock Neurotech, Inc.. F. Consulting Fees (e.g., advisory boards); Blackrock Neurotech, Inc., Neurowired LLC.. **C.M. Greenspon:** None.

Presentation Number: NANO04.09

Topic: D.02. Somatosensation – Touch

Support: DoD SCIRP SC180308
VAMR I01 RX003699
T32 EB004314

Title: Duration of peripheral nerve stimulation modifies the sensory experience and neural response in primary somatosensory cortex in humans

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Abstract: Peripheral nerve stimulation (PNS) is a powerful tool for restoring the sense of touch in individuals with sensory deficits, such as those experienced after limb loss or spinal cord injury. To understand how to produce more natural sensory percepts, we need to first explore how modulating individual PNS parameters affects the evoked sensation and cortical response. We studied the effect of varying PNS duration in an individual with an ASIA-B spinal cord injury enrolled in the Reconnecting the Hand and Arm to the Brain clinical trial. Six PNS pulse train duration conditions (0.1 s, 0.2 s, 0.3 s, 0.5 s, 1 s, and 2 s) were presented pseudorandomly to the participant's median and ulnar nerves through contacts in Composite Flat Interface Nerve Electrodes. Neural activity was recorded from two 64-channel Utah arrays located in the primary somatosensory cortex (S1). After each trial, the participant reported the perceived intensity and perceived duration of the evoked sensation on open-ended scales, and rated the perceived

naturalness on a scale of 1-10, where 10 was as natural as touch applied above injury to the participant's face. Varying duration significantly increased perceived intensity (slope-line test, $p < 0.03$), decreased perceived naturalness (slope-line test, $p < 0.001$), and increased perceived duration ratings (slope-line test, $p < 0.001$). Perceived duration varied inversely with perceived naturalness (slope-line test, $p < 0.001$). Threshold crossing features were extracted, smoothed using a 50 ms window, and normalized using z-scoring. Responsive electrodes during PNS onset or offset were defined as those that had a significant increase from baseline (paired t-test, $p < 0.05$) during the first 200 ms after PNS began or the first 500 ms after PNS stopped, respectively. Across all four sessions, the amplitude, width, and latency measures for the peaks observed at PNS onset and offset were not significantly different across the duration conditions (ANOVA $p > 0.05$). Responsive electrodes during PNS onset exhibited an average excitatory peak of 9 +/- 3 s.d. above baseline with an average peak width of 137 +/- 14 ms. This peak occurred at an average latency of 110 ms +/- 12 ms from the initial start of PNS. Responsive electrodes during PNS offset also exhibited a peak in neural activity that was on average 8 +/- 4 s.d. above baseline with a slightly larger average peak width of 168 +/- 27 ms and longer average latency times of 360 +/- 118 ms. This suggests that the timing of the onset and offset of S1 response to PNS does not depend on the duration of PNS pulse trains. These findings could help with the design of future biomimetic stimulation patterns to improve the naturalness of sensation.

Disclosures: P. Bhat: None. **B. Spilker:** None. **A. Ketting-Olivier:** None. **W. Memberg:** None. **R.F. Kirsch:** None. **B. Ajiboye:** None. **E.L. Graczyk:** None.

Presentation Number: NANO04.10

Topic: D.02. Somatosensation – Touch

Support: NIH NS122333
NIH NS107714

Title: Replay of natural neural activity to create bespoke biomimetic intracortical microstimulation for tactile feedback in humans

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Abstract: Intracortical microstimulation (ICMS) of somatosensory cortex evokes focal and salient tactile sensations, a phenomenon that we can exploit to help restore touch to those with amputation, nerve injury, or spinal cord injury. While we have excellent control over the location or intensity of the percept by changing the electrode or stimulus parameters respectively, the quality of the sensations is far less informative than natural touch; this must be improved for ICMS to provide the sensations necessary to support dexterous tactile behaviors in bionic hands. Efforts to sensitize bionic hands using electrical stimulation of peripheral nerves or somatosensory cortex have shown that stimulation encoding schemes that mimic natural tactile signals (so called biomimetic feedback) better supports interactions with objects than do non-biomimetic feedback, even reducing interference on motor decoding in closed-loop BCI tasks.

Despite these preliminary successes, biomimetic feedback has yet to produce fully realistic sensations that are comparable to natural touch experience. We posit that this is in part because previously tested biomimetic profiles, especially for indentations, are based on the averaged aggregate neural response from many electrodes and does not capture the spatiotemporal nuance of somatosensory cortex dynamics.
Consequently, we attempted to determine if approaching this objective from the other end of the spectrum, having a specific stimulation pattern for every relevant electrode, could produce more natural sensations. To achieve this, we studied one participant who was enrolled in a clinical trial of a sensorimotor BCI with electrode arrays implanted in primary sensory with residual sensation on his contralateral hand. We first recorded cortical responses to a broad range of natural tactile stimuli (indentations, vibrations, and textures). Then, we designed and implemented ICMS patterns that resembled the recorded patterns of natural cortical activation for each mechanical stimulus with multiple encoding strategies: amplitude modulation, frequency modulation, and amplitude:frequency co-modulation. In early testing, we found that amplitude modulation created more realistic sensations, compared to traditional encoding strategies, as reported by our participant.

Disclosures: C.M. Greenspon: None. **M. Bonizzato:** None. **M. Boninger:** None. **R.A. Gaunt:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Blackrock Neurotech. F. Consulting Fees (e.g., advisory boards); Blackrock Neurotech, Neurowired. **G. Valle:** None.

Presentation Number: NANO04.11

Topic: D.02. Somatosensation – Touch

Support: NIH Grant UH3NS107714
NIH Grant U01NS108922
NDSEG Fellowship

Title: Biomimetic and interleaved intracortical microstimulation improves resiliency to percept desensitization in humans

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Abstract: Intracortical microstimulation (ICMS) of the human somatosensory cortex (S1) evokes artificial tactile sensations by activating neurons that would normally respond to touch. Typically, ICMS trains consist of unmodulated single-channel stimulation with a constant amplitude and frequency. People report these vivid tactile percepts as originating from their own hand. Unfortunately, the perceived intensity of these sensations can rapidly decrease during sustained stimulation. This desensitization is a critical obstacle to using ICMS to provide reliable, natural, and uninterrupted sensory percepts for neuroprosthetics. Desensitization may occur, in part because these unmodulated ICMS trains do not resemble modulated, naturally evoked neural activity. We tested biomimetic and interleaved ICMS encoding schemes;

biomimetic ICMS mimics naturally occurring neural activity patterns and requires less charge than unmodulated stimulation to evoke equivalent percept intensities, while interleaved ICMS distributes the stimulation across multiple electrodes to minimize desensitization. Interleaved stimulation benefits from the fact that multiple electrodes evoke sensations from the same location on the hand. We hypothesized that rapidly modulating frequency and amplitude, and dividing stimulation amongst multiple electrodes, may reduce desensitization. Six different encoding strategies were tested: single and multichannel biomimetic, interleaved, biomimetic interleaved, and unmodulated single and multichannel stimulation. To test how resilient these encoding schemes are to percept desensitization, we used verbal reports from two individuals with tetraplegia each implanted with two microelectrode arrays in S1. Participants watched a clock face on a computer monitor while receiving stimulation and were asked to report how long the sensation lasted. Stimulation durations were randomly chosen from an interval between 1 to 15 s, and two repeats of each duration were tested each day. Preliminary results across three sessions show that the perceived durations of both single and multichannel unmodulated trains were typically below the actual stimulus duration. Single channel biomimetic stimulation improved the perceived duration accuracy, while both multichannel biomimetic, interleaved, and biomimetic interleaved ICMS strategies resulted in the most accurate percept duration reports. These data support the idea that biomimetic and interleaved stimulation both improve our ability to provide natural and reliable sensations for neuroprosthetics, perhaps by more accurately representing normal neural activity patterns in S1.

Disclosures: T.G. Hobbs: None. **C.M. Greenspon:** None. **C. Verbaarschot:** None. **M. Boninger:** None. **R.A. Gaunt:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Blackrock Neurotech. F. Consulting Fees (e.g., advisory boards); Neurowired, Blackrock Neurotech.

Presentation Number: NANO04.12

Topic: D.02. Somatosensation – Touch

Support: Independent Research and Development Fund from the Research and Exploratory Development Mission Area of the Johns Hopkins University Applied Physics Laboratory

Title: Electrocorticographic responses in somatosensory cortex to fingertip haptic vibrations in an individual living with ALS

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Abstract: The goal of this study was to explore how activity from the human somatosensory cortex during haptic stimulation is modulated by vibration location and amplitude. Additionally, we studied how neural responses evolved over time.Electrocorticographic (ECoG) recordings

were collected from a human participant (male) affected by amyotrophic lateral sclerosis (ALS). Two 8 x 8 subdural electrode grids (2 mm diameter electrodes, 4 mm center-to-center spacing, PMT Corporation) were implanted in the left hemisphere of the brain over sensorimotor representations for upper extremity movement and speech. We provided haptic vibration to the fingertips of the right hand for 500 ms at 300 Hz using C-3 tactors (Engineering Acoustics, Inc.) while the participant attended to his hand. On a portion of the trials, the participant verbally identified the perceived intensity of the tactile stimulation and on the other trials the participant passively experienced the vibration and did not respond.We extracted the high-gamma (70-170 Hz) and beta (13-30 Hz) power from the neural activity of the electrode array that mapped to the upper limb. We identified substantial modulation in the spectral power associated with haptic vibration amplitude and location. We also observed somatotopically organized responses in postcentral gyrus, exhibiting an inferior to superior organization from thumb to little finger across the electrode array.This work has implications for better understanding how the somatosensory cortex encodes and responds to tactile feedback over time in individuals with ALS.

Disclosures: B. Christie: None. **M.S. Fifer:** None. **L. Diaz:** None. **D. Candrea:** None. **K. Nathan:** None. **N. Thomas:** None. **S. Luo:** None. **M. Wolmetz:** None. **F. Tenore:** None. **N.E. Crone:** None. **L. Osborn:** None.

Presentation Number: NANO04.13

Topic: D.02. Somatosensation – Touch

Support: Science Fund of the Republic of Serbia (grant: IDEAS)
Innovation Fund of Serbia

Title: Neuroprosthetic device for restoring somatosensory feedback and reducing pain in patients with diabetic polyneuropathy

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Abstract: Diabetic Peripheral Neuropathy (DPN) affects 30% to 50% of the diabetic population. It is associated with chronically high blood sugar, resulting in abnormalities that cause damage to the peripheral nerve endings, with consequent loss of sensations from the foot. These patients usually also suffer from strong neuropathic pain, which is believed to be associated with aberrant sensory input, and are dealing with reduced balance and mobility.
Transcutaneous electrical nerve stimulation (TENS) holds the potential to non-invasively target the branches of nerves that innervate the area of sensory loss. We developed fully portable neuroprostheses that restore sensory feedback and suppress neuropathic pain by establishing a flow of information from the periphery to the brain via TENS. A system takes information about

the foot-ground interaction insoles and translates it into the previously chosen stimulation parameters that are delivered to the nerves innervating the foot sole when the specific parts of the insole are active. This restores lost sensation in the appropriate area in real time.
We designed a clinical study with DPN patients to understand the therapeutic effects of electrical restoration nsation lost to neuropathy. Patients were using a device during training sessions, daily throughout a two-week period. We have evaluated their performance with and without the sensory feedback during gait and while performing balance tasks and we also assessed their metabolic conof the sesumption during walking. Moreover, using standardized clinical tests we have followed if their sensory acuity and proprioceptive displacement are changing over time. The level of neuropathic pain was assessed using a visual analog scale (VAS) and neuropathic pain symptoms inventory (NPSI) questionnaires.
DPN patients were confirmed to have significant sensory loss in their feet as measured by standardized qualitative sensory testing (QST). The neuroprosthesis restored part of the lost sensation in all tested patients and improved their proprioceptive displacement. The usage of the device provided functional improvements related to movement ability. Moreover, pain was significantly reduced using a neuroprosthetic device.
These results suggest that neural stimulating prostheses are amenable to restoring lost sensation in DPN patients and have valuable functional benefits. These findings take steps towards the development of an at-home usable system for treating long-term DPN symptoms and related complications.

Disclosures: N. Katic Secerovic: None. **N. Gozzi:** None. **J. Schröder:** None. **L. Chee:** None. **S. Ilic:** None. **M. Ristic:** None. **S. Dobrijevic:** None. **A. Cimolato:** None. **G. Milic:** None. **V. Ilic:** None. **D. Stanisic:** None. **D. Gostiljac:** None. **N. Jorgovanovic:** None. **V. Dimitrijevic Sreckovic:** None. **V. Ilic:** None. **S. Raspopovic:** None.

Presentation Number: NANO04.14

Topic: D.02. Somatosensation – Touch

Support: NIH U01NS123125
NIH UH3NS107714
NIH NS122333

Title: Comparing temporal dynamics of sensations evoked by intracortical microstimulation, peripheral stimulation and natural touch in a human BCI context

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Abstract: Brain-computer interfaces (BCIs) aim to improve the quality of life of people with severe paralysis by decoding movement intentions from the brain and translating them into control signals that can be used to control robotic devices. Providing artificial sensory feedback with intracortical microstimulation (ICMS) of the somatosensory cortex substantially improves

the control of these robotic devices.
ICMS evoked sensations are reliably localized on the skin and their perceived intensity varies depending on the stimulation parameters. However, how the temporal acuity of ICMS evoked sensation compares to natural sensations remains widely unknown. Here, we investigate how the temporal dynamics of the evoked sensations are related to the dynamics of the ICMS trains themselves using a discrimination task. Four male participants with spinal cord injury participated in the study. Each had microelectrode arrays implanted in their primary somatosensory cortex for a clinical trial of sensorimotor BCI. We presented participants with two stimulation periods (with a fixed duration and stimulus amplitude) that were separated in time by a pause of varying length. Participants were asked to report whether they felt a single sensation, or two distinct sensations separated by a gap between them. Additionally, we recorded neural activity to evaluate whether the neural response in S1 relates to the psychophysical results on the perceptual task. This allows us to assess whether the participants perceptual reports could be predicted by ICMS-evoked activity in S1.
The data suggests that the temporal discrimination threshold for each participant was usually shorter than ~100 ms; though on some electrodes this threshold was as high as 400 ms. The temporal acuity of ICMS is therefore significantly higher than thresholds reported in the literature for similar experiments with touch. Next, we collected a dataset using both peripheral electrical stimulation and a pneumatic stimulator with the same experimental paradigm with healthy participants as a comparison. Additionally, two BCI participants with residual sensation in their hands to allowed for an assessment of their temporal discrimination acuity using pneumatic stimulation and peripheral electrical stimulation. Combined, these results highlight the differences between ICMS-evoked and naturally-evoked sensations and suggest that a detailed knowledge about the dynamics of evoked sensations is needed in order to optimize encoding strategies for closed-loop BCI applications.

Disclosures: R. Lienkamper: None. **C.M. Greenspon:** None. **T. Hobbs:** None. **M. Boninger:** 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.; Blackrock Microsystems. **S.J. Bensmaia:** None. **R.A. Gaunt:** 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.; Blackrock Microsystems.

Nanosymposium

NANO05: Functional Imaging and Data Analysis

Location: MCP Room S106

Time: Saturday, October 5, 2024, 1:00 PM - 4:15 PM

Presentation Number: NANO05.01

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH NINDS F31 NS108665
NIH NLM 5T15LM007059-37

Title: Ramping amplitudes of regional BOLD activity during rest follow a common sensorimotor-association axis across cortical and subcortical brain areas

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Abstract: Recent years have seen rapidly growing interest in characterizing the dynamics of BOLD fMRI time series recorded in the absence of active behavior or an externally imposed cognitive task (i.e. rest). Here, we provide evidence that the amplitude of resting BOLD fluctuations tends to increase over the course of a scan in many brain areas, and that regional differences in this ramping follow a common axis of functional specialization. We analyzed resting BOLD fMRI data from 277 healthy young adults and used a sliding-window approach to characterize how the amplitude (standard deviation) of BOLD fluctuations varied over the course of a scan in 553 cortical and subcortical brain areas. We then estimated the slope of a linear trend fit to the amplitude time series from each region. This revealed that BOLD fluctuations in cortical sensorimotor areas tend to increase in amplitude over the course of a scan, while amplitudes in higher-order cortical association areas remain stable. We observed similar trends in related time series features, including autocorrelation (i.e., "timescale") and low frequency power. Cortical trends were correlated with T1w/T2w MRI (a non-invasive index of anatomical hierarchy), as well as the primary gradient of cortical functional connectivity, and varied systematically across microstructurally-defined levels of the sensory-fugal axis of cortical organization, as well as between macro-scale functional connectivity networks. Trends in subcortical regions also follow functional hierarchies. Thalamic regions which show the strongest positive trends have strong functional connectivity to cortical association areas, while thalamic regions which show the weakest trends exhibit strong functional connectivity to sensorimotor cortical areas. We observe similar effects in the striatum and cerebellum. On average, head motion explained less than 1% of variance in amplitude time series, and amplitude trends remained significant when accounting for the level of motion in each sliding window. Global signal regression also had only a minimal effect on our results. Together, our results reveal systematic and spatially heterogeneous nonstationarities in fundamental properties of resting BOLD time series, and that these dynamics follow a common axis of functional specialization across cortical and subcortical brain areas. The spatial topography and positive trends we observe suggest that our findings may reflect fluctuations in arousal during scanning, and work is ongoing to characterize the extent to which they can be attributed to localized neural dynamics versus the influence of systemic physiological factors.

Disclosures: D.J. Lurie: None.

Presentation Number: NANO05.02

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Large-scale functional ultrasound connectivity networks in the mouse brain

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Abstract: Functional Ultrasound Imaging (fUSI) is a new neuroimaging technique that allows transcranial hemodynamic signal mapping in small laboratory animals. Through a small-scale portable probe, ultrasound penetrating deep into tissue allows imaging with a spatial resolution of 100 micrometers, making this method well-suited for imaging brain activity in rodents. The key advantages of fUSI mainly lie in its high spatiotemporal resolution, non-invasiveness, and portability. These characteristics make it an ideal complement to functional Magnetic Resonance Imaging (fMRI) for studying brain activity in preclinical species, potentially overcoming fMRI's spatial constraints and enabling neuroimaging investigations in behaving rodents. To explore the potential of fUSI and validate it against other well-established imaging techniques, we investigated its capability to map previously described resting-state functional brain networks in C57 mice. To this end, we established a robust protocol for mapping brain connectivity networks in lightly sedated mice using multi-slice acquisitions under different anesthetic conditions (n=15 mice for each condition). We designed a data processing pipeline mirroring the main steps employed in fMRI timeseries analysis for robustness and portability. Our results show that large-scale functional brain networks can be reliably mapped at the group level with fUSI with high sensitivity and reproducibility. The networks mapped included a default mode network (DMN), which we found to be anticorrelated with a latero-cortical network (LCN), as previously shown in fMRI investigations. To further validate our network findings, we compared the obtained results with the underlying axonal connectome of the mouse brain. We found that functional ultrasound networks spatially reconstitute axonal communities as previously described with fMRI. We next investigated the spatiotemporal dynamics of our resting-state datasets to see if recurring patterns of functional co-activation could also be detected by fUSI. Using this framework, we found that fUSI timeseries can be quantitatively modelled as a probabilistic occurrence of recurring co-activation modes. Overall, our results show that fUSI can be effectively used to probe the large-scale dynamics organization of brain networks in the mouse brain, comparable to other well-established imaging modalities, such as fMRI. These results will pave the way for expanding this platform to encompass more complex imaging investigations of awake and behaving mice.

Disclosures: C. Pepe: None. **J. Mariani:** None. **S. Gini:** None. **F. Ricci:** None. **G. Iurilli:** None. **A. Gozzi:** None.

Presentation Number: NANO05.03

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: ERC Grant – DISCONN 802371
CIHR postdoctoral fellowship MFE187902

Title: Probing the dynamic organization of the mouse default mode network via optogenetic-fMRI

Authors: *E. DE GUZMAN[1], A. GALBUSERA[1], B. SPAGNOLO[2], F. PISANO[4], M. PISANELLO[2], L. BALASCO[5], Y. BOZZI[5], N. SHEMESH[6], M. DE VITTORIO[2,7], T. FELLIN[3], F. PISANELLO[2], A. GOZZI[1];
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Abstract: Spontaneous fMRI activity is organized into highly dynamic spatiotemporal patterns. Within these patterns, cognitively relevant networks have been identified that have been shown to be disrupted in various brain disorders. While studies correlating fMRI with electrophysiological recordings have aimed to understand how specific brain rhythms relate to fMRI network dynamics, their findings have been inconsistent. As a result, the neural rhythms and dynamic rules that causally support fMRI network activity remain unclear. Departing from correlational research in humans, here we investigate how rhythmic modulation of the mouse medial prefrontal cortex (PFC), a hub component of the default mode network (DMN), propagates at the network level. We employed tapered fiber optogenetics to perform network-level stimulation of the PFC using either standard block-design or rhythmic oscillation. Rhythmic stimulations were performed by pulsing light, either at fixed frequency of 20Hz or jittered, and modulating the amplitude at a different envelope frequency (either 0.05, 0.1, 0.2 or 4Hz). We found that the responses to block and rhythmic stimulation were functionally distinct. Using a canonical block design, we obtained a positive and bilaterally evoked fMRI response consistent with the structural connectivity of the PFC. Rhythmic stimulation of the mouse PFC at an envelope frequency of 0.1Hz resulted in a mix of increases and decreases in functional connectivity (FC) between the PFC and other key components of the DMN. However, phase-resolved analyses revealed that the apparent reduction in FC was the expression of increased coupling throughout the DMN masked by region specific phase differences (i.e., time-lags). Using in vivo electrophysiology we probed some of these regions and found that phase differences in fMRI signal matched the phase differences in local field potential coherence (produced by the same rhythmic stimulation), supporting a neural origin for this phenomenon. Importantly, phase differences (tested at 0.05, 0.2 and 4Hz) were largely dependent on the envelope, but not carrier, frequency employed, suggesting that the time-lag of fMRI signal propagation may be a resonant property of the stimulated axonal network. Taken together, our study shows that time-averaged fMRI connectivity is strongly influenced by phase of the resulting interareal coupling, and that distributed networks like the DMN exhibit resonant-like properties in response to rhythmic stimulation. Our results shed light on the dynamic organization of fMRI network activity and its response to targeted neuromodulation.

Disclosures: E. De Guzman: None. **A. Galbusera:** None. **B. Spagnolo:** None. **F. Pisano:** None. **M. Pisanello:** None. **L. Balasco:** None. **Y. Bozzi:** None. **N. Shemesh:** None. **M. De Vittorio:** None. **T. Fellin:** None. **F. Pisanello:** None. **A. Gozzi:** None.

Presentation Number: NANO05.04

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIAAA ZIAAA000550

Title: Slow breathing increases low-frequency fMRI signal amplitude in the white matter

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Abstract: Abnormally slow breathing (or bradypnea) has been observed in various neuropsychiatric disorders including brain injury, opioid and alcohol use disorders, and chronic insomnia. There has been considerable interest in understanding how variations in breathing affect fMRI signal, yet the consequences of respiratory changes on brain function remain underexplored. A total of 15 healthy participants (7 females, 20-42 years old) were scanned in four consecutive sessions (5 min each) using an ultrafast sequence (TR = 0.385 s) with pulse and respiratory recordings to study the low frequency (LF, < 0.1 Hz) content of the fMRI signal while avoiding aliasing of high frequency cardiac and respiratory components. Participants were asked to breathe normally while viewing a static respiratory wave (session 1) or their own dynamic respiratory wave (session 2). Sessions 3 and 4 proceeded with the same setup but participants were asked to breathe slower than usual. Two-way within-subject ANOVAs were performed to assess changes in peripheral and brain responses. When instructed, participants almost halved their respiratory rate (0.22 Hz vs. 0.11 Hz, $p < 0.001$) with no significant change in heart rate. Visual feedback of respiratory signal had no significant effect, nor it had a significant interaction with slow breathing in any of the analyses ($p > 0.05$). LF power of respiratory and pulse signals significantly increased with slow breathing ($p < 0.005$). Across all conditions, we observed a significant LF temporal coupling (indexed by cross-spectrum phase) between the pulse signal and an autonomic mode network of sensorimotor, visual, and insular regions ($p_{FWE} < 0.05$), and between the respiratory signal and major vascular territories in thalamus, insula, and posterior visual and cerebellar regions ($p_{FWE} < 0.05$). Slow breathing changed the temporal order of respiratory associations with superior and middle frontal and lingual gyri ($p_{FWE} < 0.05$). Slow breathing resulted in striking increases (on average 18%) in the fractional amplitude of LF (fALFF) in all white matter territories and brainstem ($p_{FWE} < 0.05$). Increases in white matter fALFF and LF power of the respiratory signal were significantly associated ($r(13) = 0.62$, $p = 0.01$). Changes in respiratory-frontal associations implicate these regions in intentional modulation of breathing. Respiratory-related increases in white matter fALFF were different in location from regions that showed LF coupling with the respiratory signal. The findings may provide mechanistic insights into physiology of white matter fMRI signal and may be helpful in characterizing the effects of disease-related abnormal breathing on brain function.

Disclosures: E. Shokri Kojori: None. **V. Ramirez:** None. **D. Tomasi:** None. **N.D. Volkow:** None.

Presentation Number: NANO05.05

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

Support: NINDS 1R01NS109367
NINDS 1R01NS115929
NIDCD R01DC018805

Title: Enhanced structural brain connectivity analyses using high diffusion-weighting strengths

Authors: *L. YU[1], A. FLINKER[2];
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Abstract: Tractography using diffusion-weighted Magnetic Resonance Imaging (dMRI) is an emerging tool in clinical and neuroscientific research, enabling presurgical planning and the study of structural connectivity networks. Recent studies showed that with increasing diffusion-weighting strength, probed by the *b*-value, dMRI becomes primarily sensitive to the intra-axonal signal. We hypothesize that increasing *b*-values hold the untapped potential to increase the reliability of tractography. Multishell dMRI data was used from the publicly available MICRA dataset from 6 healthy participants with 5 repetitions and 6 *b*-values, below to beyond clinical standard. Whole-brain, probabilistic tractography is performed using anatomical constraints. Connectivity matrices were constructed by computing the count and density of streamlines that connect corresponding cortical areas. We first characterized the global and local change in the number and length of streamlines in long/short term range connections by fitting a polynomial mixed effect model to regress out individual differences. Focusing on critical regions for the language network, we found that the average length of streamlines connecting the Inferior Frontal Gyrus (IFG) and Middle Frontal Gyrus (MFG) significantly increased by 24.08% and 34.70% from *b*= 1200 to 6000s/mm^2. This is further reflected via increment in (a) long-range, cross-cortex connections and (b) number of interhemispheric connections. For example, the number of streamlines seeded from left IFG to left STG increased by 160.6% in *b*=6000 compared to *b*=1200, while the total number of interhemispheric connections increased from 4.4% to 7.0% in such *b*-range. We then characterized the changes of global and nodal graph network metrics. Global changes in centrality of streamline count are limited to -13.79%. Certain regions are more vulnerable to *b*-value changes. In STG and MTG, the centrality decreases dramatically, (STG -48.27% and MTG -29.49%, from *b*=1200 to *b*=6000), reflecting the heterogeneity of white matter tracts in the cortex. To quantify reproducibility, we calculated the coefficient of variation (CoV) and intraclass coefficient for global and local networks. Despite the lower SNR at high *b*-values, we do not observe loss in reproducibility of the findings. In conclusion, we observe that *b*-value is a critical experimental design factor that impacts tractography and structural connectivity analyses. Higher *b*-values are more robust in reconstructing long-range connections, and less impacted by spurious short-range connections, thereby advancing the study of structural brain connectivity.

Disclosures: L. Yu: None. **A. Flinker:** None.

Presentation Number: NANO05.06

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

Support: NIH Grant R01EB022573

Title: Characterizing individualized hierarchical brain functional networks from fMRI using self-supervised deep learning

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Abstract: Individualized brain functional networks (FNs) can effectively characterize variation in neurofunctional anatomy, providing opportunities for promoting both basic neuroscience and

translational research of neuropsychiatric illnesses. However, existing modeling methods for FNs typically identify individualized FNs at a specific scale. Inspired by evidence that the brain is a multi-scale system with a hierarchical functional organization, we develop a self-supervised deep learning (DL) method to identify multi-scale FNs with a hierarchical structure at individual level. Our DL model consists of a feature learning module, an FN learning module, and a functional hierarchy learning module. The first two modules learn informative features from fMRI data and identify FNs at the finest scale, then the learned features and FNs at finer scale are fed into the hierarchy learning module to learn the hierarchical structure between two consecutive scales, and finally the finer-scale FNs are aggregated into coarser-scale FNs according to the learned hierarchical structure. The DL model is trained using a self-supervised loss in an end-to-end way by optimizing the functional homogeneity of FNs at all scales simultaneously. Once the model is trained, it can be applied to unseen fMRI data of individuals to identify their individualized, hierarchical FNs in one single forward pass. The proposed method has been evaluated using fMRI data of 965 participants from the Human Connectome Project, and 3-scale FNs with 148, 50, 17 FNs from fine to coarse scales were identified for each participant. Experimental results have demonstrated that the FNs identified by the DL model align with well-established FNs and were highly reproducible at all scales. Furthermore, the individualized hierarchies were significantly associated with behavior measures (e.g., reading: r=0.20, grip strength: r=0.23) in a cross-validated regression analysis, indicating that they can serve as quantitative measures for charactering individualized brain functional hierarchy. Together, our method provides an effective means to characterize multi-scale FNs and the inter-scale functional hierarchy for individual subjects.

Disclosures: H. Li: None. **Y. Fan:** None.

Presentation Number: NANO05.07

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

Title: Establishing functional connectivity between functional cell types in visual cortex

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Abstract: Our aim was to establish how neurons in the visual cortex interact to form functional processing units. Although various studies have focused on identifying neural connections within diverse neural networks, several technical challenges remain *in vivo*, i.e. the need to record from large cell populations simultaneously, while also classifying cortical cell types. Here, we employed a statistical inference method, the spike-triggered average (STA), to analyse electrophysiological data from 84 neurons obtained when recording from NeuroNexus 32-channel multielectrode arrays. STA analysed the spikes triggered in a neuron in response to the activities of other neurons and established functional connectivity among neurons in the primary visual cortex (V1) in cat and wallaby. Spike-sorting was performed using the automatic spike-sorting program, Kilosort, and the manual curation tool (graphical user interface), phy, to separate spikes from different neurons based on their waveform shapes, recording depths, and spike timing. Spike-sorting allowed us to differentiate recordings from lateral geniculate nucleus (LGN) input neurons (positive spikes, PS), cortical pyramidal neurons (regular spikes, RS) and

inhibitory inter-neurons (fast spikes, FS) (Sun et al. 2021). To infer the functions of the cells, we used a data-driven technique to infer the biologically plausible receptive fields (RFs) and input nonlinearities of connected neurons using maximum likelihood methodology (Almasi et al. 2020). We found LGN neurons that had RFs with single-filter linear properties that fed into pyramidal neurons with multiple visual filters and nonlinear processing. We also identified connections between pyramidal neurons, where cells with single nonlinear filters fed into highly nonlinear multi-filter cells. In conclusion, this study employs extracellular recording techniques to correlate the responses of specific neuron types with their connectivity patterns in the visual cortex, offering insights into the intricate dynamics of the neural connectivity of visual processing. Sun SH, Almasi A, Yunzab M, Zehra S, Hicks DG, Kameneva T, Ibbotson MR, Meffin H (2021). J Physiol. 599: 2211-2238; Almasi A, Meffin H, Cloherty SL, Wong Y, Yunzab M, Ibbotson MR (2020). Cerebral Cortex 5:bhaa102.

Disclosures: Y. Shen: None. **H. Meffin:** None. **M.R. Ibbotson:** None. **A.N. Burkitt:** None. **W. Tong:** None.

Presentation Number: NANO05.08

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

Support: 5T32DA007234-37

Title: Within-subjects comparison of precision confidence mapping at 3T and 7T MRI in humans

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Abstract: Assessing the reliability of resting-state functional connectivity MRI measurements is crucial as precision functional mapping (PFM) is increasingly used to aid in personalized interventions such as transcranial magnetic stimulation (TMS) and deep brain stimulation (DBS). Image acquisition methods continue to improve alongside increased accessibility to ultra high field scanners, allowing for a finer-grained characterization of functional connectivity at higher spatial resolutions. Exploring the convergence rate of the “ground truth” in functional connectivity at standard high-field (3 Tesla) and ultra-high field 7T would aid in establishing appropriate study design criteria when conducting PFM experiments. The current study aims to compare the reliability in functional topology and topography using highly sampled resting-state MRI acquisitions at 3 Tesla (3T) and 7T MRI. Resting-state data acquired at 3T and 7T were processed using the surface-based pipelines fMRIprep version 23.2.2 [RRID:SCR_016216] and XCP-D version 0.5.2 [DOI 10.5281/zenodo.5139942]. Traditional PFM methods (Gordon et al.

2017; Laumann et al. 2015; Gratton et al. 2018) were used to calculate functional connectivity by measuring the correlations of blood oxygenation level-dependent fluctuations across the entire brain. For each magnetic field intensity, we constructed reliability curves by correlating connectivity matrices derived from incremental time segments with a proxy for a ground truth matrix created from held-out data (half of data = 35 minutes @ FD<0.2). This approach identifies the minimum amount of resting-state data required for the incremental matrices to reliably approximate the ground truth connectivity matrix, ensuring a stable assessment of functional connectivity. Preliminary data collected at 7T and 3T show that 7T MRI approaches the hold-out correlation matrix more rapidly than 3T. Using parcellated data, a hold-out correlation of 0.9 was reached with > 10 minutes of data at 7T and > 25 minutes of data at 3T. Using vertex-wise data of 91k greyordinates, correlation values of 0.7 were reached at > 20 minutes of 7T data, while data acquired at 3T did not reach this threshold within 35 minutes. These findings highlight the importance of choosing the appropriate MRI technology and data acquisition lengths to ensure accurate and reliable functional connectivity analyses, critical for advancing personalized medicine and enhancing intervention strategies in neurological and psychiatric conditions.

Disclosures: J.S.B. Ramirez: None. **G. Grimsrud:** A. Employment/Salary (full or part-time):; Turing Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Turing Medical. **J. Moser:** None. **R.J. Hermosillo:** None. **V.T. Morgan:** None. **K.B. Weldon:** None. **O. Miranda Dominguez:** None. **B.C. Tervo-Clemmens:** None. **L. Vizioli:** None. **E.S. Yacoub:** None. **D.A. Fair:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Turing Medical. **S.M. Nelson:** F. Consulting Fees (e.g., advisory boards); Turing Medical.

Presentation Number: NANO05.09

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1R01MH126699-01

Title: The BIDS connectivity project - A practical standard to report and share brain connectivity data

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Abstract: The Brain Imaging Data Structure (BIDS) has revolutionized the standardization of neuroimaging datasets, greatly enhancing data sharing, scientific transparency, and rigor within the field. Initially designed for raw MRI data, BIDS has expanded to include a broader array of imaging techniques, propelled by community contributions. Recognizing the need for standardized descriptions of advanced data derivatives, particularly in brain connectivity research, the BIDS Connectivity project was launched. This initiative aims to extend BIDS to encompass both processed data and sophisticated derivatives from various brain connectivity experiments.
The project targets standard descriptions for connectivity derivatives across six major data modalities: anatomical, diffusion-weighted, and functional MRI, PET, M/EEG, and iEEG. Such standardization is expected to bolster research capabilities by enhancing data generation, sharing, and the replication of studies using these data derivatives. It also aims to streamline neuroimaging pipelines and processing, thereby accelerating research progress.
The development of the BIDS Connectivity standards was a community-driven effort that began with a stakeholders' meeting in September 2022. This was followed by drafting new BIDS Extension Proposals (BEPs) with expert inputs, soliciting feedback from the wider neuroimaging community in Spring 2023, and conducting workshops. Over thirty experts participated in these processes, advancing five BEPs that cover diffusion voxel-wise models, diffusion tractography, connectivity matrix schema, dimensionality reduction-based networks, and brain atlas specification. These BEPs have been open for community feedback and are on track to be integrated into the main BIDS specification during 2024.
The establishment of a data-sharing standard for brain connectivity metrics represents a significant advancement toward enhancing best practices, scientific stringency, and transparency in neuroimaging. This framework will not only facilitate the integration of results from various datasets and processing pipelines but also boost interoperability among diverse brain connectivity projects. It will create opportunities for synergy across different levels of analysis, as network neuroscience aims to combine data across modalities, spatial, and temporal scales.

Disclosures: P. Herholz: None. **J. Kent:** None. **A. Heinsfeld:** None. **D. Hermes:** None. **C. Eierud:** None. **V. Calhoun:** None. **A.R. Laird:** None. **A. De La Vega:** None. **C. Gratton:** None. **R. Oostenveld:** None. **C. Pernet:** None. **E.P. Duff:** None. **A. Delorme:** None. **T.D. Satterthwaite:** None. **R.A. Poldrack:** None. **A.S. Rokem:** None. **F. Pestilli:** None.

Presentation Number: NANO05.10

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1019480
ONR Grant

Title: Deep isolation forest outlier analysis of large multimodal adolescent neuroimaging data

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Abstract: In neuroscientific data, outlier detection is critically vital for both quality control as well as clinical anomaly detection. Outliers have been found to contain clinically relevant information as well as progressing knowledge discover, especially in large datasets. Typically, heuristics or supervised learning methods are utilized for outlier detection. For comprehensive datasets, it is useful to identify anomalies in an unsupervised manner. In this work, we apply deep isolation forest to functional MRI and derived functional connectivity measures to assess outliers and compare it to typical approaches. Furthermore, we analyze the outliers to determine if they are at the sample or subject level. For subject-level outliers, we summarize the data differences in the context of functional network differences.

Disclosures: M. Wagner: None. **A. Camassa:** None. **V. Patro:** None. **K. Gano:** None. **E. Silberman:** None. **G. Cauwenberghs:** None. **T.J. Sejnowski:** None.

Presentation Number: NANO05.11

Topic: I.07. Data Analysis and Statistics

Support: Z01AA000280-18
Z01AA000281-18

Title: A Multivariate Analysis of Disturbances of Iron Metabolism in Alcohol Use Disorder

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Abstract: Clinical studies suggest a potential correlation between Alcohol Use Disorder (AUD) and systemic iron accumulation, including brain hemochromatosis. Despite the diverse nature and variability of these studies, understanding remains limited. To address this gap, we conducted a multivariate analysis to explore the effects of heavy alcohol use on iron metabolism. Our primary objective was to examine the extent of iron overload and disruptions in iron metabolism due to recent and past heavy alcohol consumption. Our secondary objective was to investigate the interactions between alcohol exposure and genetic influences on iron metabolism. We analyzed the effects of alcohol exposure level and timing on iron and related indices—transferrin, ferritin, and percent iron saturation—among 2,180 participants, including 691 AUD patients and 1,489 non-AUD individuals. Our statistical analyses included Pearson correlation and ANOVA to assess variable independence, and we used quintile-based relative risk to evaluate the impact of AUD on iron variables. The results were analyzed using restricted cubic spline regression (RCS) to model a continuous association between iron variables and AUD. Additionally, we applied Mendelian randomization using iron and AUD genome-wide association studies (GWAS) to explore causal relationships between iron metabolism and AUD. Our findings indicate a significant association between AUD and iron biomarkers. Serum ferritin levels demonstrated a high relative risk of 4.44 (95% CI: 2.81-6.98), and RCS modeling confirmed a linear relationship between serum ferritin concentration and AUD. The Mendelian randomization analysis revealed a reciprocal causal effect between iron levels and AUD, underscoring the complex interactions influencing these conditions.

Disclosures: Y. Jung: None. **K. Morisaki:** None. **M. Schwandt:** None. **C. Hodgkinson:** None. **D. Goldman:** None.

Presentation Number: NANO05.12

Topic: I.07. Data Analysis and Statistics

Support: NIMH 5R01MH121069

Title: Nonergodicity in neurocognitive dynamics of inhibitory control

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Abstract: Nonergodicity—when inferences about a group of subjects fail to generalize to the subjects individually—poses a significant and underappreciated challenge for neuroscience. We demonstrate this by examining the neurocognitive dynamics underlying inhibitory control with stop-signal task data from thousands of children in the Adolescent Brain Cognitive Development study (N=4469 subjects aged 9-10; 48% male and 52% female sex assigned at birth). To study mechanisms of inhibitory control while allowing for nonergodicity, we developed a Bayesian computational model that characterizes the latent cognitive constructs governing action execution and inhibition in the stop-signal task. This model allowed us to infer trial-level stop-signal reaction times (SSRTs), probabilities of proactivity, and anticipated stop signal delays (SSDs), for each subject. Then, we related these dynamic representations of subjects' behaviors to simultaneous BOLD fMRI activity between and within subjects. Between subjects, we correlated subject-average correct stop versus correct go contrasts with subject-average SSRTs, probabilities of proactivity, and anticipated SSDs. Within subjects, we examined how SSRT, probability of proactivity, and anticipated SSD parametrically modulated brain activity. Strikingly, between-subjects associations were reversed within subjects, revealing the nonergodic nature of these processes. Between subjects, the correct stop versus correct go contrast was generally negatively associated with SSRT, not associated with probability of proactivity, and positively associated with anticipated SSD. But within subjects, each of these three parameters showed a mixture of positive and negative associations with brain activity. SSRT was associated positively with activity in the salience and somatomotor networks and negatively with activity in the limbic network. Proactivity, as captured by both the probability of proactivity and anticipated SSD, was associated with suppression of the salience and frontoparietal networks, prominently the right anterior insula, and was associated with activation of default mode areas such as the left retrosplenial cortex. We tested the replicability of these results by resampling them at varying sample sizes. The within-subjects findings were highly stable and key results were likely to be observed in samples as small as 25 subjects. Our work emphasizes the need to consider nonergodicity and employ within-subjects analysis in the study of cognition and its brain bases.

Disclosures: P.K. Mistry: None. **N.K. Branigan:** None. **Z. Gao:** None. **W. Cai:** None. **V. Menon:** None.

Presentation Number: NANO05.13

Topic: I.07. Data Analysis and Statistics

Support: NIH R01NS130183
MJFF-010435

Title: Data-driven optimization of deep brain stimulation parameters for gait improvement in Parkinson's disease: insights into neurophysiological mechanisms

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Abstract: Deep brain stimulation (DBS) has emerged as an effective treatment for various symptoms of Parkinson's disease (PD); however, effectively managing advanced gait-related issues remains challenging and is an active area of research. Challenges include the complex nature of gait functions, variable responses to DBS settings, and the intricate neurophysiological mechanisms underlying DBS effects on gait. Additionally, DBS programming, primarily based on appendicular symptoms does not fully capture gait disorders in PD. This study addresses these challenges by identifying optimized DBS settings to enhance walking, analyzing neurophysiological impacts of DBS on gait, and exploring the neural characteristics of the basal ganglia and motor cortex. Local field potentials (LFPs) were recorded from the globus pallidus and motor cortex of three PD patients using a bidirectional, investigational device (Summit RC+S, Medtronic). Gait kinematics were captured using inertial measurement unit sensors. During clinic visits, patients' DBS settings were systematically altered, and subjects were asked to perform overground walking tasks in response to each setting adjustment. A walking performance index was developed to capture a variety of gait kinematics important in PD. Employing a data-driven approach, Gaussian Process Regression (GPR), we modeled the relationship between the DBS setting parameters and identified the optimized configurations for enhancing walking performance. The neurophysiological impact of these optimized settings on the cortical and subcortical networks was analyzed using linear mixed-effects models.
We identified DBS settings that resulted in an overall 18% improvement in walking performance for PD patients. This improvement includes a 16% increase in stride velocity, a 5% increase in arm swing amplitudes, and more symmetric walking, with a 37% and 31% reduction in step length and time variabilities, respectively. Additionally, enhanced walking performance is associated with reduced LFP signal power in the beta band of the pallidum area during the ipsilateral leg swing and the double support period ($p < 0.05$, $\chi^2 \approx 4.3$). Furthermore, increased coherence between the pallidum and the primary motor cortex in the alpha frequency band during the stance phase of walking correlated with higher performance levels (p $lt 0.05, $\chi^2 \approx 4.8$). Our findings highlight the potential of using GPR to optimize DBS parameters, underscoring the importance of personalized interventions. The neurophysiological insights from this study provide a foundation for designing adaptive DBS systems that improve gait functions in PD patients.

Disclosures: H. Fekri Azgomi: None. **K.H. Louie:** None. **J.E. Bath:** None. **K. Presbrey:** None. **J. Balakid:** None. **J. Marks:** None. **D.D. Wang:** F. Consulting Fees (e.g., advisory boards); Consulting with Medtronic, Boston scientific, and iota biosciences.

Nanosymposium

NANO06: Neural Activity Underlying Higher-Order Human Behaviors: Insights From Intracranial Recordings

Location: MCP Room N427

Time: Saturday, October 5, 2024, 1:00 PM - 4:00 PM

Presentation Number: NANO06.01

Topic: H.08. Learning and Memory

Support: NIH K99MH132873
NIH R01DA043695
NIH R21DA049243
NIH R21MH120789
NIH R01MH122611
NIH R01MH123069
NIH R01MH124115

Title: Neuronal activity in the human hippocampus links reward-prediction errors to subsequent memory

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[1]Psychiatry, [2]Neurosci., [3]Neurosurg., Icahn Sch. of Med. at Mount Sinai, New York, NY;
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Abstract: Reinforcement learning (RL) is a prominent framework for understanding why we prioritize certain decisions, tethered to the release of dopamine associated with reward-prediction errors (RPEs). Here, we utilized this framework to understand why the human brain prioritizes certain events in memory. Healthy controls (n=140) and neurosurgical patients (n=20) performed a gambling task wherein decisions resulted in both probabilistic rewards and associated image stimuli, followed by a recognition test for these image stimuli. Using computational models to estimate trialwise RPEs, we found that positive RPEs not only encouraged participants to prioritize certain choices, but also predicted successful recognition memory. Neurally, RPEs elicited high-frequency activity (HFA), a proxy for local neuronal spiking, in several regions including hippocampus, amygdala, and anterior insula. However, only hippocampal electrodes showed a strong correlation between RPE-modulated HFA during encoding and memory-modulated HFA during subsequent recognition. These findings demonstrate that neuronal spiking in the hippocampus, possibly reflecting dopaminergic input from midbrain areas, selectively prioritize memory for the reward computations that govern reinforcement learning.

Disclosures: S.E. Qasim: None. **L. Nunez Martinez:** None. **F. panov:** None. **A.E. Rhone:** None. **C.M. Garcia:** None. **B.J. Dlouhy:** None. **X. Gu:** None. **I. Saez:** None.

Presentation Number: NANO06.02

Topic: H.08. Learning and Memory

Support: MRC Grant MR-T032553-1
NIH Grant DC004290

Title: Human single neuron activity modulated during auditory working memory and at event boundaries

Authors: *J. I. BERGER[1], A. J. BILLIG[2], P. E. GANDER[1], S. KUMAR[1], K. V. NOURSKI[1], C. K. KOVACH[3], A. E. RHONE[1], C. M. GARCIA[1], H. KAWASAKI[1], M. A. HOWARD III[1], T. D. GRIFFITHS[4];
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Abstract: In order to perceive the world around us, our brains have to keep scenes in memory and detect when scenes change. Recent intracranial work utilizing visual paradigms has begun to elucidate the specific neural mechanisms involved in these processes, highlighting contributions of structures such as the hippocampus and cingulate cortex. However, it is not yet clear whether the same mechanisms are involved in the processing of acoustic stimuli. Here, we report human intracranial recordings of single neurons recorded from various brain structures while participants performed separate tasks involving either auditory working memory or auditory boundary detection. We intentionally used non-verbal stimuli to isolate these processes and avoid potential confounds of including semantic information. For the working memory task, participants were required to keep in mind a target tone on each trial and then – following a delay period – match a repeated tone to the frequency of the target. For the boundary detection task, participants listened to concatenated texture sections, each lasting several seconds and consisting of tone glides randomly overlapping in time and frequency. Glide direction and frequency excursion were fixed within a section, but one or both parameters changed from section to section – thus creating acoustic boundaries that did not vary in their overall energy or spectrum. Participants listened passively to the stimuli and were then required to listen again while detecting boundaries between acoustic events. Single neurons were isolated offline using an automated procedure with manual curation. We found neurons within the hippocampus and cingulate whose firing rates were modulated at various phases of the working memory task, including throughout the delay period and during active tone adjustment. Often, these neurons showed suppression rather than increased activity, though there was heterogeneity in response types across the population. Additionally, for the first time, we demonstrate cells in the hippocampus that respond to acoustic boundaries only when participants are actively engaged in detecting these events. Overall, these data implicate the hippocampus and cingulate cortex in auditory event segregation and working memory. Studies are ongoing to determine how widespread these processes are and how they relate to activity within auditory cortex.

Disclosures: J.I. Berger: None. **A.J. Billig:** None. **P.E. Gander:** None. **S. Kumar:** None. **K.V. Nourski:** None. **C.K. Kovach:** None. **A.E. Rhone:** None. **C.M. Garcia:** None. **H. Kawasaki:** None. **M.A. Howard:** None. **T.D. Griffiths:** None.

Presentation Number: NANO06.03

Topic: H.08. Learning and Memory

Support: Return program of the Ministry of Culture and Science of the State of North Rhine-Westphalia

Title: Identifying egocentric bearing cells in the human medial temporal lobe

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Abstract: Place, grid, and head-direction cells are prominent examples of allocentric cell types that encode spatial information relative to the external environment. In contrast, egocentric cells represent spatial information relative to the navigating subject. They encode, for example, whether a boundary or landmark is ahead, behind, to the left, or to the right of the subject (Wang et al., Science, 2018; LaChance et al., Science, 2019). Whereas allocentric cells have been widely studied over the last five decades—leading to many important insights into their tuning properties—much less is known about egocentric cells. Using human single-neuron recordings in epilepsy patients performing virtual navigation tasks, we recently identified egocentrically tuned cells in the human medial temporal lobe (Kunz et al., Neuron, 2021). These egocentric bearing cells represent egocentric directions and distances between the subject and reference points in the environment, and they appear to be particularly common in the parahippocampal cortex. Recent rodent studies identified similar cells in the rodent hippocampal formation and suggested that they are important for goal-directed navigation (Ormond & O'Keefe, Nature, 2022; LaChance & Taube, Hippocampus, 2023). However, analytically identifying egocentrically tuned cells is non-trivial and requires methods that adequately control for the unequal sampling of locations and heading directions as is the case in many empirical studies of spatial navigation. Here, we thus performed numerical simulations to study the accuracy of our method for identifying egocentric bearing cells. Our results show that this method reliably detects egocentric bearing cells; that it detects their reference points with good accuracy; and that it does not incorrectly identify allocentric place or direction cells as egocentric bearing cells. We find, in contrast, that egocentric bearing cells can be mistaken as allocentric direction cells and that egocentric bearing cells with distance tuning can be mistaken as allocentric place cells. Our findings may help investigating egocentrically tuned cells in humans and other animals, and they may help advance our understanding of how the brain accomplishes spatial navigation and spatial memory.

Disclosures: L. Kunz: None. **L. Nett:** None. **T. Guth:** None.

Presentation Number: NANO06.04

Topic: H.08. Learning and Memory

Support: DFG Emmy Noether HE8329/2-1
DFG Emmy Noether 423175063
Hertie Network for Excellence in Clinical Neurosciences
Jung Foundation, Ernst Jung Career Advancement Award in Medicine
Medical Faculty of the University of Tübingen, TüFF Program
Medical Faculty of the University of Tübingen, JRG Plus Program

Title: High-frequency band activity: Triple dissociation of neural firing, gamma oscillations and aperiodic activity

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Abstract: Intracranial electroencephalography (iEEG) provides an exceptional spatiotemporal resolution to understand the neural population dynamics underlying human behavior. Specifically, high frequency band activity (60-180 Hz; HFA), which is sometimes referred to as 'high gamma activity', constitutes an information-rich signal that has been shown to index local cortical activation and predict human behavior on a single trial level. While HFA is often regarded as a proxy of neural firing, several recent lines of inquiry highlighted that this view might be overly simplistic. Here, we combined intracranial recordings from early visual cortex in humans and mice with selective optogenetic perturbations in different behavioral settings, to understand the factors that shape the HFA response. We employed spectral parametrization to separate oscillatory from aperiodic responses and observed a strong correlation between neural firing and aperiodic activity during different arousal states. The correlation between neural firing and gamma oscillations, however, was context-dependent. Sensory input in form of visual gratings monotonically increased the oscillatory responses, while neural firing exhibited a non-linear response profile. Critically, visual stimulation left the aperiodic activity unchanged. Optogenetic inactivation of different interneuron subtypes (somatostatin-, vasoactive intestinal polypeptide- and parvalbumin-positive) revealed that a modulation of the underlying balance between excitatory pyramidal cell activity and inhibitory interneuron activity mainly modulated aperiodic neural dynamics, with a more nuanced impact on oscillatory activity. Critically, these findings were well explained by a canonical microcircuit model, which indicates that the broadband high-frequency range of electrophysiological brain activity encompasses multiple concordant processes that jointly shape the local field potential. In sum, these observations link a canonical microcircuit to large-scale population dynamics as observed in humans and provide insights into the underlying physiology of broadband high-frequency activity.

Disclosures: R.F. Helfrich: None. **J. Veit:** None. **J.D. Lendner:** None.

Presentation Number: NANO06.05

Topic: H.08. Learning and Memory

Support: McDonnell Center for Systems Neuroscience
AFOSR (FA9550-21-1-0088)
NSF (BCS-1945230, IIS-2114644)
NIH (R01MH129426)
NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
NIH/NINDS (U24-NS109103)

Title: Neural coding and dynamics of faces in the human ventral temporal cortex

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Abstract: Understanding how the human brain recognizes faces has been a central inquiry in cognitive neuroscience for decades. While neuroimaging studies have extensively explored the functional localization of face processing, the neural computations underlying face recognition remain elusive. Among the face-selective areas localized in previous fMRI studies, which typically included posterior and middle fusiform gyrus (also know as pFus and mFus), the anterior fusiform gyrus was rarely discussed. It is unclear whether the lack of face-selective response in the anterior VTC is simply due to magnetic susceptibility artifacts or the functional differences. To address this gap, we utilized human intracranial electroencephalogram (iEEG), offering high spatial-temporal resolution, to investigate the neural encoding of faces across different brain areas in the VTC. Specifically, we recorded iEEG signals from 13 drug-resistant epilepsy patients while they viewed 500 natural faces. We identified 4 regions of interest (ROIs) based on anatomical landmarks, including the middle and anterior fusiform gyrus (mFus and aFus), and the middle and anterior inferior temporal gyrus (mIT and aIT). Distinct coding models of faces have been proposed in single-neuron studies, separately in the human brain and non-human primate brain. We hence surveyed the distribution of these models across the VTC areas. First, our results revealed widespread visual responsiveness across all ROIs, with response latencies increasing anteriorly, indicating the involvement of anterior VTC in visual face processing. Second, identity-selective responses were predominantly observed in the fusiform gyrus, with a strong right lateralization in the aFus. Third, by fitting the neural responses with visual features extracted from a deep neural network (DNN), we demonstrated that both the mFus and aFus exhibited robust axis-based feature coding, also with a pronounced right lateralization in the aFus. Additionally, the mFus exhibited a preference for encoding features from the intermediate DNN layers, while the aFus favored features from the later DNN layers, suggesting a feedforward visual feature processing hierarchy in the VTC. In contrast, neither the mIT or aIT showed significant axis-based feature coding. Lastly, our results revealed a decrease in the ratio of axis coding channels relative to identity coding channels from the mFus to the aFus, implicating a progression of identity-specific processing along the visual hierarchy. Together, these findings advance the mechanistic understanding of face processing in the human VTC, filling a crucial gap in the existing neuroimaging literature.

Disclosures: R. Cao: None. **T. Xie:** None. **Y. Wang:** None. **P. Brunner:** None. **J.T. Willie:** None. **S. Wang:** None.

Presentation Number: NANO06.06

Topic: H.08. Learning and Memory

Support: NIH R00NS115918
NIH R01MH107512
NIH R01NS021135
NIH R00MH117226

Title: Intracranial slow theta rhythms and aperiodic activity predict memory outcomes from childhood through middle age

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SHAIKHOUNI[12], K. I. AUGUSTE[13], E. F. CHANG[13], P. BRUNNER[14], J. L. ROLAND[14,15], R. M. BRAGA[1], R. T. KNIGHT[16], N. OFEN[3], ***E. L. JOHNSON**[2];
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Abstract: The hippocampus is causally involved in episodic memory and posited to support memory in children. The prefrontal cortex (PFC) is central to higher-order cognition and posited to support developmental improvements in memory from childhood into young adulthood. Both regions demonstrate structural and functional changes with advancing age across adulthood that make them susceptible to neurodegenerative disease. Yet, it is not known how age affects hippocampus or PFC physiology from childhood through middle age, or whether age-related neurophysiological differences have distinct spatiotemporal profiles that predict memory ability. In this preregistered study, we analyzed intracranial EEG data from an exceptionally large cohort of neurosurgical epilepsy patients (n = 101, n seizure- and artifact-free channels = 5,691; age range = 5-55 years; 63 males). We isolated signals from each channel that have been linked to memory, higher-order cognition, and aging: slow (~1.5-4.5 Hz) and fast theta (~4.5-8 Hz) frequencies and the broadband aperiodic slope. We quantified these signals in the hippocampus and subregions of PFC during memory task-based (i.e., visual memory recognition task) and task-free (i.e., awake rest) states, mapped effects by age, and related effects to individual memory outcomes. We found that: 1) in hippocampus, faster slow theta rhythms predict superior memory recognition independent of age, and 2) in inferior frontal gyrus, the age-related slowing of slow theta rhythms predicts age-related improvements in memory recognition. Both effects were specific to theta rhythms during memory encoding and were not observed during rest, suggesting region-by-region relationships specific to task engagement. By contrast, 3) in rostral middle frontal gyrus, we observed age-related flattening of aperiodic slopes during both memory encoding and rest that predicted age-related improvements in memory recognition. These effects reveal the aperiodic slope in PFC as a potential trait-like marker of healthy brain development across the lifespan. Theta rhythms and aperiodic activity reflect distinct neurophysiological mechanisms, each of which may be targeted with brain stimulation to slow down or ameliorate age-related memory decline characteristic of many neurodegenerative diseases.

Disclosures: Z.R. Cross: None. **S.M. Gray:** None. **A.J.O. Dede:** None. **Q. Yin:** None. **P. Vahidi:** None. **E.M.B. Rau:** None. **C. Cyr:** None. **A.M. Holubecki:** None. **E. Asano:** None. **J.J. Lin:** None. **I. Saez:** None. **F. Girgis:** None. **S.U. Schuele:** None. **J.M. Rosenow:** None. **J.Y. Wu:** None. **J.S. Raskin:** None. **A. Shaikhouni:** None. **K.I. Auguste:** None. **E.F. Chang:** None. **P. Brunner:** None. **J.L. Roland:** None. **R.M. Braga:** None. **R.T. Knight:** None. **N. Ofen:** None. **E.L. Johnson:** None.

Presentation Number: NANO06.07

Topic: H.08. Learning and Memory

Title: Reward circuit local field potentials modulate risk taking

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Abstract: Risk taking behavior can be a symptom of multiple neurologic and psychiatric disorders. Neuroimaging studies suggest that reward signaling regions including orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), amygdala, and insula are involved in risk taking. However, the associated electrophysiological activity and how this may be related to reward signaling are understood. Eleven epilepsy patients with implanted depth electrodes in the amygdala, OFC, ACC, and/or insula participated. Patients completed a gambling task while local field potentials (LFPs) were recorded. Subjects were shown a playing card and after a delay were prompted to place a wager ($5 vs $20) as to whether this card was higher than a face down card. After their response the card was revealed followed by feedback about the outcome. We used cluster-based permutation testing to identify time frequency clusters that significantly differed between unexpected and expected rewards to identify LFP correlates of reward prediction error (RPE). We also used cluster-based permutation testing to identify time frequency clusters that were different prior to low versus high bets in high risk (<50% chance of winning) trials. We used two-way ANOVA with bet and risk level to classify risk features of clusters. We used linear mixed effects models to evaluate the relationship between RPE and risky decision power. A linear regression model to evaluated the relationship between risky decision power and impulsivity scores. We found clusters associated with RPE in the amygdala (p=0.0066), ACC (p=0.0092), and OFC (p=6.0E-4). Risky decisions were predicted by increased high-gamma oscillatory power during card presentation in OFC (p=0.0022) and by increased power during bet cue presentation in theta-to-beta range in OFC (p=0.0022), high-gamma in ACC (p=0.004), and high-gamma in insula (p=0.0014). Decreased OFC gamma power predicted risk adverse decisions (*p*=2.0E-4). Insula risky decision power was associated with OFC high-gamma RPE power (p=0.0048) and impulsivity scores (p=0.0047). Our findings identify and help characterize reward circuitry activity predictive of risk-taking in humans. These findings may serve as potential biomarkers to inform the development of novel treatment strategies in the future.

Disclosures: N. Hughes: None. **B. Singh:** None. **Z. Wang:** None. **D.J. Englot:** None. **S. Williams Roberson:** None. **C. Constantinidis:** None. **S.K. Bick:** None.

Presentation Number: NANO06.08

Topic: H.08. Learning and Memory

Support: ERC-2018-COG 819814
SNSF 204651
Swiss Epilepsy Foundation

Title: Neuronal population representation of human emotional memory

Authors: *D. FETTERHOFF[1], M. COSTA[1], R. HELLERSTEDT[1], R. JOHANNESSEN[2], L. IMBACH[2], D. LEDERGERBER[2], J. SARNTHEIN[3], B. A. STRANGE[1];

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Abstract: Understanding how emotional processing modulates learning and memory is crucial for the treatment of neuropsychiatric disorders and pathologies characterized by emotional memory dysfunction. We investigated how the human medial temporal lobe (MTL) supports emotional memory on a single-unit level by recording neuronal spiking activity from the hippocampus, amygdala, and entorhinal cortex during encoding and recognition sessions of an emotional memory task in patients with medically resistant epilepsy. Our findings reveal distinct representations for both remembered compared to forgotten, and emotional compared to neutral scenes in both single units and MTL population spiking activity. Additionally, we demonstrate that a distributed network of human MTL neurons exhibiting mixed selectivity on a single-unit level collectively processes emotion and memory as a network, with a small percentage of neurons responding conjointly to emotion and memory. These results expand our understanding of the neural mechanisms underlying emotional memory by focusing on the activity of individual neurons rather than signals measured at more macroscopic levels, like electrical intracranial local field potentials (LFPs) and hemodynamic responses measured with functional magnetic resonance imaging (fMRI). Using microelectrodes to record spiking activity represents a promising approach for studying emotional memory by enabling a more detailed understanding of the underlying dynamics, and further research into these neurophysiological mechanisms could lead to a better understanding of how emotional and memory processing interact during healthy and maladaptive learning. Finally, analyses of spike-LFP coupling will be presented.

Disclosures: D. Fetterhoff: None. **M. Costa:** None. **R. Hellerstedt:** None. **R. Johannessen:** None. **L. Imbach:** None. **D. Ledergerber:** None. **J. Sarnthein:** None. **B.A. Strange:** None.

Presentation Number: NANO06.09

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH128187

Title: Computational and local neural contributions to the intracranial reward positivity

Authors: *R. L. COWAN[1], T. S. DAVIS[1], B. KUNDU[2], S. RAHIMPOUR[1], B. SHOFTY[1], J. D. ROLSTON[3], E. H. SMITH[1];
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Abstract: Reward Positivity (RewP) is a reward-related potential that occurs 250 - 350 ms post-outcome. Distinct from the feedback-related negativity, RewP is a difference potential between rewarded and unrewarded outcomes. RewP has been associated with prediction error (RPE) signals, which are critical for updating value expectation (RVE) when a mismatch between expected and actual reward occurs. Scalp source localization studies mostly localize RewP to anterior cingulate cortex (ACC), but also subcortical structures and the parietal lobe. Limitations inherent in EEG localization studies motivated us to study the RewP intracranially. We sought to identify the neurocomputational underpinnings and neural generators of RewP via trial-by-trial estimation of RVE and RPE in intracranial recordings of the human brain. We study these features across subjects in the context of impulsive choice (IC): a maladaptive tendency shown to

contribute to substance use and other psychiatric disorders.We recorded intracranial and scalp potentials from 43 patients undergoing neuromonitoring for epilepsy while they carried out the Balloon Analog Risk Task (BART). We calculated IC using the Kullback-Leibler Divergence (KLD) between active and passive trial inflation time distributions. RewP was examined for broadband local-field potential (LFP), high broadband frequency (HFA: 70 - 150 Hz), an established measure of neuronal population firing, and the broadband spectrogram (spectral: 1-150 Hz). For these signals, outcome-aligned HFA and LFP were modeled as a linear combination of the RewP amplitude, impulsivity, and temporal difference learning variables, RVE and RPE, via linear mixed effects models. The KLD metric categorized our cohort as more and less impulsive choosers. From 3211 intracranial electrodes, rank-sum tests revealed RewP was encoded on 315 LFP, 297 HFA, and 1251 spectral contacts. For the HFA model, RPE (t=-3.887, p<0.001) and actual reward (t=3.532, p<0.001) were significantly predicted by RewP amplitude; with more robust RPE signals eliciting lower RewP amplitudes and greater reward increasing RewP. For the LFP model, we also saw interactions between impulsivity level, RVE, and actual reward (t=2.547, p=0.011), suggesting a modulatory effect of impulsivity on RewP. Regional analysis revealed RewP encoding in ACC for LFP (12.3%) and HFA (8.3%), as well as other subcortical regions. We extend understanding of intracranial RewP, showing associations with learning signals and modulation by impulsivity, as well as how RewP is parametrically encoded in the human brain. These findings have implications for reinforcement learning and psychiatric disorders.

Disclosures: R.L. Cowan: None. **T.S. Davis:** None. **B. Kundu:** None. **S. Rahimpour:** None. **B. Shofty:** None. **J.D. Rolston:** None. **E.H. Smith:** None.

Presentation Number: NANO06.10

Topic: H.08. Learning and Memory

Support: NINDS Intramural Competitive Postdoctoral Fellowship Award
NIH Grant R01-MH104606
NIH Grant U01-NS113198
NIH Grant RF1-MH114276
DARPA Restoring Active Memory programme (Cooperative Agreement No. N66001-14-2-4032)

Title: The direction of theta and alpha traveling waves modulates human memory processing

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Abstract: To support a range of behaviors, the brain must flexibly coordinate neural activity across regions, yet the precise mechanism for this coordination is unknown. One potential mechanism is a traveling wave, in which a neural oscillation propagates across the brain while organizing the order and timing of neural activity. Although spontaneous traveling waves are present across the brain during a range of behaviors, their functional relevance remains mysterious. Here, using direct human brain recordings from neurosurgical epilepsy patients, we

demonstrate a distinct functional role for traveling waves of theta- and alpha-band (2-13 Hz) oscillations in the cortex. Traveling waves propagated in different directions during separate cognitive processes. In episodic memory, traveling waves tended to propagate in a posterior-to-anterior direction during successful memory encoding and in an anterior-to-posterior direction during recall. Because traveling waves of oscillations correspond to local neuronal spiking, these patterns indicate that neural activity moves across the brain in different directions for separate behaviors. We then modulated the direction of traveling waves by delivering direct electrical stimulation to different cortical locations at a range of frequencies and amplitudes. We demonstrated that waves often change direction after high-frequency stimulation of the cortex. In particular, when waves propagated towards a stimulation location prior to stimulation, they were most likely to switch to the opposite direction following stimulation. Our results suggest that traveling waves play a fundamental role in flexibly organizing the timing and direction of network interactions across the cortex to support cognition. By characterizing the effects of brain stimulation on the direction of traveling waves, our results provide causal evidence in humans into how brain stimulation may be optimized to modulate traveling waves that support memory processes.

Disclosures: U.R. Mohan: None. **H. Zhang:** None. **B.G. Ermentrout:** None. **J. Jacobs:** None.

Presentation Number: NANO06.11

Topic: H.08. Learning and Memory

Support: U01NS117839
K99NS126233

Title: Prefrontal and Medial Temporal Neurons Encode Ordinal Information of Event Sequences in Humans

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Abstract: Remembering the temporal order of events is critical for episodic memory. Previous studies suggest that linking individual events into temporally associated memories relies on the medial temporal lobe and prefrontal cortex. Damage or losses to these regions can disrupt the recall of stories and real-life events in the correct temporal sequence. Despite the critical role of the medial temporal lobe and prefrontal cortex in episodic memory, little is known about how ordinal information is encoded and later retrieved in these regions. To address this question, we recorded single-neuron activity and local field potential signals with depth electrodes implanted in 11 drug-resistant epilepsy patients for diagnostic purposes. Participants first watched 25 video clips with no audio. Each clip contained four events, with visual cuts either inserted at event boundaries, away from boundaries, or without visual cuts. Participants' memory of clip content

was subsequently evaluated in two memory tests. In the scene recognition task, participants identified whether a frame was familiar or not. In the time discrimination task, participants determined which event happened first when presented with two frames associated with the tested events. Behaviorally, participants had comparable recognition memory regardless of the clip type but had more accurate order memory for frame pairs extracted from clips with visual cuts at event boundaries. We recorded 634 neurons in total. At the single cell level, we found neurons in the hippocampus (6%), amygdala (5%), and orbitofrontal cortex (7%) that transiently increased their firing rates selectively following a specific ordered event boundary (e.g., 2nd event boundary in a four-event clip) relative to clip onset, invariant to event contents. At the population level, hippocampal and prefrontal theta power after event boundaries increased along with event orders while the frequency of theta oscillations decreased. Further, transient theta phase precession was observed following event boundaries, with its strength modulated by event order. In sum, our results suggest that the medial temporal lobe and prefrontal cortex employ multiple neural coding mechanisms at both single-cell and population levels to index sequential order in memory.

Disclosures: J. Zheng: None. **E. Pavarino:** None. **M. Yebra:** None. **I. Skelin:** None. **N. Kurilenko:** None. **C. Reed:** None. **T.A. Valiante:** None. **D. Kramer:** None. **J.A. Thompson:** None. **A.N. Mamelak:** None. **G. Kreiman:** None. **U. Rutishauser:** None.

Presentation Number: NANO06.12

Topic: H.08. Learning and Memory

Support: 2022ZD0204802
2022ZD0204804

Title: Percept Cells in Human Primary Visual Cortex

Authors: *G. CHEN, R. YANG, Q. WANG, F. FANG;
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Abstract: In conscious vision, internal experiences can vary despite a consistent external stimulus, prompting intriguing questions about the neural representation of these subjective perceptions within the brain. It is generally accepted that cells in higher visual areas encode the contents of conscious vision. However, the involvement of neurons in lower visual areas, such as the primary visual cortex (V1), in encoding conscious contents, is highly contentious. In the current study, we utilized single-cell recording techniques to investigate whether there were cells in human V1 that can encode the perceived contents independently of the external stimuli.
We employed the isoluminant chromatic flicker (ICF) paradigm to dissociate perceived contents from external stimuli. In this paradigm, when two isoluminant colors, such as red and green, alternate at a frequency of 30 Hz, subjects perceive a single fused color (yellow), despite the alternating external stimuli being red and green. Subjects were asked to adjust the RGB values of a yellow stimulus until it perceptually matched the perceived yellow of the 30-Hz ICF. Subsequently, we presented 30-Hz flicker, spectrum-matched red, spectrum-matched green, and percept-matched yellow, in the spatial location aligned with the receptive fields of V1 neurons, while subjects concurrently engaged in a fixation task.
We identified 112 visually responsive neurons. Then we introduced the *response similarity index*

to quantify each neuron's relative response similarity between the 30-Hz ICF and spectra-matched red or green (spectral similarity) compared to that between the 30-Hz ICF and the percept-matched yellow (perceptual similarity). For a given neuron, if the perceptual similarity significantly exceeds the spectral similarity, it is defined as a percept cell; conversely, it is defined as a spectrum cell. We identified 30 spectrum cells and 21 percept cells, suggesting that in human V1, there were not only cells that can represent the external world, but also cells that can represent internal experiences. In addition, we found that the response waveforms of spectrum cells to 30-Hz ICF flicker stimuli and the percept-matched yellow were different, while the response waveforms of percept cells to those two stimuli were statistically inseparable during stimulus presentation.
In summary, by integrating the human single-cell recording techniques and the ICF paradigm, we identified percept cells within human V1. This discovery underscores the crucial role of V1 in directly encoding the contents of conscious perception, highlighting its significant contribution to the construction of conscious experience.

Disclosures: G. Chen: None. **R. Yang:** None. **Q. Wang:** None. **F. Fang:** None.

Nanosymposium

NANO07: Value-Based Decision Making Across Model Systems

Location: MCP Room N228

Time: Saturday, October 5, 2024, 1:00 PM - 3:45 PM

Presentation Number: NANO07.01

Topic: H.10. Human Learning and Cognition

Title: The Influence of False Memory on Rewarding Experience and Decision-Making

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Abstract: Our memories play a crucial role in many decisions. Recent evidence supports the idea that individuals recombine elements of past episodes stored in memory to evaluate the outcome of some decisions (Biderman, Bakkour, & Shohamy, 2020; Shohamy & Daw, 2015). However, memory is not infallible and can be easily distorted. Understanding the impact of false memories on decision-making is essential. To explore how rewards influence false memory and subsequent choices, we employed a modified version of the classic Deese-Roediger-McDermott (DRM) paradigm, testing the effect of different reward levels assigned to various DRM lists at the encoding stage (Deese, 1959; Roediger & McDermott, 1995; Roediger et al., 2001). In Experiment 1, participants (N = 122) studied DRM word lists with varying reward values and recalled the words after a delay during which they performed a distractor task. In Experiment 2, another sample of participants (N = 124) studied the same DRM word lists and then chose between words and point values to maximize their rewards. Words were either novel, studied, or conceptually related critical lures. We found that high-value critical lures were falsely recalled more often than low-value critical lures, but that those high-value critical lures were more likely to be identified as novel (i.e., participants properly monitored their memory for those words)

when prompted. Moreover, participants chose high-value critical lures more often than novel items that were not related to the studied lists. These findings suggest that reward can modulate memory representations to be more generalizable and consequently shape decisions that rely on such representations

Disclosures: X. Li: None. **A. Bakkour:** None.

Presentation Number: NANO07.02

Topic: H.10. Human Learning and Cognition

Title: Episodic memory is used to flexibly access features of past experience for decision making

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Abstract: Our decisions often require us to prioritize some features of our experiences over others. One way to do so is to focus on relevant features and discard those that are irrelevant. Yet learning which features to prioritize requires extensive experience. Moreover, features that are irrelevant now may become relevant in the future. These issues can be addressed by instead making decisions by sampling individual richly encoded experiences from episodic memory. Here we hypothesize that episodic memory is used to flexibly construct decision variables according to whichever features of the past are currently relevant for a choice. We test this hypothesis using a novel paradigm in which people were asked to make decisions about features that were present across multiple past experiences. Critically, making good choices in this task requires constructing a decision variable by summing the value of distinct episodes that share a common feature. Across two experiments (Exp 1: n=67; Exp 2: n=71), we find evidence that people use episodes to access features of past events on the fly during decision making. Participants' choices were better predicted by the value of subsequently recalled choice-relevant episodes relative to the veridical value of each choice (Exp 1: ELPD=16.48, SE=15.9; Exp 2: ELPD=17.78, SE=13.86; difference in cross validated expected log predictive density). Further, participants took longer to make choices that required referencing more episodes (Exp 1: β=0.05; 95% CI=[0.02,0.09]; Exp 2: β=0.06; 95% CI=[0.03,0.13]; main effect of the number of memories on decision response time). In a third experiment (Exp 3: n=50), we aimed to assess whether episodes are particularly useful when it is unclear which features should be prioritized ahead of time. To do so, we manipulated whether participants knew prior to encoding each episode which features would be needed for future choices. Only when this information was unknown did participants base choices on the value of individual episodes (Known: ELPD=-6.32, SE=10.19, Unknown: ELPD=13.14, SE=7.61) and take longer to make choices based on more episodes (Known: β=0.02; 95% CI=[-0.04,0.07], Unknown: β=0.08; 95% CI=[0.04,0.12]). Overall, these results suggest that episodic memory promotes adaptive choice when knowledge of multiple features is necessary.

Disclosures: J. Nicholas: None. **M.G. Mattar:** None.

Presentation Number: NANO07.03

Topic: H.10. Human Learning and Cognition

Support: NIA Grant RF1AG058065

Title: Age-dependent changes in hippocampal contributions to decision-making

Authors: *C. VAN GEEN[1], M. S. COHEN[2], K. M. LEMPERT[3], K. MACNEAR[4], F. M. RECKERS[1], L. ZANESKI[1], D. A. WOLK[5], J. W. KABLE[1];
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Abstract: This study investigates how changes in hippocampal-dependent processes during healthy aging affect different forms of decision-making. A sample of 86 participants (45 younger and 39 older adults) first underwent fMRI while they performed a choice task in which accurate performance relied on retrieving value information from a single past episode. The same group of people were then also scanned during a reinforcement learning (RL) task in which they learned the average value of four stimuli incrementally across repeated exposures. The traditional view of multiple memory systems posits that these two paradigms should rely on distinct neural substrates, with episodic memory in the first task recruiting the hippocampus and RL in the second task evoking neural activity in the striatum and ventromedial prefrontal cortex (O'Doherty et al., 2003; Squire & Zola, 1996). Given that we can simultaneously characterize age-related neural changes in either system, our experiment is well-positioned to test the validity of this distinction: we compare group-level performance on each of the two tasks, as well as the neural activity that underlies it, in order to measure whether age-related cognitive impairments preferentially affect one function or the other. We find that older adults were especially impaired at making decisions from episodic memory (age*reward interaction: $\beta = 0.41$, $p = 0.016$), and showed reduced activity in hippocampus and medial temporal lobe at encoding. In the reinforcement learning task, older adults accurately learned the value of the four stimuli (age*learning block interaction: $\beta = 0.004$, $p = 0.84$), and showed no differences in reward prediction error signaling in the striatum. Nonetheless, older adults did show subtle performance deficits in RL, especially at the end of learning (age difference in proportion correct choice on the final block: $t = -1.86$; $p = 0.06$). Using representational similarity analysis to assess how the hippocampus tracks distinct stimulus identities, we can link these subtle behavioral impairments to disrupted patterns of stimulus-specific neural activity. Together, these findings show that changes in hippocampal processing with healthy aging underlie both larger impairments in decisions based on episodic memory and more subtle impairments in incremental learning.

Disclosures: C. Van Geen: None. **M.S. Cohen:** None. **K.M. Lempert:** None. **K. MacNear:** None. **F.M. Reckers:** None. **L. Zaneski:** None. **D.A. Wolk:** None. **J.W. Kable:** None.

Presentation Number: NANO07.04

Topic: H.10. Human Learning and Cognition

Support: NIMH/NIH grant (1R01MH130068)
Multi-University Research Initiative Grant (ONR/DoD N00014-17-1-2961
Zuckerman Institute Alan Kanzer Postdoctoral Fellowship
Gatsby Foundation (GAT3708)
Kavli Institute for Brain Science at Columbia

Title: A Normative Account of the Influence of Contextual Familiarity and Novelty on Episodic Memory Policy for Value-Based Decision Making

Authors: *Q. LU[1], K. NORMAN[3], D. SHOHAMY[2];
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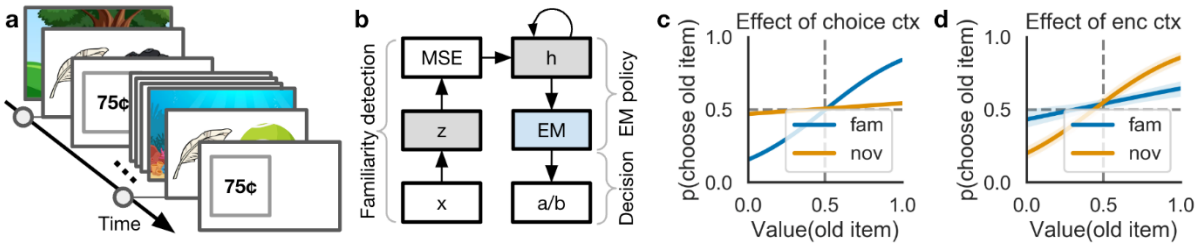
Abstract: How do humans decide when to retrieve and when to encode episodic memories to support decision-making? Empirical results have shown that familiarity and novelty of the context matter. Studies of value-based decision-making (Fig. a; Duncan and Shohamy 2016; Duncan et al. 2019), found that seeing a familiar stimulus biases subjects toward a lingering retrieval mode, while seeing a novel stimulus biases subjects toward a lingering encoding mode, even when the familiar or novel stimulus is incidental to the task. From a normative standpoint, it is unclear why the familiarity of incidental stimuli should bias episodic memory. We hypothesized that these biases could arise because the episodic memory policy - whether to retrieve or encode at a given moment - is learned in an environment where stimulus familiarity is autocorrelated in time. We present an episodic-memory-augmented neural network (Fig. b) that learns an episodic memory policy using reinforcement learning. Learning to encode was facilitated by allowing the reward obtained by retrieval to propagate back to reinforce the action of encoding this memory. As our model learns in an autocorrelated environment, empirically observed effects of contextual familiarity emerged (Fig. c, d). This is because, in an environment where familiar stimuli tend to precede other familiar stimuli, familiarity indicates that relevant episodic memories are present, making retrieval more useful. For the same reason, novelty indicates that relevant episodic memories are absent for subsequent time points, making encoding more useful. Our results suggest that the influences of familiarity and novelty are adaptive features of human episodic memory policy, reflecting autocorrelated environments.



Disclosures: Q. Lu: None. **K. Norman:** None. **D. Shohamy:** None.

Presentation Number: NANO07.05

Topic: H.10. Human Learning and Cognition

Support: Howard Hughes Medical Institute Investigator Funding
Simons Foundation
NIH grant RF1 DA056403
NSF Career Award
James S. McDonnell Foundation
McKnight Foundation
CIFAR

Title: Representations of the intrinsic value of information in mouse orbitofrontal cortex

Authors: *J. J. BUSSELL[1,2], R. P. BADMAN[3,4], C. MARTON[3,4], E. S. BROMBERG-MARTIN[5], L. F. ABBOTT[1,2], K. RAJAN[3,4], R. AXEL[1,6,2];
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Abstract: Animals are motivated to acquire knowledge. They often seek information that reduces uncertainty, even if it does not lead to increased external reward and comes at a cost. This implies that information is intrinsically valuable. However, due to its cognitive nature, the neural mechanisms that assign value to information-associated stimuli to guide knowledge seeking are poorly understood. We have therefore developed an information seeking task for mice and used it to investigate neural population representations of information-associated odor stimuli. In this task, mice choose to receive probabilistic water reward in either of two nosepoke ports, which differ only in whether they provide information via odor that reveals the reward amount but cannot be used to increase reward. Mice are cued at trial start by odor in a third port which reward ports are available, such that they learn an association between odor and predicted information. Mice strongly prefer the information port (68% mean preference, $p<0.001$, N=33). Moreover, mice exchange water to pay for information, preferring the information port even when it decreases their water reward. We fit reinforcement learning parameters to choices between information and water to model the subjective value of information (mean 38% water value exchanged for information, N=4). Thus, like humans and other animals, mice display an economically sub-optimal information bias in their decision making, behaving as if information has intrinsic value. To ask how representations of neutral odor stimuli are transformed by association with information value, we imaged neurons in orbitofrontal cortex (OFC) using miniature microscopes in mice learning the information seeking task (N=7 mice, 1138 cells). Decoding analysis and dimensionality reduction revealed a population representation of predicted information comprising 18% of OFC neurons. The difference between neural activity in anticipation of information vs. no information scaled with the time animals spent in a state of uncertainty, consistent with the information prediction representation being a scalar value signal. We observed different but overlapping populations of neurons responsive to odors predictive of information and odors predictive of water reward (18% of OFC cells each, 30% of which overlap). Moreover, a latent variable model recapitulated distinct representations of intrinsic information versus extrinsic water value in the low-dimensional dynamics of OFC activity. These data suggest that mice have evolved distinct pathways in OFC that represent the intrinsic value of knowledge and the extrinsic value of water reward.

Disclosures: J.J. Bussell: None. **R.P. Badman:** None. **C. Marton:** None. **E.S. Bromberg-Martin:** None. **L.F. Abbott:** None. **K. Rajan:** None. **R. Axel:** None.

Presentation Number: NANO07.06

Topic: H.10. Human Learning and Cognition

Title: Segmenting experience into generalizable predictive representations

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Abstract: To make effective decisions, humans must predict how our actions will influence future events. This can be achieved by learning predictive representations that encode the relationships between events. Since real-world experience is diverse, decisions must often be made in unfamiliar situations. Thus, representations must also be generalizable to facilitate novel inferences about novel futures. The present research investigates how generalizable predictive representations are learned. Given that distinct events often share common features, we hypothesized that generalization would be bolstered by learning compositional representations of the stable features of experience (feature-based) rather than specific representations of the rapidly changing events (conjunctive). To test this, human participants (N = 100) completed a task in which they made reward-based choices between multi-feature items. During training, predictive associations (start→successor) and reward value (successor→$) were defined at the feature-level, allowing either feature-based learning or conjunctive learning on items. A subsequent test phase then probed participants' abilities to infer the distal reward value of novel start items (start→$). Accurate choice at test therefore required predictive learning to be generalized (start→successor→$). We fit computational successor representations models to the choice data to identify how participants learned. Results showed a large portion of participants (p = 0.51) were best fit by the feature-based model, and that this group generalized best at test. Moreover, given the numerous potential feature mappings for each start-successor item pair, we hypothesized an inductive bias that weights learning towards semantically related features would reduce this space of possibilities for more accurate inference. This bias was captured by a free segmentation parameter in the feature-based model. Supporting our hypothesis, a higher segmentation parameter fit was associated with better generalization. In all, these findings suggest that the ability to segment experience into simplified predictive representations may underlie inference in novel settings. This form of memory may thus be important for adaptive behavior in our complex world.

Disclosures: E. Prentis: None. **A. Bakkour:** None.

Presentation Number: NANO07.07

Topic: H.10. Human Learning and Cognition

Support: NIH K99MH133994

Title: Neurcognitive development of value-guided generalization during adolescence

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Abstract: As adolescents navigate newfound independence, they encounter many new experiences that allow them to develop a richer understanding of the world around them. How do adolescents integrate this expanding knowledge to make good and bad decisions? How does ongoing brain development support the ability to generalize across multiple experiences and guide value-based inference? We tasked participants (n=98) aged 11-25 with making value-based decisions while undergoing fMRI scanning. Participants chose between pairs of every-day

objects for the chance to receive a monetary reward. Objects were sampled from 33 distinct categories which were, on average, worth different amounts of reward. This design created a latent structure of values, allowing participants to learn which categories yielded high-value and low-value payouts. We tested whether individuals generalized category value to guide decisions when they were presented with novel objects from previously learned categories. To index explicit awareness of the category value structure, participants self-reported category values after learning. We found that younger adolescents were less likely to use category values to guide trial-by-trial decision making. However, adaptive decision-making emerged with age, and older adolescents and adults were more likely to generalize category value to guide choice. Even though younger adolescents did not apply category values during decision-making, they still reported explicit awareness of the category values following the task. This reveals that younger participants knew the category values, but they did not generalize this knowledge to make adaptive decisions. Therefore, adolescents exhibited a knowledge-behavior gap, which decreased with age. Because retrieving and updating category knowledge relies on cortical systems that continue to mature during adolescence, we examined how functional activity tracked category value signals during the choice phase of the task. For adults, vmPFC activity parametrically tracked choice value, but this was not the case for adolescents. Together, these findings demonstrate that younger adolescents experience a knowledge-behavior gap: they can explicitly express value knowledge but do not apply it to guide value-based decision. Thus, even when adolescents know the best course of action to take, their decisions can deviate from their knowledge about the world. This developmental difference may reflect the late maturation of cortical circuitry, which supports the emergence of adaptive decision-making during adolescence.

Disclosures: C. Insel: None. **N. Biderman:** None. **D. Shohamy:** None.

Presentation Number: NANO07.08

Topic: H.10. Human Learning and Cognition

Support: NIH Grant R01 MH126183
NIH Grant F31 MH134620
National Defense Science & Engineering Graduate Fellowship
Brain and Behavior Foundation Grant Young Investigator Award

Title: Reinstated episodes and context differentially guide decision making across development

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Abstract: We can use past experience to guide our decisions in multiple ways. To anticipate an action's outcome, we might rely on a running average, updated incrementally across many experiences, or, without a reliable average, we might instead rely on a single, specific experience retrieved from distant memory. While prior work has characterized how incremental learning changes across development (Nussenbaum & Hartley, 2019), it remains unclear how memory-guided decision making changes with age. On one hand, we might expect memory to inform

valuation early on in development as it allows for robust learning from limited experience and is computationally simple (Lengyel & Dayan, 2008). At the same time, the structure of memory continues to change across adolescence (Keresztes et al, 2018), which could have consequences for which experiences are retrieved together (Shin & DuBrow, 2020). To characterize the development of memory-guided decision making, we had participants (N=105, ages 8-25 years old) complete a two-day multi-armed bandit task optimized to distinguish the influence of different kinds of memory on choice (Bornstein & Norman, 2017). On the first day, they learned which of three probabilistic options was most likely to yield reward. Each choice's outcome was tagged with a trial-unique image. On the second day, recognition memory trials were interleaved between choice trials. We were most interested in choice trials immediately following memory trials, those on which the prior day's images were shown. This image could evoke different experiences: either the outcome received on the trial episode or the outcomes received across the broader context. Payoffs were structured such that these two sets of experiences would bias participants towards different options. We found that participants' choices were biased by both evoked episodes and contexts ($\beta;_{episode}$= 0.09, 95% CI =[0.04,0.13], $\beta;_{context}$= 0.15, CI = [0.06,0.24]). The influence of the evoked episode marginally decreased with age, while the context's influence increased ($\beta;_{episode*age}$= -0.04, CI = [-0.08,0.00], $\beta;_{context*age}$= 0.09, CI = [0.00,0.18]). Our results suggest that memory's role in valuation is early emerging, but, as memory representations become increasingly associative with age, context exerts a stronger influence on decision making.

Disclosures: N.C. Harhen: None. **A.M. Bornstein:** None. **C.A. Hartley:** None.

Presentation Number: NANO07.09

Topic: H.10. Human Learning and Cognition

Support: NIMH R01MH124849
NIMH R21MH122863
NSF Career Award 204611
NIH Award 1S10OD02518.

Title: Control adjustment costs limit goal flexibility: Empirical evidence and a computational account

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Abstract: The ability to flexibly adjust cognition and behavior is the cornerstone of human flexibility. As our goals change or incentives increase, we adjust how we process information and act. Such flexibility requires adjustments in cognitive control states that align cognition with our current goal (e.g., changing levels of attention and response caution). However, flexibility comes at a cost: here we demonstrate that people are better at achieving a goal in isolation than when transitioning between goals. We show that this cost can be explained by inertia in transitions among control states that support goal-directed behavior. Using a classical control-demanding task (Stroop), we develop a behavioral paradigm in which people perform the same task, but adjust their control states based on changing performance goals (e.g., focus on fast vs.

accurate performance). Across five studies we demonstrate the existence of control adjustment costs: people under-shoot their target control state in environments that demand frequent movements between different target states. Further, we develop a dynamical system model that casts control adjustments as gradual movements from the current state (e.g., low attention and response caution) to the target state (e.g., high attention and response caution). We leverage sequential sampling models of decision-making to study adjustments in response threshold (caution) and evidence accumulation rate (a potential index of selective attention attention) as people move between goals that demand adjustments in these control signals. Our findings validate the core prediction of our model that control adjustment costs arise due cognitive control dynamics. We show that control adjustment costs arise in situations that demand frequent changes in control levels (N=44), and that the magnitude of the cost depends on the distance between target states (N=38) and the time allowed for the transitions (N=50). Further, we show that the costs increase with more frequent switches between states (N=55), as well as when the value of a state (i.e., associated incentives) increase (N=51). Together, this work demonstrates that control adjustment costs arise from the dynamics of transitions in the space of control signals that determine information processing and action selection. Our modeling shows how such costs can arise within a simple dynamical system which is cast as a small recurrent neural network adjusting control states based on changing goals. In so doing, our work sheds a new light on the factors that determine and constrain the flexibility of goal-directed behavior.

Disclosures: I. Grahek: None. **X. Leng:** None. **S. Musslick:** None. **A. Bonin:** None. **A. Shenhav:** None.

Presentation Number: NANO07.10

Topic: H.10. Human Learning and Cognition

Support: NIH R01 Grant MH130532
NARSAD Young Investigator Award

Title: Cognitive and computational mechanisms underlying the perceived cost of self-control

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Abstract: Failures in self-control pose a recognized challenge for both individual and public health. A growing body of cognitive and decision neuroscience research has now demonstrated that exercising goal-directed control imposes cognitive “costs” on individuals, pointing to a potential explanation for why exercising control is so difficult and often fails. We recently demonstrated that the cost of self-control can be estimated from the monetary cost choosers are willing-to-pay (WTP) to avoid temptation that could lead to self-control failures, and that these costs scale with temptation level (e.g., dieters will pay more to avoid unhealthy vs. healthy foods). Here, we sought to (1) probe the underlying algorithmic process through which control costs grow with temptation level and (2) examine how inter-individual heterogeneity in cognitive capacity and impulsivity—two factors known to influence goal-directed behavior—shape the perceived cost of self-control. Healthy dieters (n=60) completed an economic decision-making task in which they viewed images of foods that varied on temptation level, quantity, and duration of time the given food may be physically present (1-60 min). On each trial, participants reported

their WTP from a study endowment to avoid exposure to the food depicted on the trial. A realization phase followed, during which one trial was randomly selected and played out in a standard economic auction procedure. We used trial-by-trial WTP to construct a cost function mapping subjective control costs to exposure time with the food across each temptation level. Control costs ordered as expected, increasing with higher temptation, quantity and exposure time with the foods. To probe this algorithmic process, we tested if increases in temptation changed the curvature of the cost function, or whether it altered costs through a scaling mechanism. Computational modeling confirmed that the curvature remained unchanged across temptation levels and that multiplicative scaling best accounted for the observed changes in self-control costs as temptation increased. Finally, we examined how cognitive capacity and impulsivity (measured through a working-memory and delay discounting task, respectively) influenced these costs in an independent cohort of dieters (n=90). We found WTP to avoid control was higher both in participants with lower working-memory capacity and higher discount rates. Our findings point to an algorithmic process through which temptation renders it disproportionately costlier to exert self-control over time and suggest higher impulsivity and lower cognitive capacity lead to greater perceived costs of exercising self-control.

Disclosures: C. Raio: None.

Presentation Number: NANO07.11

Topic: H.10. Human Learning and Cognition

Support: NIH U19 NS107616 (to K.L.)

Title: Determinants of context-dependent valuation in artificial and biological neural networks

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Abstract: In contrast to the assumptions of normative choice theories, empirical decision-making is context-dependent, varying with factors like the composition of the choice set. This context-dependence implies a relative rather than absolute value code, consistent with relative reward representations seen in neural activity. However, it is unknown why context-dependent valuation arises and what function it serves; furthermore, the extent and distribution of relative reward coding across different brain regions is unclear. Here, we hypothesize that relative value coding: (1) arises intrinsically in neural networks trained to make value-guided choices, (2) reflects internal processing constraints, and (3) is governed by external properties of the reward environment. We trained simple deep neural networks with gradient descent to make value-guided choices; in primary simulations, value inputs were drawn from multivariate normal distributions with added environmental normal noise. Simulations were implemented in Python and analyzed in Python and Matlab. Following training, relative value coding in hidden layer units was quantified by multiple regression and the calculation of a *relative value index (RVI)*. Across simulation runs, we examined how RVI depended on both internal network (layer size, layer depth, neural nonlinearity, training loss function) and external environmental (reward covariance, input noise, reward distribution) factors. We find that relative value coding arises

naturally in deep networks trained to perform value-guided decision making, evident as a positive RVI in hidden layer units following training. RVI exhibited an anatomical gradient: in multilayer networks, the overall strength of value coding was stronger in later layers but the degree of relativity was highest in early layers, suggesting an initial contextualization of value information prior to subsequent processing. Furthermore, RVI was driven by external correlation, with stronger relativity when reward inputs were more correlated, supporting the hypothesized role of relative coding in reducing redundancy introduced by statistical structure in the world. These findings suggest that context-dependent valuation serves to implement efficient coding, optimizing performance under biological capacity constraints. Relative value coding arises naturally in artificial network optimization, governed by both internal and external factors. Ongoing work will use the layer-dependent strength of relative valuation as a framework to examine human neuroimaging data, identifying core brain regions in the hierarchical evaluation and decision-making process.

Disclosures: K. Louie: None. **S. Sinha:** None. **D. Levy:** None.

Nanosymposium

NANO08: Drug Delivery

Location: MCP Room S401

Time: Saturday, October 5, 2024, 1:00 PM - 3:00 PM

Presentation Number: NANO08.01

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 764958
FCT fellowship 2021.06297.BD
FCT project 2022.07615.PTDC
FCT project PTDC/BTM-SAL/5174/2020

Title: Engineering extracellular vesicles with microRNAs to permeabilize the blood-brain barrier

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Abstract: The scientific community actively seeks medications for neurological disorders, a leading cause of global disabilities. Yet, delivering these drugs to the brain in a non-invasively way faces challenges due to presence of the blood-brain barrier (BBB), composed of tightly

bound endothelial cells supported by pericytes and astrocytes. While vital for shielding the brain, the BBB poses a significant hurdle for drug targeting. Current strategies, like hyperosmotic solutions or physical stimuli, often entail side effects and lack precision and safety. Our research aims to transiently open the BBB to facilitate drug delivery, inspired by observations of BBB breakdown during aging, phase during which circulating factors change and affect the vessels. We utilized four microRNAs (miRNAs) associated with aging (miR-34a-5p, miR-181c-5p, miR-124-3p, miR-383-3p) to engineer plasma extracellular vesicles (EVs) from umbilical cord blood. Ensuring diversity, we used plasma from three different donors to isolate each batch of EVs. These enriched EVs were then incubated with an in vitro human BBB model obtained in Transwell systems by co-culturing endothelial cells differentiated from CD34-positive cells and pericytes. Our findings revealed that miR-383-3p-loaded EVs significantly increased BBB paracellular permeability (p value < 0.001) after 48 hours of incubation compared to control samples treated with EVs enriched with a scramble miRNA. Quantification of the immunocytochemical investigations showed a 45% decrease in claudin 5 expression, a crucial protein for BBB tight junctions, in BBB models incubated with miR-383-3p-EVs. Importantly, our strategy modulated the BBB reversibly, as evidenced by the restored paracellular permeability and claudin 5 expression in the BBB model 24 hours after removing the nanoformulations from the culture medium. We identified ATF4 and NR3C2 as the target genes of miR-383-3p, which helped elucidate how the tight junctions of the BBB were weakened. Furthermore, we investigated the effects of modulated EVs on endothelial cells using a microfluidic system-based human BBB model under flow conditions. Even under these biomimetic settings, the engineered EVs exhibited the same effects on the flow-stimulated BBB model as observed in static conditions. Our results suggest that engineered EVs hold promise as a strategy for temporarily opening the BBB, facilitating easier access for drugs to reach the brain upon injection into the bloodstream.

Disclosures: F. Tomatis: None. **S. Rosa:** None. **S. Simões:** None. **M. Barao:** None. **E. Barth:** None. **L. ferreira:** None.

Presentation Number: NANO08.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Sanofi, Sponsored Research Agreement
Canadian Institutes of Health Research 168906 & FDN 154272
Canada Research Chairs program, CRC Tier 1 in Brain Repair and Regeneration
Weston Family Foundation
Temerty Chair in Focused Ultrasound Research at Sunnybrook Research Institute
Carlsberg Foundation fellowships CF20-0379 & CF22-1463

Title: Focused ultrasound increases gene delivery to deep brain structure following the administration of a recombinant adeno-associated virus in the cerebrospinal fluid

Authors: *R. H. KOFOED[1], K. NOSEWORTHY[2], K. WU[2], L. M. VECCHIO[2], C. DIBIA[2], S. SIVADAS[2], S.-K. WU[2], K. MIKLOSKA[2], M. WHITE[2], B. ELMER[3], S. RAMACHANDRAN[3], C. MULLER[3], K. HYNYNEN[2], I. AUBERT[2];

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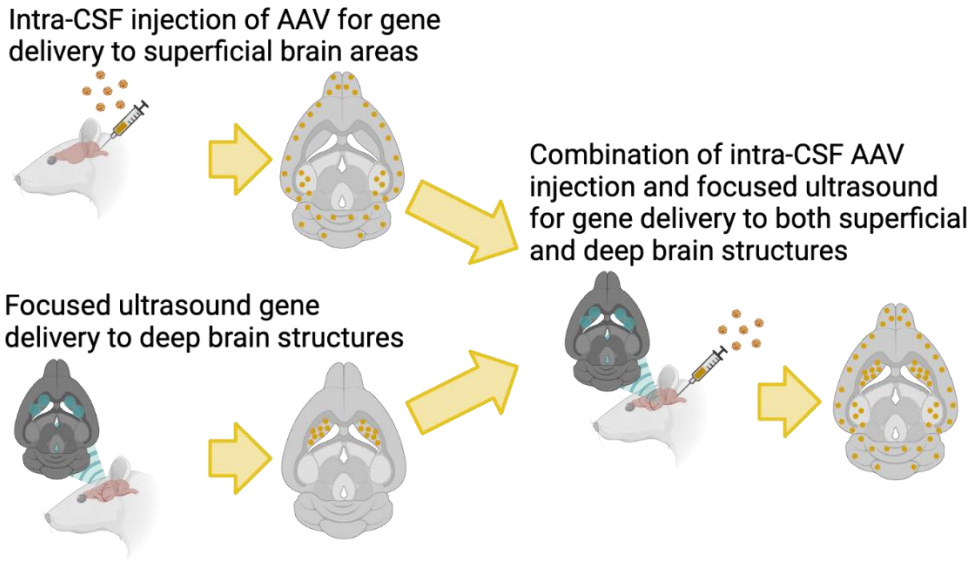
Abstract: Gene delivery via adeno-associated viral vectors can provide lasting clinical benefits following a one-time treatment. Delivery throughout the brain is needed for the treatment of neurological disorders with widespread pathology, including Alzheimer and Parkinson diseases, and amyotrophic lateral sclerosis. Most gene vectors have poor diffusion in the brain tissue. Furthermore, it is only at high intravenous doses that gene vectors can overcome the blood-brain barrier. In contrast, relatively lower doses of gene vectors injected in the cerebrospinal fluid enable significant transduction of superficial brain regions. The remaining challenge and unmet need of gene therapy is to deliver gene vectors to deep brain structures using a minimally invasive strategy. Here, we demonstrate that non-invasive focused ultrasound blood-brain barrier modulation can increase the delivery of recombinant adeno-associated virus by 5-fold to deep brain structures following injection in the cisterna magna. Delivery of adeno-associated viral vectors to the central nervous system, via administration in the cerebrospinal fluid, is being evaluated in several clinical trials for treating beta-galactosidase-1 deficiency, Batten disease, Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and spinal muscular atrophy. Our findings suggest that the efficacy of gene therapies delivered in the cerebrospinal fluid can be enhanced by targeting brain areas of interest with focused ultrasound.



Disclosures: R.H. Kofoed: F. Consulting Fees (e.g., advisory boards); Sunnybrook Research Institute. **K. Noseworthy:** None. **K. Wu:** None. **L.M. Vecchio:** None. **C. Dibia:** None. **S. Sivadas:** None. **S. Wu:** None. **K. Mikloska:** None. **M. White:** None. **B. Elmer:** A. Employment/Salary (full or part-time):; Sanofi. **S. Ramachandran:** A. Employment/Salary (full or part-time):; Sanofi. **C. Muller:** A. Employment/Salary (full or part-time):; Sanofi. **K. Hynynen:** 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.; Sanofi. **I. Aubert:** 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.; Sanofi.

Presentation Number: NANO08.03

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Dynamics of focally hyperconcentrated, ultrasound-triggered drug release for non-invasive neural circuit manipulation

Authors: *G. AYDEMIR[1], P. M. JOHNSON[1], M. S. OZDAS[1], J. HANNA[1], M. AGHILIBEHNAM[1], A. STEPIEN[2], W. VON DER BEHRENS[1,2], M. F. YANIK[1,2];
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Abstract: Central nervous system (CNS) disorders present significant challenges for effective drug delivery due to the spatial heterogeneity of neuronal circuits and spatially distributed expression of molecular targets. Recently, we developed a technology allowing focally hyperconcentrated (1,300x) drug delivery to specific brain regions, without opening the blood-brain barrier (BBB) while significantly reducing off-target effects [Ozdas et al. 2020]. In this study, we investigate the dynamics of focal hyperconcentrated drug release mechanisms using focused ultrasound (FUS) technology and ultrasound-controlled drug carriers (UC-carriers) for non-invasive drug delivery to the brain. We used an engineered two-component Aggregation and Uncaging Focused Ultrasound Sequence (AU-FUS) that employs both continuous and burst waveforms. The aggregation sequence concentrates our UC-carriers in the target area, while subsequently the uncaging sequence provides rapid, temporally precise release of drugs. The UC-carriers were manufactured using microfluidic technologies to precisely control their size, concentration, and drug-loading capacity. Subsequently, we assessed the dynamics of the UC-carrier aggregation and drug release using a custom-built microfluidic setup mimicking brain capillaries to investigate AU-FUS dynamics to enhance focal drug release. We analyzed the behavior of drug carriers under focused ultrasound by combining passive cavitation detection (PCD) and optical imaging. PCD signals from the drug carriers allow us to tune AU-FUS intensity to a regime which normally avoids BBB opening *in vivo*. The optical imaging system records the behavior of drug carriers, allowing the characterization of aggregation clusters and ratio-metric measurements to quantify the drug release. First, we characterized the aggregation dynamics and assessed the impact of acoustic pressure and flow rate on aggregation dynamics. Our findings indicate an optimal pressure range for aggregation of our drug carriers where we achieve larger size and stable aggregations, facilitating increased drug concentration in the target. Second, we used DiI fluorophore as a cargo to measure dye deposition, demonstrating the drug uncaging in real time. Ultrasound exposure duration correlated with an increase in DiI deposition. The AU-FUS sequence yielded 2000-fold more dye deposition than commonly used FUS burst waveforms. These results hold significant implications for optimizing the targeted drug delivery methods for CNS disorders.

Disclosures: G. Aydemir: None. **P.M. Johnson:** None. **M.S. Ozdas:** None. **J. Hanna:** None. **M. AghiliBehnam:** None. **A. Stepien:** None. **W. Von Der Behrens:** None. **M.F. Yanik:** None.

Presentation Number: NANO08.04

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: 1R01GM116119-01

Title: Drugs elevating $[cAMP]_i$ combined with low dose α_2-adrenergic receptor competitive antagonist rapidly reverse dexmedetomidine-based sedation and anesthesia in rats

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Abstract: The α_2 adrenergic receptor agonist dexmedetomidine (Dex) is an important sedative with analgesic and neuroprotective properties. Currently available Dex reversal agents, like the α_2-receptor antagonist atipamezole (Ati), cause serious adverse effects at the large dosages required for effective reversal; they are not used clinically. Without reversal agents, recovery from Dex-based sedation and anesthesia are slow. In this study we tested the ability of low-dose Ati, in combination with caffeine or forskolin, a drug elevates $[cAMP]_i$, to reverse Dex effects. The low dose of Ati employed should not be associated with unwanted effects. University of Chicago IACU approved this study using 16 adult female and 8 male Sprague Dawley rats (8 per group). Multiple protocols were employed. In the first protocol, a bolus of Dex was rapidly applied and the drug was allowed to equilibrate for 10 minutes before rats received either saline (as control) or low-dose Ati with caffeine. Following this procedure, rats were placed on their backs. Emergence from unconsciousness was the time for rats to recover their righting reflex (RORR). A second protocol simulated a pediatric magnetic resonance imaging (MRI) scan. Adult rats were anesthetized with dexmedetomidine for one hour followed by 30 minutes with both dexmedetomidine and propofol. At the end of 90 minutes, rats received either saline (control) or a combination of low-dose Ati, and caffeine. RORR was used as a proxy for the recovery of unconsciousness. Finally, we tested the effectiveness of low dose Ati and caffeine to reverse Dex-based anesthesia by using Dex combined with low dose $GABA_A$ or NMDA type of drugs to mimic the level of surgical anesthesia. Emergence from Dex sedation, the time for rats to RORR, decreased by ~90% when using an atipamezole dose ~20 fold lower than manufacturer's recommendation, supplemented with caffeine. Using an atipamezole dose ~10 fold lower than recommended, with caffeine, emergence times decreased by ~97%. A different stimulant, forskolin, when tested, was as effective as caffeine. For the MRI simulation, emergence times were decreased by ~93% by low-dose atipamezole with forskolin. Low dose Ati and caffeine also rapidly reversed the effects of Dex/midazolam or Dex/N_2O combination in a similar fashion. In summary, low dose atipamezole with caffeine was effective at reversing Dex-based sedation/anesthesia. Rats regained not only their consciousness and vital signs but also their balance and their ability to carry out complex behaviors. These findings suggest that low dose Ati with caffeine may permit rapid clinical reversal of Dex-based anesthesia without unwanted effects.

Disclosures: Z. Xie: None. **A. Fox:** None.

Presentation Number: NANO08.05

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant R01NS129788
Mission Connect Grant 022–105
NIH Grant T32NS126115

Title: Bioengineered human astrocyte encapsulation strategy to reduce neuroinflammation

Authors: *S. NASR ESFAHANI[1,2], R. KRENCIK[1];
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Abstract: Neuroinflammation is characterized by dynamic cellular reactivity that impacts the microenvironment with both beneficial and detrimental consequences. Current treatments aimed at reducing neuroinflammation typically involve systemically administered immunotherapies, which often suffer from lack of target specificity, resulting in off-target effects and potentially suboptimal therapeutic outcomes. Leveraging bioengineering advancements to modulate local neuroinflammation presents a promising approach for restoring normal function across a diverse range of neuropathological conditions. Astrocytes, due to their dynamic response to the microenvironment and pivotal role in the neuroinflammatory process, are ideally positioned as both initiators and targets for innovative immunomodulation techniques. To explore this potential, human pluripotent stem cell-derived astrocytes were encapsulated within an immunomodulatory alginate hydrogel to provide an indirect organoid coculture for *in vitro* studies and circumvent the immune system *in vivo*. Human astrocytes within capsules were able to secrete proteins to the external environment and become reactive in response to stimuli. Astrocyte transgenic production of human interleukin 1 receptor antagonist (hIL-1Ra) and Nuclear Factor Kappa B (NF-κB)-activated luciferase within capsules reduced reactivity of organoids to inflammatory cytokines and reported inflammatory reactivity in response to various inflammatory stimuli, respectively. Encapsulated astrocytes were also able to be stably implanted into the brain while secreting hIL-1Ra. Additionally, for the long-term delivery of hIL-1Ra into the brain, we are assessing the foreign body response (FBR) and screening a library of small-molecule-modified alginates. These alginates were recently developed in our lab to reduce peripheral FBR and are now being tested in the brain to identify formulations that could minimize the FBR. Altogether, these studies validate an indirect organoid coculture and cellular transplantation approach that delivers a novel platform to test and validate neuroinflammatory modulators.

Disclosures: S. nasr esfahani: None. **R. Krencik:** None.

Presentation Number: NANO08.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: College of Medicine
E. Malcolm and Gary Leo Dunbar Endowed Chair

Title: Optimization of PAMAM dendrimers for delivery of YWHAB siRNA as a potential therapeutic for Glioblastoma treatment.

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Abstract: Glioblastoma (GB) is a primary tumor of the brain that arises from central nervous system malignancies. Glioblastoma is usually treated with a combination of radiotherapy, chemotherapy (temozolomide), and neurosurgery. The lack of precision medicine, combined with tumor recurrence, contributes to an extremely low survival rate with existing therapy options. Small interfering RNAs, or siRNAs, have a straightforward design, are very target-specific, and work well at low dosages. This study explored the possibility of YWHAB siRNA being delivered using a new carrier method to tackle glioblastoma. The 14-3-3β protein, which is selectively increased in glioblastoma and increases malignancy, is encoded by the YWHAB gene. Previous research from our lab has demonstrated that 14-3-3β deletion significantly reduces U87MG cell spheroid formation and cellular proliferation. However, a significant challenge with most therapeutics is their inability to cross the blood-brain barrier (BBB). Poly-amido(amine) (PAMAM) dendrimers may offer a promising alternative for siRNA delivery as they confer several advantages such as crossing the BBB due to their small size, increased cellular uptake of siRNA by protection against enzymatic degradation, stable dendrimer-siRNA complexation (dendriplex) across a wider pH range, and high solubility. To optimize the dendriplex, we employed generation 4 (G4) 70/30 PAMAM dendrimers with a modified cystamine core and YWHAB siRNA in HEK293 cells. G4 70/30 PAMAM dendrimers with 70% hydroxyl and 30% surface amine groups have low cellular toxicity and high transfection efficiency. Using the dendriplex formulation, we achieved a significant knockdown efficiency of 70% for the YWHAB gene in our initial experiments conducted on HEK293 cells. In addition, we found that the maximum knockdown efficacy of dendriplex was at a concentration of 1x, as opposed to higher (2x) and lower (0.5x) concentrations. Furthermore, according to our preliminary research, the dendriplex formulation reached its maximum knockdown efficacy 120 hours (about 5 days) after transfection.

Disclosures: O. Dubey: None. **B. Srinageshwar:** None. **G.L. Dunbar:** None. **J. Rossignol:** None. **J. Bakke:** None.

Presentation Number: NANO08.07

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: the Neuroscience program
the College of Medicine
Office of Research and Sponsored Program
E. Malcolm
Gary Leo Dunbar
John G. Kulhavi Professorship in Neuroscience at CMU

Title: Effects of G4 70/30 PAMAM dendrimers in the C57 mouse brain after direct nose-to-brain drug delivery

Authors: *N. ALLAHYARZADEH KHIABANI[1], O. DUBEY[1], D. DOYLE[5], D. STORY[1], B. SRINAGESHWAR[2], J. SURMA[1], J. SAYLES[6], A. SHARMA[6], D. SWANSON[3], G. L. DUNBAR[7], J. ROSSIGNOL[4];
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Abstract: Various routes of drug administration to the brain have been explored, the blood-brain barrier (BBB) poses a significant challenge in achieving effective drug delivery for neurological diseases. Nanocarriers have appeared as a promising approach to overcome this barrier. Nose-to-brain drug delivery is emerging as a promising non-invasive approach for enhancing therapeutic efficacy in various neurological disorders. Intranasal administration offers a direct pathway for drug delivery to the brain, circumventing the BBB. The olfactory nerve route, which includes the olfactory bulbs, can provide an entrance point for NPs into the human brain. Dendrimers, which are used for drug delivery to the central nervous system (CNS), are spherical nanomaterials with extensive branching manufactured for diagnostic and therapeutic purposes. Dendrimers show potential as carriers for CNS via intranasal administration, offering reduced systemic exposure and limited side effects after in vivo administration. The present study aimed to examine the in vivo consequences of G4 70/30 PAMAM dendrimer nasal exposure in the C57 mouse brain. The treatment group received daily intranasal delivery of CY5.5-labeled G4 70/30 PAMAM dendrimers, while the control group received HBSS. Measurements were taken using the In Vivo Imaging System (IVIS) every week for a period of three weeks following intranasal treatment initiation. Following three weeks, organs, including the brain, lung, liver, and kidney, were extracted. These organs were subsequently frozen and sectioned for further analysis. The sections were imaged using fluorescence microscopy to assess the distribution of PAMAM dendrimers. The results of fluorescence evaluation revealed a significant accumulation of PAMAM dendrimers in the brain, liver, and kidney of intranasally inoculated mice. This observation suggests that intranasal administration facilitates the efficient delivery of these dendrimers to the brain and has considerable implications for the field of neuroscience and drug delivery. Specifically, it underscores the potential of intranasal delivery as a viable method for targeting the brain in the context of neurological diseases. Moreover, the ability to deliver PAMAM dendrimers intranasally opens avenues for the delivery of therapeutic agents such as genes and drugs, including promising candidates like curcumin, for treating brain tumors. Our study demonstrates the efficacy of intranasal delivery of PAMAM dendrimers in reaching the brain, highlighting its potential for therapeutic applications in neurological disorders and offering opportunities to develop innovative treatment strategies.

Disclosures: N. Allahyarzadeh Khiabani: None. **O. Dubey:** None. **D. Doyle:** None. **D. Story:** None. **B. Srinageshwar:** None. **J. Surma:** None. **J. Sayles:** None. **A. Sharma:** None. **D. Swanson:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Presentation Number: NANO08.08

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Efficient, safe, and cost-effective production of potent AAV vectors using whole plants as bioreactors, with enhanced expression and immune tolerability, validated in vivo.

Authors: *P. D. MATHUR, K. WONG, Q. XIE, F. BURNS, K. DUMAR, J. O. CONNORS, D. GIBBS;
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Abstract: The high expenses associated with producing Adeno Associated Viral (AAV) vectors in mammalian or insect cells, which contribute to unsustainable costs per dose to patients, pose significant challenges to the development of systemically administered CNS targeting AAV gene therapies. Here, we present a groundbreaking achievement: Successful production of highly potent AAVs utilizing whole plants as modular bioreactors, that is highly potent, safe, scalable, and more than 10X cheaper. Employing a agrobacterium/infiltration based transient plant-based expression system, we introduced the essential components for AAV production (Rep, Cap9, GOI with GFP as the transgene, and helper genes) into *Nicotiana Benthamiana* plants. After 5-6 days, harvested leaf material underwent homogenization, and plant AAV9-CMV-GFP was purified from the clarified lysate using downstream methods identical to conventional AAV purification. Concurrently, mammalian-AAV9-CMV-GFP was produced using standard triple transfection methods in HEK293T cells. Plant derived AAV passed a range of QC tests, displaying similar purity to that of mammalian-AAV. Notably, we obtained approximately 1e14 vector genomes from 1 kg of plant biomass and 1 L of mammalian culture. In vitro and ex vivo comparisons demonstrated that both plant and mammalian AAVs displayed comparable potency. To compare infectivity, tropism and safety we injected both AAVs in non-human primates via Intravenous and intrathecal routes. Biodistribution of both plant and HEK293T derived AAV9 was comparable in analyzed tissues. Interestingly, we found higher GFP mRNA levels in most skeletal muscle and several CNS tissues that were dosed with plant-AAV, compared to the mammalian-AAV dosed animal tissues. These results were also validated at the GFP protein level. Both Plant and mammalian-AAV did not significantly increase the GFAP levels in the brain and spinal cord tissues. Surprisingly, we observed that the plant-AAV dosed animals had significantly reduced Neutralizing Antibody levels compared to mammalian-AAV dosed animals. Our study presents the pioneering achievement of transiently producing AAVs utilizing plants as modular bioreactors. Plant produced AAVs demonstrate high potency, safety and tolerability in a clinically relevant NHP animal model with a (>10-fold) reduction in production costs for treating CNS and other genetic disorders.

Disclosures: P.D. Mathur: A. Employment/Salary (full or part-time):; Employment. **K. Wong:** A. Employment/Salary (full or part-time):; Full time. **D. Gibbs:** A. Employment/Salary (full or part-time):; employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock.

Nanosymposium

NANO09: Mechanisms and Modulators of Neuronal Development and Synaptic Specificity

Location: MCP Room N227

Time: Sunday, October 6, 2024, 8:00 AM - 9:45 AM

Presentation Number: NANO09.01

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant DP1 NS10665
NIH Grant R01 NS104055
NIH Grant F31 NS127518
Harvard Quantitative Biology Initiative

Title: Identification of growth cone RNAs and proteins regulating precision of subtype-specific cortical projection neuron development

Authors: *D. E. TILLMAN, O. DURAK, J.-Y. KIM, P. VEERARAGHAVAN, J. D. MACKLIS;
Harvard Univ., Cambridge, MA

Abstract: Cortical projection neurons (PN) have critical roles in sensory, motor, cognitive, and behavioral circuits. During development, PN build intricate circuitry by extending axons through diverse extracellular environments, then innervating specific targets located at great distances (10^3 - 10^5 cell body diameters) from their nucleus-containing cell bodies (somata). This precise navigation is regulated by growth cones (GCs): semi-autonomous, subcellular compartments at tips of projecting axons that rapidly integrate extracellular signals to control axon pathfinding. Proper GC function enables PN to form subtype-specific connectivity with synaptic targets. For example, callosal PN (CPN) innervate contralateral cortex, corticothalamic PN (CThPN) innervate specific thalamic nuclei, and subcerebral PN (SCPN) innervate locations in brainstem and spinal cord. Previous studies identified molecules in somata that control subtype-specific PN development, including the key transcription factor *Bcl11a*/*Ctip1*, which is required for precise CPN targeting, and is a highly penetrant, monogenic locus for ASD/ID. Further work identified GC-localized RNAs that regulate distinct stages of CPN circuit formation. However, molecular mechanisms linking nuclear transcription to GC RNA abundances have not been elucidated, few subtype-specific GC proteins have been identified, and little is known about how these molecules regulate precise circuit construction. Recently, our lab developed experimental and analytical approaches to purify GCs and their parent somata from specific PN subtypes in developing mouse cortex, then quantitatively "map" RNAs and proteins between subcellular compartments. Here, we utilize these approaches to identify subcellular RNA dysregulation in *Bcl11a*$^{-/-}$ CPN, quantify defects in circuit formation and adult behavior, and functionally investigate two exemplar genes with altered GC abundances: *Mmp24* and *Pcdhac2*. We also obtain proteomes from CPN GCs pre- and post-midline crossing, and from dysfunctional *Bcl11a*$^{-/-}$ CPN GCs, and functionally investigate GC proteins that function in precise CPN circuit formation. Notably, we integrate our advances in GC purification and ultra-low-input mass spectrometry to improve GC purity five-fold, and proteome sensitivity three-fold. Our investigations promise to identify molecular mechanisms controlling construction, function, and maintenance of cortical circuitry, and will deepen understanding of how proteomic regulation in distinct subtypes, stages, and/or subcellular compartments contributes to normal circuit development and disorders of nervous system circuitry.

Disclosures: D.E. Tillman: None. **O. Durak:** None. **J. Kim:** None. **P. Veeraraghavan:** None. **J.D. Macklis:** None.

Presentation Number: NANO09.02

Topic: A.05. Axon and Dendrite Development

Support: DFG Research Unit FOR5289 RobustCircuit

Title: Synaptic specificity through self-organization in the fly visual map

Authors: *R. HIESINGER;
Neurosci., Freie Univ. Berlin, Berlin, Germany

Abstract: The idea of guidance toward a target is central to the development of synapse-specific brain wiring. We now show how several thousand presynaptic growth cones self-pattern without target-dependent guidance during neural superposition wiring in Drosophila. Ablation of all postsynaptic lamina neurons or loss of target adhesion prevents the stabilization, but not the development of the pattern. Intravital imaging at the spatiotemporal resolution of growth cone dynamics in intact pupae and data-driven dynamics simulations reveal a mechanism by which >30,000 filopodia do not explore potential targets, but instead simultaneously generate and navigate a dynamic filopodial meshwork that steers growth directions. Hence, a guidance mechanism can emerge from the interactions of the growth cones being guided, suggesting self-organization as a mechanism leading to synaptic specificity in brain wiring.Reference: Agi, Reifenstein et al., 2024 Science DOI: 10.1126/science.adk3043

Disclosures: R. Hiesinger: None.

Presentation Number: NANO09.03

Topic: A.05. Axon and Dendrite Development

Support: HHMI investigator (to S.L.Z.)

Title: Projections of L2/3 glutamatergic neurons from the primary visual cortex to higher visual areas are sublayer- and cell-type-biased

Authors: V. XU[1], ***F. XIE**[2], P. MIRSHAHIDI[2], R. GORZEK[3], E. TRING[2], S. JAIN[2,4], G. FLEISHMAN[5], J. T. TRACHTENBERG[3], S. L. ZIPURSKY[2];
[1]Biol. Chem., [3]Neurobio., [2]Univ. of California Los Angeles, Los Angeles, CA; [4]Georgia Inst. of Technol., Atlanta, GA; [5]Janelia research campus, Ashburn, VA

Abstract: Understanding the relationship between cortical cell types and their axonal projection specificity is essential to uncover the molecular logic of wiring in the cortex. Recent studies using single-cell sequencing combined with anatomical tracing have shown that at high-level, cortical glutamatergic neurons comprise distinct transcriptomic subclasses that correspond 1:1 with distinct projection targets including intra-telencephalic (IT), extra-telencephalic (ET), cortical-thalamic (CT) and near-projecting (NP) neurons. Within cortical-cortical projecting (IT) neurons, however, the relationship between cell type and projection specificity appears to be more complex and is poorly understood.
As a subgroup of cortical-cortical projecting (IT) neurons, L2/3 glutamatergic neurons in the primary visual cortex (V1) project to the surrounding higher visual areas (HVAs). These closely related neurons project divergently to one or more HVAs, and they comprise a continuum of transcriptomic cell types that differentially express many wiring-related genes. The correspondence between L2/3 cell types and their HVA-projection specificity appears to be complex. Functional and tracing studies indicate projection follows topography. In addition, a projection-defined single-cell RNA-seq study has found L2/3 cells projecting to AL vs PM (AL: anterolateral; PM: posteromedial) have largely-overlapping transcriptomic signatures despite some differentially expressed genes.
Here, we combined topography mapping, AAVretro labeling, and whole-mount tissue FISH to

determine the relationship between V1 L2/3 cell types and their HVA-projection specificity while controling for topography. Our methods enabled the multi-modal profiling of gene expression, spatial location and projection of thousands of L2/3 neurons in topography-defined subregions in V1. We found that L2/3 cell types organize continuously along the pia-ventricular axis. By contrast, L2/3 cells projecting to different HVAs (LM vs RL) have spatial preferences along both the pia-ventricular and tangential axes, indicating that both type and topography contribute to HVA projection-specificity. LM-projecting neurons are enriched in upper L2/3, while RL-projecting neurons in lower L2/3. The level of this sublayer projection bias depends on topographic location. LM- and RL-projecting neurons are also enriched in different cell types along the L2/3 continuum. Overall, we found cell types organize along the cortical depth, while projection-specificity depends on both cell types and topography.

Disclosures: V. Xu: None. **F. Xie:** None. **R. Gorzek:** None. **E. Tring:** None. **S. Jain:** None. **G. Fleishman:** None. **J.T. Trachtenberg:** None. **S.L. Zipursky:** None.

Presentation Number: NANO09.04

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01 NS097161
NIH Grant T32 GM13882
NIH Grant R35 GM147179
NSF Fellowship DGE-1656518
ERC Fellowship MCSA-IF 702346
NIH Grant R35 GM147179

Title: Structural insights into the formation of repulsive Netrin guidance complexes

Authors: *E. ÖZKAN[1], J. M. PRIEST[1], E. L. NICHOLS[2], R. G. SMOCK[3], J. B. HOPKINS[4], J. L. MENDOZA[5], R. MEIJERS[6], K. SHEN[7];
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Abstract: Netrins dictate attractive and repulsive responses during axon growth and cell migration, where presence of the receptor UNC-5 on target cells results in repulsion. Here, we showed that UNC-5 is a heparin-binding protein, and determined its structure bound to a heparin fragment. Using a directed evolution platform or structure-based rational design, we were able to modulate, increase and decrease, the UNC-5-heparin binding affinity. We demonstrated that UNC-5 and UNC-6/Netrin form a large, stable and rigid complex in the presence of heparin, and heparin and UNC-5 exclude the attractive UNC-40/DCC receptor from binding to UNC-6/Netrin to a large extent. *C. elegans* with a heparin-binding deficient UNC-5 fail to establish proper gonad morphology due to abrogated cell migration, which relies on repulsive UNC-5 signaling in response to UNC-6. Combining UNC-5 mutations targeting heparin and UNC-6/Netrin contacts results in complete cell migration, as well as complete motor axon guidance defects in the circumferential commissures resulting in the uncoordinated phenotype. We also show that our

biochemical findings hold for mammalian Netrin-Heparin-UNC5 complexes. Our findings establish repulsive Netrin responses to be regulated by and mediated through a glycosaminoglycan-regulated macromolecular complex.

Disclosures: E. Özkan: None. **J.M. Priest:** None. **E.L. Nichols:** None. **R.G. Smock:** A. Employment/Salary (full or part-time):; AB Enzymes. **J.B. Hopkins:** None. **J.L. Mendoza:** None. **R. Meijers:** A. Employment/Salary (full or part-time):; Institute for Protein Innovation. **K. Shen:** None.

Presentation Number: NANO09.05

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS123290

Title: The structural basis of WFIKKN2 complex formation in the repulsive guidance of axons

Authors: *E. CORTES[1], K. NICKERSON[2], F. M. SAMMOURA[2], A. JAWORSKI[2], E. OZKAN[1];
[1]Univ. of Chicago, Chicago, IL; [2]Brown Univ., Providence, RI

Abstract: During nervous system development, newly differentiated neurons navigate a crowded environment by extending their axons towards synaptic targets, a term commonly known as axon guidance. Axon guidance is controlled by attractive and repulsive cues within the environment of the nervous system interacting with receptors present in the axonal growth cone, however, the full repertoire axon guidance molecules remains elusive. DCC (deleted in colorectal cancer) receptors are one of the major class of neuronal receptors that control the directionality of growth in neurites, known as axon guidance receptors. DCC and Neogenin interact with the secreted guidance cue Netrin, resulting in attractive growth of the axon. However, mammals have three other DCC-like receptors in total, including Punc, Nope and Prtg, with unknown roles in axon guidance. Using an interactomics screen, we have shown that these receptors interact with another secreted molecule, WFIKKN2. WFIKKN2 has repulsive effects on Nope-expressing dorsal root ganglion (DRG) sensory neurons, guiding their growth through the spinal cord periphery. Here, we have determined the first structure of a DCC-like receptor, Punc, bound to WFIKKN2. Our structure shows that Punc, and the related Nope and Prtg, interact with their guidance cue using their N-terminal immunoglobulin domains, unlike DCC and Neogenin, which bind to Netrin with their C-terminal Fibronectin type-III domains. Using our structure, we have designed point mutations on WFIKKN2 that break Nope binding, which also result in loss of repulsive guidance as tested with primary DRG neurons in vitro. Finally, we show that WFIKKN2 oligomerization might be a determining factor in activation of Nope as a guidance receptor. This work provides insights into how WFIKKN2 signals through receptor binding, serving as an axon guidance cue for divergent DCC protein family members.

Disclosures: E. Cortes: None. **K. Nickerson:** None. **F.M. Sammoura:** None. **A. Jaworski:** None. **E. Ozkan:** None.

Presentation Number: NANO09.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: Astellas Pharmaceuticals
Aligning Science Across Parkinson's ASAP-000282
Jane Coffin Childs Fellowship
Fred and Joan Goldberg Education Fund at Harvard Medical School.
NIH Grant R01NS083524
NIH Grant R01NS110395

Title: Combinatorial selective ER-phagy remodels the ER during neurogenesis

Authors: *M. HOYER[1], J. HARPER[2];
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Abstract: During neurogenesis, a refined tubular endoplasmic reticulum (ER) network is assembled via ER shaping proteins into newly formed neuronal projections to create highly polarized dendrites and axons. Previous studies suggested a role for autophagy in this ER network formation. Autophagy-deficient neurons in vivo exhibit hyperexcitability and striking axonal ER accumulations within synaptic boutons. In selective autophagy, an organelle needs to be cleared, so receptor proteins localize to the organelle and target a double membrane autophagosome to encase the organelle cargo. Then the autophagosome is targeted to a lysosome for degradation. Interestingly, the membrane-embedded FAM134B, a receptor specific to selective removal of the ER or "ER-phagy" has been genetically linked with human sensory and autonomic neuropathy. Our goal was to define the mechanisms underlying selective removal of regions of the ER network and define which ER-phagy receptors were required for regulating ER network formation in neurons. We combined a genetically tractable induced neuron (iNeuron) system for monitoring ER remodeling during in vitro differentiation with proteomic and computational tools to create a quantitative landscape of ER proteome remodeling via selective autophagy. Through analysis of single and combinatorial ER-phagy receptor mutants, we delineated the extent to which each receptor contributes to both the magnitude and selectivity of ER protein clearance. We defined specific subsets of ER membrane or lumenal proteins as preferred clients for distinct receptors. Using spatial sensors and flux reporters, we demonstrated receptor-specific autophagic capture of ER in axons, and directly visualized tubular ER membranes within autophagosomes in neuronal projections by cryo-electron tomography. Our molecular inventory of ER proteome remodeling and versatile genetic toolkit provide a quantitative framework for understanding the contributions of individual ER-phagy receptors for reshaping ER during cell state transitions.

Disclosures: M. Hoyer: None. **J. Harper:** F. Consulting Fees (e.g., advisory boards); Caraway Therapeutics, Lyterian Therapeutics.

Presentation Number: NANO09.07

Topic: A.01. Neurogenesis and Gliogenesis

Support: FCT Grant 2020.04492.BD
FCT Grant PD/BD/150343/2019
IBRO Early Career Award
ANR-21-CE14-0053 JANUS
FCT Bilateral Cooperation Transnational Project between Portugal and

France - Programa Pessoa
HORIZON-WIDERA-2023-ACCESS-04-01 under grant agreement 101160180 (PANERIS)

Title: Caffeine as a catalyst for adult brain plasticity: examining its neurogenic and synaptogenic properties

Authors: *J. B. MOREIRA[1,2,3,4], J. M. MATEUS[1,2], S. RIMBERT[5,4], M. RUSSEAU[5,4], A. M. SEBASTIAO[1,2], L. V. LOPES[1], S. LÉVI[5,4], S. XAPELLI[1,2];
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Abstract: Adult neurogenesis continues into adulthood mainly in the subventricular zone (SVZ) and hippocampal dentate gyrus (DG), where neural stem cells (NSCs) differentiate into new neurons, that are incorporated in the existing circuits, forming synapses. Caffeine, the most used psychostimulant worldwide, is a potent non-selective adenosine receptor antagonist. Despite the involvement of the adenosinergic system in the regulation of adult brain plasticity, caffeine impact on these phenomena has been largely overlooked. Therefore, our main objective was to dissect the effect of caffeine on postnatal neurogenesis and synaptogenesis. Our results indicate an effect of caffeine in the regulation of neurogenesis *in vitro*, with caffeine (125 υM) inducing a significant increase in proliferation of SVZ-derived neurospheres at DIV1, while in DG-derived neurospheres an increase in the number of mature neurons was observed at DIV7. Regarding synaptogenesis, GABA synapses are the majority in SVZ-derived new-born neurons and A2AR accumulate at synapses during the period of synaptogenesis. Acute caffeine treatment decreased the number of GABAergic synapses at DIV14, having no impact at DIV7. This was accompanied by a functional alteration, as reported with calcium imaging revealing an excitatory action of GABAergic synapses in SVZ-derived neurons, which was significantly diminished by caffeine. Therefore, caffeine decreased GABAergic synaptogenesis in SVZ cells. In DG-derived neurospheres, GABA synapses are a majority at DIV7 whist at DIV14 there is a majority of glutamatergic synapses. Caffeine also reduced GABAergic synaptogenesis at DIV7, whilst it increased glutamatergic synaptogenesis at DIV14. This study sheds light on caffeine effects in postnatal neurogenesis and synaptogenesis, giving novel insights about its effects on brain plasticity. Caffeine positively regulates neuronal maturation in a niche-dependent manner (in the DG rather than the SVZ) and has opposite effects on GABAergic and glutamatergic synaptogenesis in new-born neurons, decreasing (in the DG and SVZ) and increasing (in the DG) respectively.

Disclosures: J.B. Moreira: None. **J.M. Mateus:** None. **S. Rimbert:** None. **M. Russeau:** None. **A.M. Sebastiao:** None. **L.V. Lopes:** None. **S. Lévi:** None. **S. Xapelli:** None.

Nanosymposium

NANO10: Tauopathies and Other Neurodegenerative Disorders: New Insights and Mechanisms

Location: MCP Room S401

Time: Sunday, October 6, 2024, 8:00 AM - 11:00 AM

Presentation Number: NANO10.01

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: P01 AI077774

Title: Cryo-em reveals unique CWD elk PrP^{SC}structure

Authors: *V. BANERJEE[1], F. WANG[2], D. GORSKI[2], V. MALLMAPALLI[3], S. RAMIREZ[6], D. HARRISON[2], N. ASTUDILLOCORRAL[2], N. DE GREGORIO[2], G. FAN[4], S. PRITZKOW[2], S. A. BOOTH[7], M. L. BAKER[5], I. SERYSHEVA[4], C. A. SOTO[2];
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Abstract: Chronic Wasting Disease (CWD), a prion disorder affecting various species of cervids, remains a significant concern due to its worrisome spread to new geographic areas and its unclear zoonotic potential. Prion disorders are caused by the accumulation of misfolded prion protein (PrP^{Sc}) which behaves as an infectious agent to transmit the disease. In this report, we present the high-resolution structure by cryo-EM of the ex vivo PrP^{Sc} filaments isolated from the brain of an elk naturally infected with CWD in a North American farm. These filaments exhibit a distinctive spiral fold with a parallel in-register beta sheet (PIRBS) architecture, clearly different from rodent-adapted scrapie prion strains and other recombinant PrP filaments. The material used for structure determination was fully infectious as proven by animal bioassay. Noteworthy, CWD elk prions exhibit a similar spiral fold as that reported for GSS human prions. The resemblance between the CWD and human prion folds may have implications for the potential transmission of CWD to humans.

Disclosures: V. Banerjee: None. **F. Wang:** None. **D. Gorski:** None. **V. Mallmapalli:** None. **S. Ramirez:** None. **D. Harrison:** None. **N. Astudillocorral:** None. **N. De Gregorio:** None. **G. Fan:** None. **S. Pritzkow:** None. **S.A. Booth:** None. **M.L. Baker:** None. **I. Serysheva:** None. **C.A. Soto:** None.

Presentation Number: NANO10.02

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH R21MH120496
NIH R01NS083086
NIH R01MH125772

Title: Phosphorylation of FMRP granules alters their transport, protein synthesis and phase separation

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Abstract: Fragile X messenger ribonucleoprotein (FMRP) is an RNA-binding protein involved in mRNA transport and protein synthesis with links to autism. FMRP is a multivalent protein with the ability to interact with mRNA, ribosomes, as well as other regulatory proteins for transport and translational control in neurons. Expression of fluorescent FMRP leads to formation of punctate granular structures that coincide with ribosomes and move bidirectionally along dendrites and axons. While there is evidence that FMRP granules can undergo rapid activity-dependent trafficking to facilitate protein synthesis, what is unclear is why granules are necessary for FMRP function. Numerous models have been proposed where FMRP granules dissolve and release mRNA for translation, but evidence to support such models have been lacking. Alternatively, it has been suggested that FMRP can form membraneless compartments through liquid-liquid phase separation (LLPS). Recently, the unstructured region of the C-terminus of FMRP (including the RGG box) was identified as a low complexity region (LCR) with the ability to phase separate and self-assemble in vitro. To study whether LLPS occurs in neurons, we performed fluorescence recovery after photobleaching (FRAP) to determine exchange of FMRP within granules. We found that when translation elongation was blocked, the constitutively phosphorylated mutant of FMRP (S499D) displayed rapid recovery. Moreover, we observed that when a light-inducible clustering CRY2 domain was fused to the FMRP I304N mutant incapable of binding polysomes, it assembled with preexisting FMRP granules rather than forming independent granules or condensates. These intriguing results suggest that FMRP granules can exhibit features of phase separation in dendrites and may play a role in regulating local protein synthesis in neurons.

Disclosures: Y. Yoon: None. **S. Kharod:** None. **D. Hwang:** None. **H. Choi:** None. **P.E. Castillo:** None. **R.H. Singer:** None.

Presentation Number: NANO10.03

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH grant NS120488
Alzheimer's Association Research Fellowship AARF-22-926735

Title: Selective hyperexcitability of inhibitory neurons in human iPSC-cortical neuronal model with disease-causing tau R406W mutation

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Abstract: Selective neuronal vulnerability is a hallmark of neurodegenerative diseases. Limited attention has been given to how pathological tau selectively influences the function of neuronal subpopulations in the context of Alzheimer's disease and related tauopathies. Using a human induced pluripotent stem cell (iPSC)-derived neuronal model, we characterized changes in excitatory vs. inhibitory neurons with familial R406W tau mutation. The presence of mutant tau did not significantly alter excitatory vs. inhibitory ratio in mutant neurons compared to isogenic control. However, the fraction of inhibitory neurons with phosphorylated tau significantly increased in mutant neurons ($p<0.0001$), but remained unchanged in excitatory neurons ($p=0.99$). We further conducted patch-clamp experiments to evaluate the functional impact of mutant tau in these neurons. The presence of mutant tau not only significantly increased the ratio of spontaneously active inhibitory neurons (isogenic, 71% quiescent, 29% active; R406W, 29% quiescent, 71% active; $p=0.02$), but also enhanced the firing rates of active neurons by two-fold ($p=0.01$). No such effect was observed on excitatory neurons. Further analyses of the intrinsic firing properties revealed that mutant inhibitory neurons were able to fire action potentials at a 30 pA lower rheobase current ($p=0.002$), indicating their higher excitability than the isogenic control. Mutant inhibitory neurons had 64% increase in input resistance ($p=0.001$) and 21% decrease in cellular capacitance ($p=0.01$), compared to isogenic inhibitory neurons. Moreover, action potential waveforms fired by mutant inhibitory neurons had 9.5 mV more depolarized afterhyperpolarization potential ($p=0.01$), 5.8 mV more hyperpolarized threshold potential ($p=0.02$), and 6 mV higher amplitude ($p=0.04$). We further analyzed the impact of mutant tau on spontaneous excitatory post-synaptic currents (sEPSC) and inhibitory post-synaptic currents (sIPSCs) in inhibitory neurons. The frequency of sEPSCs doubled ($p=0.01$) and the amplitude of sEPSCs increased by 77% ($p=0.01$) in mutant neurons. In addition, the frequency of sIPSCs remained similar, but the amplitude of sIPSCs increased by two-fold ($p=0.01$). As a result, the excitation over inhibition significantly increased in mutant inhibitory neurons, which synergized with their enhanced excitability and promoted activities of these cells. In summary, tau-R406W selectively affected the intrinsic firing properties and synaptic transmission of inhibitory neurons, which led to their hyperexcitability in the human iPSC-derived tauopathy neuronal model.

Disclosures: C. Ji: None. **S.C. Song:** None. **W. Coetzee:** None. **E.M. Sigurdsson:** None.

Presentation Number: NANO10.04

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NINDS 1R21NS135426
NINDS 2R37NS076896
NIA 5T32AG066596

Title: Cellular prion protein in iPSC-derived human neurons triggers Arc and pERK1/2 increase via epidermal growth factor receptor (EGFR)

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Abstract: Synaptic dysfunction and loss are major correlates of cognitive decline in neurodegenerative disease, including Alzheimer's (AD) and prion diseases (PrD). The cellular prion protein (PrP^C) binds prion, amyloid-β, tau, and α-synuclein oligomers, resulting in the activation of macromolecular complexes and signaling at the post-synapse, yet the mechanisms driving synaptic loss are poorly understood. At early stages of prion disease (40% of the incubation period), bulk RNAseq on the hippocampus revealed elevated Arc/Arg3.1, a synaptic activity response gene. Arc protein was also increased in postmortem brain samples from prion-infected mice and sporadic and familial PrD (frontal cortex) cases. We then exposed human iPSC-derived neurons (iNs) to an anti-PrP^C antibody (POM1), which reportedly mimics prion aggregate-induced signaling. Strikingly, POM1 exposure for 2 hours induced Arc and pERK1/2, indicative of heightened synaptic activity. To identify membrane receptors and kinases driving increased Arc in human neurons, we tested POM1- or control-treated iN lysates in a phospho-kinase panel and identified a significant decrease in EGFR phosphorylated at Y1086 (pEGFR), and increased phosphorylated phospholipase C (PLC)-γ1 -Y783, suggestive of EGFR and PLC-γ1 activation. EGFR and pPLC-γ1 activation trigger calcium release from the ER and may underlie the increase in Arc and pERK1/2 levels. Moreover, we show that pharmacological inhibition of EGFR fully reversed the increase in Arc and pERK1/2Together these data support a model in which PrP^C stimulation contributes to synaptic signaling via EGFR. Understanding the signaling pathways downstream of PrP^C may lead to the discovery of novel therapeutic targets for PrD and other neurodegenerative disorders.

Disclosures: D. Ojeda-Juárez: None. **D.B. McClatchy:** None. **G. Funk:** None. **S.L. Gonias:** None. **X. Chen:** None. **J.R. Yates:** None. **C. Sigurdson:** None.

Presentation Number: NANO10.05

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Research Grant Council of Hong Kong GRF 17106018
Research Grant Council of Hong Kong GRF 17117720
Research Grant Council of Hong Kong GRF 11102422
Research Grant Council of Hong Kong Collaborative Research Fund (C1024-22G)
University Grants Committee of Hong Kong AoE/M-604/16
University Grants Committee of Hong Kong T13-605/18-W
Food and Health Bureau of the Hong Kong SAR Government HMRF 06172986

Title: Als-related fus mutation impairs the dendritic arborization and synapse development of human ipsc-derived cortical neurons

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive, lethal and incurable adult-onset neurodegenerative disease, of which Fused in Sarcoma (*FUS*) gene mutation is one of the leading genetic causes. Many FUS mutations disrupt the nuclear localization of FUS protein that

leads to the formation of pathological cytoplasmic aggregates. Understanding the pathology of FUS-related ALS (FUS-ALS) is essential for the development of therapeutic treatment. Human induced pluripotent stem cells (hiPSCs), which can be induced into human neurons, provide the opportunity to investigate FUS-ALS in a human neuronal context. While many studies using hiPSCs to investigate FUS-ALS have been reported, a vast majority of them differentiate the hiPSCs into spinal motoneurons because ALS is primarily regarded as a motor neuron disease. However, increasing evidence suggests that the cerebral cortex and cognitive functions are disrupted in ALS patients, and transgenic mice carrying ALS-FUS mutations also exhibit synaptic deficits and memory impairment. It is therefore important to determine how ALS-*FUS* mutations affect hiPSC-derived cortical neurons. Here, we employed CRISPR/Cas9-mediated genome editing to knock-in different ALS-*FUS* mutations into the nuclear localization signal. At the same time, a V5-tag was added at the C-terminus of the edited FUS protein. We demonstrated that the V5 tag could facilitate screening of the correct genome-edited clones while enabling the visualization of FUS localization in hiPSC-derived neurons. After co-cultured with rat astrocytes for 6 weeks, the hiPSCs-derived cortical neurons formed distinct synaptic puncta on dendrites. Neurons carrying the clinically severe P525L-*FUS* mutation exhibited diffuse cytoplasmic localization with no apparent aggregate formation. Notably, compared to the wild-type *FUS* control neurons, the P525L-*FUS* mutant neurons showed reduced dendrite arborization and aberrant synapse development. Collectively, our study provides new insights on ALS-*FUS* pathology by indicating that *FUS* mutation can disrupt hiPSC-derived brain neurons in the absence of cytoplasmic FUS aggregates.

Disclosures: T. Wang: None. **A.D. Flores:** None. **Q. Lyu:** None. **A. Tsui:** None. **K. Lai:** None.

Presentation Number: NANO10.06

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: IIT Gandhinagar

Title: Ptm triggered aggregation hotspots of tmem106b: implications for als and related neurodegenerative disorders

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Abstract: Transmembrane protein 106B (TMEM 106B) is a type II transmembrane lysosomal glycoprotein that physiologically participates in lysosome morphology, localization, trafficking, and pH maintenance. Recently, many studies have shown the presence of TMEM106B fibrils to be involved in the pathogenesis of various Neurodegenerative diseases (NDs). TMEM106B filaments have been observed in the post-mortem brains of persons with TDP43-proteinopathies, familial and sporadic Tauopathies, Abeta amyloidosis and synucleinopathies. The genome-wide association studies (GWAS) have also identified a significant association of TMEM106B mutations and Single Nucleotide Polymorphisms (SNPs) with the development of various NDs. From a structural point of view, CryoEM studies have hinted at the C-terminal domain of the protein being a major factor in amyloid fibril formation. However, very limited information is available about the aggregation-prone motifs of TMEM 106B and relevant post-translational modifications, except for the glycosylation. We have undertaken a detailed study to identify

aggregation-prone stretches that aggregate readily or are activated when modified post-translationally. We have exclusively focused on charge-neutralizing PTMs such as acetylation and carbamylation of lysine and phosphorylation of serine residues. Using in silico methods, we identified aggregation-prone motifs, and model peptide sequences were assembled using solid-phase peptide synthesis. These peptides were subjected to ThT aggregation assay, and aggregates formed were analyzed by an array of biophysical and microscopic methods, such as spectroscopy, CLSM, SEM, and AFM. A total of 8 hexapeptide stretches were studied, and only one of the original unmodified sequences aggregated readily. However, upon modification (acetylation and carbamylation), four additional aggregation hot spots were activated and exhibited robust amyloid fibril formation. We have further identified two aggregation-prone regions originally derived from cryo-EM structures. All of these stretches are from the C-terminal domain. The present study demonstrates the significance of lysine-based PTMs on the aggregation potential of TMEM106B, which is in line with our observations of other proteins relevant to NDs, such as tau and alpha-synuclein. These insights may further help pinpoint the early events in the TMEM106B aggregation cascade in ALS and FTLD.

Disclosures: S. Gupta: None. **K.G. Bhavsar:** None. **S. Gupta:** None.

Presentation Number: NANO10.07

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Florida Department of Health FDOH 21A21

Title: Regulation of proteostasis by sleep in Drosophila models of Tauopathy

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Abstract: Sleep and circadian rhythm dysfunctions are common clinical features of Tauopathies. Increasing evidence suggests that in addition to being a symptom, sleep disturbances can also drive the progression of neurodegeneration. Protein aggregation is a pathological hallmark of Tauopathies, however the molecular pathways behind how sleep affects protein homeostasis remain elusive. Here we demonstrate that sleep modulation influences proteostasis and the progression of neurodegeneration in *Drosophila* models of Tauopathy. We show that sleep deprivation enhanced Tau aggregational toxicity resulting in exacerbated synaptic degeneration. In contrast, sleep induction using gaboxadol led to reduced hyperphosphorylated Tau accumulation in neurons as a result of modulated autophagic flux and enhanced clearance of ubiquitinated Tau, suggesting altered protein processing and clearance that resulted in improved synaptic integrity and function. These findings highlight the complex relationship between sleep and autophagy, in regulating protein homeostasis, and the neuroprotective potential of sleep-enhancing therapeutics to slow the progression or delay the onset of neurodegeneration.

Disclosures: N. Ortiz-Vega: None. **R.G. Zhai:** None.

Presentation Number: NANO10.08

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Tau oligomer-mediated increase in mitochondrial NADPH leads to a vicious cycle favoring its pathological transmission

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Abstract: Pathological tau and mitochondrial dysfunction are conspicuous features of Alzheimer's disease (AD), but the connecting mechanism remains elusive. Mitochondrial ATP production 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, it is becoming evident that the mitochondrial pool of NADPH is critical in regulating mitochondrial functioning through the action of NAD^+ kinase (NADK2), which phosphorylates NADH to produce NADPH. However, the contribution of the mitochondrial NADK2-NADPH pathway (NNP) to AD pathogenesis remains unexplored. We found that low doses of human extracellular tau oligomers (xcTauO) increase mitochondrial NADPH in human neurons derived from neuronal progenitor cell lines in an NADK2-dependent manner. Importantly, the xcTauO-NNP pathway upregulates the expression of LRP1, a well-known tau receptor at the plasma membrane, increasing xcTauO endocytosis by neurons. Using 2-photon fluorescence lifetime microscopy (2P-FLIM) to track changes in the fluorescence lifetime of the mitochondrial-enriched coenzymes, NADPH and NADH**,** we found a significant increase in the biochemically active, enzyme-bound NADPH in human adult neurons obtained by direct conversion of dermal fibroblasts from human AD patients (iNeurons). This event occurred early during the iNeuron differentiation and coincided with the expression of tau proteoforms identified using antibodies directed against tau oligomers. To test the relevance of these observations in vivo, we applied 2P-FLIM to analyze mitochondrial activity in the PS19 live mouse brain, which naturally produces tau oligomers. Compared to WT mice, PS19 mice showed a time-dependent increase in enzyme-bound NADPH in animals as young as 1 month, nearly 5 months before cognitive decline and other neuropathological features arise in this AD mouse model. Altogether, these results suggest that xcTauO-mediated dysregulation of the NNP controls the expression of its own receptor, upregulating its uptake and likely spreading. Thus, dysregulation of the NNP could be an early contributor to AD initiation.

Disclosures: E.C. Pardo: None. **T. Kim:** None. **G. Mingledorff:** None. **V. Sagar:** None. **X.C. Sun:** None. **A. Periasamy:** None. **G.S. Bloom:** None. **A. Norambuena:** None.

Presentation Number: NANO10.09

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Insights into Neuroimmune Regulation: Dissecting the Molecular Interplay Between Tau Pathology and LC3-Associated Pathways to Single Membranes

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Abstract: Recent studies highlight the protective role of the conjugation of autophagy proteins (ATG8) to single membranes (CASM) in mitigating neuroinflammation and promoting CNS homeostasis. CASM dysfunction has been linked to the accumulation of β-amyloid (Aβ) and defects in immune receptor recycling, particularly in microglia. However, its potential impact on tauopathy remains poorly understood. This study aims to elucidate the mechanism(s) by which CASM prevents tauopathy and associated neuroinflammatory responses in a cell-intrinsic manner. Utilizing neuronal and microglial-specific deletions of CASM in PS19 and hTau mice expressing humanized tau, we will evaluate both sexes at 3, 6, and 9 months of age. Cognitive analysis using Y-maze, novel object recognition, and open-field tests will be performed, along with neuropathology. Molecular analyses will interrogate the phospho-Tau profile, neuroimmune architecture, neuroanatomy, neurodegeneration, and neuronal functional capacity. *In vitro* studies will evaluate the dependency of tau receptor recycling (FcyR, CX3CR1, RAGE, LRP1) on CASM function and the role of CASM in regulating tau seeding, using primary microglia, BV2 cells, primary rat neurons, and human iPSC-derived neurons. Preliminary data from global CASM inhibition in PS19 mice reveal that CASM is protective against aberrant tau hyperphosphorylation and prevents cognitive decline. CASM facilitates the recycling of several immune receptors, including toll-like receptors, in various cell types, such as microglia. Preliminary data on the recycling of putative tau receptors (CX3CR1, LRP1) in microglia further substantiate CASM's role in modulating tau-induced inflammatory activation. This study will improve our understanding of CASM's biological roles and provide valuable insights into the immune response to tau pathology, opening new avenues for potential therapeutic interventions in neurodegenerative diseases.

Disclosures: N. Truong: None. **B. Heckmann:** None.

Presentation Number: NANO10.10

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH grants R21 AG059391
R56 AG083436
R01 AG032611
R01 NS077239
RF1 NS120488
Alzheimer's Association [AARF-22-924783]

Title: Single-domain antibody-based protein degrader for synucleinopathies and tauopathies

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Abstract: Synucleinopathies and tauopathies are neurodegenerative disorders with α-synuclein (α-syn) or tau buildup that lack curative treatments. Antibody therapies targeting α-syn or tau aim to inhibit aggregation and enhance degradation, and struggle to cross the blood-brain barrier (BBB). This study introduces single-domain antibody (sdAb)-based protein degraders with

enhanced BBB uptake and improved capacity for α-syn or tau protein degradation.
For α-syn, protein degrader 2D8-PEG4-T was developed by linking 2D8 sdAb and thalidomide (T) with four polyethylene glycol (PEG) linkers. It targets α-syn and Cereblon, inducing α-syn ubiquitination and proteasomal degradation. In primary neuronal models, 2D8-PEG4-T prevented α-syn-induced toxicity by reducing α-syn levels via both lysosomal and proteasomal degradation. It led to superior efficacy compared to the parent unmodified 2D8 sdAb that mainly degraded α-syn through lysosomes. In the M83 synucleinopathy mouse model (n=17), 2D8-PEG4-T reduced α-syn brain imaging signal by 81% ($p = 0.0049$) vs PBS controls after 3 i.v. injections (100 μg each), whereas 2D8 was ineffective. In western blots, 2D8 reduced insoluble total and phospho-serine 129 (pS129) α-syn by 59-69% ($p<0.01$) vs PBS controls, while 2D8-PEG4-T was more efficacious (89-93% reduction, $p<0.0001$). 2D8-PEG4-T also reduced soluble total and pS129 α-syn levels by 70% ($p = 0.0072$) and 90% ($p = 0.0001$), respectively vs PBS group, whereas 2D8 was ineffective. Our study shows that 2D8-PEG4-T enhances proteasomal degradation of α-syn, while preserving unmodified sdAb 2D8's lysosomal clearance. Its improved clearance of α-syn in both in vitro and in vivo models underscore its therapeutic potential for synucleinopathies (Y. Jiang, Mol Neurodegener, in press).
For tau, the protein degrader 1D9-TP53INP2, created by linking 1D9 sdAb with a mutant LIR (LC3-interacting region) motif, targets tau and LC3 proteins on autophagosomal membranes. It facilitates tau transportation to the autophagy-lysosomal pathway for degradation. In patients' induced pluripotent stem cell (iPSC)-derived neurons with a P301L tau mutation, 1D9-TP53INP2 reduced total tau by 55% ($p=0.0003$, $n=12$) at 50 nM, vs vehicle controls, and unmodified 1D9 was ineffective at this dose. Further investigations are ongoing to evaluate the effect of 1D9-TP53INP2 on phospho-tau and efficacy in Drosophila and P301L/P301S tauopathy models.
These findings highlight the potential of small sdAbs with improved brain penetration and potency to enhance the efficacy of antibody-based therapies for synucleinopathies and tauopathies.

Disclosures: Y. Jiang: None. **C. Ji:** None. **Y. Lin:** None. **R. Pan:** None. **X. Kong:** None. **E.E. Congdon:** None. **E.M. Sigurdsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); E.M.S. is an inventor on patents application related to the initial development of anti-α-syn/tau sdAbs filed by New York University.

Presentation Number: NANO10.11

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: R01AG044404

Title: Oligomeric Tau-induced Oxidative Stress and Blood-Brain Barrier Disruption in Cerebral Endothelial Cells: Role of RhoA/ROCK Signaling Pathway

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Abstract: Dysfunction of the blood-brain barrier (BBB) plays a pivotal role in the development of Alzheimer's disease (AD). Although the microvascular deposition of oligomeric Tau (oTau) has been observed in AD brains, its direct impacts on BBB function are not fully investigated. In this study, we employed an *in vitro* BBB model using primary mouse cerebral endothelial cells (CECs) to investigate the mechanism underlying the effects of oTau on BBB function. We found that exposing CECs to oTau induced oxidative stress through NADPH oxidase, increased oxidative damage to proteins, decreased proteasome activity, and expressions of tight junction (TJ) proteins including occludin, zonula occludens-1 (ZO-1) and claudin-5. These effects were suppressed by the pretreatment with Fasudil, a RhoA/ROCK signaling inhibitor. Consistent with the biochemical alterations, we found that exposing the basolateral side of CECs to oTau in the BBB model disrupted the integrity of the BBB, as indicated by an increase in FITC-dextran transport across the model, and a decrease in trans endothelial electrical resistance (TEER). oTau also increased the transmigration of peripheral blood mononuclear cells (PBMCs) in the BBB model. These functional alterations in the BBB induced by oTau were also suppressed by Fasudil. Taken together, our findings suggest that targeting the RhoA/ROCK pathway can be a potential therapeutic strategy to maintain BBB function in AD.

Disclosures: F. Hossen: None. **J.C. Lee:** None.

Presentation Number: NANO10.12

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIH/NIA R01AG067762
NIH/NIA R01AG044372
NIH/NIA R01NS082730

Title: Interactome mapping reveals distinct mitochondrial proteins with human 2N4R Tau and oligomeric Tau

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Abstract: Tau is a multifunctional protein that regulates microtubule-dependent and -independent functions. Abnormal accumulation of misfolded and oligomeric tau is a central pathological feature of neurodegenerative tauopathies, the most common of which is Alzheimer’s disease (AD). Mitochondrial dysfunction is thought to occur early in AD. Tau accumulation can lead to mitochondrial dysfunction which might be mediated by interactions with tau. Deciphering tau interactors is critical to understanding tau-mediated cellular processes. In this work, we utilized two proximity-dependent biotinylation approaches to identify potential interactors with human 2N4R tau or oligomeric tau. To identify physiological tau interactors, BioID2 (biotin ligase) was fused to either the N-terminus (BioID2-Tau) or the C-terminus (Tau-BioID2) of full-length human 2N4R tau isoform. Control constructs were created to express only the BioID2 protein. BioID2 controls and tau constructs were expressed in mouse tau knockout primary cortical neurons. BioID2 allows for *in situ* biotin labeling of interacting proteins in living neurons. Biotinylated proteins were isolated and identified by mass spectrometry (n=3 biological replicates). To identify oligomeric tau interactors, an antibody that binds to oligomeric tau (TOC1) was used for biotinylation by antibody recognition (BAR) labeling in the inferior

temporal gyrus from AD patients (Braak V-VI). BAR was also applied to inferior temporal gyrus tissue from AD patients with primary antibody delete as control (n=3). BAR is based on directly labeling TOC1 with horseradish peroxidase and in-tissue conjugation of biotin to proximal proteins using a biotinyl tyramide substrate. Biotinylated proteins were then isolated and identified by mass spectrometry. Tau-BioID2 identified 324 proteins as candidate tau interactors, of which 118 proteins were associated with mitochondria-related pathways. While the TOC1-BAR approach identified 427 proteins as potential interactors with oligomeric tau, of which 77 proteins were associated with mitochondria-related pathways. Among the mitochondrial proteins identified with both approaches, only 6 proteins overlapped suggesting there are distinct sets of mitochondrial protein interactions with human 2N4R tau and oligomeric tau species. We validated tau protein interactions with mitochondrial proteins using two independent approaches: coimmunoprecipitation (n=3) and proximity ligation assay (n=3). This work helps expand our understanding of tau's potential roles and advances our understanding of its role in neurodegenerative diseases mediated by protein-protein interactions.

Disclosures: A. Atwa: None. **N.M. Kanaan:** None.

Nanosymposium

NANO11: Recovery Mechanisms From Brain Injury

Location: MCP Room N426

Time: Sunday, October 6, 2024, 8:00 AM - 10:30 AM

Presentation Number: NANO11.01

Topic: C.10. Brain Injury and Trauma

Support: W81XWH-18-1-0433
Indiana Spinal Cord & Brain Injury Research Grant

Title: Prospective Blood Transcriptomics Study in a Mild Traumatic Brain Injury Cohort Identifies a Number of Genes Which May Be Predictive of Post-traumatic Headache.

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Abstract: Post-traumatic headache (PTH) is a common consequence of mild traumatic brain injury (mTBI) that can severely impact an individual's quality of life and rehabilitation efforts. However, the underlying neuropathogenesis mechanisms contributing to PTH are still poorly understood. It is now recognized that cells previously considered to be restricted to the periphery, are also found in the central nervous system under conditions of injury or disease. The response by these cells to mTBI may contribute to PTH based on the balance between proinflammatory immune cell responses, including humoral factors (e.g., cytokines and the complement system) and immunosuppressive mechanisms engaged at the local and systemic level. This current study

investigated associations between pain sensitivity, psychological assessments, and transcriptomics to identify differences in a group of mTBI subjects with unresolved pain at 6 months (n=9) compared with healthy control subjects (n=10). Pain sensitivity assays were measured using quantitative sensory testing and psychological assessment questionnaires at 1-month and 6-months post-injury. Peripheral blood mononuclear cells (PBMCs) were used for bulk RNA sequencing analyses from age and gender matched healthy control subjects and mTBI subjects across time (2 weeks, 1 month and 6 months). At the transcriptome-wide level, we found that several candidate genes which were significantly upregulated or downregulated with time in subjects with unresolved persistent pain. The top ten differentially expressed genes candidates included increases in KIR2DS4, HP, NOD2, GCC1, IL1B and CR1. Downregulated genes were TMEM18, UBE2G2, RAB15 and MSR1. The upregulation of a number of these genes is known to be involved in important innate immune system processes and may play a major role in the debilitating chronicity of PTH. Better understanding of how immune cells respond to mTBI and are distinct between individuals will allow for identification of biomarkers and potential therapeutic targets.

Disclosures: J.A. Smith: None. **T. Nguyen:** None. **T. Hato:** None. **K.M. Naugle:** None. **D.K. Lahiri:** None. **F.A. White:** None.

Presentation Number: NANO11.02

Topic: C.10. Brain Injury and Trauma

Title: Characterizing the cellular immune response in human cerebrospinal fluid after intraventricular hemorrhage

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Abstract: Intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH) are often associated with a profound, but poorly characterized, cellular inflammatory response in the central nervous system. Cerebrospinal fluid (CSF) drained via an external ventricular drain as part of routine treatment is a potential source of biomarkers to monitor the inflammatory processes in the brain and predict outcomes. At the University of Maryland, these samples are banked through the Brain Injury Omics (BIO) initiative, a part of the prospective, observational Recovery After Cerebral Hemorrhage study (NCT04189471). Through BIO, we performed the first reported single nucleus RNA sequencing on intrathecal CSF samples from 7 hemorrhagic patients (1 SAH and 6 ICH) and profiled 11,191 high quality nuclei. Clustering revealed 53.8% neutrophils, 26.09% monocytes/macrophages, 17.78% lymphocytes, and 2.36% neurons. Subclusters and gene co-expression clusters of the neutrophils showed distinct populations of interferon responsive, aged CXCR4+, and activated (S100A8/9 or CD177 enriched) neutrophils. We identified canonical CD14+ monocyte and macrophage populations and PTPRG+ monocytes and FCGR3B+ macrophages with interferon-induced downstream signaling. These results reveal

the specific cellular phenotypes that constitute the inflammatory response to acute hemorrhagic injury in the central nervous system. Further characterization of these samples may lead to the discovery of prognostic biomarkers and therapeutic targets for brain hemorrhage.

Disclosures: S. Malaiya: None. **M.E. Cortes-Gutierrez:** None. **R. Serra:** None. **B. Wilhelmy:** None. **S. Yarmoska:** None. **P. Patel:** None. **J. Simard:** None. **G. Schwartzbauer:** None. **G. Parikh:** None. **S.A. Ament:** None. **P. Ciryam:** None.

Presentation Number: NANO11.03

Topic: C.10. Brain Injury and Trauma

Support: W81XWH-17-1-0424

Title: Balance deficits across concussion subtypes

Authors: *M. E. STOJAK[1], K. CAMPBELL[2], R. J. PETERKA[3], P. ANTONELLIS[3], J. CHESNUTT[4], L. A. KING[3];
[1]Neurol., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Oregon Hlth. & Sci. Univ., Portland, OR; [3]Dept. of Neurol., Oregon Hlth. & Sci. Univ., Portland, OR; [4]Dept. of Neurol. & Family Med., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Title: Balance deficits across concussion subtypes Authors: Stojak ME, Campbell KR, Peterka RJ, Antonellis P, Chesnutt JC, & King LAA recent clinical paradigm proposes classifying people with concussion into subtypes -- ocular, vestibular, cognitive, headache, and mood, based on symptom presentation. The recognition of concussion subtypes may benefit collaborative care and facilitate appropriate referrals. However, the diffuse nature of a concussive injury presents challenges. Balance is controlled across several cortical and subcortical brain regions with substantial central processing time needed for multisensory integration and motor planning. While balance rehabilitation is common for vestibular and ocular (motor) subtypes, it is conceivable that even mood, cognitive or headache subtypes may have deficits that could go untreated. **PURPOSE:** To assess central processing time via time delay involved in balance control across subtypes. **METHODS.** 124 symptomatic people with concussion (age=36±11, days since injury=50±21) and 58 healthy controls (HC) (age=37±11) were assessed using the Central Sensorimotor Integration (CSMI) Test. The CSMI measures sagittal plane sway evoked by 20-s cycles of 2° peak-to-peak pseudorandom rotations of the stance surface eyes open (SS/EO) or closed (SS/EC) or with a rotating visual surround and fixed surface (VS/EO). Time delay to coordinate balance responses was calculated. The Neurobehavioral Symptom Inventory (NSI) was used to categorize people into subtypes; cognitive (50), headache (15), mood (32), and vestibular ocular (27). Across subtypes there were no significant differences in age, symptom severity (NSI), and days since injury. An ANOVA, with Tukey adjusted pairwise differences, was used to compare groups. **RESULTS:** Relative to the HC group, the cognitive subtype had significantly longer time delays across all CSMI conditions (p's<0.01). The mood subtype had significantly longer time delays in the SS/EC (p=0.001) and SS/EO (p=0.04) conditions. The vestibular-ocular group had significantly longer time delays during the SS/EC (p=0.007) and VS/EO (p=0.006) conditions. There were no significant differences in time delay between the headache subtype and HCs (p's>0.40). **CONCLUSION:** Apart from the headache subtype, all other subtypes exhibited lengthened time

delays that may negatively impact balance control. These results support the notion that persisting balance deficits should be evaluated and treated, regardless of clinical subtype.

Disclosures: M.E. Stojak: A. Employment/Salary (full or part-time):; Oregon Health & Science University, Balance Disorders Laboratory. **K. Campbell:** A. Employment/Salary (full or part-time):; Oregon Health & Science University, Balance Disorders Laboratory. **R.J. Peterka:** A. Employment/Salary (full or part-time):; Oregon Health & Science University, Balance Disorders Laboratory. **P. Antonellis:** A. Employment/Salary (full or part-time):; Oregon Health & Science University, Balance Disorders Laboratory. **J. Chesnutt:** A. Employment/Salary (full or part-time):; Oregon Health & Science University. **L.A. King:** A. Employment/Salary (full or part-time):; Oregon Health & Science University, Balance Disorders Laboratory. 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.; Department of Defense, National Institutes of Health.

Presentation Number: NANO11.04

Topic: C.10. Brain Injury and Trauma

Support: Mitacs Accelerate IT30534

Title: Does Concussion Alter Eye-Movement Performance During Multiple Object Tracking?

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Abstract: Team-sport athletes need to attend to and track the movements of their teammates, opponents, and the object of the game (e.g. ball). This is particularly important in contact and collision sports because tracking who is friend and foe is vital both for playmaking and injury avoidance. In the lab, this tracking behavior is approximated by the Multiple Object Tracking task (MOT; e.g., Pylyshyn & Storm, 1988) for which participants are asked to track the unpredictable motion of a number of targets simultaneously amongst the motion of identical distractors. Research shows that humans can track and later reasonably identify up to 5 targets in a set of 10 items. We wondered whether concussion impairs this ability and whether these impairments are sensitive to time since injury. We also investigated whether the eye-movement strategies adopted during the tracking phase were different for participants with concussion (N=20) than for healthy (N=20) or orthopedic-injury (N=20) control participants. Participants' eye movements were tracked while they completed MOT trials containing 1-5 targets in sets of 10 items (10-nTarget distractors). On each trial, targets were revealed using color, the color was removed and item motion began and continued for 3 seconds, and at the conclusion, participants identified which items they believed were the original targets. While the concussed group did not differ from controls in the percentage of targets identified correctly (p = .126), they achieved a perfect tracking score on significantly fewer trials than controls (p = .017). Our preliminary analysis of eye-movements revealed that object-tracking models based on target position did a significantly poorer job accounting for the eye movements of concussed participants than controls (ps < .038) and that a tracking model based both on target and distractor position did a significantly better job accounting for the eye movements of concussed participants than controls, at least in trials where there was a single target (p = .017). This result suggests that

people with concussion are less able to discount distractors than controls. Future analyses will examine how these patterns relate to time since injury.

Disclosures: L.E. Brown: None. **E. Okafor:** None. **M. Reynolds:** None. **E. Pownall:** None.

Presentation Number: NANO11.05

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant W81XWH-20-1-0717

Title: The mini SCAT symptom severity index and acute concussion identification: Findings from the NCAA-DoD CARE Consortium

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Abstract: Objective: The purpose of this study is to A) rigorously evaluate the Sport Concussion Assessment Tool (SCAT) symptom list and improve the sensitivity/sensitivity by creating a mini-SCAT (mSCAT) and B) identify an added utility of additional tests/exams with mSCAT. Methods: Cohort study of collegiate athletics and military service academies. 59,901 athletes and cadets were enrolled in the NCAA/DOD CARE consortium; 5,075 diagnosed with a concussion. These analyses utilize the SCAT Symptoms and other concussion assessments in concussed versus non-concussed individuals. Results: Individual symptoms demonstrate a variety of Cohen's-d effect sizes, the smallest being Nervous/Anxious (d=0.23) and Sadness (d=0.43). The largest effects are Pressure in Head (d=2.59), Don't Feel Right (d=2.51), and Headache (d=2.85). The largest AUCs for concussion assessments are BSI Somatization Score (0.75), SCAT Symptom Severity Score (0.88), VOMS/modified VOMS (0.92). A proposed mini-SCAT (mSCAT) including the symptoms of headache, pressure in head, don't feel right, sensitivity to light, dizziness, and sensitivity to noise improves the AUC to 0.94 with a sensitivity of 87% and specificity of 88%. The only concussion test/exam which adds utility to mSCAT is VOMS, all other test/exams in CARE are non-additive in acute concussion identification. Conclusion: These results suggest a mSCAT symptom list should be considered, possibly to 6 questions for an AUC improvement from 0.88 to 0.94 with a 2% improvement in sensitivity and 12% improvement in specificity.

Disclosures: L.T. Rooks: None. **N.L. Port:** None.

Presentation Number: NANO11.06

Topic: C.10. Brain Injury and Trauma

Support: Dana Foundation
R01NS123374
R01NS082432
T32GM149364-01
3R01NS123374-02S1

Title: In Vivo Detection of Pathology at the Depths of Cortical Sulci in Sports Repetitive Head Impacts

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Abstract: Post-mortem evidence suggests the depths of sulci (DoS) are vulnerable to repetitive head impacts (RHI). Diffusion MRI (dMRI) has identified microstructural features of brain injury but has largely overlooked the juxtacortical white matter (jWM). We assessed the relationship of RHI due to heading in soccer players with dMRI in jWM at DoS. RHI has been associated with worse verbal learning; we tested the mediating role of dMRI in this relationship.
Healthy amateur adult soccer players (n=380; 18-53 years old; 30% female) and healthy non-collision athlete controls (82; 18-50; 61%) were included. In this cross-sectional analysis, we assessed the relations among estimated 12 month RHI (HeadCount) represented in quartiles (medians: 43, 300; 782, 2607) and verbal learning (International Shopping List).
3T dMRI ($2mm^3$, 109 directions, b=300, 800, 2000) was processed to extract DTI (fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD)) and NODDI (orientation dispersion index (ODI), neurite density index (NDI), isotropic water fraction (ISO)) metrics from (1) jWM subjacent to the DoS, (2) jWM subjacent to the crests of the gyri (CoG), and (3) deep WM (dWM: corticospinal tract, corpus collosum, fornix, and uncinate fasciculus). dMRI metrics at each region for each RHI quartile were compared to non-collision athletes, using generalized linear models adjusted for age, sex, and concussion history. Significant associations underwent causal mediation analysis using bootstrapping to test the significance the mediating effect of a dMRI metric on the relationship of RHI with verbal learning. Bonferroni correction was applied.
dMRI metrics in DoS jWM differed from controls in an RHI-dependent fashion. The highest RHI quartile exhibited (corrected $P<0.001$) lower FA in the frontal lobe (FL), orbitofrontal cortex (OFC), parietal lobe (PL), temporal lobe (TL), and occipital lobe (OL); lower AD in OFC, PL, TL, and OL; higher RD in FL, OFC, PL, TL, and OL; higher ODI in FL, OFC, PL, TL, and OL; and lower NDI in OFC. DoS effect sizes were larger CoG or dWM. jWM ODI in OFC partially mediated the association of greater RHI with worse verbal learning ($P=0.008$); other white matter regions had no mediation effect.
Microstructural injury related to RHI in young healthy individuals is most prominent in DoS jWM. The previously identified adverse association of RHI with verbal learning is partially mediated by OFC DoS jWM, consistent with measurable functional effects of subclinical axonal injury, demyelination, and/or inflammation. Our findings suggest a focus on DoS jWM holds potential for identifying clinically significant injury pathology in RHI.

Disclosures: B. DeMessie: None. **W. Stewart:** None. **R. Lipton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds stock and stock options in Axon, Biohaven Holdings, CoolTech and Manistee. F. Consulting Fees (e.g., advisory boards); Serves as consultant, advisory board member, or has received honoraria from: Abbvie (Allergan), American Academy of Neurology,

American Headache Society, Amgen, Avanir, Axon, Axsome, Biohaven, Biovis. Other; Receives royalties from Wolff's Headache 7th and 8th Edition, Oxford Press University, 2009, Wiley and Informa. **M. Zimmerman:** None. **M. Kim:** None. **K. Ye:** None. **T. Kaminski:** None. **R. Fleysher:** None. **M.L. Lipton:** None.

Presentation Number: NANO11.07

Topic: H.04. Executive Functions

Support: Great Plains IdEA-CTR (MN)
UNL Office of Research and Economic Development

Title: Cortical volume is not related to self-reported concussion history in college athletes

Authors: *D. SCHULTZ[1], H. C. BOUCHARD[2], M. BARBOT[3], J. LAING-YOUNG[3], A. CHIAO[7], K. HIGGINS[4], C. R. SAVAGE[5], M. NETA[6];
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Abstract: The long-term consequences of concussion are still being uncovered but have been linked to disruptions in cognition and psychological well-being. Previous studies focusing on the association between concussion history and structural changes in the brain have reported inconsistent results. We sought to examine the effect of concussion history on cortical volume with a focus on functional networks. These networks are associated with many of the functions that can be disrupted in those with an extensive concussion history. We collected structural MRI data in contact sport college athletes (n=296, 33 women) along with a self-report measure of concussion history and a baseline neuropsychological assessment, Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT). Participants were divided into two groups, a group who reported no history of concussion (n=194) and a group who reported at least one concussion prior to collection of MRI data (n=102). We examined cortical volume in these groups at the level of functional networks, and at a more spatially constrained anatomical region level. College athletes who reported concussion history did not report different baseline symptoms and did not exhibit consistent differences in cognitive performance relative to those who reported no concussion history. Concussion history was not related to cortical volume at the network or region level, even when we compared participants with two or more concussions to those with no concussion history. We did identify relationships between cortical volume in the visual network and dorsal attention network with cognitive performance. Together, these results suggest that self-reported concussion history is not associated with changes in cortical volume in young adult athletes. While it is challenging to interpret null findings, we used Baysian statistics and found moderate support for the null hypothesis that concussion history is not related to cortical volume. This pattern of results is consistent with clinical and other scientific evidence suggesting that concussion-related outcomes are associated with disruptions in brain function and connectivity as opposed to anatomical changes. Indeed, previous research has suggested that concussion history is associated with other changes in the brain (white matter, functional connectivity, electrophysiology, etc.). Our results suggest that future work to identify brain markers of concussion history may be better served by examining these measures rather than focusing on cortical morphology.

Disclosures: D. Schultz: None. **H.C. Bouchard:** None. **M. Barbot:** None. **J. Laing-Young:** None. **A. Chiao:** None. **C.R. Savage:** None. **M. Neta:** None.

Presentation Number: NANO11.08

Topic: C.10. Brain Injury and Trauma

Support: P41 EB018783 (NIH/NIBIB)
Stratton VA Medical Center
DOH-C38338GG
DOH-C37714GG
1 I21RX004410
UK EPSRC EP/T022175
UK EPSRC EP/V025724/1

Title: Objective neurophysiologic markers to aid assessment of prolonged disorders of consciousness (PDoC)

Authors: N. DU BOIS[1], *T. VAUGHAN[2], J. HILL[3], A. KORIK[4], ***T. M. VAUGHAN**[5,6], D. COYLE[4], J. R. WOLPAW[2];
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Abstract: Clinical assessments of individuals with cognitive-motor dissociation (CMD) following brain injury are both challenging and error prone. Prior studies have shown that electroencephalographic or EEG-based brain-computer interface protocols for motor-command following (MCF) and differences in the N1 and P3 components of auditory evoked potentials (AEPs) in response to an auditory oddball paradigm, can provide a more accurate, quantitative assessment of children with CMD. This study investigates if these EEG measures would aid in the assessment of adults with prolonged disorders of consciousness (PDoC); and if brain-computer interface (BCI) protocols using motor-imagery decoding tasks or latencies of AEPs can improve cognitive assessments of individuals with PDoC.
Methods: EEG data from nine individuals with PDoC, including cases of unresponsive wakefulness syndrome (UWS), minimally conscious state (MCS), and locked-in syndrome (LIS), were recorded using a 16-channel gNautilus system (g.tec). The MCF protocol included up to 12 sessions of 240 trials each. During the first six sessions, participants underwent training with and without feedback, to learn to consistently imagine moving one of two limbs, such as the left or right hand, in response to auditory cues. From the seventh session onward, this binary imagery task was associated with yes and no and applied in a closed question-and-answer task. Separately, the auditory oddball protocol included at least two sessions, approximately 10 days apart. Each session involved 2 five-minute sets of auditory stimuli: 340ms square-wave beeps at frequencies of 400 Hz (standard) or 575 Hz (deviant), along with various novel sounds, following a standard:deviant:novel ratio of 27:8:6 per set.
Results: Mean N1 AEP latencies had significant group differences due to lower latencies for the LIS and MCS groups as compared to the UWS group (LIS v UWS - $p < 0.001$; MCS v UWS - p

= 0.005). Furthermore, mean AEP latencies were found to be negatively correlated with the mean of the decoding accuracies (DA) obtained from significant runs for each participant during the corresponding motor-imagery sessions (i.e., latencies decreased as DA increased, $p = 0.011$, one-tailed).
Conclusion: The latency of the N1 AEP may aid the assessment of awareness in PDoC. The finding that N1 latencies are correlated with motor imagery DA across groups suggest that both movement-independent measures could be used complementarily to improve accuracy in detecting consciousness in adults with PDoC.

Disclosures: N. du Bois: None. **T. Vaughan:** None. **J. Hill:** None. **A. Korik:** None. **T.M. Vaughan:** None. **D. Coyle:** A. Employment/Salary (full or part-time):; CEO NeuroConcise. **J.R. Wolpaw:** None.

Presentation Number: NANO11.09

Topic: C.10. Brain Injury and Trauma

Support: NMSS Grant RG 4232A1/1
NJCBIR Grant 10.005.BIR1

Title: Functional connectivity of cognitive fatigue following brain injury and disease

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Abstract: Cognitive fatigue (CF) is a common and debilitating symptom following brain injury or disease . Here, we investigated connectivity in individuals with multiple sclerosis (MS) and individuals who had sustained a traumatic brain injury (TBI), comparing both groups to a control group. For all participants, CF was induced while neuroimaging data was acquired using a processing speed task.
The sample consisted of 31 Controls, 27 individuals with MS and 30 individuals who had sustained a moderate-to-severe TBI. The groups were matched for education (age was not matched and was controlled for statistically). CF was induced while subjects lay in an fMRI scanner and worked through 4 blocks of a modified Symbol-Digit Modalities Task (mSDMT). Participants rated their CF at baseline and after each task block. The sample was then divided into those who reported high CF and those who reported low CF by using a median split of subjects' average CF.
We deployed the Riemannian manifold-based machine learning approach to analyze task-based functional connectivity (t-FC). This technique involved categorizing static t-FC matrices according to their geodesic distance, allowing us to pinpoint distinct connectivity patterns associated with different groups of HC, MS, and TBI patients. BOLD time-series data from Brainnetome atlas parcels were extracted, excluding any structural lesions, and static t-FC matrices were computed using Pearson correlation. We obtained a vectorial representation by applying geodesic clustering to these t-FC matrices and calculated the geodesic distance from each matrix to cluster centroids (i.e., reference connectome). Then the most discriminative centroid was chosen and the reference connectome of each group was determined.

Discriminative connections illustrating FC differences between the groups were identified by comparing these reference connectomes.
Individuals with MS and TBI reported significantly more CF than the controls ($p<0.0001$), as has been previously shown. The MS and TBI groups showed a largely similar pattern of disconnection, with decreased connectivity between the precentral and postcentral gyri and inferior parietal and insular regions compared to the control group. The MS group showed increased connectivity between the thalamus and the basal ganglia as well as between the insula and basal ganglia. The TBI group showed increased connectivity mainly between and within parietal regions. That is, though the outward manifestation of CF are similar in the MS and TBI groups, the underlying patterns of connectivity differ. This suggests that different approaches should be taken to alleviate fatigue in these two populations.

Disclosures: G.R. Wylie: None. **M. Yamin:** None. **H.M. Genova:** None. **J. DeLuca:** None.

Presentation Number: NANO11.10

Topic: C.10. Brain Injury and Trauma

Support: R01 AG075802
R01 AG080001

Title: Coexistence of the CTE and Alzheimer folds of tau in the brain of a former soccer player affected by Alzheimer disease: a neuropathologic and cryo-em study

Authors: *K. L. NEWELL[1], C. QI[2], H. J. GARRINGER[1], M. JACOBSEN[1], R. VIDAL[1], S. SCHERES[2], M. GOEDERT[2], B. GHETTI[1];
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Abstract: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head impacts, often sport-related. CTE can also result from exposure to blast waves. Studies combining neuropathology and cryo-electron microscopy (cryo-EM) of cerebral tissue of an individual who had played soccer and developed dementia later in life have not been reported. Here we report a male, who played soccer for several years in childhood and as a professional between 18 and 21 years of age, and began experiencing word-finding difficulties followed by cognitive decline at age 69. At age 71, neuropsychological, neurological, and neuroimaging studies led to the diagnosis of dementia, consistent with Alzheimer disease (AD). He died at age 76. The brain weighed 1,145 grams and was moderately atrophic. The tissue was studied using histology, immunohistochemistry, and cryo-EM. The diagnosis was AD with severe neuropathologic changes scored as A3, B3, C3, according to the NIA-AA guidelines. In view of the severity of the pathology that is mostly affecting the neocortex and hippocampus in AD and that may overshadow the CTE pathology, it was important to be able to determine whether in this case the tau pathology of AD was associated with that of CTE. Both pathologies are associated with the presence of six tau isoforms with tau inclusions made of 3R and 4R tau. Tau immunohistochemistry, using antibodies AT8, anti-RD3 and anti-RD4, revealed a glial tau pathology frequently present around blood vessels located along the subependymal tissue adjacent to the ventricles. Glial tau pathology also occurred beneath the pia mater over the surface of brainstem and spinal cord and was best shown using the anti-RD4 antibody. Neuropathologically, the severity of AD pathology did not allow us to recognize the well-

described neocortical CTE changes including tau pathology at the depth of sulci and glial perivascular tau. For the CTE studies, the ideal tissue was the neocortex, amygdala and hippocampus due to the presence of a large number of ghost tangles that were best recognized by the anti-RD3 antibody. The amygdala and the hippocampus were areas where neurofibrillary tangles coexisted with RD4 immunopositive astrocytes and astrocytic processes in perivascular location. Frozen specimens of these areas were used for cryo-EM, which revealed the presence of two types of tau filaments; those with the Alzheimer fold and those with the CTE folds. This study is the first to use a combination of histology, immunohistochemistry and cryo-EM to analyze the AD and CTE comorbidities that are characterized by the coexistence of two six-tau isoforms pathologies. (equal contribution; KLN, CQI)

Disclosures: K.L. Newell: None. **C. Qi:** None. **H.J. Garringer:** None. **M. Jacobsen:** None. **R. Vidal:** None. **S. Scheres:** None. **M. Goedert:** None. **B. Ghetti:** None.

Nanosymposium

NANO12: Genetic and Circuit Mechanisms in Retinal Function and Regeneration

Location: MCP Room S106

Time: Sunday, October 6, 2024, 8:00 AM - 10:00 AM

Presentation Number: NANO12.01

Topic: D.06. Vision

Title: Single-cell cross-species analysis reveals pathways promoting regeneration in the human retina

Authors: *S. J. MILLER;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: The zebrafish is capable of regenerating neurons in the adult retina in response to injury. Retinal injury in the zebrafish results in the proliferation and differentiation of Muller glia that then serve as an endogenous stem cell population. However, in humans the ability to regenerate neurons in the retina is not currently achievable. To circumvent the human limitations in neuronal replenishment, we aimed to identify molecular pathways that may regulate the zebrafish's ability to generate endogenous stem cells to then apply this to the human. To address this aim, we performed single-nucleus RNA sequencing (snRNA-seq) of 24 postmortem retinas from glaucoma, age-related macular degeneration, and controls. The snRNA-seq revealed the upregulation of activated Muller glia genes, CLU and VIM in the diseased donors but not in the controls. To explore disconcordant pathways between the response to neuronal injury in the zebrafish and human, we compared the enriched genes between activated Muller glia. In doing so, we identified YAP1. YAP1 inhibition led to activated Muller glia proliferation and subsequential de-differentiation into stem-like cells in human retinal explants and age-accelerated retinal organoids. Lastly, we show that extended retinal cultures with YAP1 inhibitor led to the stem-like cells to express early neuronal lineage markers such as Beta-III Tubulin. Collectively, these results suggest that human activated Muller glia retina contain the capacity to

de-differentiate into an endogenous stem-like cell population that can undergo early neuronal differentiation.

Disclosures: S.J. Miller: None.

Presentation Number: NANO12.02

Topic: D.06. Vision

Support: P30 EY026878
R01 EY029323
R01 EY014454
R01 EY026555

Title: Compartmentalized pooling generates orientation selectivity in wide-field amacrine cells

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Abstract: Orientation is one of the most salient features in visual scenes. In the brain, different strategies for orientation detection have been revealed. Here, we identify a computational rule, termed compartmentalized pooling, as the key for generating dendritic orientation selectivity in B/K wide-field amacrine cells (B/K WACs) — a group of giant, non-spiking interneurons in the mouse retina defined by co-expression of Bhlhe22 (B) and Kappa Opioid Receptor (K). B/K WACs exhibit orientation-tuned calcium signals along their long, straight, unbranching dendrites, which contain both synaptic inputs and outputs. Simultaneous dendritic calcium and somatic voltage recordings reveal that individual B/K dendrites are electrotonically isolated, exhibiting a spatially confined yet extended excitatory receptive field along the dendrite, and center-surround antagonism perpendicularly. Phenomenological receptive field models demonstrate that compartmentalized pooling along the dendrite suffices to generate orientation selectivity, and center-surround antagonism shapes band-pass spatial frequency tuning. At the microcircuit level, B/K WACs receive excitation driven by one contrast polarity (e.g., ‘ON’) and glycinergic inhibition driven by the opposite polarity (e.g., ‘OFF’). However, this crossover inhibition is not essential for generating orientation selectivity. A minimally sufficient biophysical model recapitulates compartmentalized pooling of feedforward excitatory inputs with the intrinsic electrotonic property at a biophysical limit. Collectively, our results reveal a computational principle for orientation selectivity and highlight its implementation by B/K WACs, enriching the scientific understanding of diverse strategies employed across different hierarchies of the visual system to achieve orientation selectivity.

Disclosures: W. Lei: None. **D. Clark:** None. **J.B. Demb:** None.

Presentation Number: NANO12.03

Topic: D.06. Vision

Support: Purdue University Lab Start-Up

Title: The role of transcription factor Meis2 in the development of GABAergic amacrine cells in mammalian retina.

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Abstract: Of the major classes of retinal neurons, amacrine cells (ACs) exhibit the greatest diversity, with more than sixty molecularly distinct subtypes. Each AC subtype is thought to carry out a specific function necessary for the detection of a single visual feature. The genetic factors that control AC diversity during retinal development are unknown, but are important for understanding the genetic basis of AC function, morphology, and their contribution to visual behaviors. Identifying these genetic factors has been difficult, however recent single-cell transcriptomics studies have unveiled genetic distinctions between the two major groups of ACs—the GABAergic and Glycinergic ACs. One of the most distinct genetic differences between GABAergic and Glycinergic ACs is the expression of the transcription factors *Meis2* and *Tcf4*, respectively. In this study, we focused on the role of *Meis2* in GABAergic AC development. Based on the expression of *Meis2* and its known roles in the nervous system, we hypothesized that *Meis2* is necessary for the neuronal specification, survival, and morphology of GABAergic ACs. To test this, we used *Meis2* conditional knockout mice, $Meis2^{Flox/Flox}$ ($Meis2^{CKO}$) crossed with $Six3^{Cre}$ or $Ptf1a^{Cre}$ mice to selectively delete *Meis2* from the whole developing retina or AC precursor cells, respectively. In both $Meis2^{CKO}$ mouse lines, we observed a reduction in both the total number of ACs and the inner plexiform layer (IPL) thickness. Furthermore, both $Meis2^{CKO}$ mouse lines had a 60% reduction in the total number of GABAergic ACs. Finally, in both $Meis2^{CKO}$ mouse lines, we observed AC subtype differences in IPL stratification. We found thinning of the dopaminergic (TH+) AC layer of the IPL, but no changes in cholinergic (ChAT+) layers of the IPL in both $Meis2^{CKO}$ mouse lines. These results suggest that *Meis2* is necessary for both the survival and dendritic stratification of at least some of the GABAergic ACs in the mammalian retina.

Disclosures: P.C. Kerstein: None.

Presentation Number: NANO12.04

Topic: D.06. Vision

Support: Research to Prevent Blindness (RPB) Career Development Award (MT) and RPB unrestricted gift to Flaum Eye Institute, University of Rochester Medical Center.
University of Rochester Genomics Research Center (GRC)
National Institutes of Health (NIH) grant R01EY024334 and P30EY003176

Title: P2x7 drives pathophysiological remodeling of the inner retina during progressive photoreceptor loss

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Abstract: Purpose: Congenital or idiopathic retinal degeneration (RD) leads to vision impairment and blindness. The loss of photoreceptors in the outer retina triggers **pathophysiological remodeling**, a crucial but understudied phenomenon affecting the inner retina, resulting in intrinsic and spontaneous retinal ganglion cell (RGC) **hyperactivity**, reducing the signal-to-noise ratio (SNR) of remaining light responses, further degrading vision. Inhibition of hyperactivity improves image recognition in-vivo, using strategies that are currently clinically unsuitable. This study aims to uncover new druggable targets and devise therapeutics to block hyperactivity and improve vision in animal models and patients.
Methods: Rd1 mice (a retinitis pigmentosa model) were crossbred with purinergic 2x isoform 7 (P2X7) knockout mice, generating a double-mutant mouse strain. Gene and protein expression were tested using qRT-PCR, RNA-sequencing, and immunohistochemistry in retinal lysates or fixed eyeballs. Membrane permeability of Yo-Pro dyes was quantified using confocal imaging in whole-mounted living retinas. RGC activity was recorded using multielectrode arrays and patch-clamp electrophysiology.
Results: Here we show that photoreceptor loss upregulates transmembrane P2X7 receptor in the inner retina. Knocking-out P2X7 in RD mice prevents hyperactivity, while P2X7 overexpression in WT retina increases spontaneous action potential firing. RNA-sequencing indicates RD enhances the expression of membrane depolarization-related genes in a P2X7-dependent manner, including ion channels and transporters such as HCN1, CACNA1h, RYR2/3, and SLC1A2. Patch-clamp recordings following photoreceptor loss reveal robust HCN1-dependent ion current activation in Off-RGCs but not On-RGCs, which was rescued by knocking-out P2X7.
Conclusions: P2X7 is necessary and sufficient for degeneration-dependent hyperactivity in the inner retina of blind mice. Our results suggest that this effect is achieved through downstream activation of several ion channels and transporters, most significantly HCN1 channels in Off-RGCs. Targeting P2X7 in photoreceptor degeneration could be a viable therapeutic approach to antagonize pathophysiological remodeling, reduce spontaneous hyperactivity, and improve the SNR of light responses.

Disclosures: L. Afrima: None. **R.H. Kramer:** None. **M. Telias:** None.

Presentation Number: NANO12.05

Topic: D.06. Vision

Support: NIH Grant R01 EY034662
NIH Grant T32 EY025202

Title: Genetic tuning of intrinsically photosensitive retinal ganglion cell subtype identity to drive visual behavior

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Abstract: Light is a profoundly important regulator of circadian rhythms of physiology and behavior across a wide range of organisms. Light information is relayed via diverse retinal ganglion cell types to approximately 50 distinct targets in the brain. The melanopsin-expressing,

intrinsically photosensitive retinal ganglion cells (ipRGCs) represent 6 of the approximately 40-50 retinal ganglion cell types present in the mouse retina. M1-M6 ipRGCs are defined by a distinct complement of subtype-defining morphological, physiological, and transcriptional characteristics. However, how this cellular diversity is achieved is largely unknown. Brn3b (Pouf42) is a transcription factor involved that is poised to influence gene expression programs defining properties of ipRGCs. In this study we tested the hypothesis that Brn3b actively shapes the morphological, physiological, and transcriptional identity of ipRGC subtypes. We compared gene expression patterns, melanopsin expression, morphological properties as well as ipRGC-driven behaviors in mice where Brn3b is conditionally removed (Brn3bcKO animals) or overexpressed (Brn3bOE) in ipRGCs. Our results indicate that Brn3b expression levels in ipRGC correlates with, and actively regulates, the levels of melanopsin mRNA and protein. Using TRAP-seq we found that Brn3b is a central regulator of transcriptional programs that define ipRGC subtype identity. Additionally, we found that Brn3b plays a key role defining morphological properties such as soma size and dendritic development of ipRGC subtypes. Finally, we analyzed the axonal projections patterns to the main ipRGC targets in the brain as well as ipRGC-driven behaviors in Brn3bcKO, Brn3bOE, and control littermate mice. We found that ipRGC-projection patterns as well as ipRGC-driven behaviors were altered in Brn3bcKO and Brn3bOE mice. Altogether these findings indicate that Brn3b define the transcriptional identity, the morphological properties and behaviors driven by ipRGC subtypes.

Disclosures: M. Aranda: None. **J. Bhoi:** None. **O. Payan Parra:** None. **T. Yamada:** None. **Y. Yang:** None. **T.M. Schmidt:** None.

Presentation Number: NANO12.06

Topic: D.06. Vision

Support: MRC

Title: Transplantation of human embryonic stem cell-derived cone photoreceptors partially reverses retinal remodelling and restores function in the Aipl1$^{-/-}$ mouse model of end-stage Leber Congenital Amaurosis

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Abstract: Photoreceptor degeneration is a leading cause of untreatable sight-loss. We have previously demonstrated restoration of retinal function following transplantation of human pluripotent stem cell (hPSC)-derived cone photoreceptors (hCones) in the *Rd1* model of rod-cone dystrophy. However, for photoreceptor replacement therapy to be effective, it must work for a variety of disease types. Here, we sought to determine if it is possible to rescue the *Aipl1*$^{-/-}$ mouse model of Leber Congenital Amaurosis (LCA). This model exhibits extremely rapid and widespread photoreceptor degeneration, with near complete loss by postnatal day (P)18; this rapidity affects the initial formation of photoreceptor-bipolar cell synapses, presenting a particularly severe case for rescue.

Following transplantation of hCones into AIPL1 Foxn1$^{nu/nu}$ retinas, hCones expressed markers of mature photoreceptors including pre-synaptic proteins. Host cone bipolar cells under the hCone cell mass extended dendrites towards transplanted photoreceptors and demonstrated an upregulation of post-synaptic machinery, providing evidence of nascent synaptic connectivity. Ex-vivo Multielectrode Array recordings demonstrated robust rescue of light responses in regions below the transplanted cell mass, in the form of light-evoked micro-ERGs and fast transient changes in the firing rate of downstream Retinal Ganglion Cells. A variety of different response profiles were observed, over four different light levels, which are similar to those identified in cone only mouse retina, but not in aged-matched degenerate controls. Synaptic blockers reversibly eradicated all light-evoked mERGs and light responses, confirming they originated from glutamatergic transmission between hCones and host Bipolar cells in the outer retina. Mice showed some improvements in optomotor head tracking behaviour.
Together with our previous findings, this suggests that photoreceptor replacement therapy may be feasible even in the most severe cases of retinal degeneration, where extensive remodelling of the host retina has already occurred, providing support for the use of photoreceptor cell therapy in severe LCA and in other similar advanced states of degeneration.

Disclosures: C.A. Procyk: None. **A. Melati:** None. **J.J. Delicata:** None. **J. Liu:** None. **M.J. Branch:** None. **M. Tariq:** None. **M. Moshtagh Khorasani:** None. **E.L. West:** None. **A.J. Smith:** None. **R.A. Pearson:** None. **R. Ali:** None.

Presentation Number: NANO12.07

Topic: D.06. Vision

Support: R01 EY030565

Title: Visual circuits underlying sexually biased threat anticipation

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Abstract: The ability to anticipate potential threats in nature provides a clear evolutionary advantage. In mammals, the visual system is one of the primary senses used to respond and to detect changes in the environment. However, whether and how the visual system may influence the ability to anticipate environmental threats is unknown. In this work, we tested the hypothesis that the melanopsin (Opn4)-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) are critical for the ability to anticipate the future appearance of a previously experienced threat. For this, we developed a novel Visual Threat Anticipation (VITA) paradigm, in which we exposed control and melanopsin knock-out (Opn4$^{-/-}$) mice to a threatening visual "looming" stimulus (Exposure Phase). After two days, we returned animals to the identical context and measured their anticipatory behavior (i.e. VITA) in the absence of any "looming" stimulus to determine whether they associated that context with the prior threat exposure (Test Phase). We found that male Opn4$^{-/-}$ animals lacked VITA, while non-sexually receptive Opn4$^{-/-}$ females (i.e. Diestrus or Metestrus, estrus cycle stages) showed increased VITA. Using a c-Fos induction screening, we identified the Perihabenular Region (PHb) as candidate ipRGC-central target for driving VITA. Chemogenetic manipulation of PHb-ipRGCs and GABAergic PHb neurons induced opposite effects on VITA behavior in males and females. Our results suggest

that ipRGCs mediate VITA behavior through a retina-PHb circuit in a sex- and estrous cycle dependent manner.

Disclosures: M. Aranda: None. **E. Min:** None. **L. Liu:** None. **A. Schipma:** None. **T.M. Schmidt:** None.

Presentation Number: NANO12.08

Topic: D.06. Vision

Support: NIH Grant EY032119
NIH Grant EY034004
Unrestricted Grant from RPB

Title: Diversity and collicular projection patterns of retinal ganglion cells in tree shrew

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Abstract: Tree shrew is an emerging small animal model for studying complex visual processing. Tree shrews have a cone-dominated retina with a region of high acuity, and midbrain structures such as the superior colliculus (SC) and the lateral geniculate nucleus with distinct laminae - features attributable to a highly developed visual system resembling primates. Yet, little is known about the functional diversity of retinal ganglion cells (RGCs) and their output projections to subcortical areas, in particular, the SC that is implicated in visuomotor tasks such as gaze, attention, and motion processing.Here, we sought to characterize the morphological and functional diversity of RGCs and map the projection pathways from the retina to the SC in tree shrew. We used an AAV2-retro virus construct to retrogradely target RGCs projecting to the SC for expressing a fluorescent protein and a red shifted Channelrhodopsin (ReaChR). Immunofluorescent labeling of brain sections and retinal wholemounts indicate successful uptake of virus in the superficial layers of SC and transduction of RGCs in the retina, respectively. Fluorescence fundus imaging was performed to determine level of transduction in RGCs in vivo. Retinas from centrally injected animals were biopsied and spiking activity of hundreds of RGCs to a battery of visual stimuli was measured over a multielectrode array. Receptive field measurements indicate multiple ON and OFF RGC types, with mosaics of RGCs with small receptive fields and low-pass temporal integration and mosaics of larger receptive fields with more band-pass temporal integration. A sizable fraction of RGCs was direction selective (~15%) while a smaller fraction was orientation selective (<10%). ReaChR responses identified by short temporal latency combined with receptive field measurements and post-hoc immunolabeling and anatomical tracing, indicate both monostratified and bistratified RGCs (including direction selective RGCs) project to the SC. This study reveals for the first-time, mosaics of multiple functionally distinct RGC types, along with direction- and orientation-selective RGCs, in the tree

shrew retina. The study also reveals the subset of RGC types that project to the SC, highlighting the retinal influence in shaping functional specializations in the superior colliculus.

Disclosures: S. Roy: None. **M.O. Bohlen:** None. **J. Cruger:** None. **N.C. Shultz:** None. **M.A. Sommer:** None. **D. Fitzpatrick:** None. **G.D. Field:** None.

Nanosymposium

NANO13: Neural Substrates of Working Memory and Sustained Representations

Location: MCP Room N228

Time: Sunday, October 6, 2024, 8:00 AM - 10:00 AM

Presentation Number: NANO13.01

Topic: H.05. Working Memory

Support: National Natural Science Foundation of China (32271089)
Shanghai Pujiang Program (22PJ1414400)
Ministry of Science and Technology of China (STI2030-Major Projects 2021ZD0203701)

Title: Generalization principles of working memory in human parietal and prefrontal cortices

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Abstract: Working memory, the ability to flexibly maintain and manipulate information to serve goal-directed behavior, is fundamental to human higher intelligence. It has been argued that working memory possesses a highly constructive nature, wherein mnemonic information is abstracted through shared perceptual or structural knowledge to facilitate efficiency and learning. For instance, reordering numbers based on their magnitude and sorting pictures based on their visual similarity are two distinct memory manipulation tasks, but their underlying task structure remains similar, that is, organizing items into a specific sequence. However, it remains unclear whether the brain utilizes a generalizable neural code to solve these tasks. In this study, we addressed this question by investigating the generalization principles for both stimulus and goal information in working memory. Human participants completed a memory manipulation task in two distinct circular stimulus spaces: location and object. In the location task, participants mentally rotated spatial locations by a cued rotation angle (0, ±60, ±120, ±180 degrees). In the object task, participants first acquired the structure of the circular object space by learning the transitional relations between objects drawn from the space, and during the main task, mentally updated objects according to symbolic cues indicating the distances between objects (0, ±1, ±2, ±3 steps). In other words, the two tasks shared similar stimulus and goal structures but with distinct stimulus and cue sets. Leveraging functional MRI (fMRI) and multivariate pattern analysis, we observed that the posterior parietal cortex (PPC), but not the early visual cortex, exhibited representations of both goal and manipulated stimulus in both location and object tasks, indicating PPC as a domain-general brain region for working memory manipulation.

Furthermore, combining principal component analysis and subspace decoding analyses, we found that the goal structure of the two tasks could generalize to each other within PPC, while the subspaces of the manipulated stimulus remained independent. Additionally, we found successful generalization of goal structure in multiple subregions of the lateral prefrontal cortex, albeit with a later temporal onset and weaker representational strength. In summary, our results reveal distinct computational principles for the generalization of information maintained in working memory: while PPC maintains all task-relevant information, it utilizes a generalizable neural code for representing goals and a separable neural code for representing stimuli.

Disclosures: D. Shi: None. **Q. Yu:** None.

Presentation Number: NANO13.02

Topic: H.05. Working Memory

Support: EU Horizon 2020 no 754513
EU Horizon 2020 no 703456

Title: Attention re-allocation and item maintenance in human working memory

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Abstract: Working memory, keeping information in mind for short periods of time and using it in a goal directed manner, is essential to meaningfully interact with our environment. Yet, while being central to everyday life it is still hugely controversial how working memory is implemented in the human brain or how optimal functioning can be supported in disorders. Here we present novel findings from human electrophysiology indicating that while slow oscillatory activity is relevant for generalised attention allocation, oscillations in the beta range are key in actual item maintenance. By focusing on beta activity we are able to differentiate working memory contents while slow oscillatory activity supports long-range network formation. Furthermore, an induced fast rhythmic signal was uniquely able to track the locus of attention.We combined a working memory retro cue paradigm with a novel approach to invisibly tag visual stimuli (RFT, rapid invisible frequency tagging) that can be read out using electrophysiology and recorded participants in the MEG (magnetoencephalography, n=40). During every trial participants were simultaneously presented with a lateralised face and Gabor grating. They encoded the items and had to retain them both for a short period of time until a cue told them which one they were going to be tested on. Participants were instructed to only maintain the cued item after this retro cue. A probe stimulus in the same location as the cued item was presented after the post cue retention period and participants responded with a match/non-match button press. Importantly, the two items were invisibly tagged with distinguishable frequencies (62 and 66Hz) and we were able to successfully read out those tags using MEG. The frequencies showed significant amplitude modulation by attention during the second time window indicating a reliable marker for attention allocation. Furthermore, slow oscillatory activity in the alpha range showed lateralised item maintenance which was unperturbed by the tagging signal. While the described findings were generalised to item maintenance and attention allocation, oscillatory activity in the beta frequency was uniquely able

to distinguish between the two item conditions. Beta oscillations differentiated the maintenance of faces from the maintenance of Gabor grating orientation in working memory, i.e. during their absence from the environment.Our findings are well in line with literature on invasive animal models highlighting the relevance of beta oscillations for item maintenance in working memory and offer a new approach for tapping into the neural correlates of human working memory.

Disclosures: B. Berger: None. **T. Minarik:** None. **O. Jensen:** None.

Presentation Number: NANO13.03

Topic: H.05. Working Memory

Support: NIH R01 EY017077

Title: Decoding Neural Activity in Working Memory Tasks Using Machine Learning Models

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Abstract: In this presentation, we applied machine learning and deep learning techniques to decode neural activity during primate working memory tasks, specifically focusing on an oculomotor delayed response task. We achieved a decoding accuracy exceeding 90%, indicating high precision in neural information encoding. Data were collected from the prefrontal and posterior parietal cortices of juvenile and adult male rhesus monkeys. To enhance our models' effectiveness, we utilized advanced statistical methods for feature selection from time-series spike data and optimized hyperparameters to improve generalizability to new data. Our results highlighted that even simple computational models such as logistic regression, support vector machines, and shallow neural networks are capable of accurately predicting neural activity based on aggregated neuron data. Notably, we also demonstrated predictive capabilities in the posterior parietal cortex neurons—areas not typically associated with working memory. Additionally, we employed continuous-time recurrent neural networks that emulate natural neuronal dynamics, further reinforcing our models' reliability and providing deeper insights into the neural mechanisms underpinning working memory. Overall, our study confirms that machine learning and deep learning are powerful tools for analyzing neural recordings, offering profound implications for understanding the neural basis of working memory and potentially extending to other cognitive functions.

Disclosures: J. Gerstenberger: None. **C. Chen:** None. **S. Pu:** None.

Presentation Number: NANO13.04

Topic: H.05. Working Memory

Support: MH131678

Title: Neural evidence for decision-making underlying attractive serial dependence

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Abstract: Recall of stimuli is biased by recent stimulus history, manifested as an attractive bias toward, or a repulsive bias from, a previous stimulus. This is known as the serial dependence effect. A recent two-stage model of serial dependence (Sheehan & Serences, 2022) proposes that a repulsive bias emerges during the encoding stage of the current stimulus, where encoding is influenced by sensory adaptation to the previous stimulus; an attractive bias, on the other hand, arises from the (post-perceptual) decision-making stage, when the memory representation for the current trial is read out. Neural evidence for a repulsive bias at encoding has been found in several studies. For example, in Hajonides, van Ede, Stokes, Nobre, & Myers (2023), two samples were presented sequentially in each trial and human observers were cued to recall one of them at the trial end while neural activity was recorded with magnetoencephalography. During encoding the neural representation of the current stimulus was biased repulsively by both the cued item from the previous trial (henceforth “previous target”) and the previously encoded sample from the current trial ("sample 1”). In contrast, at the behavioral level, sample 1 repulsively biased the report, but the previous target exerted an attractive bias. Here we assessed whether this discrepancy between neural effect and behavioral report may be rooted in the decision-making stage. We re-analyzed the data from this study but focused on the memory recall period. Whole-head multivariate decoding showed that the neural representation of the current target was attractively biased toward the previous target (both shortly after the probe onset and before participants completed recall), but there was no evident bias from sample 1. A follow-up searchlight decoding analysis isolated this attractive bias to a right-central cluster of sensors, in line with a role for higher-order decision processes. Our results suggest that the priors that influence post-perceptual decision-making are updated by the previous trial’s target, but not by stimulus information from the current trial.

Disclosures: J. Shan: None. **J.E. Hajonides:** None. **N. Myers:** None.

Presentation Number: NANO13.05

Topic: H.05. Working Memory

Support: Grant-in-Aid for Scientific Research from MEXT (22H02737)

Title: thalamic computation enables tracking of short-term sensory history

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Abstract: The brain continuously extracts statistical features across previously encountered sensory inputs (sensory history) and incorporates them into a stable, yet flexible internal model. Despite this model being the basis of our ability to interpret ambiguous sensory information for decision making (perceptual inference), how it is implemented is unclear. Schmitt *et al* (Nature 2017) suggest that “higher-order” thalamic nuclei can stabilize neuronal representations within the cortex. To explore how the thalamus may support sensory-history integration into the internal model we focused on interactions between the posterior parietal cortex (PPC), its thalamic counterpart the pulvinar nucleus (PUL) and thalamic reticular nucleus (TRN). Optogenetic

inhibition of either PUL or PPC prevented the inclusion of experiential history in current decision making in a mouse auditory sensory-history task. To better understand how sensory-history is represented in these areas we conducted recordings with Neuropixels targeting PPC, PUL and TRN in awake head-fixed mice exposed to a controlled auditory sensory experience while manipulating these areas optogenetically. Machine-learning based analysis of neuronal activity recorded in the PPC was able to decode sensory-history information while optogenetic suppression of the PUL significantly reduced decoding accuracy, consistent with the PUL controlling stability of cortical representations. Supporting previous studies that suggest modulation of inhibition is essential for stabilizing short-term neuronal representations (Kim & Sejnowski Nat Neuro 2021), optogenetic tracing revealed that putative inhibitory cell types in the PPC received a significantly greater proportion of the input from the PUL, indicating that PUL could increase the stability of PPC representations of sensory-history by the same means. To characterize this stabilization input and determine how/when it is engaged, we used a model explainer tool (SHAP), to rank individual neuron's contribution to predicting sensory-history. This approach revealed a strong contribution from putative TRN inputs to the PUL suggesting a key role for this inhibitory input. Further, network motif analysis of PUL-TRN connections uncovered an architecture consistent with a biological comparator that suppresses the PUL in response to shifts in sensory experience statistics. Based on these findings, we propose a system that allows updating of sensory-history representations within the PPC by selective destabilization controlled *via* the PUL/TRN tracking environmental patterns, thereby updating the internal model and enabling effective inference.

Disclosures: P.S. Hosford: None. **H. Mei:** None. **C. Hayde:** None. **L.I. Schmitt:** None.

Presentation Number: NANO13.06

Topic: H.05. Working Memory

Support: Translational Biomedical Sciences Program
Cognitive Neurophysiology Laboratory

Title: Dual-task walking uncovers distinctive EEG effects on encoding and retention stages of working memory in healthy young adults: A Mobile Brain-Body Imaging (MoBI) study.

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Abstract: Introduction: Working memory (WM) comprises a series of metacognitive neural processes to temporarily encode, maintain and use information to execute a desired action, all essential for the correct execution of activities of daily living. The interactions of these neural dynamics are not fully understood. Furthermore, walking while simultaneously executing a cognitive task has been shown to modify behavior, gait and neural resources. However, it is unknown if variations in cognitive load when walking or sitting alters behavior and neural processes during a WM task. To our knowledge, no studies have utilized the MoBI technology to examine cognitive-motor interaction (CMI) and variations in cognitive load on WM. Our

objective was to characterize the effects of CMI and variations in memory load on WM in healthy young adults. We hypothesize that the interaction between cognitive and motor systems will uncover previously hidden WM traits. Understanding how WM neurophysiology is modulated by walking in a healthy population can provide a platform to later study those at risk of disease. **Methods:** 33 neurotypical young adults (18 (54.5%) females and 15 (45.5%) males with a mean age 22.12 ± 4.35 and 16.18 ± 2.9 years of education) completed a Delayed Match-to-Sample task (DMTS) using congruent or incongruent stroop-like stimuli, while sitting or walking on a treadmill. MoBI was utilized to simultaneously obtain behavioral, high density electroencephalography and gait recordings. Demographic information and a baseline cognitive score (MoCA) were also obtained. Data were analyzed with Matlab^{R}. **Results:** Participants had an overall accuracy d' = 3.88, with behavioral improvements when walking (d' sitting = 3.755 ± 0.650, walking = 4.041 ± 0.659, t_{33} = 2.734, P = 0.010, Cohen's d = 0.476) and a decrement when exposed to incongruent stimuli independently from the motor condition (d' congruent = 3.923 ± 0.528, incongruent = 3.768 ± 0.697, t_{33} = 2.577, P = 0.015, Cohen's d = 0.449). No interaction was found between motor condition and congruency. Stride length between single and double tasking did not change. EEG amplitude reductions were found between sitting and walking at frontal, central and parietal scalp regions, during the encoding (conflict resolution - N200) and the retention (delay) period of WM (walking-sitting Cluster based Monte-Carlo permutation test - t_{33} = - 2.5, P = 0.009). **Conclusion:** CMI using the MoBI uncovered hidden characteristics of WM encoding and maintenance in a young healthy cohort. Changes were observed in behavior and spatiotemporal EEG amplitudes associated with conflict resolution and semantic rehearsal processes of WM whether sitting or walking.

Disclosures: E. Avendano: None. **E.G. Freedman:** None. **E. Mantel:** None. **S. Nasimjonova:** None. **J.J. Foxe:** None.

Presentation Number: NANO13.07

Topic: H.05. Working Memory

Support: NIH Grant R01MH063901
Swiss National Science Foundation Grant P500PB_214404

Title: Causal evidence for a role of alpha oscillations in working memory maintenance

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Abstract: Working memory (WM) is the brain's ability to temporarily hold and manipulate information when it is no longer present in our environment. Previous studies showed that WM is supported by interactions between frontal-parietal areas and posterior sensory regions. In particular, the lateral prefrontal cortex (LPFC) provides top-down control over sensory WM representations. The intraparietal sulcus (IPS) has also been shown to be involved in maintaining WM information. The neural mechanisms behind such frontal-parietal control remain underspecified. Different brain oscillations may establish the inter-areal communication within these cognitive networks. A previous study used repetitive transcranial magnetic stimulation (rTMS) in a retro-cue task, to show that theta and alpha oscillations respectively prioritize and suppress WM representations (Riddle et al., 2020). Using computational modeling and MEG we

have also shown that posterior alpha oscillations provide a phase-coding mechanism for maintaining information in WM (Pagnotta et al., 2024). Here, our goal was to assess the role of theta and alpha oscillations in WM maintenance, to differentiate their role in maintenance from their involvement in retro-cue dynamics. We used rTMS in a delayed-response task. Subjects (N=10) were shown a lateralized display of 3 circles (stimulus: 0.2 s) and had to remember their colors and positions. After memory delay (2 s), one circle was cued by position and subjects were asked to report its color. Subjects adjusted a probe to match the value of the relevant feature held in WM and placed 6 responses over a 360-degree field of colors. This allowed us to derive behavioral measures of both accuracy (error) and precision of the WM representation, from the distribution of the subject's responses. Subjects completed 240 trials of the task (8 blocks). In each trial during the second half of delay, online rTMS was delivered to either left LPFC or IPS. ROIs were identified on the subject's anatomical whole-head image, collected in a preliminary MRI session. Each rTMS consisted of a train of 4 biphasic pulses, with stimulation frequency of either 6 Hz (theta), 10 Hz (alpha), or arrhythmic (control). Our results did not show any effects of rhythmic stimulations on accuracy. However, we found increased precision when alpha rTMS was applied to IPS, in contralateral presentation trials. These results suggest that, while LPFC is involved in the manipulation of WM information but not maintenance, the IPS serves as controller over WM maintenance via alpha oscillations. Further, the fact that accuracy was not affected by rTMS to IPS suggests that this area does not store feature information locally.

Disclosures: M. Pagnotta: None. **M. D'Esposito:** None.

Presentation Number: NANO13.08

Topic: H.05. Working Memory

Support: NIH RF1NS127129
NIH R01DC107979
NSF 2014217

Title: Neural dynamics for working memory and evidence integration during olfactory navigation in Drosophila

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Abstract: In order to navigate towards an uncertain goal location, animals must accumulate evidence about the location of a goal and store this information in working memory. Here we identify a population of local neurons in the fan-shaped body of *Drosophila* that exhibits working memory and evidence integration dynamics during odor-guided navigation. Imaging from these neurons during virtual navigation reveals a bump of activity that is activated by odor, but can outlast the odor stimulus by several seconds. Persistent bump activity is associated with continued movement in the direction adopted during odor, arguing that these neurons represent a directional working memory signal for navigation. Bump position remains fixed during closed-loop navigation, consistent with it representing an allocentric goal rather than an egocentric heading, but can slowly remap when the fly rotates repeatedly during odor. When the fly navigates a virtual odor plume, bump activity slowly ramps up with successive odor encounters, indicating that it integrates odor information over time. These dynamics are not observed in a

different population of local neurons, although both populations are modulated on slow timescales by the fly's engagement in the navigation task. Silencing of the first local neuron population impairs both behavioral evidence integration and persistent upwind heading driven by trains of odor pulses. Our work identifies a small group of genetically-identified neurons that integrate and store stochastic sensory evidence to support navigation in complex natural environments.

Disclosures: N. Kathman: None. **A. Lanz:** None. **K. Nagel:** None.

Nanosymposium

NANO14: Learning and Memory: Episodic, Physiology and Pharmacology

Location: MCP Room S404

Time: Sunday, October 6, 2024, 8:00 AM - 10:45 AM

Presentation Number: NANO14.01

Topic: H.07. Long-Term Memory

Support: National Science Foundation under Grant BCS-2001025

Title: Disentangling common, depression-related, and anxiety-related internalizing symptoms in relation to REM sleep, slow-wave sleep, and episodic memories

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Abstract: Background: Altered sleep characteristics, including increased Rapid Eye Movement (REM) sleep and decreased Slow-Wave Sleep (SWS) are putative risk factors for high internalizing symptoms. Prior research has shown associations between REM sleep and emotional memory consolidation, and between SWS and neutral memory consolidation, although the evidence is mixed. This study examines emotional and neutral aspects of episodic memories as mechanisms through which microstructures of REM and SWS perpetuate common, depression-related, and anxiety-related internalizing symptoms. **Methods**: Healthy adults encoded scenes featuring either negative objects (e.g., a snake) or neutral objects (e.g., a chipmunk), and indicated whether objects were old or new compared to what they encountered during encoding. All participants completed one night of laboratory-monitored polysomnography, and reported internalizing symptoms using the Beck Depression Inventory, Beck Anxiety Inventory, and State-Trait Anxiety Inventory. **Results**: Increased REM peak amplitude (the absolute highest amplitude reached by eye movements) was significantly associated with remembering negative objects better than neutral objects (β;=0.28, p=.012), which in turn predicted more severe anhedonia (β;=0.26, p=.016). Reduced slow-wave frequency (the number of slow waves per second) was numerically related to increased false alarms for negative objects (β;=-0.25, p=.081), which in turn significantly predicted more severe depressive general distress (β;=0.24, p=.014) and mixed general distress (β;=0.20, p=.034).

Discussion: Targeting maladaptive emotional memory could serve as an effective intervention for internalizing symptoms exacerbated by sleep disturbances, particularly for depression-related symptoms.

Disclosures: X. Niu: None. **K.E.G. Sanders:** None. **D. Denis:** None. **T. Cunningham:** None. **G. Zhang:** None. **E.A. Kensinger:** None. **J.D. Payne:** None.

Presentation Number: NANO14.02

Topic: H.07. Long-Term Memory

Support: NBRI grant

Title: Sparse coding of episodic memory in the human hippocampus is related to the excitability of neurons at encoding

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Abstract: Sparse coding is the predominant theory of episodic memory representation in the human hippocampus. It holds that individual neurons represent few memories, and individual memories are represented by few neurons. Prior studies detected a sparse coding signal in epilepsy patients using intracranial recording during a recognition memory task (Urgolites et al., 2022; Wixted et al., 2018). The spike count distributions for targets and lures were visually compared using quantile-quantile (QQ) plots and statistically compared by computing differences in standard deviation, skewness, and kurtosis between the two distributions. The sparse coding account was supported by a significant increase in these statistics for targets relative to lures. Using an independent dataset of single neuron recordings from the medial temporal lobe (Chandravadia et al., 2020), we aimed to (1) replicate these findings and (2) further investigate the relationship between sparse coding, recognition memory performance, and neuronal allocation. The data were originally collected by Rutishauser et al. (2010, 2015). At learning, participants were presented with 100 images of objects (e.g., phones, animals, and complex natural scenes) for 1 or 2 s each. Later, they completed a recognition test, indicating whether images were old (50 targets) or new (50 lures). Normalized spike counts were calculated for each neuron for every trial, and the distributions for targets and lures were compared. There were 1,066 neurons recorded from the amygdala and 736 from the hippocampus. Within each session and for each item, mean spiking before and during item presentation at encoding was calculated. Items were labeled as high or low spiking using a median split both before and during encoding. Neurons were labeled as "excitable" in response to a specific item if the spiking changed from low before the item was presented to high during item presentation at learning. The sparse coding signal was evident in QQ plots for the hippocampus but not the amygdala (replicating prior work). In addition, this signal was selectively detected in the hippocampus for remembered targets vs. lures (standard deviation difference: 0.08, $p = 0.01$; skewness difference: 1.77, $p = 0.03$; kurtosis difference: 42.38, $p = 0.06$). Additionally, the sparse coding signal was evident only for excitable neurons associated with remembered targets vs. lures (standard deviation difference: 0.23, $p < 0.001$; skewness difference: 3.74, $p < 0.001$; kurtosis difference:

84.72, p = 0.01). The sparse coding signal is related both to the “excitability” of neurons at encoding and to successful recognition at retrieval.

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

Presentation Number: NANO14.03

Topic: H.07. Long-Term Memory

Support: NSFC Grant 32071060

Title: Voluntary and reflexive components of oculomotor behavior for recognition memory in macaque monkeys

Authors: *J. LIU[1], Z. JIN[2], R. YANG[2], J. CAI[3], S. PAN[4], M. CAO[5], H. WANG[1], S. KWOK[2]; [1]East China Normal Univ., Shanghai, China; [2]Duke Kunshan Univ., Kunshan, Jiangsu, China; [3]King's Col. London, London, United Kingdom; [4]Duke Univ., Durham, NC; [5]Johns Hopkins Univ., Baltimore, MD

Abstract: Recognition memory enables us to discriminate whether an event has occurred in the past and its underlying processes can be reflected in oculomotor behavior. Past research has suggested that eye movement viewing behavior reflects human participants’ conscious memory decision whereas one’s pupil size is modulated by a pupil old/new effect. The former reflects a voluntary and conscious process whereas the latter implies an involuntary and automatic process beyond conscious control. Here we set out to examine these two physiological processes using high-sampling rate eye tracking on macaque monkeys performing a three-alternative forced-choice (3AFC) delay-matching-to-sample (DMTS) task. Single-unit neuron recording was conducted on the macaque’s posterior parietal cortex (the precuneus) for elucidating the two processes’ respective neural substrates. Five male rhesus macaques each completed 15 sessions (~ 6,800 trials in total) of a 3AFC DMTS task while their eye movements were recorded. We examined the eye-memory relationship by considering (1a) the proportion of duration and number of fixations, (1b) saccades, and (2) pupillometry to study the voluntary and reflexive components of the recognition memory task. In a second experiment, extracellular neuronal responses from the precuneus are recorded and analyzed to assess the neural correlates for these distinct components. By comparing between correct and incorrect responses, we found that fewer fixation and saccadic events ($P<0.05$), shorter saccadic and fixation duration ($P<0.05$), and increased pupil diameter ($P<0.05$) for correct responses compared to incorrect responses. Neuronal responses are shown as a function of these physiological and other behavioral parameters. Our study suggests eye movement patterns and pupillometry show a physiological link to recognition memory performances, revealing voluntary and reflexive components in the process.

Disclosures: J. Liu: None. **Z. Jin:** None. **R. Yang:** None. **J. cai:** None. **S. Pan:** None. **M. Cao:** None. **H. Wang:** None. **S. Kwok:** None.

Presentation Number: NANO14.04

Topic: H.07. Long-Term Memory

Support: National Natural Science Foundation of China (General Program grant 32071060)

Title: Whole cortex neural replay of temporal order memory triggered by inter- and intra-hippocampal ripples

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Abstract: Replay has emerged as a promising mechanism for episodic memory retrieval and consolidation, wherein patterns of neural activity recapitulate past or possibly anticipate future trajectories. While evidence for neural replay of the "where" (location) and "what" (object) sequences has been found in both rodents and humans, another key aspect, namely the replay of the "when" or "time," remains poorly understood. To address this gap, here we ask whether neural replay can capture temporal information independently of specific content in mnemonic processes. We investigated this by using intracranial electroencephalography (iEEG) data collected from 18 epileptic human volunteers (mean age: 24.67 ± 8.21), while they performed a temporal order judgment task that required them to recall the order of events from just watched videos. Employing a single-shot protocol with unique movie clips presented only once, our L1-logistic regression classifier aimed to capture the neural representation of general time rather than the specific visual elements. Using the temporally delayed linear model (TDLM) framework (Liu et al., 2021), in which the strength of replay is reflected in the magnitude of the temporal dependence among reactivation levels, we observed rapid, bi-directional replay during both online memory retrieval (sequenceness: 0.014 vs. permutation threshold: 0.013) and subsequent offline resting period (sequenceness: 0.012 vs. permutation threshold: 0.011), with a compress factor ranging from 2.7 to 12.5. Furthermore, the total strength of bi-directional replay during both phases could predict the participants' metacognitive abilities (Pearson correlation, Online retrieval: $r = 0.54$, $p = 0.02$; Offline resting: $r = 0.55$, $p = 0.02$). Moreover, by correlating the occurrence time of hippocampal sharp-wave ripples (SWRs) with reconstructed temporal order, we found that replay can be represented by chaining multiple SWRs together in addition to within-SWR replay among a subset of 8 participants who had implanted electrode(s) in the hippocampus. Our findings of this intra-SWR (one sample t-test: $t = 4.88$, $p < 0.001$) and inter-SWR (one sample t-test: $t = 3.89$, $p < 0.001$) cortical replay events enhance our understanding of hippocampal-cortical communication, and they also shed light on the complex multi-scaled nature of temporal structure in human episodic memory.

Disclosures: X. Zhu: None. **H. Jiang:** None. **C. Li:** None. **D. Santos-Pata:** None. **Z. Wang:** None. **S. Zhang:** None. **S. Kwok:** None.

Presentation Number: NANO14.05

Topic: H.07. Long-Term Memory

Support: Wellcome Trust/Royal Society Sir Henry Dale Fellow 206682/Z/17/Z
Wellcome Trust Principal Research Fellowship 224444/Z/21/Z
UK DRI Dementia Research Institute
Gatsby Foundation

Title: Homecage-based unsupervised novel object recognition in mice

Authors: N. KEJZAR[1], S. HO[1,2], S. BURTON[3], ***L. KATSOURI**[3], J. M. O'KEEFE[3], M. BAUZA[3], J. KRUPIC[1,2];
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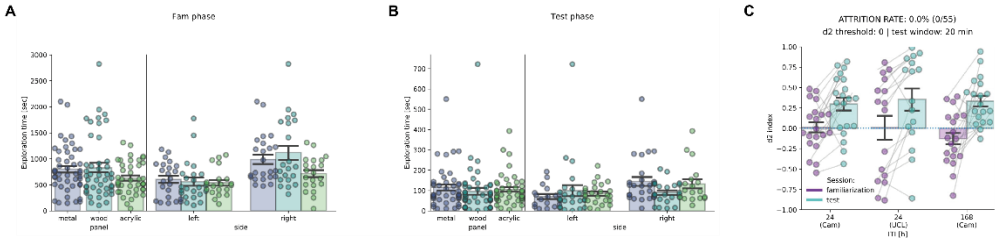
Abstract: Novel object recognition (NOR) task is commonly used for testing hippocampal and parahippocampal-related memory in rodents. It is based on an animal's natural tendency for increased exploration of novel objects relative to familiar ones and does not require food or water restrictions for motivation. However, its implementation is often challenging due to the high variability associated with exploratory behaviours. Here, we present an unsupervised NOR task implemented in a homecage (smart-Kage). In the 24-hour sampling phase mice were presented with two distinct objects, with which they could interact by touch, vision, and smell. The sampling phase was followed by either 24-hour (n=20 mice Cambridge, n=16 UCL) or 7 days (n= 19 mice) inter-trial intervals. In the test phase, mice were presented with a duplicate of a previously presented object and an unfamiliar one. The object presentation was randomised to balance the object identities and location. First, we confirmed no bias in object identity or location preference during both phases (Fig. A-B). Next, we showed that mice could recall familiar objects after an interval of 24 hours and 7 days (Fig. c, discrimination index (d2; sampling vs test): 24h, 0.013±0.064 vs 0.298±0.08, $p<0.005$; 7 days, -0.126±0.074 vs 0.332±0.064, $p<0.005$). Importantly, tests conducted in two different facilities displayed comparable results despite a degree of variability in mouse behaviour across facilities (Fig. C; d2 (sampling vs test): 24h, 0.01±0.15 vs 0.355±0.134, $p<0.005$). We envisage the smart-Kage automated NOR task will enable higher throughput and more standardised memory testing in mice.



A-B, Left: Time mice spent exploring the three materials used as object; Right: Exploration time of objects when presented on the left or right side. Paired Student's t test with B-H correction, all with $p>0.05$. **C,** Mice on average explored the two objects similarly in sampling phase (purple) and showed significant preference for novel objects in test phase (mint). Dashed line denotes equal preference for both objects. Paired Student's t test with B-H correction.

Disclosures: N. Kejzar: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge

Phenotyping Ltd. **S. Ho:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd. **S. Burton:** None. **L. Katsouri:** None. **J.M. O'Keefe:** None. **M. Bauza:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd. **J. Krupic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd.

Presentation Number: NANO14.06

Topic: H.08. Learning and Memory

Support: NIH grant R01NS110806
NIH grant R01AG074226
NIH grant R01DA054977
NSF grant 1422438

Title: Spectral dynamics θ and slow γ oscillons in behaving rats

Authors: *Y. DABAGHIAN;
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Abstract: Spectral dynamics θ and slow γ oscillons in behaving rats
M. Zobaer[1], N. Lotfi[1], C. Behera[1], C. Domenico[2], L. Perotti[3], D. Ji[2], Y. Dabaghian[1]
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Oscillons are recently discovered, high-resolution constituents of the extracellular fields. In particular, the most robust, highest-amplitude θ-oscillons are salient in actively behaving rats, bearing some resemblances to the familiar θ-waves. Slow-γ oscillons can be viewed as qualitative counterparts of the conventional slow-γ waves [1]. However, the similarities apply mostly to the ballpark, averaged, lento-changing characteristics, such as mean frequencies, amplitudes, and bandwidths. In addition, hippocampal and cortical oscillons exhibit many novel, intricate, behavior-attuned, transient properties that suggest a new vantage point for the brain rhythms' structure, origins and functions. For example, the fact that oscillons are frequency-modulated waves with speed-controlled parameters opens a surprising, "FM" perspective on the information exchange in hippocampo-cortical network. This observation also links electrophysiological data to oscillatory models of brain waves, which can be pictorially illustrated using a toy model of neuronal synchronization. In particular, it can be shown that the synchronicity level in physiological networks is fairly weak, that the oscillatory dynamics is coupled with the stochastic component of the LFP, and so forth. Overall, we suggest that oscillons represent the actual temporal architecture of synchronized neural dynamics, whereas the conventional θ and γ waves are only approximations to the physical oscillatory motifs.
[1] M. S. Zobaer, N. Lotfi, C. M. Domenico, C. Hoffman, L. Perotti, D. Ji & Y. Dabaghian. *Theta oscillons in behaving rats*, https://www.biorxiv.org/content/10.1101/2024.04.21.590487v1

Disclosures: Y. Dabaghian: None.

Presentation Number: NANO14.07

Topic: H.08. Learning and Memory

Support: CU-ZI-MR-S-0025
MSCA 101068893-MemUnited

Title: Differentiation of object and movement representations in the hippocampus and in domain-specific regions after sequence learning

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Abstract: There is evidence that exposure to regularities can shape object representations in the medial temporal lobe (Schapiro et al., 2012). Specifically, it was shown that regularities can increase the representational similarity between pairs of objects that appeared nearby in time. Here we investigated representational shaping as a consequence of sequence learning in different memory domains. Specifically, we examined how temporal proximity in the sequence influenced representational shaping in regions in the medial temporal lobe (hippocampus, parahippocampus and perirhinal cortex) as well as domain-specific cortical regions (primary motor cortex; M1 for motor memory and lateral occipital complex for object memory). In the study, all participants (N=23) learned two sequences while fMRI data was acquired (1 week interval between sessions). Depending on the task, participants either encoded a four-element sequence of finger movements (motor sequence encoding; serial reaction time task) or objects (object sequence encoding; 1-back task). To investigate changes in the representation of individual movements and objects over time, the same objects/movements were presented in a random order before and after sequence exposure. Baseline objects or movements that were not part of a sequence were also included for comparison. For each ROI and timepoint, the multi-voxel pattern of activation evoked by individual objects and movements was extracted and pattern similarity between pairs of different movements or objects was computed. For each task, we computed correlations (1) between members of the sequence and (3) between baseline items that were not part of the sequence. The results showed that in all ROIs there was a reduction in pattern similarity between different movements representations (RM ANOVAs with time (pre vs. post) and pair (baseline vs. sequence); main effect of time, all ps <.05), irrespective of whether movements were part of the sequence or not (time x pair interaction, all ps < .1). Interestingly, the decrease in pattern similarity in the (posterior) hippocampus and M1 between non-consecutive movements in the sequence was significantly larger as compared to baseline and consecutive movements (Fig. 1), suggesting greater differentiation of representations of non-adjacent movements after sequence learning. Higher levels of differentiation in both M1 and the hippocampus were related to higher performance gains during motor sequence learning. For the object task, pattern similarity between different objects decreased over time in all ROIs except M1 (effect of time, all ps <.05) and to a similar extent for different pairs (time x pair, all ps < .1).

Disclosures: N. Dolfen: None. **W. Fias:** None. **L. Davachi:** None.

Presentation Number: NANO14.08

Topic: H.08. Learning and Memory

Support: Brain Initiative Award 1RF1MH123247-01
NIH Grant 1R01NS113889-01A1
NIMH Grant ZIA-MH002838

Title: Cross-hierarchy propagating waves coordinate sensory stimulus encoding and memory retrieval

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Abstract: Our brain must balance exteroceptive sensory sampling with internal mnemonic processes to efficiently directing behavior within complex environments. In mouse forebrain, switching between two modes facilitating sensory encoding and memory-related processes respectively is marked by repeated, brain-wide cascades of multi-seconds. However, it remains unclear whether a similar phenomenon exists in humans and is linked to network-level brain dynamics. In this study, we showed that spontaneous pupil dilations at rest are associated with the cascade dynamic in mouse spiking data whereas infra-slow (<0.1 Hz) propagating waves from low-order sensory-motor (SM) regions to high-order default mode network (DMN) in human fMRI, thereby linking the two types of infra-slow global brain activity across two species. Similar to the mouse spiking cascades, the SM-to-DMN fMRI waves persist when subjects performing a visual memory task. To determine how these waves may affect the sensory and memory functions, we quantified the sensory encoding efficacy through a deep learning model to decode semantic information from stimuli-evoked fMRI signals, and then memory encoding and recall through behavioral responses. We found that both sensory and memory functions were systematically modulated across the fMRI wave cycles: the semantic and memory encoding exhibited similar changes, peaking during SM-activated phase, whereas memory recall showed an opposite trend, peaking at DMN-activated phase. These seconds-scale modulations of sensory and memory processing resembled those observed in mice over the spiking cascade cycles. Collectively, these results suggested that quasi-periodic global brain activity represents an evolutionary conserved feature of mammalian brain physiology that coordinates sensory and memory functions. The finding may provide a new perspective into the nature of behavioral variability during cognitive tasks.

Disclosures: Y. Yang: None. **D.A. Leopold:** None. **J.H. Duyn:** None. **X. Liu:** None.

Presentation Number: NANO14.09

Topic: H.08. Learning and Memory

Title: The Combination of E2027 (Irsenontrine) and Donepezil Hydrochloride Increases Supernatant Acetylcholine Concentrations in Human iPS-derived Neurons and Improves Memory Deficits in Rat Models

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Abstract: Cyclic nucleotide-specific phosphodiesterases (PDEs) play essential roles in signal transduction by regulating the intracellular levels of cAMP and cGMP. Among the PDE family, PDE9 is a highly cGMP-specific hydrolyzing enzyme. PDE9 inhibition may be effective in dementia as PDE9 is expressed widely in the brain and modulates important neurotransmitter systems. The NO-cGMP axis plays an important role in the regulation of acetyl choline (ACh) release in the brain [Prast and Philippu (1192); Guevara-Guzman et al. (1994)], therefore, the combination of PDE9 and cholinesterase inhibitors (PDE9Is and ChEIs, respectively) may synergistically increase ACh concentrations in the brain, suggesting a novel therapeutic option. E2027 (Irsenontrine) is a PDE9I created by Eisai [Ishani S. Landry et al. (2022)]. In this study, we investigated effects of the combination of PDE9Is and ChEIs on ACh concentrations in human iPS cell-derived neurons. In addition, its effects on cognition were examined using rat models. We used E2027, as PDE9I, and donepezil HCl as ChEI. We generated human neural stem cells (long-term self-renewing neuroepithelial-like stem cells) from 201B7, an iPS cell line of Kyoto University. Human neurons were differentiated from neural stem cells for ~28 days, and on the final day, we added compound(s) into culture wells and harvested supernatants and cell lysates 1, 24, and 72 hours later. ACh was determined by LC-MS/MS. cGMP was measured by LC/MS-MS or an enzyme immunoassay kit. Effects on cognitive function were evaluated in the rat novel object recognition task.We observed choline acetyl transferase positive neurons in neural cell populations by immunocytochemistry, and K+-dependent Ach release from neurons. Intracellular cGMP was increased in an E2027 concentration-dependent manner in the presence of YC-1 (guanylate cyclase). Supernatant Ach levels were increased in an E2027 concentration-dependent manner. A synergistic increase of supernatant ACh was observed in the combination of E2027, YC-1 and donepezil HCl (interaction, $p < 0.0001$). Co-administration of E2027 and donepezil HCl at sub-pharmacologic doses had a significantly greater effect on the novel object recognition task than did donepezil HCl alone in both the natural forgetting model and muscarinic receptor antagonist scopolamine-induced memory impairment model. Donepezil HCl did not affect the cGMP-increasing effect of E2027 in rat CSF.E2027 promoted ACh release from human cholinergic neurons, and when combined with donepezil hydrochloride, ACh concentrations were increased in culture supernatants synergistically. This combination treatment also improved memory in rats.

Disclosures: M. Ando: A. Employment/Salary (full or part-time):; Eisai Co. Ltd. **K. Yamazaki:** A. Employment/Salary (full or part-time):; Eisai Co. Ltd. **E. Takahashi:** A. Employment/Salary (full or part-time):; Eisai Co. Ltd. **S. Kotani:** A. Employment/Salary (full or part-time):; Eisai Co. Ltd. **T.R. Patel:** A. Employment/Salary (full or part-time):; Eisai Ltd. **A. Bradford:** A. Employment/Salary (full or part-time):; Eisai Ltd. **K. Horie:** A. Employment/Salary (full or part-time):; Eisai Inc. **J. Gartlon:** A. Employment/Salary (full or part-time):; Eisai Ltd..

Presentation Number: NANO14.10

Topic: H.08. Learning and Memory

Title: Modulation of fear extinction by single-dose psilocybin application is sex-specific in stress-susceptible and resilient mice

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Abstract: After exposure to a traumatic event, susceptible individuals can develop post-traumatic stress disorder (PTSD), characterized by intrusive re-experiencing of the fearful event, impaired fear safety learning as well as altered cognition. Due to the disorder's sex-dependent comorbidities and symptom complexity, a majority of PTSD patients do not respond to available pharmacological or psychological interventions, indicating that current treatment procedures are low in clinical efficacy. The overall increasing incidence suggests the urgent need for new treatment options. Here, we used a trauma model in male and female mice with repeated memory retrievals, eliciting intrusive re-experiencing aspects of the traumatic event, leading to long-term behavioral and cognitive changes. Subsequently, we classified animals of the two sexes based on the freezing behavior into subgroups of resilient and susceptible phenotypes, respectively. Mice then underwent a cued fear conditioning paradigm with two tones at different frequencies, whereby only one of them was paired with a mild electric foot-shock to address discriminative safety learning. Upon fear extinction paradigm, we treated half of the two classified animal subgroups with saline or psilocybin. In contrast to female mice, the male cohort treated with psilocybin showed significant overall facilitation of extinction learning and improved tone discrimination abilities, suggesting the advantageous effects of the drug on extinction memory acquisition and/or memory retrieval in PTSD-like male phenotypes. Overall, using a clinically transferable rodent model of PTSD, our work demonstrates differential sex- and stress-subgroup dependent modulation by psilocybin in fear extinction and discrimination ability following an aversive associative learning paradigm.

Disclosures: L. Islami: None. **O. Ordowski:** None. **A. Justen:** None. **C. Salazar Sandoval:** None. **M. Meinhardt:** None. **M. Kuchar:** None. **B. Lutz:** None.

Presentation Number: NANO14.11

Topic: B.05. Synaptic Plasticity

Support: NIH U19 NS107616

Title: Efficient coding emerges via spike-timing-dependent plasticity in an E-I network

Authors: *B. SHEN[1], S. SONG[2], R. C. FROEMKE[3], K. LOUIE[1], P. W. GLIMCHER[1]; [1]Neurosci. Inst., [2]IonLab, [3]Otolaryngology, NYU Grossman Sch. of Med., New York, NY

Abstract: Lateral inhibition is a prevalent neural circuit architecture, prominent in sensory processing and linked to higher cognitive functions like attention and decision making. A crucial role for lateral inhibition is thought to be efficient coding of information, driven by its ability to eliminate redundant information across input channels. Yet, it remains unclear how such architecture arises and whether it adapts to environments with varying degrees of redundancy. Here, we examine how lateral inhibition motifs arise in neural networks with empirically derived, biological learning rules. Specifically, we quantified how the level of input correlation shapes network structure in a randomly connected spiking network of excitatory (E) and inhibitory (I) neurons governed by spike timing-dependent plasticity (STDP) rules. Excitatory units implemented a classic Hebbian STDP rule, where synapses are potentiated after pre-post

synaptic spike sequences and depressed after post-pre sequences. In contrast, inhibitory neurons implemented a recently discovered symmetric plasticity kernel, with synapses always potentiated regardless of the order of the pre and post spikes (Vogels et al. Science 2011; Field et al. Neuron 2020). In high redundancy environments, where two inputs are correlated over time and therefore share a large amount of information, the network forms long-range E-to-I connections between the two input regions, establishing lateral inhibition that is mediated through local I-to-E connections. In contrast, in low redundancy environments, where the two inputs are independent, the network exhibits reduced long-range E-to-I connections but maintains local I-to-E connections, favoring independent coding of the two inputs. These findings suggest that lateral inhibition motifs can spontaneously arise when the neural network passively receives redundant inputs; furthermore, the degree of lateral inhibition adapts to the degree of information redundancy in the interacting environment. More broadly, this work sheds light on the origins and plasticity of efficient representations in neural circuits, highlighting the essential role of brain-environment interactions in cognitive development.

Disclosures: B. Shen: None. **S. Song:** None. **R.C. Froemke:** None. **K. Louie:** None. **P.W. Glimcher:** None.

Nanosymposium

NANO15: Molecular Mechanisms of Neurodevelopmental Disorders and Autism

Location: MCP Room N426

Time: Sunday, October 6, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO15.01

Topic: A.07. Developmental Disorders

Support: DeNardo Education and Research Foundation Grant

Title: Ctbp1 mutation affects cell adhesion and migration in early stages of neurodevelopment

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Abstract: Brain development is initiated in the embryo and, subsequently, adult characteristics are acquired upon maturation of the nervous system. The main cellular processes occurring in the early stages of neurodevelopment include neurogenesis, cell migration, cell differentiation, synaptogenesis, neuronal cell death, and synaptic rearrangement. These processes must occur in a precise sequence both spatially and temporally, any disruption of these processes having a lasting impact on brain development. Genetic, environmental, and experiential factors can influence neurodevelopment and lead to clinical manifestation of such disorders. Therefore, understanding the underlying factors and the biological mechanism(s) that lead to such manifestations is crucial to identifying and mitigating risks that may affect neurodevelopmental outcomes. A newly emerging neurodevelopmental disorder involving hypotonia, cerebellar ataxia, developmental delay, and tooth enamel defects (HADDTS) has been recently studied.

The disorder has been linked to a de novo heterozygous missense mutation in the C-terminal binding protein 1 (CTBP1) gene (NM_001328.2: c.1024C >T, p.Arg342Trp) and is often associated with early onset of profound cerebellar atrophy in patients. To better understand the neurodevelopmental defects manifested in patients with HADDTS, we used induced pluripotent cells (iPSC). Isogenic iPSC lines were generated to avoid the variability in patient-derived cell lines due to genetic or pathological damages and environmental factors. Isogenic cell lines harboring heterozygous (R342/W342) and homozygous (W342/W342) mutations were produced using the CRISPR/Cas9 methodology. Transcriptome analysis of differentiated neurons from isogenic cell lines harboring heterozygous and homozygous mutations for the pathogenic CTBP1 p.R342W allele indicated that several genes involved in the cell adhesion, neurodevelopment, and signaling pathways were downregulated. We hypothesized that the pathogenic mutation in *CTBP1* causes various defects in the early stages of neurodevelopment. To correlate transcript data with the phenotypic defects, we developed a 2D cell culture model and a 3D model using neurospheres to understand cell adhesion, migration, and neurite formation in the mutant cells. The results obtained from our experiments supported our hypothesis. We observed morphological differences in neurite growth in differentiated neurons. The mutant neural stem cells (NSCs) exhibited low adhesion compared to the parental NSCs. Cell migration in the homozygous and heterozygous cells was lower compared to the parental isogenic wild-type cell line.

Disclosures: S. Lee: None. **V. Selvamani:** None. **G. Chinnadurai:** None. **U. Ezekiel:** None.

Presentation Number: NANO15.02

Topic: A.03. Stem Cells and Reprogramming

Support: Telethon Grants Project GGP19181

Title: CACNA1A loss of function mutations impair neurogenesis in different ways in human iPSC-derived models

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Abstract: CACNA1A encodes the pore-forming α1A subunit of the voltage-gated CaV2.1 calcium channel. This channel is found primarily on presynaptic terminals, dendrites, and neuroendocrine cells in the brain, and it is critical for regulating synaptic function. Many loss of function mutations have been identified in CACNA1A as cause of different neurological diseases, including various forms of ataxia, epilepsy, and migraine. However, the molecular mechanisms underlying these disorders are little known, and specific therapeutic approaches are lacking. The aim of this study was to investigate the effects of CACNA1A loss of function mutations on neuron development and function by using human-derived in vitro models. Accordingly, we generated by CRISPR/Cas9 iPSCs carrying two CACNA1A variants causing episodic ataxia type 2: Y1854X, selectively affecting the CaV2.1[EFa] isoform, and F1491S, affecting both CaV2.1[EFa] and [EFb] isoforms. Mutated and isogenic control iPSC lines were

used to generate neuronal cultures by differentiation protocols passaging through embryoid bodies, neural rosettes, neural progenitors (NPCs), and neuronal networks stages. Morphological, molecular, and functional tests highlighted different neurodevelopmental defects caused by the two mutations. Cells carrying F1491S showed an impaired neuronal induction at very early stages, with mutated NPCs appearing with altered features, including morphology, transcriptomic signatures, and cell migration. Such cells failed to differentiate in neuronal networks as demonstrated by the impairment in neurites outgrowth, axon specification, and neurons to glia specification. By contrast, cells carrying Y1854X behaved apparently normal in terms of neuronal induction and maturation but showed an altered electrophysiological activity on HD-MEA recordings, with a complete lack of synchronous network events. Our findings highlighted novel roles of CACNA1A in neuronal induction and differentiation, besides confirming its relevance in synaptic communication. Importantly, the iPSC-derived neuronal models developed in this study will pave the way for future therapeutics testing for neurological disorders involving CACNA1A.

Disclosures: I. Musante: None. **L. Muzzi:** None. **F. Jaudon:** None. **R. Ruggeri:** None. **L. Cingolani:** None. **F. Zara:** None. **P. Scudieri:** None.

Presentation Number: NANO15.03

Topic: A.07. Developmental Disorders

Title: Consequences and therapeutic targeting of tyrosine kinase receptor regulatory variants in epilepsy

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Abstract: Profiling tissue directly from epileptic foci has identified new genetic variants and potential drivers of intractable epilepsy. Somatic variants in the receptor tyrosine kinase pathways (RTK) have emerged as a common hit in studies of pharmacologically resistant epilepsy including *EGFR, PDGFRA, FGFR2* and *FGFR1*. However, little is known about whether these RTK variants are capable of directly driving focal cortical dysplasia (FCD) and neural activity phenotypes associated with subpopulations of intractable epilepsy. Here, we aimed to develop a model of EGFR-amplifying regulatory variants in cortical neurons, enabling us to mechanistically study their impact on neuronal function and structure. To accomplish this, we utilized converging approaches to directly induce EGFR overexpression including activation of epilepsy-linked enhancer variants through epigenome editing (CRISPRa). A central finding from this work was that EGFR activation in developing cortical neurons produced hallmarks of FCD1a such as reduced soma size, altered dendritic morphology and hyperexcitability. Interestingly, some of these phenotypes were rescued by treatment with a brain penetrant EGFR inhibitor. Ongoing studies explore whether regulatory variants in other RTKs, such as PDGFRA, lead to similar phenotypes. Beyond RTK-specific variants, we seek to identify ways that regulatory variants impact chromatin architecture and transcriptional signatures of epilepsy.

Implications for treatment with brain penetrant RTK inhibitors and epigenetic therapies will be discussed.

Disclosures: M. Weinman: None. **P.P. Mathkar:** None. **M. Snuderl:** None. **M.C. Weston:** None. **J. Stafford:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nzumbe Inc..

Presentation Number: NANO15.04

Topic: A.07. Developmental Disorders

Support: NIH Grant HD103360
NIH Grant NS122316
DOD Grant W81XWH-21-1-0247

Title: Autism- and epilepsy-associated EEF1A2 mutations lead to translational dysfunction and development delay in iPSC derived neurons

Authors: *M. S. MOHAMED[1], R. SAM[2], L. COLE[3], A. CHEN[3], S. REYMAN[4], E. KLANN[4]; [1]New York Univ. Ctr. For Neural Sci., New York, NY; [2]NYU, New York, NY, ; [3]New York Univ., New York, NY, ; [4]New York Univ., New York, NY

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 endogenous 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, translation is also disrupted in the G70S mutants with polysome profiling showing altered initiation rates to compensate for decrease elongation. Whole-cell electrophysiological recordings show increased input resistance, resting membrane potential and firing rates in EEF1A2 mutant neurons. Mutant neurons also display altered morphology with increased length but decreased number of neurites. RNA-Seq and ribosome footprint profiling show decreased translational efficiency of genes that play a role in ion transport, synapse organization, synapse assembly and memory. These processes which are involved in late-stage neuronal development and maturation have decreased translational efficiency in EEF1A2 mutant neurons indicating that there is significant delay in the maturation of these 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.

Disclosures: M.S. Mohamed: None. **S. Reyman:** None.

Presentation Number: NANO15.05

Topic: A.07. Developmental Disorders

Support: Internal Funding / Yale University Department of Neurosurgery

Title: A supervised machine learning algorithm predicts microcephaly-associated genes based on their- spatiotemporal expression patterns in developing human brain.

Authors: Z. MOYNIHAN[1], A. PEKSEN[2], B. GULTEKIN[2], A. ERCAN-SENCICEK[2], E. DENIZ[3], K. BILGUVAR[2], M. GUNEL[2], *T. BARAK[1,2];
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Abstract: Primary hereditary microcephaly (MCPH) is a neurodevelopmental disorder characterized by a notably small brain size at birth, occurring in approximately 1 in 30,000 to 250,000 live births globally. Despite its relatively frequent occurrence, the molecular mechanisms of microcephaly remain poorly understood due to its heterogeneous etiology. To address this, we employed a novel technique using a supervised machine learning algorithm that leverages spatiotemporal single-cell RNA sequencing data from the developing human brain. This approach facilitated the prediction of genes associated with MCPH, which we named the Primary Microcephaly Score (PMS). Our analysis covered gene expression across 22 anatomical locations and 7 developmental time points using publicly available scRNAseq data. We analyzed 912 known microcephaly-associated genes, both primary and secondary, from the OMIM database, categorizing them into five distinct clusters. The majority of MCPH-related genes were grouped in a specific cluster, cluster-4, which exhibited a unique signature indicative of their potentially overlapping functions. To refine our predictions, we utilized the k-Nearest-Neighbors Conditional-Density Resampled Estimate of Mutual Information (kNN-DREMI), a method designed for quantifying statistical dependencies between genes in scRNAseq data. This analysis generated a pseudogene value by calculating the per-cell median expression of 29 known MCPH-genes, ranking the genes and scoring them based on their similarities to the MCPH gene expression pattern. We validated the performance of PMS using 3,798 OMIM genes, where PMS effectively identified primary microcephaly genes with an area-under-curve (AUC) of 0.99 and genes in cluster-4 with an AUC of at least 0.96, demonstrating the high effectiveness of PMS in predicting MCPH- and cluster-4 associated genes. Cluster-4 genes showed the highest expression in dividing cells in scRNAseq data, a finding further corroborated using an independent dataset, the Prenatal LMD Microarray in the Allen Brain Atlas. Our computational approach was further tested by knocking down one of the top-ranking genes, RAD51, in a Xenopus tropicalis model, which resulted in a small head phenotype in 2-day post-fertilization (dpf) old tadpoles, confirming its role in microcephaly. These results underscore the efficacy of our computational approach in classifying microcephaly-associated genes, significantly enhancing our understanding of the etiology of MCPH and potentially aiding in the development of targeted diagnostic and therapeutic strategies.

Disclosures: Z. Moynihan: None. **A. Peksen:** None. **B. Gultekin:** None. **A. Ercan-Sencicek:** None. **E. Deniz:** None. **K. Bilguvar:** None. **M. Gunel:** None. **T. Barak:** None.

Presentation Number: NANO15.06

Topic: A.03. Stem Cells and Reprogramming

Support: Rosamund Stone Zander Translational Neuroscience Center
BCH ECRAC grant

Title: High-throughput phenotypic screen combining optogenetics, machine learning and iPSC-derived neuronal models to investigate cannabidiol (CBD) and CBD analogs as an anti-seizure strategy

Authors: *W. AFSHAR SABER[1], Z. YANG[2], N. TEANEY[2], F. GASPAROLI[3], K. D. WINDEN[2], J. HUBBS[2], M. SAHIN[2];
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Abstract: Tuberous sclerosis complex (TSC) is a neurocutaneous syndrome that is characterized by benign tumors in multiple organs and associated with neurological symptoms such as epilepsy and behavioral disorders. Rapalogs have been approved to treat several manifestations of TSC, but the neurological symptoms associated with TSC have been more recalcitrant to treatment. In addition, two trials of rapalogs for behavioral and neuropsychiatric symptoms associated with TSC have shown no effect. Therefore, there is an urgent need to understand the pathogenesis of the abnormalities in neuronal connectivity that lead to TSC-associated epilepsy and identify novel therapeutic targets that can be used to treat these disorders. To answer this need, we combined the use of human induced pluripotent stem cells, optogenetics and machine learning to establish a high-throughput drug screening platform. We reprogrammed patient somatic cells ($TSC2^{+/-}$) into hiPSCs and genetically modified these hiPSCs to establish the full allelic series. Additionally, we used CRISPR-Cas9 to stably express calcium indicator in the safe harbor locus (AAVS1) and used the NGN2 transcription-factor based method to establish a neuronal *in vitro* model of TSC to identify disease-associated changes to neuronal morphology and neuronal excitability. Subsequent to collecting the changes in fluorescence associated with calcium activity, we developed an improved acquisition and analysis pipeline based on micromanager and python (https://pymmcore-plus.github.io/pymmcore-plus/) which will be publicly available. This initiative stems from our desire to enable wider adoption and distribution of these open-source software. Together with the use of an automated, incubated stage and perfect focus, this system enables larger screens while selecting multiple positions per well in an unbiased manner. Additionally, we have successfully developed a deep learning model for fast and reproducible segmentation of hiPSC-derived neurons. This approach facilitates the automated extraction of calcium activity with a single-cell resolution. In this context, we tested chronic and acute rapamycin treatment, Torin, cannabidiol (CBD) and CBD analogs and identified compounds that rescued the functional TSC disease phenotypes. This strategy provides an unbiased quantitative imaging platform for therapeutic development in TSC and a framework for phenotype discovery and drug screening in other neurological disorders.

Disclosures: W. Afshar Saber: None. **Z. Yang:** None. **N. Teaney:** None. **F. Gasparoli:** None. **K.D. Winden:** None. **J. Hubbs:** None. **M. Sahin:** None.

Presentation Number: NANO15.07

Topic: A.07. Developmental Disorders

Support: ERC
SFARI

Title: Developmental excitation/inhibition imbalance disrupts social circuit function via activity-dependent transcriptional remodeling

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Abstract: Autism and related developmental disorders encompass a wide range of heterogeneous conditions with an overall prevalence of 1 % in the human population. Although autism varies in symptomatology and severity, its two main core symptom domains (i.e. social and communicative impairment, as well as restricted and repetitive behaviors) appear to be consistently affected across the spectrum. However, how diverse etiological mechanisms converge to produce the relatively narrow set of behavioral and etiopathological manifestations that characterize autism remains unclear. A popular theory posits that an imbalance between excitatory and inhibitory (E/I) activity might contribute to the etiology of autism. However, controversy exists about whether E/I imbalances observed in animal models and clinical populations causally contribute to autism pathology or are instead a compensatory or epiphenomenal phenotype. To reconcile these views, here we test the hypothesis that transient E/I imbalance during early development is sufficient to alter the developmental trajectory of the brain, leading to lasting autism-relevant phenotypes. We find that chemogenetically-induced E/I imbalance in the mouse neocortex during early development leads to permanently impaired sociability but largely preserved cognitive and motor-sensory functions. Corroborating the developmental specificity of these results, control studies in which the same manipulation was applied to adolescent mice did not result in any behavioral alteration. Social alterations are associated with transcriptional dysregulation of autism-risk ion channels and synaptic genes, thus putatively implicating activity-dependent transcriptional mechanisms in the generation of the observed phenotypes. Transcriptomic dysregulation furthermore resulted in enduringly altered electrical properties of pyramidal neurons, suggesting altered maturation of principal neurons. Circuit level investigations in adult mice using resting state fMRI show that developmental E/I imbalance profoundly disrupts functional connectivity in limbic fronto-hippocampal components of the social brain but preserved coupling in sensory regions. Notably, hypoconnectivity of prefrontal regions with dopaminergic nuclei is also highly predictive of social disruption in manipulated animals. Our results, reconcile conflicting findings in the field and support a developmental reconceptualization of the E/I imbalance theory of autism whereby developmental changes in E/I are sufficient to produce autism-related phenotypes via epigenetic dysregulation of autism-relevant synaptic genes.

Disclosures: A. Stuefer: None. **L. Balasco:** None. **G. Colombo:** None. **S. Gini:** None. **L. Coletta:** None. **B. D'Epifanio:** None. **F. Rocchi:** None. **C. Montani:** None. **F. Alvino:** None. **D. Sastre Yagüe:** None. **A. Galbusera:** None. **M. Aldrighetti:** None. **S.M. Bertozzi:** None. **P. Lau:** None. **F. Papaleo:** None. **G. Iurilli:** None. **Y. Bozzi:** None. **L. Cancedda:** Other; funder and scientific consultant @IAMA therapeutics. **M.V. Lombardo:** None. **A. Gozzi:** None.

Presentation Number: NANO15.08

Topic: A.07. Developmental Disorders

Support: Roy J. Carver Charitable Trust Grant # 23-5683

Title: Evaluating fetal neocortical development in germ-free mice in response to maternal IL-17A administration

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Abstract: Maternal inflammation during pregnancy has been identified as an environmental factor that can trigger the pathogenesis of neurodevelopmental disorders (NDDs) in offspring. The mechanisms behind this trigger are still not fully understood. However, previous research in mice has shown that interleukin-17 (IL-17) is potentially a key mediator in this phenomenon. A commonly used physiological marker of proper neurological development is the patterning of the cerebral cortex. Abnormalities in the somatosensory cortex are correlated with behavioral phenotypes in mice that resemble those of NDDs. Notably, the gut microbiota is key for the differentiation and expansion of T helper 17 cell that produce IL-17, and some studies have shown that maternal microbial composition dictates the ability of IL-17 to influence offspring neurodevelopment and behavior. Our study aims to determine if IL-17A is sufficient to cause NDD-like malformations in the fetal brain in the total absence of maternal microbes. We used pregnant germ-free (GF) mice and administered either 1 µg recombinant IL-17A or 0.1 mL sterile saline (vehicle) intraperitoneally once daily from gestational day (GD) 10.5 through GD15.5. Fetal brains were collected on GD 16.5 and postfixed in 10% formalin before being sectioned coronally and immunohistochemically (IHC) stained for TBR1, a marker for excitatory neurons, and DAPI. Counts of TBR1+ cells and TBR1 fluorescence intensity were measured to determine layer patterns across the cortical plate. DAPI staining was used to measure layer thickness and total cortical plate thickness. Additionally, Spatial Light Interference Microscopy (SLIM), a label-free imaging technology, was used to quantify layer thickness. This technique, which for the first time is used in prenatal samples, allows researchers to overcome limitations of traditional IHC. Our preliminary findings indicate that IL-17A does not trigger the emergence of malformations as measured by TBR1 patterning and overall cortical thickness, suggesting that IL-17A alone may not be sufficient for triggering fetal neurodevelopmental abnormalities in the absence of maternal microbes.

Disclosures: I. Chalen: None. **S. Wang:** None. **A.M. Otero:** None. **R. Gonzalez-Ricon:** None. **C.A.B. Popescu:** None. **A.M. Antonson:** None.

Presentation Number: NANO15.09

Topic: A.07. Developmental Disorders

Support: Samsung Grant SSTF-BA2102-09
NRF Grant 2019M3C7A1031537
NRF Grant 2020H1D3A1A04104610
NRF Grant 2023R1A2C3003378
NRF Grant RS-2023-00217798
NRF Grant RS-2023-00265581
KBSI Grant 2021R1A6C101A390

Title: Gut microbiome changes improves social behavior in autism mouse model through epigenetic and transcriptional changes at single-cell level

Authors: *M. SIM, N. JUNG, T.-K. KIM;
Pohang Univ. of Sci. and Technol., Pohang, Korea, Republic of

Abstract: The gut microbiome dysbiosis has been suggested as a pivotal mechanism for neurodevelopmental disorders such as autism spectrum disorder (ASD). The gut microbiome changes are environmental, strongly suggesting the involvement of the epigenetic modifications in the ASD patients' brains. However, the epigenetic alterations driven by gut microbiota in ASD have not been elucidated. It has been shown that *Lactobacillus reuteri* (*L. reuteri*) and its metabolite, the tetrahydrobiopterin (BH4) improved social deficits in various ASD mouse models (Sgritta M. et al. 2019, Buffington SA. et al 2020). Here, we investigated the epigenetic and transcriptomic mechanisms involved in improved social behavior by *L. reuteri* and BH4 in Cntnap2 knockout (KO), the genetic ASD mouse model, at single-cell level. BH4 treatment (20mg/kg) for 4 weeks from P21 (n=3) rescued the sociability deficit in Cntnap2 KO. We performed single-nuclei transposase-accessible chromatin sequencing (snATAC-seq) using the hypothalamus of these mice along with PBS-treated control group (n=3, pooled) The snATAC-seq analysis revealed 11 different cell types, of which three were excitatory and two were inhibitory neurons. The differential peak analysis exhibited 6240 to 14000 open regions and 41300 to 62000 closed regions in neurons of BH4-treated compared to PBS-treated KO mice. The differential motif usage analysis discovered BH4-induced chromatin opening for motifs of FOX family in excitatory neurons and POU family in inhibitory neurons, suggesting a novel role of the FOX and POU family in microbiota-driven behavior changes. In addition, we treated PBS or *L. reuteri* (10^8CFU/100ul/mouse) to WT and Cntnap2KO mice for 4 weeks after weaning and observed that *L. reuteri* treatment rescued the social deficit in social novelty and reciprocal social interaction (RSI) of KO mice (n=26-30 per group). To identify the epigenetic mechanisms and its molecular consequences in social deficit rescue, we performed snRNA and snATAC-seq on the hypothalamus of the three groups (n=2-3 pooled per replicate, 2 replicates for each group). The snRNA-seq analysis identified 36 clusters, of which 13 were inhibitory, and 12 were excitatory neurons. The differential gene expression analysis to identify target genes regulated by epigenetic changes in the hypothalamus of *L. reuteri*-treated KO mice and snATAC-seq analysis

are on going. In summary, our research suggests the association between the epigenetic alterations in the brain and the improvement of social behavior in the ASD mice.

Disclosures: M. Sim: None. **N. Jung:** None. **T. Kim:** None.

Nanosymposium

NANO16: Therapeutic Strategies in Parkinson's Disease

Location: MCP Room N228

Time: Sunday, October 6, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO16.01

Topic: C.03. Parkinson's Disease

Support: R01 HD110389-01

Title: Cortical correlates of gait automaticity in Parkinson's disease: impact of medication

Authors: *P. BURGOS[1], F. B. HORAK[1], C. BATISTA[2], P. CARLSON-KUHTA[3], A. RAGOTHAMAN[2], V. V. SHAH[1], D. ENGEL[3], M. MANCINI[1];
[1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Neurol., Oregon Hlth. and Sci. Univ., Portland, OR; [3]Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Cortical correlates of gait automaticity in Parkinson's disease: impact of medication Pablo Burgos, Fay B. Horak, Carla Silva-Batista, Patricia Carlson-Kuhta, Anjani Ragothaman, Vrutangkumar Shah, David Engel, Martina Mancini **Background:** Walking automaticity is critical for independent mobility in daily life. It has been proposed that walking impairment in people with Parkinson's disease (PD) is due to a shift in locomotor control from healthy automaticity to compensatory executive control (i.e., increased activity in the prefrontal cortex). While Levodopa has been shown to improve some features of gait control, its role in walking automaticity remains unclear. The objective is to investigate the effects of Levodopa on cortical correlates of gait automaticity. **Methods:** Nine individuals with idiopathic PD (age=71±6 years, disease duration=9±4 years, MDS-UPDRS-III=34±12 score) were assessed in the Off and On medication states, and 10 Healthy controls (HC, age=70±6) were assessed. They completed the tasks of 1) Walking 2 minutes, 2) Walking 2 min + Motor Dual Task (Normal Arm motion), 3) Walking 2 minutes + Motor Dual Task (Incongruent Arm motion), 4) Walking 2 min + Cognitive Dual Task (CPT). The cortical activity was assessed with a portable fNIRS system (Artinis Medical Systems). Specifically, a 36-channel (26+10) arrangement with 6 short channels, consisting of 18 transmitters and 10 detectors/receivers, will cover prefrontal, motor, supplementary-motor (SMA), parietal, and occipital cortices bilaterally.
Results: We found significant differences between HC and PD in On state in the occipital activity (levels of oxygenated hemoglobin) during Walking DT (task 2, p=0.008, T-test). Where there was a lower activation for PD group. We also found differences between On and Off states of participants with PD in the SMA activity during the same task (task 2, p=0.03, T-test paired). The SMA activity was higher in the On levodopa state compared with the Off state and HC values.

Conclusions: Our preliminary results suggest that Levodopa can modulate cortical activity when walking in people with PD. Significant changes were observed in the SMA and occipital activity. As we are actively collecting data, the next step will be to correlate cortical changes with changes in gait performance from Off to On state in PD.

Disclosures: P. Burgos: None. **F.B. Horak:** None. **C. Batista:** None. **P. Carlson-Kuhta:** None. **A. Ragothaman:** None. **V.V. Shah:** None. **D. Engel:** None. **M. Mancini:** None.

Presentation Number: NANO16.02

Topic: C.03. Parkinson's Disease

Title: Spinal cord stimulation reduces low frequency oscillations in the subthalamic nucleus of a Parkinson's disease patient with gait abnormalities

Authors: J. SLACK, ***A. P. YADAV**;
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Abstract: Spinal cord stimulation is a promising method for treating gait disorders associated with Parkinson's Disease (PD). However, the neurophysiological effects of SCS on supraspinal brain regions in not fully understood. Results in PD animal models show that SCS reduces low-frequency beta oscillations in the motor cortex and striatum. Here, we investigated whether SCS reduces beta oscillations in the subthalamic nucleus (STN) of a patient with PD undergoing SCS. We recorded local field potentials (LFPs) from bilateral STN electrodes in a PD patient with freezing of gait (FOG) symptoms before and 3 days after implantation of SCS percutaneous leads. LFP recordings were sampled at 250Hz and acquired by the Medtronic Percept PC system while the patient performed six straight-line walks (SLWs) with and without SCS. Three SCS programs were tested from low to high frequency and at a control condition pre-programmed by the manufacturer. Motion capture (MoCap) using IMUs was performed to align LFP signals during walking intervals. Data were exported in JSON file format and imported into MATLAB for analysis. All recordings were preprocessed and bandpassed between 1-70Hz. For each treatment condition, Welch's power spectral density (PSD) estimate of LFP was performed for individual SLWs with 50% overlap. Band power of each SLW was calculated by integrating the PSDs across the beta band (13-35Hz) and broad band (2-60Hz). For comparison across treatment conditions, the ratio of the beta band and broad band power was measured. Statistical analysis was performed in GraphPad Prism using one-way ANOVA with multiple comparisons on SLW-averaged beta power for each STN contact. We found that in addition to gait improvement, STN beta power of both hemispheres was significantly reduced during SCS for all programs compared to pre-surgery (left STN: 0.4068 ± 0.0751; right STN: 0.7431 ± 0.0278) with the program providing the most decrease (left STN: 0.2258 ± 0.0174, $p<0.001$; right STN: 0.5418 ± 0.0454, $p<0.001$) of 44.49% and 27.09% in the left and right STN respectively. Reduction in beta power was also observed post-surgery in the OFF SCS condition (left STN: 0.2572 ± 0.05, $p<0.001$, 36.77%; right STN: 0.6058 ± 0.0676, $p<0.001$, 18.48%) suggesting a chronic effect of SCS. These results demonstrate that SCS-induced gait improvements can be explained by the significant reduction in STN beta oscillations following SCS implantation. Thus, STN beta oscillations could serve as a useful biomarker to evaluate the therapeutic effect of SCS on PD symptoms.

Disclosures: J. Slack: None. **A.P. Yadav:** None.

Presentation Number: NANO16.03

Topic: C.03. Parkinson's Disease

Title: Application of a Smartwatch for the Distinction of Deep Brain Stimulation Efficacy with Machine Learning Classification

Authors: *R. LEMOYNE[1], T. J. MASTROIANNI[2];
[1]Independent, Running Springs, CA; [2]Cognition Engin., Pittsburgh, PA

Abstract: The smartwatch offers a convenient means for evaluating the efficacy of deep brain stimulation for the treatment of Parkinson's disease. Equipped with an inertial sensor system the smartwatch can provide highly insightful quantified data with respect to the unique response of a subject with Parkinson's disease to deep brain stimulation. Additionally, the wireless capabilities of the smartwatch enable the recorded inertial sensor signal data to be transmitted to the Internet for post-processing anywhere in the world, such as for consolidation of the inertial sensor signal data for machine learning classification. The efficacy of deep brain stimulation is ascertained according to disparate scenarios of deep brain stimulation through machine learning classification according to the inertial sensor signal derived from a smartwatch. These findings evolve the impact of the smartwatch as a wearable and wireless system in conjunction with machine learning for the determination of deep brain stimulation to treat movement disorders, such as Parkinson's disease.

Disclosures: R. LeMoyne: None. **T.J. Mastroianni:** None.

Presentation Number: NANO16.04

Topic: C.03. Parkinson's Disease

Title: Nanoscale Magneto-mechanical-genetics of Deep Brain Neurons Reserving Motor Deficits in Parkinsonian Mice

Authors: *W. SHIN;
Yonsei university, Seoul, Korea, Republic of

Abstract: Here, we introduce the magneto-mechanical-genetic (MMG)-driven wireless deep brain stimulation (DBS) using magnetic nanostructures for therapeutic benefits in the mouse model of Parkinson's disease (PD). Electrical DBS of the subthalamic nucleus (STN) is an effective therapy for mitigating Parkinson's motor symptoms. However, its broader application is hampered by the requirement for implanted electrodes and the lack of anatomical and cellular specificity. Using the nanoscale magnetic force actuators (m-Torquer), which deliver torque force under rotating magnetic fields to activate pre-encoded Piezo1 ion channels on target neurons, our system enables wireless and STN-specific DBS without implants, addressing key unmet challenges in the DBS field. In both late- and early-stage PD mice, MMG-DBS significantly improved locomotor activity and motor balance by 2-fold compared to untreated PD mice. Moreover, MMG-DBS enabled sustained therapeutic effects. This approach provides a

non-invasive and implant-free DBS with cellular targeting capability for the effective treatment of Parkinsonian symptoms.

Disclosures: W. Shin: None.

Presentation Number: NANO16.05

Topic: C.03. Parkinson's Disease

Support: NIH R21NS129147
DoD HT94252310406

Title: The impact of exercise on electrophysiological activity of the STN in Parkinson's disease

Authors: M. MILLER KOOP[1], **A. ROSENFELDT**[5], A. BAZYK[5], V. BERKI[1], S. DAVIDSON[2], S. J. NAGEL[6], J. Y.-C. LIAO[3], B. L. WALTER[7], *J. ALBERTS[4,1]; [1]Biomed. Engin., Cleveland Clin., Cleveland, OH; [2]Cleveland Clin., Brook Park, OH; [3]Neurol., Cleveland Clin., Cleveland, OH; [4]Cleveland Clin., Chagrin Falls, OH; [5]Cleveland Clin. Fndn., Cleveland, OH; [6]The Cleveland Clin., Cleveland, OH, ; [7]Cleveland Clin. Found, Cleveland, OH.

Abstract: Despite evidence of the benefits of exercise as an adjunct to treating PD, there is an absence of data regarding how, or if, exercise may benefit more advanced people with PD (PwPD) with deep brain stimulation (DBS). The overall aim of this project is to determine the effects of high-intensity exercise at traditional exercise durations (40 mins) on pathological neural activity in the subthalamic nucleus (STN). To achieve this aim, we have leveraged the sensing technology in Medtronic's Percept device and our effective forced-exercise (FE) cycling paradigm in advanced PwPD with DBS.Fifteen PwPD with DBS, off-stimulation and off anti-PD medication, will complete two 40 mins cycling sessions on a recumbent bike: 1) voluntary exercise (VE-cadence 60rpms or less) and one forced exercise (FE- cadence of 80-90 rpms performed on a motorized recumbent bike augments the self-selected pace if needed). Bilateral STN local field potentials (LFP) data pre-(at rest), post-(at rest)- and during cycling will be recorded. Power in traditional spectral frequency bands (alpha, beta, and gamma) will be evaluated to determine significant changes in neural activity during and post-exercise sessions relative to baseline (pre-exercise) values and to determine if the mode of exercise (FE versus VE) demonstrates different effects on neural activity. Preliminary data during a FE session from a representative PwPD with DBS demonstrates that high-intensity exercise at traditional durations (40 mins) is feasible in advanced people PwPD with DBS. In addition, STN-LFP recordings during the 40 mins FE cycling revealedpathological neural activity (beta band power), was reduced (average - 42%) compared to baseline values and remained suppressed throughout the 40-mins exercise session. After cessation of exercise, the resting state beta power immediately rebounded to baseline values. At one minute post-exercise, beta band power decreased below baseline values and remained decrease for the remainer of the 5-minute post-exercise recording.Preliminary data suggest that high-intensity FE cycling at traditional durations (40-mins) is feasible in more advanced people with PwPD with DBS and suggest that exercise has a similar effect as DBS as both attenuate resting state beta band power. Further work will determine how these neural changes correlate with functional improvements and the impact of the mode of exercise (VE versus FE). Data from this study will provide unprecedented insight

into the neural mechanism(s) underlying the positive effects of exercise and offer much needed clinical exercise guidance for advanced PD patients.

Disclosures: M. Miller Koop: None. **A. Rosenfeldt:** None. **A. Bazyk:** None. **V. Berki:** None. **S. Davidson:** None. **J. Alberts:** Other; JLA has authored intellectual property associated with the forced exercise bike..

Presentation Number: NANO16.06

Topic: C.03. Parkinson's Disease

Title: Meta Analysis shows exercise improvement in motor scores, but not cognitive scores in persons with Parkinson's Disease

Authors: *S. O. AHMAD[1], J. K. LONGHURST[2], D. L. STILES[2], L. DILLON[2], E. SHROBA[2]; [1]Occup. Sci. and Occup. Therapy, St. Louis Univ., Saint Louis, MO; [2]St. Louis Univ., Saint Louis, MO

Abstract: Background: Parkinson's disease (PD) is a debilitating neurodegenerative disorder affecting millions of people worldwide. PD results in motor and cognitive dysfunction. While there is no proven cure for PD, it is widely agreed upon that aerobic exercises and occupations can help slow the progression of the disease and keep some motor-related symptoms at bay (Ahmad et.al.,2023. The most effective forms of exercise to slow the progression of motor symptoms in Parkinson's disease have also been studied. (Ahmad, et.al., 2023).**Research Question:** This research article aims to compare the differences in outcomes of exercise on Motor versus Cognitive outcomes in Parkinson's Disease, as evaluated by Meta-analysis. **Methods:** Key terms were Parkinson's Disease and exercise terms. These search terms were then entered to electronic databases: Ovid MEDLINE, SCOPUS, and CINAHL in from March 2018 to May 2023. An ancestral bibliography was also performed. **Results:** Two reviewers screened the title and abstract records (n=528) found in the initial search. The review identified 54 studies which met inclusion criteria for meta-analysis. The first meta-analysis, which included all 54 studies, found a positive overall effect of either motor exercise or cognitive exercise on patients with PD, where the overall d-index was 0.29. This was statistically significant with a CI95% of $0.23 < \mu < 0.35$, $p < .05$, as using this approach, a positive effect was indicated, as the CI lies to the right of, and doesn't include zero. Intervals that include zero are non-conclusive and non-significant. The homogeneity analysis was significant, $Q (53) = 230.18$, $p < .05$, revealing that there was more variability in the d-indexes than would be expected due to sampling error alone. The second meta-analysis, which only focused on motor exercises and included 45 of the original 54 studies, also found a positive overall effect on patients with PD. The overall d-index was 0.38, and was statistically significant with a CI95% of $0.32 < \mu < 0.45$, $p < .05$. The homogeneity analysis was also significant, $Q (44) = 206.35$, $p < .05$. The third meta-analysis, which focused only on cognitive exercise and included 19 of the original 54 studies, found a negative overall effect on patients with PD ($d = -0.02$), and was not significant, CI95% of $-0.12 < \mu < 0.08$, $p > .05$, as the CI includes zero. Additionally, the homogeneity analysis was not significant, $Q (18) = 2.85$, $p > .05$.

Disclosures: S.O. Ahmad: None. **J.K. Longhurst:** None. **D.L. Stiles:** None. **L. Dillon:** None. **E. Shroba:** None.

Presentation Number: NANO16.07

Topic: C.03. Parkinson’s Disease

Support: BrainSafe

Title: Parkinson's Disease motor rehabilitation with Brain Computer Interface Treatment

Authors: M. SEBASTIAN-ROMAGOSA[1], W. CHO[2], R. ORTNER[2], K. MAYR[2], ***C. GUGER**[2];
[1]g.tec Med. Engin. Spain SL, Barcelona, Spain; [2]g.tec Med. Engin. GmbH, Schiedlberg, Austria

Abstract: Background
Parkinson's disease (PD) is a degenerative neurological disorder that mainly affects people over the age of 60. It starts slowly and mainly affects motor function, causing symptoms such as tremor, rigidity and problems with movement, balance and coordination. While there's no cure for PD, its symptoms can be managed with medication or surgery. In addition, physiotherapy and exercise, such as dancing, may be beneficial, although it's uncertain whether certain types of exercise are more effective than others.
Today, neurofeedback technology, also known as brain-computer interfaces (BCIs), can provide an objective tool for measuring motor imagery (MI), creating new opportunities for "closed-loop" feedback. Several review articles have analyzed MI interventions with BCI in various neurological disorders, but have never been applied to people with PD.
This clinical trial will investigate the safety and efficacy of BCI-based therapy for motor rehabilitation in PD patients.
Methods
Four patients with PD were enrolled in this study. All completed 24 BCI sessions. The BCI-MI based system used for this study is called recoveriX (developed by g.tec medical engineering GmbH). This system combines in real-time MI, Virtual Reality avatar and Functional Electrical Stimulation (FES) in order to create a closed loop of motor learning. During the BCI treatment patients were asked to perform MI tasks of the ankle and wrist dorsiflexion. Assessments were performed to evaluate changes before and after therapy. The functional scales used were Movement Disorders Society - Unified Parkinson's Disease Rating Scale section III (MDS-UPDRS-III), 10 Meter Walking Test (10MWT), Parkinson’s Disease Questionnaire 39 (PDQ39), and Modified Fatigue Impact Scale (MFIS).
Results.
The results show that patients were able to increase their motor function by -3.5 points [-6.25 to -1.75] on the MDS-UPDRS-III. This functional improvement was followed by an increase in coordination and gait ability in the 10MWT of -0.08 s [-0.18 to -0.07]. Patients also reported a reduction in fatigue as assessed by the MFIS, -2.5 points [-6.25 to 6.25] and better performance in daily activities assessed by the PDQ39, -4.5 points [-14 to 2].
Conclusions
This is the first time that BCI technology has been used to treat PD. These preliminary results demonstrate the safety and efficacy of the treatment in a very small sample of patients. PD patients improved motor functions, gait ability, reduced the fatigue and increase the global functionality. However, these results are from four PD patients, so the authors believe that this approach should be further validated in larger studies involving more patients.

Disclosures: M. Sebastian-Romagosa: A. Employment/Salary (full or part-time):; g.tec medical engineering Spain SL. **W. Cho:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **R. Ortner:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **K. Mayr:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **C. Guger:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH, g.tec medical engineering Spain SL.

Nanosymposium

NANO17: Glial and Immune Cell Mechanisms in Stroke and Injury

Location: MCP Room S106

Time: Sunday, October 6, 2024, 1:00 PM - 4:00 PM

Presentation Number: NANO17.01

Topic: C.08. Ischemia

Support: NIH RO1 NS110755-01A1 (G.B)
Univ. of Pittsburgh Department of Neurology Start-up funding (GB)

Title: Targeted deletion of Na^+/H^+ exchanger 1 (NHE1) in astrocytes improves cerebral vascular integrity and regulation in stroke brain

Authors: *G. BEGUM[1], O. CAPUK[1], M. AVUNOORI[1], S. METWALLY[1], E. BERTHOLD[1], R. MUDUGANTI[1], V. FIESLER[1], C. SNEIDERMAN[2], L. M. FOLEY[3], T. K. HITCHENS[3], G. KOHANBASH[2,5], D. SUN[1,6], S. CHAPARALA[4];
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Abstract: Dysfunction of perivascular astrocytes following ischemic stroke contributes to impaired neurovascular coupling, the blood brain barrier integrity, and reduced cerebral blood flow (CBF). The underlying mechanisms remain poorly understood. In this study, we investigated protective effects of astrocyte function in stroke brains by selective deletion of astrocytic Na^+/H^+ exchanger isoform 1 (NHE1) using the inducible *Gfap-Cre*$^{ERT2+/-}$*;Nhe1*$^{f/f}$ (*Nhe1*$^{i\Delta Astro}$) mice. Wild-type (WT) stroke mice after transient middle cerebral artery occlusion (tMCAO) displayed perivascular gliosis and loss of pericyte coverage in micro vessels, which was correlated with impaired cerebrovascular regulation with failed increase in CBF in response to vasoactive compound acetazolamide (ACZ) in arterial spin labeling (ASL) MRI. In contrast, *Nhe1*$^{i\Delta Astro}$ mice exhibited significantly reduced perivascular gliosis and preserved vessel pericyte coverage. ASL MRI measurements showed preserved cerebrovascular regulation with increased CBF in *Nhe1*$^{i\Delta Astro}$ ischemic peri-lesion areas in response to ACZ. Single cell RNA sequencing (ScRNAseq) analysis of cerebral vessels from WT and *Nhe1*$^{i\Delta Astro}$ stroke brains revealed selective upregulation of genes and pathways involved in vascular repair in endothelial (EC) and mural cells, especially ephrinB signaling pathway genes, in the EC cluster of *Nhe1*$^{i\Delta Astro}$ stroke brains. The EphB4 receptor was the most significantly upregulated gene in EC

cluster of *Nhe1*$^{i\Delta Astro}$ stroke brains. Immunofluorescence analysis showed WT stroke brains showed decreased ephrinB2 ligand expression in the peri-EC astrocytic endfeet. In contrast, strong ephrinB2^{+} signals were detected at the endfeet-EC contact points of *Nhe1*$^{i\Delta Astro}$ stroke brains. Taken together, our data suggest that perivascular astrocytic NHE1 plays a role in stroke-induced vascular damage. Inhibiting astrocytic NHE1 promotes increased expression of ephrinB factors and EphB4 receptors, thereby facilitating vascular repair and remodeling.

Disclosures: G. Begum: None. **O. Capuk:** None. **M. Avunoori:** None. **S. Metwally:** None. **E. Berthold:** None. **R. Muduganti:** None. **V. Fiesler:** None. **C. Sneiderman:** None. **L.M. Foley:** None. **T.K. Hitchens:** None. **G. Kohanbash:** None. **D. Sun:** None. **S. Chaparala:** None.

Presentation Number: NANO17.02

Topic: C.09. Stroke

Support: Deutsche Forschungsgemeinschaft DFG, FOR 2795 “Synapes Under Stress”, PE1193/6-2

Title: Neuronal and astroglial sodium-calcium exchanger-1 contributes to calcium and sodium changes in neurone and astrocytes during spreading depolarisations after stroke

Authors: *S. HAMZEITAJ[1], C. ROSE[2], G. C. PETZOLD[3,4];
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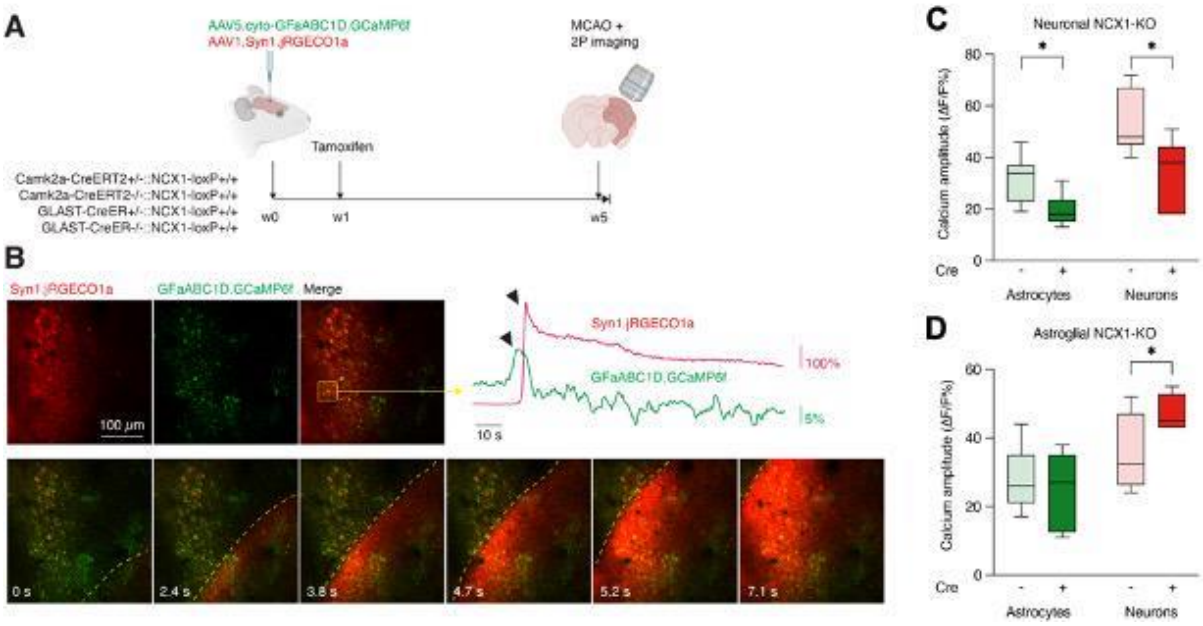
Abstract: Acute ischemic stroke leads to an imbalance of ionic and neurotransmitter gradients in neuron and glial cells, which triggers the occurrence of periinfarct depolarizations (PIDs) in the penumbra that negatively affect infarct size and clinical outcome. The cellular pathways that differentially govern intracellular changes in calcium and sodium in neurons and astrocytes during PIDs remain incompletely understood, as are the downstream consequences for extracellular glutamate release. Here, we investigated the role of plasmamembrane sodium-calcium exchanger-1 (NCX1) in these changes in a mouse model of acute stroke. To this end, we generated conditional knockout mice deficient for NCX1 either in excitatory neurons or astrocytes. Calcium, sodium and glutamate changes during post-strokePIDs were measured using astrocyte-expressed GCaMP6f, neuron-expressed jRGECO1a, astrocyte-expressed SF-iGluSFnR and the sodium indicator ING2, respectively, using in vivo multiphoton microscopy. We found that neuronal deletion of NCX1 resulted in significantly attenuated intracellular calcium changes in astrocytes and neurons, whereas intracellular sodium changes were amplified. This led to attenuated extracellular glutamate transients and a higher threshold for PIDs. In contrast, astroglial deletion of NCX1 surprisingly resulted in amplified intracellular changes of calcium and sodium in both astrocytes and neurons, and in stronger extracellular glutamate transients. Moreover, preliminary in vivo MRI data suggest that stroke volumes at 72 h post-stroke were lower in neuronal NCX1 knockout mice, but unchanged in astroglial NCX1 knockout mice compared to controls. Taken together, our data suggest that neuronal NCX1 functions in the reverse mode during PIDs, resulting in intracellular calcium accumulation in exchange for extracellular sodium transport, ultimately leading to glutamate release and PID promotion.



Disclosures: S. Hamzeitaj: None. **C. Rose:** None. **G.C. Petzold:** None.

Presentation Number: NANO17.03

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

Support: NIH R01NS130916

Title: Investigating the Reversal of GLT1 Glutamate Transporter during Metabolic Stress

Authors: *P. THAPALIYA[1], G. ULLAH[2];
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Abstract: Excess glutamate in the synaptic cleft and extracellular space causes excitotoxicity and damage to the cells. Therefore, rapid removal of glutamate from the extracellular space is required for normal neuronal functioning and survival. Although glutamate transporters are expressed by both neurons and astrocytes, astrocytes are primarily responsible for glutamate uptake. Substantial evidence shows that during severe metabolic stress, glutamate transporters operate in reverse mode, causing the release of glutamate from astrocytes instead of uptake. However, the exact conditions leading to the reversal of glutamate transporters and how these conditions vary from one brain region to another are not well-understood. Here, we develop a mathematical model to explore the effect of various intra- and extracellular ion concentrations on the mode of operation of glutamate transporters and how these modes feedback to the homeostasis of different ions, such as Na+, K+, and pH during metabolic stress events with different intensities.

Disclosures: P. Thapaliya: None. **G. Ullah:** None.

Presentation Number: NANO17.04

Topic: C.08. Ischemia

Support: NIH R21

Title: Neuropathological hallmarks during the chronic phase of ischemic stroke in mice and humans

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Abstract: Improvements in acute stroke treatment, including EVT and critical care management, have increased survival rates post-stroke. However, many stroke survivors have significant neurological deficits, and stroke remains a leading cause of long-term disability. Despite this, the chronic progression and long-term sequelae of ischemic stroke pathology remains understudied. We examined long-term neurobehavioral recovery and progressive neuropathology of young (3months) and middle-aged (14 months) adult C57Bl/6 mice at 3 and 6 months after a 60-minute transient middle cerebral artery occlusion (MCAO) or sham surgery, as well as post-mortem brain samples from patients with a chronic infarct. In the young cohort, depression-like behavior persisted for 6 months post-stroke (PS) ($p = <0.0001$), whereas cognitive function progressively worsened from 3 to 6 months as seen by a continual reduction in the discrimination index of the NORT (6-months, $p=.0063$). Deficits in memory retention in mice 6-months PS were observed using the fear-conditioning test ($p=.0084$). Brain atrophy was evident by MicroCT and MRI imaging at 2 and 6 months PS. Furthermore, histopathology revealed significant demyelination (mouse, $p=.0361$; human, $p =.0001$), and increased microglial and astrocyte activation ($p=.0059$) in the hippocampus and frontal cortex of both human and mice chronically after stroke. Interestingly, we also observed microglia activation proximal to apoptotic neurons in the brains of stroke mice, as shown by TUNEL assay. TUNEL co-labeling with a neuronal marker also showed the same neuronal apoptosis in human samples of chronic stroke in the hippocampus. Disease-associated microglial (DAM) phenotypes were marked by both increased proliferative status and senescent-like phenotypes, including elevated production of cytokines and other proinflammatory molecules. Microglia also showed increased lipid content and an altered redox state as measured by flow cytometry. Nanostring analyses on mouse brains shows upregulation of DAM and neuroinflammatory pathways up to 6 months PS. In the human brains, we found increased levels of amyloid plaque in stroke cases compared to controls ($p=.0677$). Our findings demonstrate that ischemic stroke may accelerate inflamm-aging in the brain, which induces premature senescence within the chronic infarct microenvironment. Cellular senescence and chronic neurodegenerative signatures likely result in poor cognitive function that may progressively worsen post-stroke. These chronic events may allude to a neurodegenerative etiology in the long-term post-stroke, and may offer viable targets for delayed treatment strategies.

Disclosures: R. Khan: None. **T. Do:** None. **G. Guzman:** None. **P. Devlin:** None. **J. Ahn:** None. **H. Koochak:** None. **S.P. Marrelli:** None. **C. Tan:** None. **M.E. Maniskas:** None. **V. Venna:** None. **L.D. McCullough:** None. **R.M. Ritzel:** None.

Presentation Number: NANO17.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Innovation Fund Grant 3129-00023B

Title: Whole-brain mRNA imaging unveils the dynamics of neuroinflammation after stroke

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Abstract: The dynamic role of neuroinflammation in diverse neurological disorders is still poorly characterized but could represent a promising avenue for therapeutic interventions. Microglia are known to exhibit morphological as well as gene expression changes in response to diverse stimuli depending on tissue context. However, how their activation signature propagates across the brain in response to stroke has remained unknown. Here, we aimed at characterizing brain-wide microglial responses to ischemic stroke in the mouse. We established a whole-brain three-dimensional (3D) light sheet fluorescence in situ hybridization (ISH) and immunohistochemistry (IHC) imaging method to visualize, map and quantify changes in microglial gene expression markers. Both female and male mice were subjected to permanent middle cerebral artery occlusion (pMCAO), and their brains were labelled for microglial (i.e. Iba1, Hexb) and vascular markers (CD31, SM22a) at mRNA as well as protein levels. Following tissue clearing, intact brains were visualized using light-sheet fluorescent microscopy. We show local accumulation of morphologically altered microglia and rearrangement of cerebral vasculature at the lesion site 7 days after occlusion. Stroke led to propagation of the inflammatory signal to distant brain regions, indicating broad effects that are not necessarily confined to the lesioned area. This study introduces a novel whole-brain 3D imaging platform to track the status of microglia during disease progression which could be widely utilized in preclinical pharmacological research of neuroinflammation.

Disclosures: L. Lydolph Larsen: A. Employment/Salary (full or part-time):; Gubra. **T. Topilko:** A. Employment/Salary (full or part-time):; Gubra. **A. Parka:** A. Employment/Salary (full or part-time):; Gubra. **U. Roostalu:** A. Employment/Salary (full or part-time):; Gubra. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra. **K.L. Lambertsen:** None.

Presentation Number: NANO17.06

Topic: C.09. Stroke

Support: NIH Grant R01NS104117
NIH Grant R01109221

Title: Combination treatment by lipid mediators provides long-lasting neuroprotection by targeting pro-homeostatic microglial and astrocyte genes after experimental stroke

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Abstract: Neuroprotection to attenuate or block the ischemic cascade and salvage neuronal damage has been extensively explored for treating ischemic stroke. This study focuses on the neuroprotective bioactivity of docosanoid mediators Neuroprotectin D1 (NPD1) and Resolvin D1 (RvD1) and their combination in experimental stroke. These lipid mediators are biosynthesized "on-demand" in response to stroke onset to restore homeostasis and functional integrity to resolve neuroinflammation. Recently, we have shown that NPD1+RvD1 improved behavior when they were allowed to survive for 7 days after a stroke. This study established whether neuroprotection induced by NPD1+RvD1 persists with chronic survival. In addition, we investigated whether the administration of NPD1+RvD1 affects the expression of microglia and astrocyte-specific genes. Male Sprague-Dawley rats (270-360g) were anesthetized with isoflurane/nitrous oxide and received 2h of middle cerebral artery occlusion (MCAo) by intraluminal suture. Neurological status was evaluated at 60 min, and on days 1, 2, 3, 7, 14, 21, and 28, a grading scale of 0-12 was employed. Rats were treated with NPD1 (222 µg/kg) and RvD1 (222 µg/kg) or vehicle (n=6-8 / group). Treatments were administered IV (NPD1 at 3h and RvD1 at 3h 15 min) and vehicle (saline at 3h) after the onset of MCAo. Thirty days after MCAo, rats were perfused with 4% paraformaldehyde, and an *ex vivo* brain MRI was conducted using 11.7 T MRI. Molecular targets of NPD1 and RvD1 are defined at 24 h after MCAo. Physiological variables were stable and showed no significant differences between groups. Combinatory treatment improved behavioral scores on days 1, 2, 3, 7, 14, 21, and 28 by 32, 35, 39, 40, 46, 48, and 50% compared to the vehicle group. NPD1+RvD1 significantly reduced lesions (computed from T2WI) in the cortex (0.4% vs. 5.1%), subcortex (0.2% vs. 1.5%), and total lesion (0.6% vs. 6.5%) compared to the vehicle group, respectively. Transcriptomic analysis revealed differentially regulated genes by NPD1+RvD1 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*). Our results indicate that NPD1+RvD1 exerts potent, long-term neuroprotective effects in the model of focal cerebral ischemia in rats. Uncovered genes are likely to enhance homeostatic microglia, modulate neuroinflammation, activate NPC differentiation, maturation, and synapse integrity, and contribute to cell survival. Our findings strengthen the notion that combinatory treatment might be of clinical usefulness for treating acute stroke.

Disclosures: L. Belayev: None. **A. Obenaus:** None. **M.M. Reid:** None. **L. Khoutorova:** None. **J. Ji:** None. **N.G. Bazan:** None.

Presentation Number: NANO17.07

Topic: C.10. Brain Injury and Trauma

Support: United States Department of Defense USAMRAA award W81XWH2010665 through the Peer Reviewed Alzheimer's Research Program
NIH National Center for Advancing Translational Sciences U18TR004146 and ASPIRE Challenge and Reduction-to-Practice awards
National Institutes of Health (NIH) award, R01MH128866 by National Institute of Mental Health

Title: Impact Induces Phagocytic Defect in Reactive Microglia

Authors: *R. YU[1], E. A. ROGERS[2,5], T. B. BEAUCLAIR[3], P. MANCHANDA[1], C. BEVERIDGE[3], C. RANDOLPH[3], R. SHI[4], G. CHOPRA[1];
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Abstract: Traumatic brain injury (TBI) is a leading cause of mortality and morbidity around the world. TBI significantly increases the lifetime risk of neurodegenerative diseases, such as Alzheimer's Disease (AD). TBI patients' brains have shown increased load of amyloid β (Aβ) plaques, a hallmark for AD onset. Our hypothesis is that microglial cells lose their ability to engulf Aβ leading to TBI related AD. While the association of TBI and AD is increasingly strong in both clinical and preclinical investigations, there is a lack TBI models to relate cellular response as a platform for drug screening. We have developed an *in vitro* impact model for primary cells using to represent impact injury on a chip (*Sci. Rep*. 12, 11838, 2022). This "TBI-on-a-chip" model was used to characterize the molecular and cellular changes following impact. **We discovered that impact reduced the phagocytosis of Aβ in murine microglia, along with increased inflammation at 7-days post impact (DPI).** Impacted microglia adopted amoeboid morphology and secreted increased amounts of nitric oxide (NO), suggesting an inflammatory response. To quantify phagocytosis, we used the pH-sensitive human amyloid-beta 1-42 ($A\beta^{pH}$) developed in our lab that only fluoresces when it is taken up by the cells in the phagolysosomes (*Chem Sci.* 12, 10901-10918, 2021). Using both flow cytometry and immunofluorescence imaging, we found that primary microglia reduced $A\beta^{pH}$ uptake at 7 DPI, which was surprising because inflammation is usually correlated with increased microglial phagocytosis. Given that lipid metabolism is increasingly implicated in brain trauma and neurodegeneration, we used Multiple-Reaction Monitoring profiling, a mass spectrometry technique, to evaluate the changes in lipids secreted by microglia and histiotypical networks at 7 DPI. We found many lipid species from the sphingomyelin, glycerophospholipid, and phosphatidylserine classes were significantly affected by impact, which are known to play important roles in the resolution of neuroinflammation and the pathogenesis of neurodegeneration. The "TBI-on-a-chip" model can also do neuronal recording and we believe it will be a useful drug screening tool to modulate impacted microglial response and to help develop novel immunomodulatory drug candidates.

Disclosures: R. Yu: None. **E.A. Rogers:** None. **T.B. Beauclair:** None. **P. Manchanda:** None. **C. Beveridge:** None. **C. Randolph:** None. **R. Shi:** None. **G. Chopra:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Agilent Instrument In-Kind.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meditati Inc., BrainGnosis Inc..

Presentation Number: NANO17.08

Topic: C.08. Ischemia

Support: NIH/NINDS NS076620
NIH/NCATS KL2 TR002317

Title: Role of type I interferon signaling in ischemic preconditioning-induced microglial responses

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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. Elucidating the mechanisms of IPC is a critical challenge in identifying stroke therapeutics. 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 I IFN receptor (IFNAR1) in microglia is required for both IPC-induced axonal protection and interferon stimulated gene (ISG) expression. Prior work also showed that exposure of cultured primary microglia to either IFN-beta or ischemia/reperfusion results in phosphorylation of signal transducer and activator of transcription 1 (STAT1), a key signaling kinase downstream of IFNAR1. Therefore, we hypothesized that type I interferon signaling is critical for microglial responses to ischemia and STAT1 may be a key mediator of this signaling pathway. Here we report the impact of systemic STAT1 knock-out on microglial responses after IPC. 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 changes in microglial sub-clusters and gene expression changes due to IPC. We describe global transcriptomic changes in microglial subpopulations in response to IPC and how STAT1-deficiency alters both the distribution of microglial subpopulations and the microglia cluster-specific transcriptional profiles in IPC. We provide additional data demonstrating how ischemia/reperfusion and innate immune signaling affect STAT1 phosphorylation signaling dynamics in microglia in vitro. We show that phosphorylation is dependent on both TLR4 and IFNAR1. Additionally, we present data suggesting that STAT1 phosphorylation is triggered by activation of TLR4 on a delayed time course compared to stimulation with either IFN-beta or ischemia. Our in vitro data suggest that TLR4 activation may concurrently lead to type I IFN signaling in microglia via either autocrine/paracrine mechanisms through the release of type I IFNs or via an alternative ligand-independent pathway directly activating IFNAR1. The findings 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 in IPC. Overall, the results support a central role for STAT1 as a key mediator of microglial type I IFN signaling.

Disclosures: A. McDonough: None. **C. Lee:** None. **R.A. Arnold:** None. **B. Schweitzer:** None. **J.R. Weinstein:** None.

Presentation Number: NANO17.09

Topic: C.10. Brain Injury and Trauma

Support: National Natural Science Foundation of China (no. 81925031 and 81820108026)
Guangzhou Science and Technology Program Key Projects (202007030001)

National Natural Science Foundation of China (no. 82103775)
National Natural Science Foundation of China (no. 81972967)

Title: Microglia drive transient insult-induced brain injury by chemotactic recruitment of CD8+ T lymphocytes.

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Abstract: The crosstalk between the nervous and immune systems has gained increasing attention for its emerging role in neurological diseases. Radiation-induced brain injury (RIBI) remains the most common medical complication of cranial radiotherapy, and its pathological mechanisms have yet to be elucidated. Here, using singlecell RNA and T cell receptor sequencing, we found infiltration and clonal expansion of CD8+ T lymphocytes in the lesioned brain tissues of RIBI patients. Furthermore, by strategies of genetic or pharmacologic interruption, we identified a chemotactic action of microglia derived CCL2/CCL8 chemokines in mediating the infiltration of CCR2+/CCR5+ CD8+ T cells and tissue damage in RIBI mice. Such a chemotactic axis also participated in the progression of cerebral infarction in the mouse model of ischemic injury. Our findings therefore highlight the critical role of microglia in mediating the dysregulation of adaptive immune responses and reveal a potential therapeutic strategy for non-infectious brain diseases.

Disclosures: J. Chen: None. **Y. Tang:** None.

Presentation Number: NANO17.10

Topic: C.08. Ischemia

Support: NIH/NINDS RF1 NS088555

Title: Sex- and age-based differences in skull bone marrow and dura immune cell populations at baseline and after ischemic stroke

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Abstract: Background Stroke is a leading cause of death and disability, and elderly females have both the highest rate of stroke and the worst stroke outcomes. Recent studies show that the meninges play a key role in brain immunity, and immune cell populations in this location may shift to pro-inflammatory states with age. Acutely after stroke, there is an upregulation of hematopoiesis in the skull bone marrow (BM) adjacent to the infarct. However, no studies to date have examined immune activity in the skull and dura at subacute and chronic time points after stroke - periods that may contribute to long term cognitive decline, and how cell populations change with sex and age. Therefore, the objective of this study is to characterize immune cell populations in the skull BM and dura in aging and after stroke. Methods Adult (7-15-months, n=6/group) male and female C57BL/6 mice were used to establish methods, while a

preliminary cohort of aged (>18 months, n=4/group) male and female C57BL/6 mice underwent a 30-minute transient middle cerebral artery occlusion (tMCAo) or sham surgery. Mice were sacrificed and femur BM, skull BM, and dura were collected. Samples were processed, stained for 8 general leukocyte markers (CD45, CD19, TcRβ, CD4, CD8, CD11b, NK1.1, Ly6G), and analyzed with flow cytometry. Data were gated in FlowJo and analyzed with 2-way and 3-way ANOVA with multiple comparisons (α=0.05) in GraphPad Prism. Results In the dura of the adult uninjured cohort, there was a significantly higher proportion of B cells ($p<0.05$) and lower proportion of innate immune cells ($p<0.01$) in females compared to males. In this uninjured cohort, males had a significantly lower proportion of B cells in the femur BM ($p<0.01$), and a higher proportion of T cells in the skull BM ($p<0.01$) compared to females. In the dura of the aged stroke cohort, B cell representation decreased ($p<0.01$) and T cell representation increased ($p<0.001$) after stroke in females only. In males, there was a significant decrease in T cell representation ($p<0.01$) in the dura. In the skull BM, but not femur BM, there was a significant decrease in overall $CD19^+$ cell number after stroke ($p<0.05$). Further analysis with larger sample sizes is ongoing to confirm these results. Conclusions This study aimed to characterize differences in immune cell populations in the dura, skull BM, and femur BM based on age, sex, and stroke injury. Ongoing studies are further characterizing cell phenotypes in each of these locations. Determining the origin of potential pro-inflammatory cell populations after stroke will allow us to develop targeted therapies to reduce harmful inflammation without affecting beneficial cell phenotypes.

Disclosures: A. McAtee: None. **M. Byrd:** None. **J. Turchan-Cholewo:** None. **M.K. Malone:** None. **K. Cotter:** None. **E. Winford:** None. **A.M. Stowe:** None.

Presentation Number: NANO17.11

Topic: C.10. Brain Injury and Trauma

Support: R01 NS115876

Title: Lysosomal Myelin Accumulation in Mononuclear Phagocytes contributes to Inflammation and Autophagy Impairment after Traumatic Brain Injury

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Abstract: Excessive inflammation in resident microglia and infiltrating macrophages contributes to the poor prognosis of traumatic brain injury (TBI). This exacerbated pro-inflammatory response is part of an intricately intertwined network of dysregulated pathways denoted as secondary injury. The central role of mononuclear phagocytes in orchestrating this response after TBI has been investigated for decades and yet an effective medication is missing. Intriguingly, many of the perpetual proinflammatory traits of these cells are in common with their counterparts contributing to the pathogenesis of atherosclerosis and multiple sclerosis that in both cases is inextricably related to lipid metabolism. Therefore, we asked the question whether the lipid content of monocytes of the injured brain is distinct from a healthy brain. First, our

MASS-based imaging data (DESI/MSI) demonstrated a significant buildup of the storage lipids such as cholesteryl esters in the vicinity of TBI lesion suggesting upregulation of the intracellular lipid storage organelles, lipid droplets. Immunofluorescence (IF) staining for lipid droplet coating protein, perilipin-3, on coronal brain sections from mice subjected to controlled cortical impact (CCI) confirmed lipid droplet accumulation in Iba1+ cells (common monocyte marker) after TBI. Next, we used FACS to isolate microglia and macrophages from CCI and sham-injured mice brains and submitted them to LC/LC-MS lipidomic analysis. The results showed a significantly higher accumulation of storage lipids like triglycerides in the mononuclear phagocytes after injury and the effect is more pronounced in the macrophages than resident microglia. Moreover, IF staining showed colocalization of neutral lipids in the lysosomes (LAMP2) of these cells. Accordingly, we submitted lysosomal enrichment samples from both CCI and sham injured perilesional cortical tissue to LC/LC-MS lipidomic analysis and the result resembles the cell-specific lipidomic analysis. Excessive lysosomal lipid accumulation is known to impair the normal function of lysosomes and can explain our observation that monocytes coaccumulate neutral lipids and autophagosome cargo receptor SQSTM1/P62 as a sign of autophagy flux inhibition that can be a result of lysosomal dysfunction. Moreover, the abundance of ceramides and sphingomyelin suggested myelin as a prevalent target to be phagocytosed in the microenvironment of TBI lesion. The in vitro myelin exposure of bone marrow-derived macrophages confirmed the effect of myelin on lysosomal dysfunction and autophagy flux inhibition which suggests that regulation of myelin uptake can be a therapeutic approach in TBI.

Disclosures: A. Mehrabani-Tabari: None. **S. Thapa:** None. **O. Pettyjohn-Robin:** None. **C. Sarkar:** None. **S. Kachi:** None. **J. Jones:** None. **M.M. Lipinski:** None.

Presentation Number: NANO17.12

Topic: C.09. Stroke

Support: NINDS R01NS129977 to Dr. Fudong Liu

Title: Guardians of the ischemic brain: brain endothelial CD200 signalling

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Abstract: Stroke stands as a prominent contributor to health-related, global mortality and enduring disability. Neuroinflammation plays a pivotal role in exacerbating ischemic damage, characterized by microglial activation, the infiltration of peripheral immune cells into the ischemic brain, and high levels of inflammatory cytokines. The inflammatory cascade is tightly controlled by several intrinsic inhibitory signaling pathways, among which CD200-CD200R axis is critical. CD200, a cell membrane glycoprotein primarily expressed on neurons and endothelial cells, interacts with its receptor CD200R on immune cells to suppress inflammatory response upon pathogenic stimuli. We hypothesize that brain endothelial CD200 barricades the infiltration of circulating leukocytes into the ischemic brain after stroke. Brain endothelial CD200 specific knockdown (KD) mice were generated through the administration of AAV-BR1-Cre virus to CD200 flox mice. One month after the virus injection, the mice underwent a 60-minute transient middle cerebral artery occlusion (MCAO). Three days after stroke, inflammatory responses were

assessed by flow cytometry, ELISA, immunohistochemical staining, and two-photon imaging. Stroke outcomes were evaluated by brain histological changes and neurobehavioral tests. We observed that CD200 KD mice exhibited significantly more robust inflammatory responses compared to CD200 flox mice. Two-photon imaging revealed exacerbation of leukocyte adherence to endothelial wall in CD200 KD vs. control mice. Additionally, CD200 KD mice displayed significantly larger infarct volumes and worse neurological deficits. Collectively, these findings suggest that the brain endothelial CD200 signaling acts as a primary barrier against the infiltration of circulating immune cells into the ischemic brain, thereby conferring neuroprotective effects.

Disclosures: A. Misrani: None. **C. Ngwa:** None. **K. Manyam:** None. **L.D. McCullough:** None. **F. Liu:** None.

Nanosymposium

NANO18: Cross-Modal Processing in Humans

Location: MCP Room S404

Time: Sunday, October 6, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO18.01

Topic: D.08. Multisensory Integration

Support: NICHD R01HD111546-01
VCU CCTR Grant 2020

Title: Somatosensory processing in children born preterm

Authors: *V. CHU[1], O. ROLIN[2], R. PERERA[3], J. S. THOMAS[4], S. DUSING[5];
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Abstract: Children born preterm have a 40-50% risk of developing mild to moderate motor delays (Spittle & Orton, 2014; Holsti et. al., 2002, Goyen & Lui, 2009). This study aims to examine somatosensory development in these children who are at particularly high risk for motor delays. Our somatosensory test of grip force (STOG) uses specially engineered standardized crayons with different breaking strengths. The ability to use crayons of low breaking strength reflects a child's ability to modulate force based on somatosensory feedback. Our somatosensory test of reaching (STOR) measures children's accuracy in reaching towards visually guided targets vs somatosensory guided targets (Chu & Dusing, 2022). Participants born full term (n = 33) and preterm (n = 40), between 7 to 52 months (corrected age for preterm), were recruited from local communities and health systems. In STOG, force threshold (FT) refers to the breaking force of the most fragile crayon the child could use. For STOR, we quantified the reach accuracy towards stickers on their body that they cannot see (somatosensory targets) and towards stickers in their visual field (visible target). Statistical analyses were completed in SPSS using regression analysis, with age and birth status factors. The STOG measure found FT significantly decreased

with age (n=29, p=0.025) for children under 24 months but the relationship was not significant for children over 24 months (n=44, p=0.628). While there was no significant between group difference in FT based on birth status, a greater proportion of preterm children demonstrated abnormal FT (17.1%) compared to full-term children (8.8%). The STOR measure found reach accuracy for somatosensory targets increased with age ($p<0.001$) with the preterm group displaying lower reach accuracy compared to full term (p=0.024). Reach accuracy to visible targets showed a statistically significant increase with age ($p<0.001$), but no difference was observed between birth status groups (p=0.342). These preliminary results found developmental trends for both improving force control and somatosensory-guided reaching in young children. It was interesting to note that the development of visually guided reach was similar between full-term and preterm children, yet there appears to be a delay in somatosensory guided reach in children who were born preterm. Further, difficulty with force control may be more prevalent among preterm children, though further research is needed. A better understanding of early somatosensory development and relevant assessments will provide practitioners the ability to intervene earlier when children experience challenges in somatosensory processing.

Disclosures: V. Chu: None. **O. Rolin:** None. **R. Perera:** None. **J.S. Thomas:** None. **S. Dusing:** None.

Presentation Number: NANO18.02

Topic: D.08. Multisensory Integration

Support: NSERC
SSHRC

Title: Behavioural and EEG Measures of Multisensory Integration and Sensory Sensitivity in Autism

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Abstract: Background: Up to 94% of autistic individuals report differences in sensory processing. This includes differences in hyper- and hypo-sensitivities to sensory information in multiple sensory modalities, as well as the ability to integrate sensory information across modalities. While there is an abundance of evidence of these differences in sensory processing, behavioural findings are not entirely consistent. However, previous imaging studies have shown that even when little to no behavioural differences in multisensory integration are observed, differences in the neural mechanisms underlying integration are still seen. Current study: In this study, we examined whether there is an effect of autism spectrum disorder diagnosis on multisensory integration and sensory sensitivity using a speeded response task paired with electroencephalography (EEG) measures. Methods: Autistic children (*n*= 9; 11.4 years, data collection ongoing) and typically developing (TD) children (*n*= 15; 11.9 years) were presented with auditory pure tones, visual Gabor patches, or a combination of both, all embedded in audiovisual white noise and were instructed to respond as quickly as possible when they detected a stimulus. Stimuli were presented at the participants' unisensory 50% detection threshold, determined via a psychophysical staircase procedure. Results: Only a small effect of diagnosis on accuracy gain and on the magnitude of violations of Miller's race model was found. However,

preliminary analysis suggests there are neural differences in parietal and occipital regions between the two groups. Additionally, a small effect of diagnosis on behavioural measures of sensory sensitivity was found. Conclusion: Taken together, these results suggest that neural differences for multisensory integration may exist in autistic compared to TD children, even when behavioural performance is well matched.

Disclosures: M. Luszawski: None. **C. Hare:** None. **J. Shannon:** None. **Y. Li:** None. **R.A. Stevenson:** None.

Presentation Number: NANO18.03

Topic: D.08. Multisensory Integration

Support: MSCA-PF MultiMeans 101105251
ERC Starting Grant Multsens 309349
ERC Advanced Grant MakingSense 101096659

Title: Audiotactile encoding of music in the human brain

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Abstract: To make sense of our multisensory world, the brain has to integrate signals coming from a common source but segregate those from separate sources. Little is known about how the brain merges information from audition and touch, with previous research using experiments far removed from everyday experience. We leveraged music as a natural medium for audiotactile integration. Music carries complex naturalistic features such as beat (tempo of music) and envelope (changes in sound amplitude over time); further, touch provides information about these features, enhancing the immersion in music. We composed polyphonic (two-voice) piano pieces and modulated vibrotactile signals with the same beat, and using the acoustic envelope of one of the two piano voices. In a multi-day EEG-fMRI study, participants (N=12) experienced piano voices in auditory, tactile or audiotactile contexts, with vibrations applied to their index fingers. In the multisensory setting, auditory and tactile streams were either congruent or incongruent. First, we examined whether the brain encodes beat and envelope information from audition and touch via shared neural representations. In EEG, ridge-regression models successfully reconstructed auditory and tactile beat features; further, we found significant cross-modal temporal generalisation. Conversely, we successfully reconstructed envelope features only in audition and found no temporal generalisation across modalities. Similarly, fMRI identified common activations across audition and touch only for beat (in the parietal opercula and plana temporalia). Second, we examined whether a concurrent vibrotactile signal amplifies the encoding of auditory beat and envelope. In EEG, beat reconstruction increased for audiotactile relative to auditory signals. Remarkably, envelope reconstruction was more accurate for audiotactile congruent than incongruent and audio-only signals. In fMRI, we found non-linear profiles of audiotactile integration in the Heschl's gyri and posterior insulae for envelope information. Further, audiotactile envelope congruency recruited the posterior insulae. We found similar results in complex polyphonic scenarios, suggesting that congruent vibrotactile signals

enhance the segregation and amplification of auditory streams amidst distractors. Our results offer original insights into how the brain automatically tracks naturalistic information across the senses. We show that touch clearly conveys beat information but is rather poor in contributing envelope information; yet, tactile envelope information modulates auditory processing and supports auditory scene analysis in the human brain.

Disclosures: A. Ferrari: None. **G. Degano:** None. **U. Noppeney:** None.

Presentation Number: NANO18.04

Topic: D.08. Multisensory Integration

Support: NIH Grant EY019049
NIH Grant MH116990
NIH Grant DC008983
NIH Grant DC020887
NIH Grant NS132912
NIH Grant MH116990

Title: Cross-modal enhancement of fear responses: the role of parabigemino-tectal projections

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Abstract: Fear reactions to environmental threats are crucial for animals' survival. Integration of sensory cues across different modalities associated with a threat can significantly enhance animals' perception and quick responses. However, the neural circuit-level mechanisms underlying the augmentation of fear responses under simultaneous multimodal sensory inputs remain poorly understood. We report that in mice, bimodal looming stimuli combining coherent auditory and visual signals elicit more robust fear-like reactions than unimodal stimuli. These include intensified escape and prolonged hiding, indicating elevated fear states. These varied responses depend on the activity of the superior colliculus (SC), while its downstream nucleus, the parabigeminal nucleus (PBG), predominantly influences the hiding behavior. PBG neurons temporally integrate auditory and visual signals and enhance the salience of looming stimuli by potentiating SC sensory responses through their feedback projections to the visual layer of SC. These results suggest a distinct subcortical pathway that integrates multisensory signals to cross-modally enhance threat perception and amplify fear responses.

Disclosures: J. Huang: None. **B. Peng:** None. **Z. Li:** None. **L.I. Zhang:** None. **H.W. Tao:** None.

Presentation Number: NANO18.05

Topic: D.08. Multisensory Integration

Support: NIH Grant NINDS 1R01NS109367
NIH Grant NINDS 1R01NS115929
NIH Grant NIDCD R01DC018805

Title: A prefrontal network for audio-visual integration during movie watching

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Abstract: Living in a multisensory world, our brains need to explore the best strategy to fuse diverse information across sensory streams. However, how the spatiotemporal neural signatures associated with this process under naturalistic settings are not well understood. To investigate this, we collected intracranial electrocorticography (iEEG) in 19 participants while they watched a 12-minute movie. The movie contained a rich combination of auditory and visual information allowing for an investigation of how the sensory streams are processed and integrated dynamically. We first separated the movie into four bimodal situations (English condition, foreign language condition, other sound condition, and silent condition), which allowed for a characterization of neural responses (high-gamma band 70-150 Hz) locked to auditory and visual events. Our event-related analysis showed a significant neural enhancement within the ventral lateral prefrontal cortex (vlPFC) and auditory cortices in English and foreign language conditions while the dorsolateral prefrontal cortex (dlPFC) and visual cortices were only activated in the foreign language condition. We then used an unsupervised clustering analysis (across significant electrodes), which replicated this spatial dissociation between the vlPFC and dlPFC associated with auditory and visual processing, respectively. Leveraging the high temporal resolution of iEEG we employed an encoding model analysis based on auditory features (spectrogram and wav2vec features) and visual features (Gabor and vision transformer features) extracted from the movie. Results showed that the vlPFC and dlPFC significantly encode the auditory and visual-related information separately, demonstrating the differentiated representations in the prefrontal cortex. Further, we found evidence for multimodal integration in anatomically restricted regions of the prefrontal cortex. Together, our findings reveal an anatomical separation within the prefrontal cortex supporting sensory processing and multisensory audio-visual integration.

Disclosures: F. Zhou: None. **A. Khalilian-Gourtani:** None. **P. Dugan:** None. **W.K. Doyle:** None. **O. Devinsky:** None. **D. Friedman:** None. **A. Flinker:** None.

Presentation Number: NANO18.06

Topic: D.08. Multisensory Integration

Title: Audiovisual estimation of time-to-contact

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Universitaires, Toulouse, France; [3]Maison des Sci. de l'Homme de Toulouse (MSHS-T), CNRS, Univ. de Toulouse, Toulouse, France

Abstract: The arrival time of moving objects, also known as time-to-contact (TTC), must be estimated every day. For example, as drivers or pedestrians, we have to estimate the TTC of the vehicles around us in order to move safely. Numerous studies have examined the performance of TTC estimation. However, the majority of these studies only tested visual (V) TTC, whereas auditory (A) cues may also be used to estimate TTC. In addition, studies off TTC estimation have mostly focused on objects moving at constant velocity, conditions in which the visual performance of TTC is very good. However, at accelerated velocity, the visual system poorly detects the acceleration, and therefore the TTC of accelerated objects is poorly estimated. It is in this context of accelerated speed that auditory cues are most likely to compensate for the visual deficiencies, allowing better audiovisual (AV) performance of the TTC estimation than in unisensory modalities. The aim of our study was therefore to investigate TTC estimation performance with stimuli that could be moving at constant or accelerated velocity and using the V, A and AV modalities.
Twenty participants (mean age = 24.2 years; SD = 3.1; range = 19-30; 13 women) were tested for their TTC estimation. The stimulus of the task consisted of a looming ball moving in a corridor. After a visible and/or audible motion, the ball and/or sound disappeared for a variable occlusion time. Participants had to press a button to indicate the estimated TTC. The ball could move at constant or accelerated velocity. The constant error (CE), corresponding to the mean difference between the estimated and actual TTC was then calculated and analysed with an ANOVA.
Errors were larger (i.e., more negative or more positive) in the A condition, compared to V and AV, for the shortest and longest occlusion times. Responses were anticipated in the AV condition compared to the V condition, for the shortest and longest occlusion times. At accelerated velocity, the TTC is overestimated in all conditions, compared to the constant velocity level, confirming the lack of detection of the object's acceleration. Notably, the addition of auditory cues to the visual information helps the participants in reducing the overestimation. Our study provides evidence that TTC estimation is not exclusively visual but can also be based on auditory cues. More precisely, TTC estimation can benefit from multisensory cues depending on certain parameters of occlusion time and acceleration level.

Disclosures: S. Leblond: None. **R. Baures:** None. **F. Roux:** None. **J. Tardieu:** None. **C. Cappe:** None.

Presentation Number: NANO18.07

Topic: D.08. Multisensory Integration

Support: ERC advanced grant: MakingSense 101096659

Title: Attention control in multisensory perception

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Abstract: To form a veridical percept of the multisensory world, our brain needs to flexibly integrate signals from common sources and segregate those from independent sources. A recent fMRI study (Ferrari&Noppeney, 2021) has shown that modality-specific attention influences multisensory perception via two distinct mechanisms along sensory pathways. In early sensory cortices (V1-3), attention to vision increases the precisions and hence weight of the visual signals in sensory fusion. In parietal cortices, it controls the late read out of perceptual estimates by flexibly combining auditory and visual signals according to their task-relevance. The dissociation of these two mechanisms was enabled by a novel pre-post cueing paradigm. On each trial, observers were presented with auditory and visual spatial signals in synchrony, but at variable spatial disparities. A prestimulus attention cue manipulated the sensory precision, but indicating the sensory modality observers had to attend to during stimulus presentation. A post-stimulus report cue after stimulus presentation indicated the task-relevant modality that needed to be reported. The current study (N=13; 7 males, mean ages 24.92, range 20 to 35 years) adapted this paradigm to MEG to temporal resolve the influences of pre-stimulus attention and post-stimulus report on the integration of audiovisual signals into spatial representations. Combining psychophysics and MEG multivariate pattern analyses, we show that prestimulus attention influences the weighting of sensory signals after stimulus presentation and in early time windows after the post-report cues. Additional response-locked analyses suggest that only in later time windows does the brain read out perceptual estimates that combine audiovisual signals according to their task-relevance. Our findings unveil the temporal dynamics of the multifaceted interplay of multisensory perception, causal inference and attentional control.

Disclosures: K. Dong: None. **J. Schoffelen:** None. **U. Noppeney:** None.

Nanosymposium

NANO19: Next Generation of Brain Computer Interfaces

Location: MCP Room S103

Time: Sunday, October 6, 2024, 1:00 PM - 4:00 PM

Presentation Number: NANO19.01

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS NS122333
NIH NINDS NS125270

Title: Kinematic and kinetic signals in primate motor cortex

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Abstract: Humans excel at object manipulation - the hand conforms to the object's shape and size during reaching, and the fingers apply precise forces to hold and move the object after

grasping. This behavior is complex and requires control over various physical variables: kinematic, describing posture and movement of the hand, and kinetic, describing forces. Previous studies have suggested a more robust encoding of hand postures compared to grasp force in the motor cortical responses of both humans and non-human primates. However, those conclusions were drawn based on the classification of a low number of grasping postures and discreet forces, neglecting the continuous nature of hand movements and changes in applied force. To understand the interaction between posture and force signals in the motor cortex during naturalistic behaviors, we conducted a parallel set of experiments with healthy non-human primates and humans with tetraplegia. In the first set of experiments, we trained non-human primates to grasp sensorized objects with an instructed force level. The object changed size and orientation to evoke different movements. Throughout the experiment, we recorded hand kinematics across 28 degrees of freedom together with the manual forces applied to the object. In the second set of experiments, a human participant was instructed to attempt grasping objects of varied sizes and orientations in a virtual environment with different levels of force. In both experiments, we recorded corresponding neural responses from the motor cortex using chronically implanted Utah electrode arrays. We first characterized the tuning of individual neurons in monkey and human motor cortices to object shape and target force. We have found that individual neurons were modulated to the time course of the task and that the representations of object and force were intermixed. We then built linear and non-linear decoders of continuous kinematic and kinetic traces. Our findings demonstrated that the motor cortex exhibits a robust representation of grasp force, comparable to the representation of the individual components of movement, such as hand orientation, or aperture. Moreover, our investigation into the relationship between neural representations of force and kinematics revealed non-linear dependence of grasp force on the hand posture. These results carry significant implications for our understanding of manual motor control and the creation of the next generation of decoders for brain-controlled bionic hands that can permit dexterous object manipulation.

Disclosures: E. Okorokova: None. **J.E. Downey:** None. **L.E. Miller:** None. **S.J. Bensmaia:** None. **A.R. Sobinov:** None.

Presentation Number: NANO19.02

Topic: E.05. Brain-Machine Interface

Support: Simons Foundation
Grossman Center for the Statistics of Mind

Title: Decoding implications of the strangely shaped manifold of motor cortex activity

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Abstract: Decoders for motor-based intracortical brain-computer interfaces (iBCIs) frequently assume that the neural state (a vector of rates for each neuron) is constrained to a manifold that can be approximated by a covariance matrix or its corresponding subspace. It is often further assumed that there exist behavior-correlating neural dimensions within the manifold that can be leveraged for decoding, and that a patient can control their iBCI by learning to freely move the neural state around the portion of the manifold containing these dimensions. Yet recent scientific advances suggest different constraints. Motor-cortex activity traces complex task-specific trajectories through high-dimensional neural space. The resulting manifold is sparse - even within the subspace of the data most states are never visited - and may contain no neural dimensions that consistently correlate with to-be-decoded variables. These properties are expected if neural trajectories have low trajectory tangling, which is indeed a property of motor cortex activity in most tasks. In this view, a highly nonlinear manifold is an emergent property of an underlying flow field that instantiates noise-robust dynamics. From this perspective, neural trajectories themselves are apt descriptors of the underlying flow field and its implied manifold. We designed an interpretable decode algorithm, MINT (for Mesh of Idealized Neural Trajectories), to explore whether decoding benefits from embracing the constraints implied by this emerging perspective. MINT was highly performant when benchmarked against other decode algorithms across a variety of tasks and datasets. MINT performed better than other interpretable methods in every comparison we made and outperformed expressive machine learning methods in 37 of 42 comparisons. Yet MINT is a simple, computationally efficient method that relies on explicitly assumed, rather than implicitly learned, constraints. Thus, its high decoding accuracy suggests the assumptions on which it rests may be a particularly good fit to the properties of data. These properties are relevant to the design of any motor-cortex-driven decode algorithm, and have strong implications regarding when and how decoders can be expected to generalize.

Disclosures: S.M. Perkins: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on US Patent 11,429,847, which is licensed by Blackrock Neurotech. **E.A. Amematsro:** None. **J. Cunningham:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on US Patent 11,429,847, which is licensed by Blackrock Neurotech. **Q. Wang:** None. **M.M. Churchland:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on US Patent 11,429,847, which is licensed by Blackrock Neurotech.

Presentation Number: NANO19.03

Topic: E.05. Brain-Machine Interface

Support: Whitehall Grant 2022-12-071
NSF CAREER 2145412
DoD NDSEG Fellowship

Title: Neural variability during neurofeedback adaptation

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Abstract: Variability, a ubiquitous feature of neural activity, plays an integral role in behavior. However, establishing a causal relationship between neural signals and behavior is difficult. By defining a mathematical mapping between neural spiking activity and behavior, we investigate the role of spiking variability in adaptation during a brain-machine interface (BMI) behavior. Recent BMI evidence demonstrates that creating novel neural patterns is harder than repurposing existing patterns to respond to changes in external input. However, what limits the ability to repurpose, or adapt, patterns under different magnitudes of change is less well-characterized. Here, we present evidence that shared variance in neural spiking activity is a neural feature that reveals differences in learnability between easy and hard adaptation conditions. We further demonstrate how shared variance tracks adaptation and can predict maximum adaptation. Our study illuminates the limitations in neural changes underlying behavior within a neurofeedback paradigm.

Disclosures: H. Stealey: None. **Y. Zhao:** None. **H. Lu:** None. **E. Contreras-Hernandez:** None. **Y. Chang:** None. **P.N. Tobler:** None. **J.A. Lewis-Peacock:** None. **S.R. Santacruz:** None.

Presentation Number: NANO19.04

Topic: E.05. Brain-Machine Interface

Support: Meta Reality Labs
NDSEG Fellowship
NSF M3X EAGER 2124608

Title: Shaping human-machine interactions in closed-loop, co-adaptive interfaces

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Abstract: Neural interfaces present opportunities to augment human functionality, but are still difficult to learn and control. Neural interfaces convert user neural signals into control signals for an external device, such as a computer cursor or robotic limb, via a decoder algorithm. Designing these interfaces to be co-adaptive, by leveraging closed-loop user and decoder adaptation, presents opportunities to improve interface usability and personalization (Taylor 2002; Orsborn 2014). However, co-adaptive interfaces are hard to engineer due to their complex two-learner dynamics, and we currently lack principled tools to design and optimize these systems. We present new, experimentally-validated computational approaches for analyzing and synthesizing co-adaptation. Our findings suggest principled co-adaptive approaches can influence user learning via decoder manipulations, opening new ways to achieve high-performing, individualized neural interfaces. Treating the user and decoder as two agents in a dynamic game,

we built a framework to analyze and probe user-decoder co-adaptation. We modeled the user and decoder as each adapting to an individual cost function that minimized task error and individual effort. Analyzing this model yielded two decoder parameters that affected user-decoder dynamics: learning rate and penalty terms. We then measured the effect of these decoder parameters on system performance and user adaptation in a myoelectric interface. Healthy human participants (N=14) controlled a computer cursor using a 64-channel surface electromyography (sEMG) electrode to follow a 2D continuous trajectory. Decoders were randomly initialized and adapted according to the game theoretic decoder cost in 5-minute trials. We saw evidence of co-adaptation in our myoelectric interface experiment, with users and decoders jointly adapting within trials to improve task performance. We leveraged control theory to estimate user encoders - the transformation from task space to user muscle activity - and to quantify user-decoder dynamics. We found that our experimental results corroborated our game-theoretic framework predictions about how decoder parameters influence system performance and user adaptation. Mismatches between user and decoder learning rates disrupted performance and co-adaptive dynamics. Shifts in decoder penalty terms influenced user behavior without impacting performance. This work presents new tools for predictably influencing user-decoder dynamics in co-adaptive interfaces. Our framework opens up new paths to design robust, personalized neural interfaces that fully harness the promise of user and decoder adaptation.

Disclosures: M. Madduri: None. **M. Yamagami:** None. **S. Li:** None. **S. Burckhardt:** None. **S. Burden:** None. **A.L. Orsborn:** F. Consulting Fees (e.g., advisory boards); Scientific Advisor for Meta Reality Labs.

Presentation Number: NANO19.05

Topic: E.05. Brain-Machine Interface

Support: Horizon Europe 2023 - Marie Sklodowska Curie Action - Doctoral Network Grants - ReWire Project

Title: Comparison of algorithms to decode simultaneous arm and hand kinematics from intracortical signals

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Abstract: Deciphering continuous motor functions through intracortical signals poses a contemporary challenge in brain-machine interfaces (BMI), with recent advancements favouring nonlinear decoders [Willsey, 2022, Nat. Commun.; Temmar, 2024, bioRxiv]. Existing studies predominantly focus on decoding either arm movements [Collinger, 2013, The Lancet] or constrained hand movements [Nason, 2021, Neuron], primarily concerning trajectories and velocity derivatives.
Here, we aimed at comparing the performance of state-of-the-art linear and non-linear models in simultaneously predicting continuous arm and hand kinematic outputs from multiunit spiking signals. To this end, we collected intracortical and kinematic data in a non-human primate

performing a task of reaching and grasping of different objects in a versatile robotic platform allowing for natural movements [Barra, 2020, JNE]. Offline, we investigated the performance of a range of regression models in translating intracortical signals into different arm-hand kinematic outputs encompassing trajectories, speed, and joint angles. We also investigated whether predicting the principal components (PCs) of kinematic activity improved decoding performance, and finally compared the use of different cortical areas as decoder inputs. We found that relatively non-sophisticated non-linear models exhibited very good performance similarly to complex counterparts while also outperformed the linear models. Moreover, we found that trajectories as well as velocities and joint angles could be predicted well and that the use of kinematic PCs did not improve the prediction. Finally, performance increased the more cortical areas were included as input. Our findings have implications for real-time BMI applications, emphasizing the feasibility of expanding the scope of decoded outputs for more effective control of neuroprosthetic devices. Overall, our study contributes to advancing the field of BMIs by elucidating effective strategies for decoding intracortical signals, paving the way for enhanced motor function restoration in individuals with paralysis.

Disclosures: F. Serdana: Other; Horizon Europe 2023 - Marie Sklodowska Curie Action - Doctoral Network - ReWire Project.. **V. Mendez:** None. **E. Losanno:** None. **M. Badi:** None. **S. Micera:** None.

Presentation Number: NANO19.06

Topic: E.05. Brain-Machine Interface

Support: NIH New Innovator Award (1DP2NS111817-01)
COBRE CNSF Pilot Grant 5P20GM103645-09

Title: Enhancing reach to grasp movement decoding using visual scene information

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Abstract: Perceptual information about the body and environment is used across brain-wide networks to compute complex goal-oriented behaviors. In the frontal motor areas, neural ensembles retain perceptual and cognitive signals presumably used in the end stages of computation for upcoming movements. For example, human precentral gyrus ensembles not only encode attempted reaching arm movements but also embody movement direction when arm movements are just passively viewed, suggesting that aspects of visual scenes persist into motor cortex. How scenes influence movement encoding is not understood. Here, we propose the "synergistic environment effector" (SEE) predictive framework to identify how the state of the environment, as well as the effector (i.e., upper limb) are represented in neural activity. Using the SEE framework in a non-human primate reach and grasp task we found that adding scene information (from video images of the task) improved movement decoding. We trained monkeys to reach and grasp objects using a rotating apparatus that presented four possible target objects simultaneously. Each object afforded either a power or precision grip in either a horizontal or vertical orientation (for a total of 16 unique grip, orientation, and position combinations). During the task, a light first indicated the target object, followed by a 'go' cue. Forelimb movement and

object positions were tracked using multi-camera, markerless motion tracking (DeepLabCut). An additional camera tracked the eye position. Neural activity was simultaneously recorded using microelectrode arrays implanted in primary motor and dorsal premotor cortices.Using a recurrent neural network architecture to decode movement trajectories from single unit activity, we assessed how decoding accuracy changed when scene information was included in the decoder. Contextual information included the position of all the objects for each scene, but did not indicate the movement target. Our results demonstrate that adding contextual information from the environment significantly improves decoding performance, and even enables high accuracy decoding in low-neuron count regimes.This work has strong implications for brain-computer interface applications. Our results shown that surrogate sensory signals (i.e. video) can be used to provide context for and facilitate the interpretation of neural activity patterns. Further, these findings suggest that perceptual signals can update motor behavior in motor cortical circuits even at the last stages of computation in cortical networks.

Disclosures: N. Tolley: None. **M. Makwana:** None. **J.P. Donoghue:** None. **C. Vargas-Irwin:** None.

Presentation Number: NANO19.07

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS NS122333
NIH NINDS NS125270

Title: Towards neural basis of manual coordination in human motor cortex

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Abstract: Manual interactions with objects involve coordinated movement of many joints of the hand, from the wrist to the tips of the fingers--an ability critically dependent on motor cortex (MC). Although at the level of spinal motor neurons the movements of the hand are encoded in terms of actuating muscles, it is unclear how this representation is abstracted as it ascends the neuraxis. In this study, we investigate human hand movement control, ranging from isolated single digits to coordinated movement of the fingers and wrist, leveraging a clinical trial that involves five participants implanted with electrodes in MC. We have found that movement in one direction, such as flexing a finger, is encoded as a separate neural trajectory from the return movement, such as extending the finger back to a neutral posture. Furthermore, we observed that movement direction encoding depends on the starting position. We examined the coordinated control of multiple digits by having participants attempt to shape their hands into several different postures. Decoders trained on isolated digit movements failed to predict the correct hand posture during multi-digit movements, and neural responses in MC did not simply reflect a superposition of the MC responses of constituent isolated digit movements. These results suggest that MC encodes muscle-like signals for hand control as opposed to joint-based representation. Next, we asked the participants to attempt moving fingers together with their wrists. We found that decoding wrist orientation was possible only during active wrist movement, indicating

velocity-based control. Contrary to our expectations, we discovered that decoding of attempted finger movement was reliably strong irrespective of changes in participants' attempted wrist posture. Moreover, a given classifier performed well both within and across posture conditions, suggesting that MC representations of wrist and fingers are linearly separable. Together, these results provide a detailed characterization of the neural basis of coordinated manual behavior in humans and a foundation for decoding manual behavior from MC for intracortical brain-computer interface applications.

Disclosures: A.M. Emonds: None. **E. Okorokova:** None. **J.E. Downey:** None. **L.E. Miller:** None. **S.J. Bensmaia:** None. **A.R. Sobinov:** None.

Presentation Number: NANO19.08

Topic: E.05. Brain-Machine Interface

Title: Shared neural manifolds improve speech decoding across individuals

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Abstract: Recent developments in neural recording technologies have revolutionized language neurobiology and motor speech enabling brain-computer interfaces (BCI) that restore speech functions. Real-time speech decoding models, typically customized to individual data from intact sensorimotor cortex, face challenges in generalizing, particularly for aphasia patients whose language networks are disrupted by lesions. To address this, we developed group sequence-to-sequence (seq2seq) models trained on datasets from individuals with normal language function, using stereo-electroencephalography to decode activity from distributed speech hubs. This approach significantly reduced phoneme error rate (PER) for all subject (n=7) on held-out trials when initialized with the group-level manifold. Building on these insights, we explored these models' clinical applicability for aphasia with transfer learning techniques to adapt shared latent neural dynamics from a group model of patients with sensorimotor cortex coverage to a single-subject model with differential coverage. Despite the incomplete sampling of necessary cortical areas involved in encoding articulatory kinematics, this adaptation decreased PER from 61% to 36% showcasing the potential of using pre-learned neural states for phoneme mappings to enhance single-subject decoding performance. This use of shared latent mappings presents a robust approach to adapt and apply aggregated neural encodings to situations where there is missing or incomplete sampling of cortical dynamics, extending BCI utility to those with significant cortical impairments. We used group-level dynamics to simulate artificial electrode densities across critical regions such as the posterior superior temporal, subcentral, and inferior frontal cortices, to infer optimal design of neural implants for assistive communication. Furthermore, our approach supports a causality analysis through region and lesion-based assessments to determine the necessity and sufficiency of specific cortical areas. This comprehensive methodology not only advances BCI design and neural decoding techniques but also has profound implications for personalized medicine and rehabilitative strategies, significantly enhancing the quality of life and communication capabilities for individuals with speech impairments.

Disclosures: A. Singh: None. **N. Tandon:** None.

Presentation Number: NANO19.09

Topic: E.05. Brain-Machine Interface

Support: NSF Grant 1926576
NSF Grant 2223822
NSF GRFP Grant 1841052
NIH Grant T32NS007222
NIH Grant R01NS105132
Dan and Betty Kahn Foundation Grant AWD011321
University of Michigan Robotics Institute

Title: Restoring simultaneous wrist and finger movements with a brain-machine interface and functional electrical stimulation

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Abstract: Brain-machine interface (BMI) controlled functional electrical stimulation (FES) is a promising treatment for restoring hand movements to people with spinal cord injury. This method uses a BMI to infer desired control from the brain and then FES to reanimate the paralyzed hand. One challenge with restoring hand movements is that wrist and finger movements are closely linked. Multiple finger-related muscles are located in the forearm with tendons that cross the wrist. It is currently unclear how well we can use BMI and FES to continuously control two correlated degrees-of-freedom (DOF) especially because BMIs typically treat each DOF independently.

To investigate this, one rhesus macaque (Monkey N) was implanted with microelectrode arrays targeting the hand area of motor cortex. We trained the monkey to use a 2-DOF manipulandum, flexing their fingers grouped together or their wrist, to acquire virtual targets. Neural activity, finger flexion, and wrist flexion were recorded during the task. This monkey and one other (Monkey R) were also implanted with intramuscular electrodes in forearm muscles controlling the fingers and wrist. Temporary arm paralysis was induced using a lidocaine nerve block of the median, radial, and ulnar nerves at the elbow. Stimulation was delivered through the intramuscular electrodes using the Networked Neuroprosthesis system (Makowski et al., 2021). Using the virtual hand task data, we trained a ReFIT Kalman filter model (RKF) to predict both finger and wrist flexion velocities from neural activity in real-time. Monkey N was able to use the RKF to control the virtual hand in a BMI task, achieving a 93.9% success rate over 3 experiments. In addition, Monkey N was able to use the BMI after nerve block, using a RKF trained prior to nerve block. We observed decreased success rate, but performance could be regained through an extra ReFIT step after nerve block.

Using FES without a BMI, we used a target-based control strategy to move the monkey's own nerve blocked hand to perform the 2-DOF virtual hand task. Across two sessions with both

monkeys, this strategy achieved an average 85.8% success when targets were limited to the middle 50% of the available range of motion in each DOF. Trial success was largely impacted by fatigue, with the available range of motion decreasing by an average of 62.4% within 10 minutes. Additionally, stimulation targeting one joint often evoked movements on both joints and limited the available range of motion. Ultimately, this approach achieved a high degree of success in a functional range of movements and could be paired with a BMI, such as the demonstrated RKF method, to restore finger and wrist movements after spinal cord injury.

Disclosures: M.J. Mender: None. **L. Cubillos:** None. **J. Costello:** None. **H. Temmar:** None. **M. Kelberman:** None. **D. Wallace:** None. **A. Ward:** None. **J. Lam:** None. **M. Willsey:** None. **Y. Saadeh:** None. **N. Ganesh Kumar:** None. **T.A. Kung:** None. **P.G. Patil:** None. **C.A. Chestek:** None.

Presentation Number: NANO19.10

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5R01NS105132

Title: Regenerative Peripheral Nerve Interfaces (RPNIs) and Implanted Electrodes Enable Continuous Control of Simultaneous Degrees of Freedom

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Abstract: Prosthetic limbs and pattern recognition systems can enhance functionality for people with upper limb amputation. Commercially available systems rely on surface electromyography (EMG) and are limited to sequential control of single movements. Intramuscular EMG electrodes have been shown to record stable and independent control signals, enabling reliable control of multiple degrees-of-freedom (DoF) (Dewald et al., 2019). However, following amputation there may not be sufficient residual innervated muscles available to provide complex movements of the fingers and wrist. Regenerative Peripheral Nerve Interfaces (RPNIs) surgically provide new muscle targets to the peripheral nerve and amplify efferent nerve activity to compensate for absent muscles needed for prosthetic control (Vu et al., 2020). Here we demonstrate the performance of simultaneous control of multiple continuous DoF for hand and wrist rotation. Two participants with transradial amputation (P1, P2) had intramuscular bipolar EMG electrodes (Synapse Biomedical) placed in their RPNIs and residual muscles. For P1, electrodes were placed in 3 previously created RPNIs (1 median, 2 ulnar nerve) and 5 residual muscles. For P2, electrodes were implanted at the time of RPNI creation (4 median, 1 radial nerve) and in 7 residual muscles (incl. wrist supinator & pronator). Both participants were instructed to follow a virtual hand moving in 2 DoF: hand open/close and wrist rotation. EMG signals were collected from implanted electrodes and used to calibrate continuous Kalman Filters (KFs). Participants then used these KFs in closed-loop control of the 2 DoF hand/wrist to acquire targets simultaneously. Performance was evaluated for total trial time (TTT) and orbiting time (OT),

time spent outside the target after first entering it. P1 also calibrated and used a 4 DoF decoder (index, middle-ring-small fingers, 2 DoF thumb) using a recurrent neural network (RNN) architecture. During online control, P1 achieved average TTT of 3.68 seconds and OT of 2.45 seconds. For P2, who had electrodes targeted toward wrist rotation, TTT was 2.24 seconds and OT was 1.77 seconds. During preliminary control of the 4 DoF RNN decoder, P1 was able to control all 4 DoF to achieve different hand postures, although online target metrics have not yet been collected. RPNIs and implanted electrodes have enabled people with transradial amputation to control simultaneous DoF continuously. Simultaneous hand and wrist control is necessary for prosthesis users to perform coordinated movements. Furthermore, providing patients with individual finger control may increase hand function far beyond opening/closing pre-programmed grips.

Disclosures: D.M. Wallace: None. **J.R. Costello:** None. **A.K. Vaskov:** A. Employment/Salary (full or part-time):; Employed in a leadership role by Blue Arbor Technologies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds equity in Blue Arbor Technologies, Inc.. **A.J. davis:** None. **D.H. Gates:** None. **P.S. Cederna:** A. Employment/Salary (full or part-time):; President of Blue Arbor Technologies Inc., a company that makes prosthetic control systems. No support or products from Blue Arbor Technologies were provided or used for the performance of this study.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds equity in Blue Arbor Technologies, Inc., Inventor of intellectual property that is related to this work and has been optioned by Blue Arbor Technologies, Inc. **C.A. Chestek:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of intellectual property that is related to this work and has been optioned by Blue Arbor Technologies, Inc..

Presentation Number: NANO19.11

Topic: E.05. Brain-Machine Interface

Support: EIC 2021-TransitionChallenges-01-01 ReverseParalysis 101057450
EIC-2021-Pathfinderchallenges-01-02 NEMO-BMI 101070891

Title: Adaptive decoder for ECoG based brain-spine interface to control upper / lower limbs in a patients with spinal cord injury

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Abstract: Background: Restoring voluntary movements after spinal cord injury can be achieved through brain controlled spinal cord stimulation. Real-time decoding of epidural electrocorticography (ECoG) recordings is applied to control epidural electrical stimulations (EES) below a spinal cord injury to restore arm or leg movements in motor disabled subjects. Decoding motor intentions reliably while integrating feedback from the stimulation requires adaptive algorithms. **Methods:** Two participants with spinal cord injury were implanted with the Brain-Spine Interface (BSI) system in the context of the STIMO-BSI and UP2 (NCT04632290 and NCT05665998, clinicaltrials.gov) studies. Both studies were designed to evaluate the safety and preliminary efficacy to restore natural motor control of upper and lower limbs, and improve neurological recovery with training. We designed and tested two different decoding paradigms to translate brain signals into movement probabilities to control stimulation amplitudes: 1) a dynamic hierarchical decoder or 2) parallel independent decoders. Both strategies can be independently and incrementally trained during online BSI experiments, while using the evolving model. The decoding models are modular and adaptable. The number of classification states, and their hierarchical organization are configured according to the use case, encoded by graphs to specify the complementary and antagonistic movements that can be activated simultaneously or not. The online model updates allowed to compensate for day-to-day variability but required supervised training, implying cues given to the patient by a supervising expert. Unsupervised domain adaptation techniques were tested in retrospect to compensate for drift and day-to-day variability. **Results:** Models to control different joint movements could be calibrated online within a few minutes. The iterative nature of the algorithm enabled constant refining of the models without impairing the training of the participant. The decoder used for the STIMO-BSI project allowed to distinguish between the hip, knee, and ankle activities (bilateral), trigger stepping events during gait, as well as modulate the amplitude of the steps during walking to restore a natural and smooth control of the lower limb movements. The decoder used during the UP2 clinical trial was able to distinguish between the different upper limb joints, allowing the patient to control each joint not only sequentially, but also in combined complex movements. Finally, domain adaptation strategies in the processing pipeline allowed models calibrated on a single day to achieve stable decoding performance over several months.

Disclosures: T. Aksenova: None. **F. Martel:** None. **M. Justino:** None. **R. Souriau:** None. **A. Bleuzé:** None. **T. Collin:** None. **I. Sakr:** None. **V. Spagnolo:** None. **S. Karakas:** None. **F. Sauter-starace:** None. **S. Chabardès:** None. **J. Bloch:** None. **G. Courtine:** None. **H. Lorach:** None. **G. Charvet:** None.

Presentation Number: NANO19.12

Topic: E.05. Brain-Machine Interface

Support: DARPA HAPTIX

Title: Motor control of individual digits of a robotic hand using only signals recorded from the peripheral nerves

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Abstract: Advances in the engineering of robotic limbs have resulted in hand/upper extremity prostheses with near anatomic weight and size, and up to 22 potential degrees of freedom of movement. Despite this important progress, the uptake and continued use of robotic prostheses by upper limb amputees remains disappointing. Abandonment of robotic prostheses by users is reported to be as high as 50% in some studies. The poor acceptance of robotic limb prostheses by upper limb amputees has been attributed to limited utility and embodiment, due to constraints in the interface between the robotic prosthesis and its user. Specifically, current state-of-the-art prosthetic control interfaces rely on muscle-based signals, which utilize the residual forearm muscles to control gross motions including forearm rotation, wrist flexion/extension, and digit flexion/extension. Importantly, muscle-based prosthetic control cannot predictably afford independent digit control for a variety of reasons related to the anatomy and physiology of the amputation stump. Furthermore, existing robotic prostheses do not provide feedback to the user in the form of naturalistic tactile, kinesthetic, and proprioceptive sensations. We have designed a system for the delivery of two key factors, independent digit control and physiologically congruent sensory feedback, to provide users with "dexterous" robotic hand control. We endeavor to boost the utility and embodiment of robotic limbs sufficiently to permit their routine acceptance and use among upper limb amputees. Our ultimate goal is for robotic limbs to become the standard-of-care for upper limb restoration, attaining insurance reimbursement and economies of scale.

Disclosures: E. Keefer: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fasikl Incorporated. **J. Cheng:** 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.; Nerves Incorporated. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nerves Incorporated. **Z. Yang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fasikl Incorporated.

Nanosymposium

NANO20: Sex Hormones in Cognition and Decision Making

Location: MCP Room N227

Time: Sunday, October 6, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO20.01

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Simons Junior Fellow

Title: Estrogenic gain control of reward prediction errors during reinforcement learning

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Abstract: Despite the broad influence of gonadal hormones throughout the brain, little is known about how these hormones influence cognitive behaviors and their underlying neural substrates. Exogenous estrogenic hormones are known to modulate dopamine signaling in the nucleus accumbens, which is thought to instantiate reward prediction errors (RPEs) for reinforcement learning, raising the intriguing possibility that hormones might influence reinforcement learning. Here we show that endogenous estrogenic hormones that fluctuate over female rats' reproductive cycles enhance reinforcement learning by increasing the dynamic range of dopamine signaling in the NAcc, producing a multiplicative gain on RPEs, particularly for large RPEs. We trained rats to perform a temporal wagering task with different reward states. Rats adjusted how quickly they initiated trials across states, balancing effort against expected rewards. In the high estrogenic hormone stage (proestrus), females showed greater sensitivity to reward states, which we show is driven by enhanced encoding of dopamine RPEs in the NAcc that increase or decrease the perceived value of the environment. During proestrus, dopamine transporters were reduced in expression, and computational modeling showed that reduced reuptake could increase the gain of RPEs. Genetic suppression of estrogen receptors in midbrain dopamine neurons eliminates hormonal modulation of behavior. Thus, estrogenic hormones control the rate of reinforcement learning by regulating dopamine reuptake, providing a mechanism by which hormones influence neural dynamics for motivation and learning.

Disclosures: C. Golden: None. **A. Martin:** None. **D. Grewal:** None. **A. Mah:** None. **T. Yamaguchi:** None. **D. Lin:** None. **C.J. Aoki:** None. **C.M. Constantinople:** None.

Presentation Number: NANO20.02

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIMH R01MH127820
NIMH R01MH104559
BBRF NARSAD Young Investigator Grant 31140

Title: A translational neuroeconomic approach to study sex differences in regret processing

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Abstract: The incidence of depression is two times higher in women than in men and can manifest different symptomatology, including negative rumination. However, the biological and psychological mechanisms involved in these sex differences remain unclear. Negative rumination is multifactorial and could include components of regret processing, but our understanding of changes in regret sensitivity in depression is poorly characterized. This includes exploring whether regret, even if unpleasant, could carry positive utility. New approaches in neuroeconomics have enabled the ability to capture and model elements of regret processing across species. Regret describes recognizing that alternative actions could have led to better outcomes. Recently, we and others operationalized this mental process using a novel neuroeconomic decision-making paradigm and discovered behavioral and neurophysiological

evidence of regret-related counterfactual thinking in rodents.
We leveraged our naturalistic foraging task - Restaurant Row - which has been translated for use in mice, rats, and humans to explore the behavioral properties governing sensitivity to regret. We discovered that there exist fundamentally distinct types of regret depending on the nature of specific action-selection processes. Economic choice violations due to rejecting high-value offers (type I) vs. accepting low-value offers (type II) can give rise to altered decisions on subsequent trials - a behavioral readout of counterfactual sensitivity - compared to alternative non-violation scenarios that control for disappointment and error of one's own agency. Here, we characterized sex differences in regret sensitivity between male and female C57BL6J mice using this model. We found a significant sex difference in counterfactual sensitivity to type II but not type I events: male mice displayed greater sensitivity compared to female mice. We previously linked increased type II sensitivity to individual differences in resilience to stress and discovered that overexpression of female-specific molecular regulators of stress-resilience could selectively enhance this form of regret in female mice only, potentially restoring a decision-making vulnerability in females.
This work highlights how characterizing separable aspects of regret can enhance our understanding of the psychological mechanisms underlying sex-specific decision-making proclivities and uncover computational processes mediating the perception and influence of one's prior actions.

Disclosures: R. Durand-De Cuttoli: None. **S.J. Russo:** None. **E.J. Nestler:** None. **B.M. Sweis:** None.

Presentation Number: NANO20.03

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant DA042111
NIH Grant DA048931
NIH Grant DA056221

Title: Stimulant effects on the dopamine system are sexually dimorphic and sensitive to ovarian hormone cycles

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Abstract: Dopamine release in the nucleus accumbens (NAc) regulates decision-making and attention-related behaviors, where deficits in this process has been linked to various neuropsychiatric disorders including attention deficit hyperactivity disorder (ADHD). Stimulants, such as amphetamine and methylphenidate, are widely prescribed for ADHD due to their ability in enhancing dopamine system function. Importantly, repeated stimulant use can lead to the development of substance use disorder (SUD), further impacting decision-making and attention. Sex-specific disparities in striatal dopamine release underlie sex differences in the development and expression of ADHD, SUD, and other neuropsychiatric disorders. While there has been long-standing recognition that biological sex impacts the pervasiveness and prognosis

of these disorders, there remains a critical gap in understanding the underlying mechanism. Here, we investigate how biological sex and circulating hormones affect dopamine system function in the context of cognition and decision-making. First, we define the mechanisms underlying enhanced stimulant responses in female mice and show how ovarian hormonal cycles contribute to these effects. We show that the dopamine system is highly sensitive to the ovarian hormone cycles where both dopamine release and clearance through the dopamine transporter are enhanced during the estrus phase of the estrous cycle. Amphetamine and methylphenidate were more effective at increasing dopamine levels in the NAc in females, an effect that was most pronounced during estrus. Also, amphetamine has unique effects on dopamine release that are highly sensitive to hormonal cycles and are not seen with methylphenidate. Thus, for each drug, this enhancement occurred via different pharmacodynamic mechanisms. Lastly, we provide behavioral evidence that sex differences in dopamine system function underlie sex-specific strategies in decision-making tasks. The results of these studies provide valuable mechanistic insights into the sex differences observed in response to stimulant medications, shedding light on potential avenues for therapeutic interventions that account for hormonal influences on cognition and decision making.

Disclosures: B.A. Christensen: None. **J. Tat:** None. **E. Holmgren:** None. **S.D. Emerson:** None. **M.Z. Leonard:** None. **L. Zheng:** None. **A. Carr:** None. **S. Lago:** None. **E.S. Calipari:** None.

Presentation Number: NANO20.04

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01 MH123661
R01 NS120851-01A1

Title: Enhanced cognitive flexibility in female mice in a novel touchscreen set shift task

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Abstract: Disorders of cognitive flexibility show significant sex and gender biases, implicating sex differences in the ability to shift between choices or strategies. Understanding how sex influences individual differences in cognitive (in)flexibility remains a critical knowledge gap in our fundamental understanding of brain variability and the etiology of mental illness. To probe the origins of sex differences in cognitive flexibility, we have developed an operant touchscreen Set Shift task optimized for mice. We found robust sex-biased individual differences in cognitive flexibility. Female mice completed significantly more rule shifts with fewer errors than males. However, regardless of sex, faster reaction times were correlated with better task performance, suggesting both sex biased and non sex biased individual differences in cognitive flexibility. To explore how different mechanisms of learning and decision making contribute to sex differences in cognitive flexibility, a reinforcement learning drift diffusion model was fit to Set Shift

behavior. Effects of pre-commitment (bias) and learning rate were stronger in females, corroborating our prior finding of stronger side biases and faster learning rates in female mice during bandit decision making tasks. To directly compare sex differences in the current Set Shift task to this previous behavior, we transitioned this cohort of animals to our previously tested touchscreen restless bandit task. Assessing mouse behavior during both tasks reveals correlations between cognitive flexibility and behavioral strategies. Consistent with the idea that sex differences in decision making occur due to differences in processing loss outcomes, Lose-Shift during bandit was correlated with rule shifts completed during Set Shift in females only. Taken together, sex differences in cognitive flexibility may be related to individual differences in bias and outcome processing.

Disclosures: N. Glewwe: None. **E.M. Dastin-van Rijn:** None. **E.M. Giglio:** None. **E. Knep:** None. **D. Mueller:** None. **C. Chen:** None. **B. Ebitz:** None. **A.S. Widge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Unlicensed intellectual property related to the topic of this poster. F. Consulting Fees (e.g., advisory boards); Consulting income from Abbott. **N.M. Grissom:** None.

Presentation Number: NANO20.05

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01DA049795

Title: Effects of g-protein coupled estradiol receptor-1 activation on reward preference and stimulated dopamine release in male and female rats

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Abstract: Estradiol (E2) receptor activity has been shown to play a sex-specific role in modulating the brain's reward pathway. Specifically, it has been demonstrated that E2 signaling enhances the rewarding properties of cocaine in females. Three E2 receptors, ERa, ERß, and G-Protein Coupled Estradiol Receptor 1 (GPER1), may be mediating these effects. Surprisingly, research from our lab has shown that activation of GPER1, using a GPER1 agonist G1, intra-dorsolateral striatum (DLS) attenuates the development of a preference for rewarding substances, as measured by a two-bottle choice preference test (2BC) for 0.1% saccharin (SACC) versus plain water. In intact animals, a concentration of 20% G1 intra-DLS prevented the formation of a preference for SACC in males, while a concentration of 30% G1 enhanced the formation of a preference for SACC. Acute systemic G1 administration did not affect gonadectomized animals of either sex, but chronic G1 administration enhanced SACC preference in males. The DLS is thought to be responsible for enhanced cue reactivity and compulsive drug taking after repeated drug use. E2 has been shown to modulate dopamine (DA) signaling in the dorsal striatum, enhancing both stimulated and drug-induced DA release in females but not in males. As such, it may be that GPER1 receptor activation differentially modulates dopamine signaling in the DLS of males and females and drives the sex-specific patterns of preference formation for saccharin. In our second study, gonadectomized male and female rats were implanted with three electrodes: a 16-channel carbon fiber working electrode targeting the DLS, a bipolar stimulating electrode targeting the medial forebrain bundle, and a guide cannula for a reference electrode in the

contralateral cortex. Animals underwent weekly test sessions for a total of three weeks. Each test session consisted of three injections of the designated treatment (Peanut Oil Control, G1, or E2), each 30 mins apart, with recordings of stimulated releasing occurring 10 mins after each injection. For G1, animals received three treatments with G1 of 10 µg/kg, each with a cumulative dose of 30 µg/kg. For E2, animals received one injection of 16.67 µg/kg and then oil injections for the following two treatments. After each treatment, two different electrical stimulations were applied to the bipolar stimulating electrode: 60 Hz 30 pulses and 60 Hz 60 pulses. Our preliminary data suggest that administration of G1 enhances stimulated DA release in the DLS of male but not female rats. These findings highlight the sex- and site-specific effects of GPER-1 signaling on reward preference.

Disclosures: C. Turner: None. **J.B. Becker:** None.

Presentation Number: NANO20.06

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: DK130246

Title: Hungry for more: Sex and estrous cycle dependent effects on cue-triggered food-seeking in obesity-prone and resistant rats

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Abstract: Studies in rodents indicate that there are inherent enhancements in Pavlovian motivation and striatal function in obesity-prone (OP) vs obesity-resistant (OR) male and female rats. However, there are sex differences both within and across OPs and ORs. For example, while both male and female OPs show enhanced conditioned approach compared to ORs, the magnitude of this effect is greater in OP females tested during metestrus/ diestrus (M/D) vs proestrus/ estrus (P/E). This effect requires both estradiol and progesterone (Alonso-Caraballo & Ferrario, 2019). However, the degree to which other forms of Pavlovian motivation also vary with the cycle is unknown. Here, we examined how the expression of Pavlovian-to-Instrumental Transfer (PIT) varies across the cycle in OP and OR females. Rats first underwent Pavlovian conditioning where one cue was always paired with food pellet delivery (CS+) and a second cue was never paired with food (CS-). Next, rats learned to lever press for this same food pellet (Bio-Serv; banana flavored pellet). During testing, the ability of the CS+ vs CS- to invigorate active lever pressing in the absence of food was measured. Using vaginal cell cytology, estrous cycle phase was monitored throughout initial training and during PIT testing. In initial studies we found robust expression of PIT in OP and OR females tested in the M/D phase of the cycle, but a complete absence of PIT when these same rats were tested during P/E. In addition, the magnitude of PIT was similar in OP vs OR females. In males, activity of nucleus accumbens (NAc) calcium-permeable AMPA receptors (CP-AMPARs) is required for the expression of PIT (Derman & Ferrario, 2018). Thus, results in females could suggest that the expression of CP-AMPARs varies with the estrous cycle. Furthermore, in acute slices from male OPs, inhibition of synaptic input from the medial prefrontal cortex (mPFC) to the NAc increases CP-AMPAR-mediated synaptic transmission (Fetterly et al., 2023). Thus, ongoing studies are also investigating whether *in vivo* chemogenetic inhibition of mPFC inputs to the NAc is sufficient to

enhance PIT in male OPs. Altogether, these studies will provide insight into the neurobiology of Pavlovian motivation in male and female rats.

Disclosures: L. Raycraft: None. **M. Ortego-Dominguez:** None. **C.R. Ferrario:** None.

Presentation Number: NANO20.07

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant DA726129
NIH Grant DA041781
NIH Grant DA042581
NIH Grant DA042499
NIH Grant DA041883
NIH Grant DA045463
WM Keck Fellowship

Title: Sex-specific effects of inflammatory pain on fentanyl use and dopamine neuron dynamics are modulated by ovarian hormones

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Abstract: Opioid analgesics are commonly used for pain management despite their potential for abuse. Evidence suggests that the risk for opioid misuse under conditions of pain may vary based on gender/sex, but the biological basis of this relationship is unclear. To examine this, a cre-dependent calcium indicator (jGCaMP7c) was targeted to the ventral tegmental area (VTA) of male and female TH-Cre+ rats (2-3 mo.) and optic fibers were implanted in the VTA or nucleus accumbens (NAc) to monitor VTA dopamine (VTA-DA) cell activity at the cell bodies or terminals, respectively, with wireless *in vivo* fiber photometry. Rats were implanted with IV catheters, received hind-paw injections of Complete Freund's Adjuvant (CFA) to produce inflammatory pain, and trained to self-administer fentanyl (2-5 µg/kg/infusion) during five 2-hr sessions/week for 3 weeks. Pain time-dependently enhanced fentanyl self-administration and associated responses from ventral tegmental area dopamine (VTA-DA) neurons in males, but not females. In females with pain, ovariectomy (OVX) produced a male-like phenotype such that, fentanyl use and corresponding VTA-DA neuron activity, were increased, suggesting that ovarian hormones protect against these neuroadaptations. Interestingly, systemic administration of estradiol (E2; 20 µg/kg, i.p.) failed to alter fentanyl self-administration or VTA-DA activity in OVX females but rather, suppressed fentanyl intake and VTA-DA activity in gonadally intact males. Local administration of estrogen receptor antagonists (MPP dihydrochloride hydrate, 30 µg/µL; PHTPP, 800 ng/µL) into the VTA revealed that fentanyl-evoked VTA-DA responses are suppressed through E2-dependent signaling at estrogen receptor subtype beta, not alpha. Together, these findings demonstrate that VTA estrogen receptor beta signaling has an inhibitory

influence over opioid-evoked VTA-DA neuron activity that contributes to maladaptive patterns of opioid use under conditions of pain.

Disclosures: J. Higginbotham: None. **J. Abt:** None. **R.H. Teich:** None. **T. Lintz:** None. **J. Dearman:** None. **J. Moron-Concepcion:** None.

Presentation Number: NANO20.08

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH DA053830 (to EMD)
NIH K99 DA058049 (to EMD)
NIH U54 DA016511 pilot (to EMD)
NIH L60 DA059097 (to EMD)
NIH R01 DA054271 (to JMO)
NIH R01 DA051650 (to JMO)
MUSC COMETS (to JMO)

Title: Influence of biological sex on neuronal activity dynamics in prefrontal cortex during heroin-seeking reinstatement

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Abstract: A significant contributor to the failure of therapies to translate for prevention of relapse in substance use disorders may be insufficient consideration of sex as a biological variable, with females historically underrepresented in research studies. As it is a key regulator of relapse, promising treatment targets may emerge from resolving the dysregulated prefrontal cortex (PFC) activity that emerges with SUDs. To gain greater resolution on the sex differences and PFC neuronal activity underlying relapse, in vivo two-photon calcium recordings of prelimbic-PFC pyramidal neuron activity were taken from head-fixed mice during heroin-seeking reinstatement tests. Aligning with prior reports, females exhibited greater reinstatement responding compared to their male counterparts in response to all triggers (i.e., drug-associated cues, drug prime injections, and stressors). Regardless of sex or trigger, great heterogeneity was observed in PFC neuronal activity during reinstatement. In both sexes, four unique clusters of activity dynamics emerged during reinstatement, with three excitatory and one inhibitory cluster emerging during the lever press epoch. However, nuanced sex differences characterized each cluster. Females exhibited a greater increase in fluorescence in the same excitatory cluster, regardless of trigger, wherein calcium spiking positively correlated with lever pressing. Females also had a greater ratio of neurons in collective excitatory clusters, and the number of neurons in excitatory clusters positively correlated with reinstatement responding. Conversely, males had a greater ratio of neurons in the inhibitory cluster, which negatively correlated with reinstatement responding. Females, who tend to show greater relapse vulnerability within the context of stress, also exhibited greater changes in fluorescence in all clusters during stress-induced reinstatement, with more neurons recruited to the excitatory clusters. Regardless of sex, the same neurons tended to show different activity in response to different triggers, but a lower percentage of neurons remained stable in females. Ongoing experiments using single-cell optogenetics aim to

assess causal roles for these described PFC activity dynamics in reinstatement behaviors. Given the role of the PFC in executive function, the findings from these experiments may have more widespread implications for a range of neuropsychiatric disease states, which are often characterized by sex differences.

Disclosures: E. Doncheck: None. **J.M. Otis:** None.

Presentation Number: NANO20.09

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIDA Grant R01DA053282

Title: Elucidating how sex and menstrual cycle phase influences valuation for risky rewards

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Abstract: Previous research shows that healthy adult women experience upticks in risk tolerance surrounding ovulation, when estrogen is highest during the menstrual cycle. Such shifts in risk-taking propensity may have evolutionary benefits for mating and survival. But questions remain about: 1) what is the neural mechanism through which this behavioral shift occurs; and 2) what implications might this shift have for risk-related behaviors in other domains of life, such as substance use? In Study 1, tying repeated neuroimaging to phases of the menstrual cycle in adult women (n=7 biological females; avg. of 5.6 MRI sessions per person, total of 39), we examined how within-person fluctuations in ovarian hormones intersect with changes in neural valuation of risky rewards longitudinally using a validated economic decision-making task. As expected, salivary measures of the ovarian hormones estradiol and progesterone tracked with menstrual cycle phase. Both menstrual cycle phase and changes in hormone levels correlated with the percentage of risky choices subjects made in the risky decision-making task. Furthermore, model-based analyses of the neural data suggested this may be due to increased sensitivity to risky rewards in the ventromedial prefrontal cortex, a canonical value region innervated by estrogen-sensitive dopaminergic pathways. These data are consistent with prior preclinical data suggesting estrogen heightens reward and drug-cue sensitivity, and that these effects may be sustained to contribute to aggregate sex differences. Therefore, in Study 2, using daily ecological momentary assessments (EMA) over 28 days in men (n=42 biological males) and women (n=25 biological females) with substance use disorder, we probed whether any sex-based differences exist in drug craving, drug-cue exposure, and subsequent drug-use events in subjects' natural environments. These data showed that higher-than-usual drug cue exposure predicted future (next-day) drug use, and that this effect was stronger in females, indicating heightened drug cue/reward sensitivity in this group. Collectively, our findings suggest changes in risk-taking behavior over the menstrual cycle may be due to ovarian hormone interactions with the dopaminergic system and this interaction may further influence sensitivity to environmental cues such as drug cues in people with addiction. This work paves the way for addiction research to identify behavioral and neural risk markers for drug-reuse in women, a population that generally experiences greater rates of relapse.

Disclosures: F.M. LoFaro: None. **S. Grunevski:** None. **E. Alvarez:** None. **J. Kong:** None. **A.B. Konova:** None.

Nanosymposium

NANO21: Regulation of Reward-Related Behavior

Location: MCP Room S401

Time: Sunday, October 6, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO21.01

Topic: G.03. Motivation

Support: IReSP/INCa-21-Addiction
NIH/NIDA Intramural Research Program to Jean Lud Cadet

Title: Neuroinflammatory processes: a potential risk factor for binge eating disorder

Authors: *C. HILDENBRAND[1,2], N. ADJEI[3], A. DAIWILE[3], M. MAJCHRZAK[4], C. DECRAENE[4,5], J. ULYSSE[3], A. BARBELIVIEN[4], P. ROMIEU[4], J. L. CADET[3], K. BEFORT[4]; [1]Univ. of Strasbourg, Strasbourg, France; [2]Lab. de Neurosciences Cognitives et Adaptatives (LNCA), UMR7364, Ctr. Natl. de Recherche Scientifique (CNRS), Strasbourg, France; [3]Mol. Neuropsychiatry Res. Br., Natl. Inst. On Drug Abuse/ NIH, Baltimore, MD; [4]Lab. de Neurosciences Cognitives et Adaptatives (LNCA), Univ. de Strasbourg, UMR7364, Ctr. Natl. de Recherche Scientifique (CNRS), Strasbourg, France; [5]Inst. des Neurosciences Cellulaires et Intégratives, Strasbourg, France

Abstract: Binge eating disorder (BED) is the most prevalent eating disorder. Binge episodes are characterized by the consumption of large amounts of high-calorie food over a short period, which is accompanied by a sense of loss of control. BED is co-morbid with obesity and mood disorders. Several studies have suggested that neuroinflammation occurs in reward-related brain structures following bingeing on food high in sugar. In addition, preclinical studies in rat have highlighted cognitive consequences of binge-sucrose intake; these include increased anxiety levels and poor memory performance, with some inconsistent results. We have thus proposed that molecular mechanisms altering central inflammatory processes might be responsible substrates for behavioral adaptations observed following maladaptive feeding behaviors. We tested this hypothesis using a model of BED in which juvenile male rats had intermittent (2h/d, 3d/week) access to 10% sucrose solution in a two-bottle choice paradigm. Bingeing behavior was assessed as significantly higher sucrose intake during the two hours of access in the intermittent, compared with a continuous, access group. Following 6 weeks of access, anxiety was assessed in the elevated plus-maze and the open field tests, and memory performance was evaluated using object and place recognition, Morris water maze, and fear conditioning tests. In a parallel cohort, we used quantitative PCR to measure the expression of genes involved in neuroinflammatory processes in the brain. We also used RNA-sequencing technology to provide a more panoramic view of gene expression in reward-related brain structures. We found no significant differences in the behavioral tests. In contrast, our molecular transcriptional data identified significant changes in the expression of important gene networks related to feeding

and neuroinflammation following sucrose bingeing in the medial prefrontal cortex and ventral hippocampus. Taken together, our results indicate that neuroinflammatory processes triggered by binge-sucrose might represent risk factors for the development of BED and associated comorbidities. These molecular networks might serve as targets for therapeutic interventions against binge eating disorder.

Disclosures: C. Hildenbrand: None. **N. Adjei:** None. **A. Daiwile:** None. **M. Majchrzak:** None. **C. Decraene:** None. **J. Ulysse:** None. **A. Barbelivien:** None. **P. romieu:** None. **J.L. Cadet:** None. **K. befort:** None.

Presentation Number: NANO21.02

Topic: G.03. Motivation

Title: A nutritional blend of taurine, vitamin B6, B9 and B12 improves motivated behaviors in humans

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Abstract: Motivation is a key driver in achieving goals and performing daily tasks, it is characterized as a series of cost–benefit valuations, in which we weigh the amount of effort we are willing to spend for a particular reward – which can be modulated by several factors (e.g. sleep; obesity; aging etc.).It has previously been established that higher glutathione levels (GSH) in the Nucleus Accumbens are predictive of better and steady performance over time in motivated tasks requiring effort in both humans and preclinical models (Zalachoras et al., 2022). These findings indicate the importance of brain antioxidants for the regulation of motivated behaviors that require effort.We explored the potential of certain nutrients found in food and diets to enhance GSH production to identify possible solutions to sustain motivated behaviors. We performed a luminescent-based assay for the detection and quantification of GSH in primary astrocytes *in vitro*, which are believed to have a more efficient GSH system compared to neurons. The cells were pre-treated for 48-hrs with potential nutrients identified in literature. We discovered that taurine was able to efficiently increase GSH production, but only when levels of vit B9 were sufficient and ideally in a specific ratio with taurine. Additionally, following oxidative stress, we observed a decrease in spare capacity and coupling efficiency of astrocytic mitochondria, indicating oxidative damage to the organelle. To assess the protective effects on mitochondria, we then tested the same combination of taurine and a specific ratio of B9 in astrocytes treated for 48 hours. This test demonstrated a similar effect in protecting the organelles against oxidative stress.To provide convincing evidence regarding the efficacy of a nutrient blend consisting of taurine, along with vitamin B9, B6, and B12, in enhancing effortful motivated behaviors in humans, we conducted a double blinded cross-over controlled study involving young adults aged 25-40 years (n=44). Participants were supplemented with the active blend or control for a duration of 4 weeks in each period. The motivated performance was assessed using the Monetary Incentivize Delay Task coupled with a handgrip as a physical effort. The performance in the task increased across sessions, nonetheless, supplementation with the active blend showed a significant improvement in motivational performance after 14 days

supplementation start and after 28 days in the second supplementation period compared to placebo. This study in humans demonstrated how nutritional supplementation can sustain brain health and modulate behaviors, such as motivated and goal-oriented performance.

Disclosures: L. Trovo: A. Employment/Salary (full or part-time):; Société des Produits Nestlé SA, Nestlé Research, 1000 Lausanne 26, Switzerland. **S. Sultan:** A. Employment/Salary (full or part-time):; Société des Produits Nestlé SA, Nestlé Research, 1000 Lausanne 26, Switzerland. **R.G. Jamora:** 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.; University of Philippines. **V. Anlacan:** 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.; University of the Philippines, Manila.

Presentation Number: NANO21.03

Topic: G.03. Motivation

Support: Quinnipiac University College of Arts and Sciences Grant-in-Aid Funding

Title: Examining the effects of orexin receptor 1 and orexin receptor 2 antagonism on effort-based decision making in male and female rats

Authors: H. G. VAN BLARCOM[1], J. N. MILLER[2], T. M. PANTALENA[2], ***J. L. HAIGHT**[2]; [1]Biol., [2]Psychology, Quinnipiac Univ., Hamden, CT

Abstract: Orexin, a neuropeptide exclusively produced by cells in the hypothalamus, modulates various brain functions upon release. Previous studies have demonstrated that activation of orexin receptors in the VTA leads to an increase in dopamine release in the mesolimbic pathway, and as a result can encourage motivated and reward-seeking behaviors. Orexin peptides interact with two receptors, orexin receptor 1 (OXR1) and orexin receptor 2 (OXR2). Previous research has illustrated that OXR1 antagonism can decrease motivation in rodents by decreasing effort to obtain a favorable food reward, however the effects of OXR2 antagonism on motivation are not well understood. In this experiment, the OXR1 antagonist SB-334867 (20mg/kg i.p) and the OXR2 antagonist TCS-OX2-29 (20mg/kg i.p.) were used to assess the effects of select orexin receptor antagonism on an FR5 model of effort-choice behavior in male and female rats. In this model, rats must choose between a preferred, high-effort option (5 lever presses for a 45mg chocolate-flavored pellet) or a low-effort option (freely available rat chow). Following OXR1 antagonism, both male and female rats showed decrease lever pressing behavior. In addition, male rats showed decreased chow consumption, while female rats did not. Following OXR2 antagonism, no changes in behavior were observed. These data suggest that pro-motivational orexin signaling selectively functions through OXR1 activation. In addition, OXR1 antagonism may selectively produce an anorexic-like state in male rats at the dose given.

Disclosures: H.G. Van Blarcom: None. **J.N. Miller:** None. **T.M. Pantalena:** None. **J.L. Haight:** None.

Presentation Number: NANO21.04

Topic: G.03. Motivation

Support: NIDA grant DA047233
NIDA grant DA007359

Title: Modulating levels of dFOSB alters nucleus accumbens medium spiny neuron activity in vivo to salient stimuli

Authors: *T. MARKOVIC, A. GODINO, L. HOLT, A. M. MINIER-TORIBIO, V. KONDEV, T. M. GYLES, E. M. PARISE, H. LI, E. J. NESTLER;
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Abstract: dFOSB is a key transcription factor that mediates gene expression changes in the nucleus accumbens (NAc) in response to chronic stimuli. The NAc is composed of GABAergic medium spiny neurons (MSNs) that express either dopamine receptor 1 (D1) or dopamine receptor 2 (D2). Previous work in rodents showed that cocaine induces dFOSB in D1 MSNs, chronic stress induces the protein in D2 MSNs in stress-susceptible but in D1 MSNs in stress-resilient animals, while natural rewards induce dFOSB in both. This cell-type-specific regulation of dFOSB expression in the NAc correlates with differential effects of the protein on synaptic properties of MSNs: dFOSB decreases excitatory synaptic strength and increases silent synapses onto D1 MSNs, with opposite effects seen for D2 MSNs. However, no studies have investigated how changes in dFOSB expression levels in the NAc alter the in vivo activity of MSNs. To address this, we injected D1-Cre and D2-Cre mice with Cre-dependent AAVs that express a calcium sensor and epigenome-editing tools to either induce or repress endogenous dFOSB in the NAc. We recorded in vivo activity of D1 and D2 MSNs using fiber photometry in response to social reward, saccharin reward, foot shock, and drug rewards. We found that manipulation of dFOSB primarily altered MSNs responses to salient stimuli such as foot shock and cocaine conditioned place preference (CPP). In fact, decreasing dFOSB in D1 MSNs attenuated foot shock-induced calcium transients, while decreasing dFOSB in D2 MSNs enhanced them. Similarly in a cell specific manner, decreasing dFOSB in D1 MSNs and increasing dFOSB in D2 MSNs decreases social interaction. In addition, decreasing dFOSB in D1 MSNs only blocks cocaine CPP and attenuates neuronal activity aligned with entrance to cocaine paired side. These findings of opposite in vivo modulation of D1 vs. D2 MSN activity by dFOSB demonstrate how dFOSB influences circuit activity and shed light on cell-autonomous mechanisms controlling behavioral responses. Lastly, to assess translational potential of dFOSB we utilized novel lipid nanoparticle (LNP) gene therapy technology to systemically deliver dFOSB during cocaine CPP. Using this approach, we found that LNP driven overexpression of dFOSB augmented drug seeking behavior of non-reinforcing does of cocaine. Taken together these findings demonstrate the dFOSB's bi-directional modulation of behavioral responses to salient stimuli such as cocaine and it's potential for translational utilization.

Disclosures: T. Markovic: None. **A. Godino:** None. **L. Holt:** None. **A.M. Minier-Toribio:** None. **V. Kondev:** None. **T.M. Gyles:** None. **E.M. Parise:** None. **H. Li:** None. **E.J. Nestler:** None.

Presentation Number: NANO21.05

Topic: G.03. Motivation

Support: BBRF Young Investigator Grant 30616
NIH Grant MH129040
NIH Grant DA041482

Title: Midbrain circuits coordinate novelty responses and adaptation with repeated exposures

Authors: E. WILLIAMS[1], B. O'MEARA[2], T. G. FREELS[3], R. ZHAO-SHEA[4], A. R. TAPPER[5], ***S. MOLAS**[6];
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Abstract: Exposure to novel stimuli drives goal-directed approach/avoidance behaviors necessary for survival. In a safety environment, these orienting novelty responses undergo rapid adaptation after repeated presentations and familiarity. The neural circuits that govern novelty responses and habituation learning are currently a hot topic in systems neuroscience, as disturbances in these fundamental processes contribute to numerous neurodevelopmental and neuropsychiatric disorders. A growing body of literature indicates that reward-related midbrain circuits, the majority of which tie to dopamine (DA) transmission, promote assessments to novelty and the adaptation to changing environments. Using fiber photometry recordings of genetically-encoded calcium sensors and other biosensors time locked to mouse behavior, we have identified specific patterns of DA transmission encoding novelty to stimuli of different valence. In addition, we reveal that the interpeduncular nucleus (IPN) of the midbrain encodes adaptation with repeated exposures. Finally, combining activity recordings and optogenetic tools, we unravel that IPN inhibitory outputs innervating the laterodorsal tegmental nucleus, a region that provides strong excitatory inputs to the mesolimbic system, control the novelty response. Altogether our results are paramount for the identification of new circuits that may be implicated in numerous disorders associated with impaired response to novelty.

Disclosures: E. Williams: None. **B. O'Meara:** None. **T.G. Freels:** None. **R. Zhao-Shea:** None. **A.R. Tapper:** None. **S. Molas:** None.

Presentation Number: NANO21.06

Topic: G.03. Motivation

Support: Nîmes university

Title: Higher motivation for foods than cocaine in the absence of 5-HT4 receptors

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Abstract: In cells of the nucleus accumbens, activation of a cAMP signaling pathway is a means of transforming an immediate reduction of drugs' rewarding effect into a durable dependence. After recruiting cAMP-response element binding protein (CREB)-binding protein, the resultant phosphorylated CREB (pCREB) favors the expression of some genes (FosB, ΔFosB) to the

detriment of others (methyltransferase G9a of histone), from where come changes in neuron morphology. Serotonin (5-HT, 5-hydroxytryptamine) volume transmission trough different receptors acts on cAMP signaling and modulates the activity of the reward neural pathways. Here, we examine how the absence of one of the 5-HT receptor subtypes, the Gs-coupled serotonin 4 receptors (5-HT_4Rs), impacts morpho-functional effects of cocaine. Cocaine failed to increase the levels of both cAMP and pCREB in the accumbens in the 5-HT_4R knockout (KO) mice. The resultant expression of FosB and ΔFosB was attenuated. Under basal conditions, the mRNA levels of the G9a in the accumbens were higher in mutants than wild-type animals. A reduced number of dendritic spines in the accumbens was also observed in the mutants. Mutants are less motivated to self-administer cocaine but more motivated to consume food following chronic restriction. Hence, high vulnerability to overeating and low cocaine dependence are associated with low cAMP-dependent pathway activity and reduced numbers of dendritic spines in the nucleus accumbens in the absence of 5-HT_4 receptors.

Disclosures: V. Compan: None. **S. Choquart:** None.

Presentation Number: NANO21.07

Topic: G.03. Motivation

Support: NIH Grant RO1-NS113104-01
NIGMS Grant R25-GM078441

Title: The role of the mesolimbic reward system in pair bond formation and related behaviors in male and female prairie voles (Microtus ochrogaster)

Authors: *K. GOSSMAN[1], S. YUE[2], C. HYBL[3], A. KIRCKOF[4], C. LOWE[5], Z. WANG[6], A. S. SMITH[7];
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Abstract: Humans and numerous other species live in complex social environments, requiring many important decisions to be made in the context of social interactions. Social relationships rely on our ability to make context-appropriate decisions, particularly those in committed partnerships or pair bonds. It is suggested that there are many benefits to the formation and maintenance of social relationships. Within these relationships, individuals must show commitment behaviors or selective affiliation toward a current partner and rejection of potential alternative partners. Unlike other rodent models, the prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent that forms pair bonds, or bonds between breeding pairs. Prairie voles also maintain pair bonds through partner-directed affiliative behavior and stranger aggression. The mesolimbic reward system is one network thought to regulate pair bond-related behaviors in voles as voles display partner-seeking behavior during separation, selective social preference, and other behaviors that suggest a partner represents a rewarding and motivational social cue. Surprisingly, only a few mesolimbic regions have been studied in the context of vole pair bonding, with most work focused on dopamine (DA) within the nucleus accumbens (NAc).

However, research has also demonstrated that the anterior cingulate cortex (ACC) and the basolateral amygdala (BLA) regulate various aspects of social attachment with the use of DA. Particularly in vole, it is suggested that the ventral tegmental area (VTA) is the theoretical hub of this network that produces much of the DA within this network, however, it has yet to be established how DA signaling within the VTA is regulated and how the VTA regulates downstream network activity. The VTA contains glutamate, GABA, and DAergic neurons, as well as receives a variety of inputs, such as corticotrophin-releasing factor (CRF) which is thought to regulate DA. Thus far, our studies have shown that CRF and GABA antagonist administration in the VTA in male and female voles promoted a partner preference after a 1hr cohabitation, a period typically insufficient for such preference formation in voles. Electrophysiology recordings also demonstrated that there was an increase in spontaneous action potentials of VTA DAergic neurons when a CRF bath application was applied. We plan to incorporate the immunotoxin, anti-DAT-SAP, to assess if GABA and CRF regulate VTA DAergic projections to the NAc to regulate pair bond-related behaviors. Lastly, we will assess the structural connectivity and cell phenotypes of the VTA to the ACC, NAc, and BLA and the changes of such during bachelor vs. pair bonded state.

Disclosures: K. Gossman: None. **S. Yue:** None. **C. Hybl:** None. **A. Kirckof:** None. **C. Lowe:** None. **Z. Wang:** None. **A.S. Smith:** None.

Nanosymposium

NANO22: Mechanisms of Attention Dynamics in the Human Brain

Location: MCP Room N427

Time: Sunday, October 6, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO22.01

Topic: H.01. Attention

Title: A novel method for estimating tonic and phasic pupillary dynamics in humans

Authors: *J. M. GROOT, M. MITTNER;
Dept. of Psychology, UiT - The Arctic Univ. of Norway, Tromso, Norway

Abstract: The dilation of the human pupil is a physiological metric commonly used by researchers in psychology and neuroscience. Psychosensory pupil responses are predominantly mediated by norepinephrine (NE) originating from the locus coeruleus (LC). In recent years, the hypothesized role of LC/NE-dependent neuromodulation in cognitive processes such as exploration-exploitation tradeoffs and attention regulation is increasingly recognized. However, standard methods for analyzing pupil dilation often fail to incorporate the two distinct operative modes of the LC: tonic discharge versus phasic bursts of activity. Here, we present a novel method that leverages the continuous nature of pupillary data to model the underlying tonic fluctuations. We used a Bayesian optimization algorithm to fit multiple iterations of B-spline basis functions through high-prominent negative peaks in the pupil signal. To estimate the magnitude of phasic responses, we convolved task events using a canonical pupil response function and fitted single-trial linear regression models with a non-negative least-squares solver

to constrain coefficients to be positive. The algorithms are implemented in an open source and user-friendly Python package titled Pypillometry (github.com/ihrke/pypillometry). We evaluated the utility and validity of the novel method using an existing dataset investigating the interplay between mind wandering, executive functioning, and behavioral variability with a fast-paced finger-tapping task (N=100, 750ms inter-stimulus intervals). We demonstrate that the modeled tonic fluctuations are unaffected by the artificial accumulation of transient evoked dilatory responses that are evident in fast-paced paradigms, thereby outperforming traditional estimates based on single-trial averaging in fixed windows. These results emphasize that disentangling tonic and phasic components of pupil dynamics in humans is imperative for understanding their behavioral relevance and has significant value for the non-invasive investigation of covert cognitive and attentional processes.

Disclosures: J.M. Groot: None. **M. Mittner:** None.

Presentation Number: NANO22.02

Topic: H.01. Attention

Support: NIA Grant R01AG075000

Title: Task-based functional connectivity of locus coeruleus-salience network and attentional distractibility in young adults

Authors: *Y.-Y. CHEN[1], B. KATZ[2], I. KIM[4], T.-H. LEE[1,3];
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Abstract: The locus coeruleus (LC) is the primary source of norepinephrine (NE) in the brain, which is crucial for selective processing toward salient or goal-relevant stimuli (Mather et al., 2016). Recent studies suggest that this LC-NE system is intrinsically connected to the salient network (SN), which comprises the dorsal anterior cingulate cortex (dACC) and anterior insula (aINS), and this plays an important role in processing selectivity. For example, the strength of connectivity between the LC and SN is associated with the level of attentional distractibility across various age groups (Lee et al., 2020; Neal et al., 2023). However, these recent findings were based on non-task-related, intrinsic functional signals. To further demonstrate the importance of the LC-SN circuit in the attentional process, we examined the LC-SN dynamics while participants were engaged in attentional tasks. We recruited forty-four young adults (*M* = 20.55 years) and presented them with two different distracting tasks. The attention network test (ANT) requires participants to determine the direction of a central arrow surrounded by distracting flankers. The place discrimination task (PDT) asks to distinguish target place images (buildings/houses) in the presence of distractors (faces). The LC was set as a seed for a seed-based functional connectivity analysis using the general psychophysiological interactions (gPPI) approach. The whole-brain functional connectivity results revealed that the distraction score in the ANT was positively correlated with connectivity between the LC-dACC and LC-aINS, whereas it was negatively correlated with connectivity to the medial prefrontal cortex. Additional dynamic causal modeling (DCM) was applied to characterize the direction of connectivity in the ANT, suggesting that the distracted condition modulated the connectivity from LC to SN, but not vice versa, indicating a bottom-up modulation. Subsequent sensitivity analyses using dACC and

aINS as regions of interest in the PDT confirmed the robustness of LC-dACC connectivity (r = 0.309, p = 0.042), but not LC-aINS connectivity (r = -0.057, p = 0.714), was positively associated with distraction score in the PDT. Together, these findings suggest that stronger LC-dACC connectivity was reliably associated with attentional distractibility regardless of task characteristics. The current study only included younger adults, future studies can be applied to different age groups to investigate changes in LC-SN connectivity and attentional distractibility.

Disclosures: Y. Chen: None. **B. Katz:** None. **I. Kim:** None. **T. Lee:** None.

Presentation Number: NANO22.03

Topic: H.01. Attention

Title: Modeling dynamical transitions between on- and off-focus states using Hidden-Markov models

Authors: *M. MITTNER[1], J. M. GROOT[2], S. WIENTJES[3], B. U. FORSTMANN[4];
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Abstract: Previous research has shown that large-scale brain networks such as the default-mode network (DMN), the executive network and the dorsal-attention network (DAN) are involved in mind-wandering (MW). In particular, both relative activity of these networks but also their dynamic functional connectivities seem to be highly predictive of MW (Mittner et al., 2014; Groot et al., 2020). However, current state-of-the-art methods for predicting MW based on neural data are lacking an explicit model for the temporal evolution and dynamic switches between on-task and MW states. Based on a theoretical model of mind-wandering that postulates that attentional shifts are modulated by norepinephrinergic (NE) activity (Mittner et al., 2016), we investigate attentional switches using data from a combined fMRI and pupillometry study (N=27; Groot et al., 2022). This model assumes the existence of a latent off-focus state, characterized by transiently elevated tonic NE activity, which mediates transitions between on-task and MW. Because of the transient nature of this state, it is difficult to measure with standard methods. To overcome this problem, we implemented a modified Hidden-Markov model (HMM) fit to behavioural and pupillometric data that allows to model dynamic task transitions between latent states. Crucially, the model is also informed by self-reported MW in the form of thought-probes, and therefore provides interpretable latent states with specific signatures. By projecting the sequence of latent states extracted by the HMM into the fMRI data, we can extract specific brain signatures of on-task, MW and most importantly, the elusive off-focus state. We find that our analysis provides important insights into the dynamics of mind wandering and contributes to disentangling the enigmatic involvement of the DMN and its subnetworks in MW. Finally, we apply the model to a separate dataset to validate its generalizability and robustness.

Disclosures: M. Mittner: None. **J.M. Groot:** None. **S. Wientjes:** None. **B.U. Forstmann:** None.

Presentation Number: NANO22.04

Topic: H.01. Attention

Support: National Science Foundation BCS-2043740 to M.D.R.
University of Chicago Research Computing Center

Title: Common neural activation and co-fluctuations underlie auditory and visual sustained attention

Authors: *A. CORRIVEAU[1], J. KE[1], M. D. ROSENBERG[1,2,3];
[1]Psychology, Univ. of Chicago, Chicago, IL; [2]Neuroscience Institute, University of Chicago, Chicago, IL; [3]Institute for Mind and Biology, University of Chicago, Chicago, IL

Abstract: Auditory and visual sustained attention ability is related within individuals and can be predicted by relationships between distributed brain regions. However, fluctuations in sustained attention occur on the order of seconds, a time scale better-captured by dynamic brain measures, such as BOLD activity co-fluctuations. We compared neural mechanisms—both fMRI activation and activity co-fluctuations—involved in maintaining attention to sounds and images. Adults performed a continuous performance task in which streams of trial-unique sounds and images were presented simultaneously during fMRI. Across two MRI sessions, participants were tasked with pressing a button when relevant stimuli (either sounds or images) belonged to a frequent category (90%) and withholding responses to infrequent stimuli (10%). Visual and auditory task analyses included 46 participants each (38 shared). BOLD activity was averaged within 400 functionally-defined regions of interest (ROIs), yielding 400 ROI activation time series. Pairwise co-fluctuation (edge) times series were calculated as the element-wise product of z-scored ROI time series. To identify regions and edges involved in attentional lapses, we contrasted trial-evoked activation and co-fluctuations to correct vs. incorrect responses to infrequent stimuli. To identify regions related to fluctuations in attentional state, activity and co-fluctuations were related to a parametric response time variance time course (VTC) regressor, which characterizes moments of low (engaged “in-the-zone”) and high variance (disengaged “out-of-the-zone”) pressing. Group-level analyses were tested within auditory and visual sessions separately. Max-T and network-based statistic correction were used for activation and co-fluctuation analyses, respectively. Contrasting activation on infrequent trials (correctly withheld responses - incorrect presses) revealed temporal parietal regions more active on correct trials in both auditory and visual sessions. Activity in ventral attention ROIs positively related to the VTC in auditory and visual sessions. Auditory and visual sessions also shared more overlapping edges than chance ($ps<.001$) whose co-fluctuation was significantly related (631 positive, 293 negative) to changes in VTC. 622 of 924 edges (67.3%) related to VTC were not predicted by ROI time courses, suggesting edges carry additional information about attentional state fluctuations missed by activation alone. These results identify perceptual modality-agnostic predictors of sustained attention performance and demonstrate unique predictions from edge co-fluctuations.

Disclosures: A. Corriveau: None. **J. Ke:** None. **M.D. Rosenberg:** None.

Presentation Number: NANO22.05

Topic: H.01. Attention

Support: NIH (NEI): 5P30EY008126-33

Title: Tracking task-specific activity during multitasking with ultrafast, high-field fMRI reveals serial queuing of information processing in the human brain

Authors: *Q. YUE[1,2], A. NEWTON[3], R. MAROIS[1,4,5];
[1]Vanderbilt Univ., Nashville, TN; [2]Shenzhen Univ., Shenzhen, China; [3]Vanderbilt Univ. Med. Ctr., Nashville, TN; [4]Vanderbilt Vision Res. Ctr., Nashville, TN; [5]Vanderbilt Brain Inst., Nashville, TN

Abstract: The human brain is heralded for its massive parallel processing capacity, a distinctive feature from conventional computing infrastructures with their serial mode of information processing. Yet, considerable evidence suggests that there is a central bottleneck of information processing distinct from perceptual and motor stages that limits our ability to carry out two cognitively demanding tasks at once, resulting in the serial queuing of task information processing. The neural instantiation of this central bottleneck has remained elusive, however, because of the absence of a methodology with the combined spatial and temporal resolution necessary to track the flow of information processing as it courses through the brain. Here we show the feasibility of using ultrafast (199ms TR), high-field fMRI (7T) with multivariate analyses to distinguish brain activity between two arbitrary sensorimotor response selection tasks as human participants (N=26, 19-29 years old, 6 males) performed the tasks when they were overlapping (300ms SOA) or not (1500ms SOA). The behavioral results revealed a classic delay in the response time of the second of the two tasks when they were overlapping. Correspondingly, we observed the postponement of Task 2, but not Task 1, activity of the duration of the response time costs at short SOA in a subset of multimodal fronto-parietal areas corresponding to the multiple-demand network (MDN) while earlier sensory stages were largely unimpeded. Moreover, that network coupled with modality-specific motor areas to determine the functional characteristic of the central bottleneck. These results provide direct neural evidence for serial queuing of information processing under overlapping dual-task conditions and identify the neural substrates of the central bottleneck. The findings also demonstrate the feasibility of using ultra-fast fMRI and multivariate analyses to temporally track the flow of information processing in the human brain.

Disclosures: Q. Yue: None. **A. Newton:** None. **R. Marois:** None.

Presentation Number: NANO22.06

Topic: H.01. Attention

Support: NIH NS098981

Title: Isolating stimulus-driven and task-driven network dynamics during visual recognition using large-scale intracranial recordings in humans.

Authors: *M. J. MCCARTY[1], O. WOOLNOUGH[2], E. MURPHY[3], N. TANDON[4];
[1]MD Anderson UT Hlth. Grad. Sch., Houston, TX; [2]Vivian L Smith Dept. of Neurosurg., UTHealth Houston, Houston, TX; [3]Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [4]Neurolog. Surgery, McGovern Med. Sch. at UT Hlth., Houston, TX

Abstract: The recognition of a visual object, such as a word or a face, integrates several cognitive operations: hierarchical visual processing of incoming sensory stimuli, rapid retrieval of stored categorical representations learned over time, and attentional modulation based on task demand. As such, this process involves the convergence of stimulus-driven and task-driven dynamics, hypothesized to influence neural dynamics in category-selective regions of the ventral

occipitotemporal cortex (vOTC).
In this study, we utilized intracranial electroencephalographic recordings in 25 patients (14 female) performing an object recognition task where task demands were directly modulated. Visual stimuli of different categories (Faces, Words, Scenes, or Animals) were presented. Across different trial blocks, 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 the category the stimulus belonged to (e.g., the category "fruit or vegetable words" for which an exemplar is "apple"). This task design enables the isolation of stimulus-driven and task-driven dynamics when the same stimuli are presented, but task demands shift.
We found broadband high gamma activity (BGA; 70-150Hz) within vOTC exhibited early and sustained modulation by stimulus category. In contrast, BGA dynamics in inferior frontal cortex showed significant early modulation by task condition, and were later followed by a similar modulation in intraparietal regions. These BGA changes evolved over time, with the earliest onset of task modulation occurring ~100ms after stimulus onset. To further understand the effect of changing task demand within vOTC, we used a d' index to identify electrodes that exhibit significant category-selectivity within the ventral occipitotemporal cortex (vOTC). We found significant low-frequency phase locking between category-selective vOTC electrodes and distinct inferior frontal regions that was dependent on task condition.
Through quantifying the rapid spatial and temporal dynamics between frontal and ventral regions involved in this task, we identify a potential mechanism by which the frontal cortex modulates vOTC activity based on changing task demands.

Disclosures: M.J. McCarty: None. **O. Woolnough:** None. **E. Murphy:** None. **N. Tandon:** None.

Presentation Number: NANO22.07

Topic: H.01. Attention

Support: ERC Horizon 2020 program (INSENSE 804630)

Title: Spatial attention resolves perceptual ambiguity: Evidence from N2pc and similarity analysis of EEG, concurrent EEG/MRI, and convolution models

Authors: *C. HICKEY, D. ACUNZO, D. GRIGNOLIO, O. FERRANTE;
Univ. of Birmingham, Birmingham, United Kingdom

Abstract: The N2pc is a robust EEG/MEG index of the deployment of spatial attention. Early interpretation suggested that the brain activity underlying the N2pc implemented a mechanism that protected neural representation of attended information by suppressing representation of unattended stimuli. However, a body of subsequent results have closely linked the N2pc to processing of target stimuli in ways that are difficult to reconcile with this ambiguity resolution hypothesis. Here, we use representational similarity analysis of EEG, concurrent EEG/MRI, and modelling data to show that when participants attend to one of two objects, N2pc amplitude is closely predicted by similarity of the neural responses to these two objects when they are presented in isolation. Analysis of EEG results show that the N2pc is best predicted by neural ambiguity that immediately precedes the onset of N2pc. Analysis of MRI results allows us to both track the instantiation of ambiguity resolution in visual cortex and to identify attentional

control structures elsewhere in the brain. Finally, results from analysis of convolution models show that stimulus-evoked similarity in shallow, biologically plausible models most accurately predicts human N2pc. The N2pc appears strongly determined by representational ambiguity and the competition for neural resources.

Disclosures: C. Hickey: None. **D. Acunzo:** None. **D. Grignolio:** None. **O. Ferrante:** None.

Presentation Number: NANO22.08

Topic: H.01. Attention

Support: NSF Grant 2120539
Searle Scholars Program

Title: Shared theta-rhythmic neural activity coordinates attentional and working memory processes.

Authors: *P. J. CAVANAH[1], I. C. FIEBELKORN[2];
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Abstract: Visuospatial attention (VSA) and visual working memory (VWM) are related cognitive processes that are crucial to everyday tasks (such as driving a car). Recent research has separately revealed that behavioral performance in certain VSA or VWM tasks is theta-rhythmic. That is, both our attentional focus and our working memory representations fluctuate at a frequency within the theta band (3-8 Hz). We hypothesize that these theta-rhythmic fluctuations during VSA and VWM could be due to a shared neural mechanism for the coordination of cognitive resources. The implication being that the same neural populations may subserve the coordination of VSA and VWM, helping to mediate representational conflict within and between foci in VSA and VWM. Here, we used human electroencephalography (EEG) to examine whether shared or separate rhythmic processes coordinate the sampling of external information during VSA and the sampling of internally stored information during VWM. We conducted two experiments (n = 23 and n = 20). In each, we included task conditions that isolated VSA and VWM, as well as task conditions that required both VSA and VWM (i.e., dual-task conditions). The behavioral results indicate that VWM load (on dual-task trials) interferes with VSA. This interference was particularly strong when the to-be-detected stimulus (VSA) matched the to-be-remembered item (VWM) that was previously presented at the same spatial location. Such behavioral results provide further evidence of shared neural resources between VSA and VWM. To address our primary hypothesis, we measured the frequency-specific phase of neural activity just prior to VSA or VWM target onset and quantified accuracy and response time as a function of phase. The results from both experiments show that the phase in the theta/alpha band (4-12 Hz) modulates perceptual (VSA) and memory (VWM) performance. We demonstrate statistically significant frontal and posterior clusters of this phase-behavior modulation that are strikingly similar across VSA-alone, VWM-alone, and dual-task trials (with either VSA or VWM being probed). We further demonstrate that the specific phases at which behavioral performance is best and worst are the same for VSA and VWM. To summarize, we demonstrate a robust modulation of behavioral performance by frequency-specific phase, with similar spatial and spectral characteristics across the different task conditions. These findings are consistent

with a shared rhythmic sampling process that can be turned either outward during VSA or inward during VWM.

Disclosures: P.J. Cavanah: None. **I.C. Fiebelkorn:** None.

Presentation Number: NANO22.09

Topic: H.01. Attention

Title: Integrating eye-tracking and EEG to study mind-wandering onset and offset in a self-report reading task

Authors: *H. SUN[1], A. OLSZKO[1], L. ALLEN[1], N. SINGH[1], A. M. SHOUDT[1], J. A. RUUD[1], M. D. ROSENBERG[2], D. C. JANGRAW[1];
[1]Electrical & Biomed. Engin., Univ. of Vermont, Burlington, VT; [2]Univ. of Chicago, Chicago, IL.

Abstract: Mind-wandering (MW) is a prevalent cognitive phenomenon, yet understanding its occurrence remains challenging due to no technique for identifying the onset and duration of MW. To tackle this challenge, we recruited a total of 21 adults without neurological or reading disabilities to participate in a free-viewing reading task that required them to self-report the words they were reading when their MW began and ended. During the task, we monitored and recorded their gaze movements, which we used to translate reported words into times, allowing us to examine the onsets and offsets of MW. The estimated duration of MW follows a Gamma distribution (κ=1.12, θ=7.34). For eye-tracking features, we observed that during MW periods, subjects blinked more frequently and spent a longer time on each word ($p < 0.001$), which is consistent with those reported in previous studies (Steindorf and Rummel, 2020). Simultaneously, we recorded EEG signals and epoched based on estimated MW onset and self-report moments. Control epochs were randomly selected from non-MW periods. We conducted event-related spectral perturbation comparisons between target and control epochs. We observed a significant increase in delta-band power over medial frontocentral and parieto-occipital sites 2 seconds prior to the self-report, suggesting that external stimuli were suppressed during moments of meta-awareness. These results replicate findings from a previous study using a thought-probe paradigm (Polychroni et al., 2022). However, unlike the previous study that reported an increase in faster oscillatory bands (theta and alpha) seconds prior to the though probe (during MW), we noticed increased alpha-band power over bilateral frontal, central, and right temporoparietal sites 1-2 seconds prior to the estimated onset of MW. We believe this discrepancy stems from the different temporal periods investigated. We looked specifically at the start and end of MW, whereas they examined ongoing MW episodes. Our eye-tracking and EEG findings suggest that our novel self-report task paradigm holds promise for studying the onset and offset of MW. Next, we will study frequency changes throughout the entire reported MW period to gain further insights into its dynamic nature.

Disclosures: H. Sun: None. **A. Olszko:** None. **L. Allen:** None. **N. Singh:** None. **A.M. Shoudt:** None. **J.A. Ruud:** None. **M.D. Rosenberg:** None. **D.C. Jangraw:** None.

Nanosymposium

NANO23: Regulatory Mechanisms and Influences on Neurodevelopment Across Species

Location: MCP Room S103

Time: Monday, October 7, 2024, 8:00 AM - 9:45 AM

Presentation Number: NANO23.01

Topic: A.02. Postnatal Neurogenesis

Support: HORIZON-WIDERA-2023-ACCESS-04-01 under grant agreement 101160180 (PANERIS)
ERASMUS+ Programme Strategic Partnerships (2023-1-PL01-KA220-HED-000160284)
ISN Career Development Grant
IBRO Early Career Award
PTDC/MED-PAT/2582/2021
UIDB/04138/2020
UIDP/04138/2020

Title: Novel targets in Rett syndrome and chronic stress: regulation of adult neurogenesis by cannabinoids and physical exercise

Authors: R. S. RODRIGUES[1,2], D. LOURENCO[1,2], J. B. MOREIRA[1,2], J. M. MATEUS[1,2], A. BARATEIRO[3], S. PAULO[1,2], S. H. VAZ[1,2], F. F. RIBEIRO[1,2], S. SÁ-SANTOS[3], A. M. SEBASTIAO[1,2], A. M. FERNANDES[3], S. SOLÁ[3], L. PINTO[4], C. P. FITZSIMONS[5], ***S. XAPELLI**[1,2];
[1]Inst. de Medicina Mol. João Lobo Antunes, Lisboa, Portugal; [2]Inst. de Farmacologia e Neurociências, Faculdade de Medicina, Univ. de Lisboa, Lisboa, Portugal; [3]Res. Inst. for Medicines (iMed.ULisboa), Fac. of Pharmacy, Univ. of Lisboa, Lisboa, Portugal; [4]Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Medicine, Univ. of Minho, Braga, Portugal; [5]Swammerdam Inst. for Life Sciences, Fac. of Science, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Adult neurogenesis, the process of generating new neurons in the adult brain, is influenced by various physiological and pathological conditions, including Rett Syndrome (RTT) and chronic stress. This work explored novel neurogenic targets involving the regulation of adult neurogenesis by cannabinoids and physical exercise, highlighting potential therapeutic interventions for RTT and stress-related conditions. RTT is a rare neurodevelopmental disorder associated with MECP2 gene mutations that affect neurogenesis and neuronal development. Cannabidivarin (CBDV), a non-psychotropic cannabinoid currently undergoing phase 2 clinical trials for medical use in humans, has been reported to bind to TRPV1. In this work, female RTT mouse models treated with CBDV before symptom onset showed improvements in cognitive deficits and motor coordination, although locomotion and anxiety-like behavior remained unaffected. Interestingly, using the neurosphere assay, CBDV was found to promote cell survival, proliferation, and neuronal differentiation via TRPV1, leading to the cell cycle exit of neural stem/progenitor cells (NSPCs). CBDV-responsive cells exhibited a TRPV1-dependent calcium influx, implicating calcium signaling in NSPC fate and neuronal maturation. These

results highlight a potential therapeutic role for CBDV in RTT and suggest further investigations for repurposing CBDV as a treatment for this disorder. While chronic stress is a significant risk factor for neuropsychiatric conditions like depression, adult hippocampal neurogenesis (AHN) has emerged as a promising target for addressing stress-related disorders. Therefore, we investigated the effects of modulating cannabinoid type 2 receptors (CB2R), which also lack psychotropic effects, in combination with physical exercise (PE) in chronically stressed animals. We found that CB2R inhibition, when combined with PE, significantly ameliorated stress-induced emotional changes and cognitive deficits. This combined strategy also positively influenced AHN dynamics, increasing rates of cell proliferation and differentiation of newborn neurons while reducing neuroinflammation and elevating hippocampal levels of brain-derived neurotrophic factor (BDNF). These findings demonstrate the critical role of CB2Rs in mediating the beneficial effects of PE in counteracting chronic stress. Our work emphasizes the need to understand cannabinoid and physical exercise mechanisms, offering a framework for future therapeutic strategies combining lifestyle interventions with endocannabinoid modulation to treat pathological conditions.

Disclosures: R.S. Rodrigues: None. **D. Lourenco:** None. **J.B. Moreira:** None. **J.M. Mateus:** None. **A. Barateiro:** None. **S. Paulo:** None. **S.H. Vaz:** None. **F.F. Ribeiro:** None. **S. Sá-Santos:** None. **A.M. Sebastiao:** None. **A.M. Fernandes:** None. **S. Solá:** None. **L. Pinto:** None. **C.P. Fitzsimons:** None. **S. Xapelli:** None.

Presentation Number: NANO23.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant RF1MH12460501

Title: Early postnatal growth charts of GABAergic cells and microglia unveil distinct spatiotemporal patterns in the developing mouse brain

Authors: *J. K. LIWANG[1], F. A. KRONMAN[1], J. A. MINTEER[1], Y.-T. WU[2], D. J. VANSELOW[1], Y. BEN SIMON[3], M. TAORMINA[3], S. MANJILA[1], H.-J. PI[1], D. PARMAKSIZ[1], S. WAY[3], H. ZENG[3], B. TASIC[3], L. NG[3], Y. KIM[1];
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Abstract: In the mammalian central nervous system, γ-aminobutyric acid-containing (GABAergic) neurons undergo neurogenesis and migration until programmed cell death occurs during early postnatal development, contributing to the establishment of the brain's inhibitory balance. Microglia, the brain's resident immune cells, also interact with GABAergic neurons during synaptic pruning and circuit maturation. For that reason, disruptions in this system are heavily implicated in neurodevelopmental conditions and disorders. However, our understanding of the typical developmental patterning of GABAergic cells and microglia in various brain regions is limited due to the lack of whole brain cellular resolution datasets during developmental periods. Additionally, we do not have high-resolution 3D atlases of the developing mouse brain to enable integrated analyses of whole brain imaging data. Therefore, we first created high-resolution 3D atlases of the early postnatal mouse brain (epDevAtlas) using whole brain imaging and Allen CCFv3 anatomical labels at postnatal days (P) 4, 6, 8, 10, 12, and

14, and determined the volumetric growth of different brain regions. We utilized 11 different cell type-specific transgenic animals to validate and refine anatomical labels. By implementing epDevAtlas, our findings revealed spatiotemporal heterogeneity in both GABAergic and microglial cell densities across cortical and subcortical brain regions. The developmental trajectory of somatostatin-expressing interneurons differed between sensory and association cortices, whereas vasoactive intestinal peptide-expressing interneuron densities showed no significant changes. In contrast, microglial populations expanded proportionally with brain growth and demonstrated differential spatial distributions during early postnatal brain maturation. Remarkably, microglia showed selective density increases in sensory processing areas that correlate with the emergence of individual sensory modalities. Overall, this study provides valuable insights into the region-specific development of GABAergic circuits, including the involvement of microglia, in the developing mouse brain. Furthermore, we established an open-access resource and web visualization (https://kimlab.io/brain-map/epDevAtlas) with the cell type data and 3D atlases from this project for the scientific community. Our aim is to utilize these cell type mapping methods to characterize other diverse neuronal and non-neuronal cell types, advancing our understanding of brain cell types and their roles in neurodevelopmental and neuropsychiatric disease processes.

Disclosures: J.K. Liwang: None. **F.A. Kronman:** None. **J.A. Minteer:** None. **Y. Wu:** None. **D.J. Vanselow:** None. **Y. Ben Simon:** None. **M. Taormina:** None. **S. Manjila:** None. **H. Pi:** None. **D. Parmaksiz:** None. **S. Way:** None. **H. Zeng:** None. **B. Tasic:** None. **L. Ng:** None. **Y. Kim:** None.

Presentation Number: NANO23.03

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant T32AG000222
NIH Grant T32GM007753
NIH Grant 1F31NS134253-01A1

Title: Pannexin-1 activity regulates fetal cortical development

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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. 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 ionic conductance at depolarized potentials compared to wildtype channels. Mutant channels further display increased ATP flux compared to WT channels. In utero electroporation of mutant PANX1 disrupts cortical histogenesis in both mice and ferrets, and results in aberrant gyration in ferrets. Knock-in of patient mutations in mice demonstrates altered progenitor proliferation, suggesting a role for PANX1 in progenitor cell cycle exit and cell fate determination. Through this study, we aim to further our understanding of how early electrical coupling and ion flux in the cortex shape key developmental processes.

Disclosures: N.K. Hylton: None. **D.J. Kang:** None. **C. Heffner:** None. **S. Golinski:** None. **K. Soriano:** None. **D. Exposito-Alonso:** None. **J.E. Neil:** None. **R. Andersen:** None. **M. Talukdar:** None. **E.M. Degennaro:** None. **S.K. Akula:** None. **S. Murray:** None. **R.S. Smith:** None. **C.A. Walsh:** None.

Presentation Number: NANO23.04

Topic: A.01. Neurogenesis and Gliogenesis

Title: Suppression of 4E-BP-mediated translation disrupts cortical neuron development in mice

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Abstract: Mammalian corticogenesis is a highly complex process specified by precise sequential waves of gene expression. Proper translational control is essential for timely and accurate gene expression after transcription, and dysregulated translational control is implicated in several neurodevelopmental disorders. 4E-BPs are crucial regulators of translation that inhibit the binding of the eukaryotic translation initiation factor eIF4E to the 5’ mRNA cap structure and play critical roles in defining the proteome. However, little is known about the impact of disrupted 4E-BP-mediated translational control in embryonic cortical development. Here, we examined the effects of suppressing 4E-BP-mediated translation on cortical pyramidal neuron morphogenesis and migration in mice. To decrease translation, we expressed a constitutively active 4E-BP1 (4E-BP1F113A) in embryonic migratory neurons destined to become layer 2/3 pyramidal neurons in the medial prefrontal cortex by in utero electroporation. Brains were

collected at postnatal day (P) 0, P14, and P21 for histological analysis of neuronal morphology and placement. Whole-cell patch clamp recording was performed at P21 to examine the impacts on neuronal function. Neurons expressing 4E-BP1F113A exhibited significantly decreased soma size compared to control neurons at P0, P14, and P21. The shape of the 4E-BP1F113A-expressing neurons was more elongated, as evidenced by reduced circularity and roundness, and resembled that of migrating neurons in locomotion mode during corticogenesis. By P0, 88.9% of control neurons were found in the cortical plate, with 31.3% detected in the outermost region (upper cortical layer precursor). In comparison, 65% of 4E-BP1F113A-expressing neurons were found in the cortical plate, with only 6.3% detected in the outermost region. 32% of the 4E-BP1F113A-expressing neurons were found in the subplate and intermediate zone (white matter precursor), while only 6.7% of control neurons were found in these regions. Mispositioned 4E-BP1F113A-expressing neurons were also evident in the P14 and P21 cortices. Consistent with the morphological abnormalities, neurons expressing 4E-BP1F113A displayed decreased capacitance, more depolarized resting membrane potentials, and early action potential firing and depolarization block. Overall, our study shows that constitutive activation of 4E-BP1 disrupts neuronal morphogenesis, migration, and function and highlights an important role of 4E-BP-mediated translation during corticogenesis.

Disclosures: M. Kim: None. **J. Hull:** None. **L.H. Nguyen:** None.

Presentation Number: NANO23.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS113516

Title: Arx regulates multiple aspects of cortical interneuron development

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Abstract: Mutations in aristaless-related homeobox (*ARX*) are associated with a spectrum of neurodevelopmental disorders including developmental epilepsies, intellectual disabilities, and autism spectrum disorders, with or without brain malformations. Aspects of these disorders have been linked to abnormal cortical interneuron (cIN) development and function. To further understand the role of ARX in cIN development, we investigated the function of ARX and its associated gene regulatory network by interrogating multiple *Arx* conditional knockout mice and an *Arx* poly-alanine tract mutant mice. Our data indicate ARX functions in the ganglionic eminence subventricular zone is critical for cIN differentiation and migration. Single cell transcriptomics and ChIP-seq, combined with functional studies, identified ARX-regulated genes important for cIN differentiation and migration. Our data provide new insights into how different mutations in a single transcription factor can result in a spectrum of clinical phenotypes.

Disclosures: Y. Lim: None. **S.K. Akula:** None. **J.A. Golden:** None.

Presentation Number: NANO23.06

Topic: A.10. Development and Evolution

Support: HHMI
NIH TR01 DC018691
NSF GRFP

Title: Cell-type specific, vocal learning specialized gene expression in neurons and glia from zebra finch song nuclei before and after behavior

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Abstract: Vocal Learning, the ability to reproduce heard sounds using a vocal organ, is phylogenetically rare, having independently evolved in several lineages of mammals and birds. In oscine songbirds, vocal learning is produced by a series of discrete brain regions called song nuclei. The primary song nuclei are the robust nucleus of the arcopallium (RA), HVC (proper name), the lateral magnocellular nucleus of the anterior nidopallium (LMAN), and Area X (AX). These song nuclei strongly resemble a mammalian motor control circuit with a descending corticospinal projection (HVC and RA) and a cortico-striatal-thalamic loop circuit (LMAN and AX). Further, the brain regions surrounding the song nuclei are strongly active during locomotion. Decades of work has shown that the song nuclei contain bulk patterns of gene expression not observed in the surrounding locomotor circuit (AR, PVALB, etc.), indicating strong molecular specialization for the production of learned song under baseline conditions. The song nuclei are further specialized in their dynamic response to song behavior, upregulating the immediate early gene DUSP1 which does not similarly respond to locomotion in the surrounds after locomotion. To resolve the gene expression specializations of the oscine song system at the level of specific cell types, here we micro-dissected the four primary song nuclei and associated surround regions from male zebra finches after sleeping overnight, singing, or hopping in a rotating wheel and then performed single nucleus RNA sequencing with the dissected tissues (n=3-5 samples per region*behavior combination). From the sleeping bird data we found that neurons from all song nuclei were strongly specialized with >4,000 significantly differentially expressed genes identified compared to surrounds. Most prominently, transcripts for the RNA binding protein RBFOX1 were dramatically elevated in all four song nuclei. Glia were less broadly specialized, with both oligodendrocytes and astrocytes appearing most specialized in RA. Specifically, RA astrocytes exhibited downregulation of APOA1 and RA oligodendrocytes exhibited upregulation of PCDH15. Integrating the data from the birds following behavior, we identify a second set of >4,000 genes differentially induced by singing in song nuclei compared to the hopping induced gene expression in the surround regions. These results provide new insight in the molecular basis of vocal learning and its evolution in oscine songbirds.

Disclosures: M. Davenport: None. **S. Marcus:** None. **C. Lee:** None. **E.D. Jarvis:** None.

Presentation Number: NANO23.07

Topic: A.10. Development and Evolution

Support: HHMI

Title: High-quality tetrapod genomes reveal convergent and divergent promoter evolution associated with specialized RBFOX1 brain expression in vocal learning bird- and human-lineages

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Abstract: Vocal learning is a convergent trait observed in a few vertebrate lineages, including human and songbirds, and is necessary for spoken language. Concurrent with the present work, a large single nucleus RNA sequencing (snRNAseq) study recently found that the RNA binding protein fox1 homologue (RBFOX1) gene is dramatically upregulated in the neurons of zebra finch which produce this behavior. Further, preliminary cross-species snRNAseq analyses revealed that RBFOX1 has the highest convergent differential expression in human deep layer motor cortex neurons and the analogous songbird RA. Here, we analyzed the high-quality reference genomes to reconstruct the genomic sequence evolution in the RBFOX1 promoters. Using progressive Cactus, we aligned the RBFOX1 gene with its 30kb up/down stream sequences parsed from the annotated genome assemblies of 132 tetrapod species generated by the Vertebrate Genomes Project (VGP) and Telomere-to-Telomere (T2T) consortium. Scanning alternative RBFOX1 promoters across tetrapod animals, we discovered complex lineage-specific evolutionary histories. Considering first the oscine songbirds, we identified diverse A/T homopolymers expanded immediately upstream of a transcription start site (TSS) which were found in all oscine species and absent in the suboscines, parrots, and most other birds. Consistent with this, we observed little-to-no expression of RBFOX1 inside- or outside- the vocal learning regions of the budgerigar brain. Interestingly, in vocal learning hummingbirds, we also found an expanded A/T homopolymer at the exact homologous location as in oscines despite 75 mya separation. Hummingbirds, unlike oscines, also evolved a second A/T homopolymer upstream of a different RBFOX1 TSS which also contained a convergently evolved A/T homopolymer in all mammals. All of these A/T homopolymers are predicted to form FOXP2 binding sites based on transcription factor binding analysis. Considering the human lineage, a third TSS we find human-unique 5x CCG repeats which are absent in apes (2-4x CCG) or the other vertebrates (0-4 CCG). This human 5x CCG expansion produces a novel predicted EGR1 binding site and can be found in the ancient genomes of both Neanderthal and Denisovans. Consistent with this, using a publicly available cross-species snRNAseq dataset we found that this human-specific promoter sequence was associated with increased downstream transcription compared to non-human primates. These findings suggest that the RBFOX1 promoter is a common evolutionary target in vocal learning species that may have led to the highly specialized expressions in vocal learning brain circuits.

Disclosures: C. Lee: None. **M.H. Davenport:** None. **E.D. Jarvis:** None.

Nanosymposium

NANO24: Astrocyte: Neuron Interaction in Health and Disease

Location: MCP Room N426

Time: Monday, October 7, 2024, 8:00 AM - 11:30 AM

Presentation Number: NANO24.01

Topic: B.09. Glial Mechanisms

Support: LUNDBECK NIH GRANT

Title: Optogenetic control of prefrontal state and activity via metabotropic signalling in astrocytes

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Abstract: The neural network dynamics, which underlie our decisions and behaviours are constantly altered by transitions in our cortical states, but how these states emerge and how they regulate the neural computations, remain fundamentally unresolved. Correlative studies have been done, linking neural cortical and subcortical fluctuations to biometric read-outs, such as pupil dilations, but causal behavioral effects of state transitions have been hard to probe due to the lack of non-adversarial optogenetic tools. This project is focus on the prefrontal cortex (PFC), which integrates processed information from higher-order cortico-thalamic pathways with input from subcortical neuromodulators, and then projects regulatory feedback upon other cortical regions. Through this, the PFC state facilitates computational transitions across the brain and supports a variety of functions, including decision-making, fear conditioning and attention. Traditionally, neuromodulators, such as Dopamine and Norepinephrine, have been proposed as key regulators of cortical states, however, recent studies reported intriguing correlations between astrocytic actvity and biometric read-outs for state. As astrocytes respond to neuromodulators via G protein-coupled receptors, and sustains activity with temporal dynamics more consistent with cognitive state transitions, it thus presents itself as an intruiguing link between neuromodulation and PFC state regulation. However, the causal effect of astrocytic activity on the neural network dynamics remain obscure, as the molecular tools for monitoring and manipulating astrocytic cAMP-levels have lacked. Recently, genetically encoded biosensors and optogenetic actuators of cAMP became available, and our lab produced AAV-based molecular tools for monitoring and manipulating astrocytic cAMP. With the combined use of surgically implanted microprims and dual color 2-photon microscopy, we monitored spontaenous cAMP fluctuations in the PFC of behaving mice, and modelled their interplay with the neural dynamics. In addition, we optogenetically elevated astrocytic cAMP-levels to causally assess the exerted effect on these neural network dynamics, while correlating with biometric read-outs and behavior. We observed a causal regulatory effect both on neural ensemble transitions, manifold trajectories, biometric read-outs, as well as the response to aversive stimuli. Hence, we demonstrate that astrocytic cAMP-activity regulates transitions in neural network dynamics through a direct causal effect, and suggests a novel central regulator of cortical state.

Disclosures: R. Herlo: None. **R. Yuste:** None. **H. Hirase:** None.

Presentation Number: NANO24.02

Topic: B.09. Glial Mechanisms

Support: FAPESP #2018/07027-5
FAPESP #2023/01652-3

Title: Astrocytic activation modulates neuronal activity in the Supraoptic Nucleus of the hypothalamus

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Abstract: The regulation of plasma osmolarity is crucial for maintaining homeostasis. Besides peripherical mechanisms that controls hydromineral balance, there are some central osmoreceptors that act as sensors to very small variations in plasma osmolarity, and projects to nuclei that can release hormones on blood vessels to restore osmolarity levels. One of these nuclei is the supraoptic nucleus (SON), located in the hypothalamus, that is composed of osmossensitive magnocellular neurons and releases vasopressin and oxytocin into the circulation. Recent evidence suggests that glial cells, specifically astrocytes, are also involved on SON osmossensitivity and could contribute to magnocellular neuronal excitability. However, the exact mechanism through which astrocytes modulate SON activity remains unclear. Here, we aimed to understand if astrocytes can affect SON neurons excitability and whether osmotic changes impact astrocytic function. Employing whole-cell patch clamp recordings, optogenetics, and two-photon imaging in genetically modified mice, we first observed that specific astrocyte activation increased SON magnocellular neuronal activity. Furthermore, exposure to hypertonic solutions (both high sodium concentration and mannitol solutions) led to elevated intracellular calcium levels in SON astrocytes. To investigate the physiological significance of astrocytes been activated by high sodium concentration, and if astrocytes activation modulates SON neurons activity, one astrocyte and one SON neuron recorded at the same time in an osmotic challenge with sodium overload. It was observed a simultaneous depolarization, with astrocytic depolarization (-89 ± 0.5 mV vs -87 ± 0.6 mV, n = 14; $p < 0.005$) preceding neuronal activation (60 ± 10 sec vs 131± 19 sec; n = 6 pars, $p<0.006$). These findings underscore the role of astrocytes in central osmoregulation, suggesting they might serve as mediators in the neuronal response to high osmolarity situations, thus providing insight into the intricate network regulating plasma osmolarity and homeostasis.

Disclosures: K.M. Santos: None. **M.P. Silva:** None.

Presentation Number: NANO24.03

Topic: B.09. Glial Mechanisms

Title: Astrocytic GPCR Signaling In The Hippocampus Modulates Inferential Fear Memory Through L-Lactate

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Abstract: Humans have the ability to generalize information learned from one experience and apply it to another situation. Both the hippocampus and mPFC play key roles in the encoding of new information using established associative memory schema, but it remains unclear whether this brain region represents or computes cognitive short-cuts to support associative inferential learning and memory. We expand the Pavlovian fear conditioning paradigm to test our hypothesis that following paired auditory-visual stimuli training, the direct visual-foot shock fear condition learning can cause indirect auditory inference fear memory. In this study mice perform experiments using two elements (e.g., A, auditory stimuli and C, electric foot shock) with overlapping elements (e.g., B, visual stimuli) that exist within direct relations (e.g., A-B and B-C). The foundation of transitive inference is the establishment of these direct relations (e.g., A-B, B-C). By recording the neuronal Calcium activity with jGCaMP7s, we show that recruitment of the hippocampus during reasoning increases with the relational demands required for the reasoning process. Moreover, in the testing days, auditory stimulation evoked marked visual cortex activation, indicating that reactivation in the visual cortex supports auditory inferential fear memory retrieval. Given the accumulated evidence suggests the role of astrocytic L-lactate signaling in schema memory, and astrocyte-neuron interactions in brain computation we tested our hypothesis that astrocytic activation of ACC-projecting HPC is necessary for developing inferential auditory fear. We manipulated the HPC astrocytes by expressing the G-coupled receptors hM3q and hM4Di in mice. We show that Gq pathway activation enhanced the inferential fear memory. In contract, Gi pathway activation impair it. Through retrograde virus tracing, we found that the activity of ACC-projecting HPC neurons was enhanced after astrocyte Gq activation during inferring memory retrieval, which was associated with an increase in L-lactate levels in HPC. Moreover, locally injecting L-Lactate into HPC rescued the Gi-impairing inferential memory. In conclusion, our data suggested that the memory is not only the individual records from direct experience of the event, but including the information obtained from multiple independent events. Medial prefrontal cortices projecting hippocampus contributes to reasoning by integrating new experiences into existing memory networks. Astrocytic G-protein-coupled receptor and L-lactate signalling play key roles in modulating the inference reasoning auditory fear.

Disclosures: Z. Fu: None.

Presentation Number: NANO24.04

Topic: B.09. Glial Mechanisms

Support: NIH Grant EY031248
NIH Grant EY030747

Title: Astrocyte subtypes differently regulate differentiation and neurite development of human neural progenitor cell-derived neuronal cells

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Abstract: Astrocytes exhibit multiple functions in the CNS including providing secreted factors that aid in neuronal development and function. Some of these functions of astrocytes also differ depending on their distribution within the CNS. For example, optic nerve head (ONH) astrocytes (ONHAs) are important for maintaining the proper function of the optic nerve and possibly in guiding retinal ganglion cell (RGC) axons to the ONH, which distinguishes them from other astrocytes elsewhere in the retina. At the same time, astrocyte function also changes during development. Human neural progenitor cell-derived neurons were co-cultured with either postnatal day 2 (P2) or adult murine astrocytes sourced from either the ONH or from the retina (without the ONH). Human neural progenitor cells remain undifferentiated and proliferative in the presence of growth factors, such as epidermal growth factor and basic fibroblast growth factor, but they can be terminally differentiated upon growth factor withdrawal. The goal of the present study was to determine, whether different types of astrocytes by themselves can induce the differentiation of neural progenitor cells. Differentiation was quantified by measuring neurite development and extension as well as the expression of neuronal marker proteins, such as neural cell adhesion molecule and βIII tubulin, and of neuritic and synaptic proteins. All of the four types of astrocytes tested induced the differentiation of neural progenitor cells to varying degrees, but co-culture with P2 ONHAs and adult retinal (non-ONH) astrocytes resulted in the most pronounced differentiation and neurite extension. Furthermore, P2 ONHAs upregulated the expression of several neuronal markers. These data suggest that P2 astrocytes secrete factors that more effectively induce the differentiation of neural progenitor cells to a neuronal phenotype and that the co-culture system represents a suitable in vitro model system to study region-specific neuron-glia interactions.

Disclosures: R.S. Duncan: None. **F. Abukunna:** None. **N.J. Frede:** None. **P. Koulen:** None. **K.E. Kador:** None.

Presentation Number: NANO24.05

Topic: B.09. Glial Mechanisms

Support: Natural Sciences and Engineering Research Council of Canada RGPIN-2016-05538

Title: Sex-differences in the contribution of spinal cord astrocytes to the sensitization of ascending pain pathways

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Abstract: Pathological pain is associated with changes in the strength of synaptic connections within ascending nociceptive pathways of the nervous system. In particular, the long-term potentiation (LTP) of synapses between primary sensory neurons and spinal cord dorsal horn neurons has been closely linked to the development of pain hypersensitivity in vivo. More recent investigations have shown that non-neuronal cells play a fundamental role in the regulation of neurotransmission and synaptic plasticity. Within the brain, astrocytes are known to provide neurons with metabolic support necessary to sustain their high energy demands. These cells also produce and release active molecules that directly modulate neuronal activity, and which

contribute to the development of LTP in hippocampal and cortical areas. On the other hand, much less is known about the specific role of astrocytes in the induction of spinal cord LTP, or their role in initiating the sensitization of ascending pain pathways. My thesis project uses a combination of electrophysiological and pharmacological techniques to study how astrocytes regulate synaptic plasticity at the level of the spinal dorsal horn. Acute spinal cord explants were produced from adult mice and transferred to a recording chamber; primary sensory afferents were stimulated with a bipolar electrode, and a recording electrode was inserted into the superficial dorsal horn to measure postsynaptic responses. Explants were then treated with pharmacological inhibitors targeting different aspects of astrocyte function. After drug wash-in, baseline responses were recorded and spinal LTP was induced via low frequency stimulation of presynaptic afferents. Experimental results thus far show that inhibition of astrocytic gap junctions prevents LTP induction in explants isolated from both male and female mice. Interestingly, spinal LTP is partially recovered in the presence of gap junction inhibitors when the recording solution is supplemented with exogenous lactate. In separate experiments, I demonstrate that LTP can also be disrupted by directly inhibiting astrocytic lactate shuttling, but this effect is only observed in male explants. Our results suggest that, in males, spinal cord astrocytes contribute to the development of LTP via the production of lactate, which may serve as a metabolic substrate and/or a signaling molecule for nearby neurons. Future work will focus on addressing the observed sex-differences in spinal lactate shuttling, as well as translating our ex vivo findings into behavioural experiments assessing pain sensitivity in vivo.

Disclosures: D. Rodriguez: None. **R.P. Bonin:** None.

Presentation Number: NANO24.06

Topic: B.09. Glial Mechanisms

Support: Brain Canada Future Leaders
CIHR
NSERC

Title: Diversified astrocyte developmental programs are modulated by primary ciliary signaling

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Abstract: Astrocyte diversity is greatly influenced by local environmental modulation. Here, we report that the vast majority of brain astrocytes across the entire brain possess a singular primary cilium, a specialized signaling antenna localized to cell soma. Comparative single-cell transcriptomics reveals that primary cilia mediate canonical Shh signaling to modulate astrocyte subtype-specific core features in synaptic regulation, intracellular transport, energy and metabolism. Independent of canonical Shh signaling, primary cilia are important regulators for astrocyte morphology and intracellular signaling balance. Dendritic spine analysis and transcriptomics reveal that perturbation of astrocytic cilia leads to disruption of neuronal development and global intercellular connectomes in the brain. Ultimately, mice with primary ciliary deficient astrocytes show behavioral deficits in sensorimotor function, sociability, learning and memory. Our results uncover a critical role for primary cilia in transmitting local cues that drive the region-specific diversification of astrocytes within the developing brain.

Disclosures: J. Guo: None.

Presentation Number: NANO24.07

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01MH127163-01
Department of defense grant W911NF-21-1-0312
The Brain & Behavior Research Foundation (NARSAD Young Investigator Award 28616)
The Whitehall Foundation (2020-08-35)
McDonnell Center for Cellular and Molecular Neurobiology (22-3930-26275U)

Title: Astrocytic signaling mediates the pro-cognitive property of α7nAChRs

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Abstract: The α7 nicotinic acetylcholine receptor (α7nAChR) has become a major drug target in attempts to alleviate cognitive deficits associated with schizophrenia - accounting for a third of all drugs tested in schizophrenia clinical trials over the past 15 years. Yet, the exact cellular and molecular mechanisms by which α7nAChRs support cognitive functions remain elusive, in part due to an exclusive focus on neurons. We previously showed that astrocytic α7nAChRs control the daily availability of D-serine, the endogenous co-agonist of synaptic N-methyl D-aspartate receptors (NMDARs). However, the behavioral relevance of astrocyte-based α7nAChR signaling is unknown, and the respective contribution of astrocytic and neuronal α7nAChR-mediated signaling to cognitive functions has not been explored. Here, we found that mice selectively lacking α7nAChRs from astrocytes exhibited profound deficits across the MATRICS (measurement and treatment research to improve cognition in schizophrenia)-defined cognitive domain, including semantic memory, social cognition, vocalization and spatial learning. This coincided with sharply declined D-serine levels in the brain of these mice and, importantly, supplementing D-serine through drinking water restored behavioral performance. In addition, in line with our past findings that α7nAChR-mediated astrocytic supply of D-serine is time-of-day dependent, we found that the behavioral impairments of astrocyte-specific α7nAChRs KO (Astro-α7KO) mice are absent in the light phase (Zeitgeber Time 6, ZT6). By stark contrast, neuron-specific α7nAChRs KO (Neuro-α7KO) mice showed no behavioral alterations in any assays tested. Interestingly, the deficits of Astro-α7KO mice were also limited to the cognitive domain, with no alterations in ambulation, sleep architecture, or other behaviors canonically associated with the negative symptoms of schizophrenia. Using two-photon laser scanning microscopy, we show that α7nAChR stimulation with a specific agonist (PNU-282987) increased calcium activity in astrocytes, which was abolished in the presence of a selective antagonist (methyllycaconitine, MLA) or in Astro-α7KO mice, consistent with the high calcium permeability of α7nAChRs. Conversely, calcium activity in Astro-α7KO mice was diminished compared to control and Neuro-α7KO mice, together unveiling the contribution of α7nAChRs to astrocyte intracellular dynamics. Taken altogether, our findings fuel a model in which α7nAChRs exert their pro-cognitive properties via astrocytic signaling, rather than neuronal

signaling, which could have rippling consequences on α7nAChR-targeted therapeutic approaches.

Disclosures: Y. Wu: None. **K. Lefton:** None. **S. Walsh:** None. **T. Papouin:** None.

Presentation Number: NANO24.08

Topic: B.09. Glial Mechanisms

Support: NSF HRD 1401026
NSF IOS 1755341
NSF IOS 1755033

Title: Expression and Function of SNARE Proteins in Chick Embryonic Astrocytes in Culture

Authors: *T. I. AMANFO[1], V. A. TALABATTULA[2], M. T. MOORE[3], R. DZAKPASU[4], M. TEMBURNI[2];
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Abstract: Expression and Function of SNARE Proteins in Chick Embryonic Astrocytes in Culture Tobenna Amanfo[1], Venkata Ajay Narendra Talabattula[1], Michael Moore[2], Rhonda Dzakpasu[3,4] andMurali Temburni[11]Delaware Center for Neuroscience Research and Department of Biological Sciences, Delaware StateUniversity, Dover DE 19901[2]Delaware Institute of Science and Technology, OSCAR Imaging Facility[3]Department of Physics and [4]Department of Pharmacology and Physiology, Georgetown University,Washington, DC 20057Astrocytes have been shown to modulate network activity by releasing gliotransmitters like glutamate,D-serine, and ATP. Glutamate sensing at tripartite synapses via mGluRs elevates local calcium withinthe astrocyte. With sufficient activation, the localized calcium elevation crosses a threshold causing acalcium-induced calcium release (CICR) within the astrocyte leading to glutamate exocytosis..Although there is some evidence the presence of SNARE proteins in astrocytes is not fully confirmed.We demonstrate the presence of the vesicular SNARE protein Synaptobrevin/VAMP2 in chickembryonic optic tectum and forebrain astrocyte cultures in vitro. To test the function of VAMP2 inastrocytes, we expressed a truncated VAMP2 peptide which lacks the vesicular membrane domainalong with the extracellular glutamate sensor iGluSnFR using a lentiviral vector. This truncatedVAMP2 peptide is expected to act as a dominant negative, preventing the docking of thegliotransmitter vesicles to the membrane. Our results demonstrate that blocking VAMP2 diminishedglutamate release from astrocytes in culture upon treatment with Ionomycin, a calcium ionophore. Wealso demonstrate the presence of another SNARE complex protein, Syntaxin1 in these chickastrocytes.This study is supported by NSF Research Initiation Award (HRD 1401026) and NSF IOS NeuralSystems Awards (IOS 1755341 and 1755033).

Disclosures: T.I. Amanfo: None. **V.A. Talabattula:** None. **M.T. Moore:** None. **R. Dzakpasu:** None. **M. Temburni:** None.

Presentation Number: NANO24.09

Topic: B.09. Glial Mechanisms

Support: National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006)—A Multiscale integrated approach to the study of the nervous system in health and disease (DD 1553, 11.10.2022)
National Recovery and Resilience Plan (NRRP), project CN00000041– National Center for Gene Therapy and Drugs based on RNA Technology (DD no. 1035, 17.06.2022)
AFOSR AstroDyn (FA9550-19-1-0370)
AFOSR AstroColl (FA9550-21-1-00352)
Marie Skłodowska-Curie Actions -ITN-2020 ASTROTECH (GA956325)

Title: Aquaporin4-supramolecular level of aggregation influences collective migration of astrocytes

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Abstract: The water channel AQP4 is natively expressed in brain astrocytes as aggregates of tetramers, called Orthogonal Arrays of Particles (OAPs). While it has been reported that AQP4-mediated water flows can trigger cytoskeleton biomechanically at the individual cell level, much is still unknown regarding its role in collective cell migration. To address this, we investigated the migratory ability of primary cultured brain astrocytes isolated from both WT and OAP-null mouse models, under control and pro-inflammatory conditions upon IL-1β/TNF-α-treatment. We also examined water transport rates and gap-junctional interconnectivity. We found that reactive astrocytes exhibit low levels of gap junction (GJ)-forming protein connexin-43 (Cx43) and a significant dysregulation of the cytoskeleton network compared to controls, which instead displayed similar connectivity and intact cortical actomyosin fibers. Reactive and OAP-null control astrocytes exhibited reduced water transport rates compared to WT controls. Wound healing assays revealed that under control conditions OAP-null cells almost entirely healed the scratch, unlike WT cells, while reactive astrocytes remained nearly immotile, independently of genotype. Particle Image Velocimetry (PIV) analysis revealed distinct migratory behaviors: under control conditions, both genotypes moved horizontally, but OAP-null cells displayed a unique ability to efficiently close the gap with lined-up fronts and no cell repulsion. In contrast, reactive astrocytes remained immobile, with WT cells displaying random protrusions and OAP-null cells moving slightly backward. Similar results were observed in transfected GL261 glioma cells, where OAP-transfected cells exhibited reduced migration and invasiveness in wound healing and Boyden chamber assays, along with decreased MMP9 activity. Overall, this study highlights the role of AQP4 level of aggregation in modulating collective cell motion, independent of GJ connectivity and water membrane kinetics. Furthermore, by generating an AQP4-deficient, non-migrating, and weakly coordinated gliotic phenotype it sheds light on the effects of chronic gliosis *in vitro* and identifies potential new target mechanisms for diagnosising or treating brain tumors.

Disclosures: B. Barile: None. **N.J. Mennona:** None. **G. Mogni:** None. **M.G. Mola:** None. **K.M. O'Neill:** None. **A. Cibelli:** None. **A. Memeo:** None. **V. Benfenati:** None. **W. Losert:** None. **G.P. Nicchia:** None.

Presentation Number: NANO24.10

Topic: B.09. Glial Mechanisms

Support: Leon Levy Fellowship in Neuroscience
NIMH R01 MH110136
NIMH R56 MH115281
NIMH R01 MH108962
NIMH T32 MH019524
NINDS U19 NS107616
NINDS R25 NS107178

Title: Endogenous Protective Mechanisms of Astrocyte Networks

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Abstract: In the central nervous system (CNS), metabolic resources in astrocytes promote tissue survival in response to focal neuronal stress. However, our understanding of the extent to which these resources are mobilized remains incomplete. Previously we found that during neurodegeneration in glaucoma, astrocyte gap junctional networks expand to link over 1 cm of adjacent cells and redistribute bioenergetic resources from unstressed to stressed tissue. While resource donation improves axon function and visual acuity, it renders the donating tissue susceptible to further stress (Cooper et. al., 2020, PNAS). Now, we are focused on revealing the breadth and potential specificity of astrocyte networks to identify which CNS regions support each other - possibly across larger spatial networks than afforded by initial investigations in the visual system. Through the development of a novel astrocyte-network-tracing AAV, we use whole-brain tissue clearing to examine the global reach of astrocyte gap junctional networks originating from small populations of astrocytes. We infected astrocytes via stereotaxic injections in the prefrontal cortex (PFC), barrel cortex, motor cortex, visual cortex, somatosensory cortex, hippocampus (HPC), and striatum. Although we have not yet found a significant sex difference (C57/BL6 mice; n > 55 each males and females), we do find that astrocyte networks originating in different brain regions exhibit vastly different volumes (n > 5 stereotaxic injections per brain region, always infecting only one region per mouse; networks range from 3-20x the initially infected tissue volume, but are consistent when the same brain region is infected in different mice). Surprisingly, astrocyte networks do not link all cells adjacent to the initially infected area. Instead, they link specific brain regions, even ones over 1cm apart. For example, astrocytes in motor cortex communicated through a chain of astrocytes connected by gap junctions as far as contralateral visual cortex. We have confirmed that the network mechanism is limited to gap junctions by infecting astrocyte-specific conditional double

Cx30/43 gap-junction knockout mice (*Slc1a3cre-ERT2 x Cx43fl/fl x Cx30fl/fl*; HPC, PFC, barrel cortex, and striatal networks are restricted to infected regions in cKO but not littermate controls; n = 4 mice per condition). Further, astrocyte networks are plastic. Following whisker trim, barrel cortex astrocyte networks shrink by over 50% in volume (n = 5 mice per condition). We hope this work functions as a platform for future exploration of astrocytes beyond individual cells, but as networks influencing homeostatic function and disease progression.

Disclosures: M.L. Cooper: None. **M. Selles:** None. **B. Lai:** None. **A.S. Saab:** None. **S.A. Liddelow:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AstronauTx Ltd.. F. Consulting Fees (e.g., advisory boards); BioAccess Fund, Tambourine, Synapticure. **M.V. Chao:** None.

Presentation Number: NANO24.11

Topic: B.09. Glial Mechanisms

Support: R01NS129788
Mission Connect 022-105
Paula and Rusty Walter and Walter Oil & Gas Corp Endowment at Houston Methodist

Title: Human Astrocytes Accelerate and Maintain Synchronized Network Activity Across Neural Organoid Ensembles

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[1]Dept. of Neurosurg., [2]Houston Methodist Res. Inst., Houston, TX; [3]Biol., Indiana Univ. Purdue Univ. Indianapolis, Houston, TX

Abstract: It is well established that rodent astrocytes promote neural network maturation in the nervous system through a combination of pro-synaptogenic components and neuroprotective mechanisms. Do human astrocytes have similar capabilities in promoting human neural network activity? To address this question, we optimized and tested a human-specific model with bioengineered neural organoids composed of rapidly matured astrocytes and glutamatergic neurons from human pluripotent stem cells (hPSCs) (i.e., Asteroids, Cvetkovic et. al.). We found that Asteroids more rapidly exhibit organoid-wide synchronous burst activity compared to neuron-only organoids as measured by GCaMP-based calcium imaging and multi-electrode array (MEA) analysis. To determine which astrocyte-derived components underlie this effect, we tested candidates identified in RNAsequencing and proteomic screens of Asteroid-conditioned media. A subset of these candidates was sufficient to accelerate neural network activity of neuron-only organoids. Next, we tested whether astrocyte-mediated neuroprotection maintains network activity via improved viability. We found that Asteroids have improved viability in suboptimal media and are protective against glutamate-induced excitotoxicity when compared to neuron-only organoids. Given improved network formation and maintenance, we tested if astrocytes enable generation of functionally interconnected ensembles of organoids. Ensembles of multiple interconnected Asteroids (i.e., Asteroid Belts) revealed synchronous burst activity within 24 hours with physical projections extending into neighboring tissue. Taken together, our work highlights the importance of astrocytic mechanisms in rapidly generating and maintaining human neural networks. The robust and rapid functional interconnectivity of neural networks

delivers a new human *in vitro* experimental approach that is expected to extend to models of various distinct regional networks and disease context.

Disclosures: M.D. Patel: None. **S. Lavekar:** None. **P.J. Horner:** None. **R. Krencik:** None.

Presentation Number: NANO24.12

Topic: B.09. Glial Mechanisms

Support: F31NS124107
R01HD1000074
R01DA048822

Title: Estradiol mediates astrocyte-neuron communication in the hippocampus

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Abstract: <There is emerging research about the effects of neurotransmitters on astrocytes and their subsequent release of gliotransmitters. However, little is known about how other signaling molecules, such as hormones, impact astrocyte signaling. Estradiol (E2) is an important hormone that regulates neuronal activity and brain function. However, whether E2 specifically signals to astrocytes in situ and the functional consequences on astrocyte-neuron communication remains unknown. The present study analyzed the impact of estradiol signaling on astrocyte responsiveness and the regulation of astrocyte-neuron communication in the hippocampus. The study pays particular attention to possible variations that may exist depending on the sex of the animal. Using RNAscope, we determined that estrogen receptors (ERα and ERβ) are expressed in hippocampal astrocytes in both female and male mice. Through a combination of confocal calcium imaging and electrophysiological recordings we have shown that hippocampal astrocytes respond to E2 with calcium elevations and subsequent glutamate release that activates NMDA receptors in pyramidal neurons in both sexes. Furthermore, E2 signaling modulates synaptic transmission. Taken together, these results demonstrate the existence of estradiol-mediated astrocyte-neuron communication, revealing that E2 interacts with astrocytes and play a role in regulating brain physiology.>

Disclosures: J. Goenaga: None. **C. Perez de Nanclares:** None. **M. Hall:** None. **P. Kofuji:** None. **P.G. Mermelstein:** None. **A. Araque:** None.

Presentation Number: NANO24.13

Topic: B.09. Glial Mechanisms

Support: CIHR PJT-173468

Title: Relationship between astrocyte calcium and cerebral blood flow changes during awake-sleep cycles

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Abstract: Changes to free astrocyte calcium regulates local cerebral blood flow. Astrocyte calcium activity also changes during awake-to-sleep transitions, yet whether astrocytes help regulate the changes to cerebral blood flow that are observed when mice switch between awake, NREM and REM sleep states, remains unclear. Using in vivo two-photon fluorescence imaging in head-fixed, unanesthetized mice (10-12 weeks old), we monitored astrocyte calcium activity and diameter changes of penetrating arterioles in the retrosplenial or the barrel cortex to better understand how astrocyte calcium signals correlate with cerebral blood flow during awake-sleep cycles. We used a chronic cranial window approach with bone removal and brain nano-injection of AAV2/5 to express jGCaMP8m to astrocytes (GfaABC1D promoter, 4 weeks recovery). To determine the physiological state, we continuously measured the size of the pupil and the movement of whisker during two-photon imaging, which, when assessed in combination, are a reliable indicator of the awake state (dilated pupil with active whiskers) and sleep (small pupil with less active whiskers). We commenced imaging when sleep pressure was highest (7-8am). Under this condition, each mouse regularly fell asleep after approximately 1-1.5 hours under the microscope, followed by periods of alternating wakefulness and sleep occurrences every few minutes. We found in the quiet awake mouse, the magnitude of penetrating arteriole diameter and endfoot Ca^{2+} changes were weakly correlated. However, vessel diameter and astrocytes calcium activity concomitantly increased when each mouse woke up, revealing a strong correlation, with no sex difference. Cross-correlation analysis of diameter versus endfoot Ca^{2+} revealed the strongest correlation was positive X=0 (no time delay). However, we failed to see the enhanced hemodynamic changes when the mouse falls asleep previously reported. Specific IP3R2 knockdown in astrocyte reduce the increase of the endfoot Ca^{2+} level and the magnitude of arteriole diameter from sleep to awake transitions partially. Together, our data demonstrate cerebral blood flow regulation during sleep to awake cycle is functionally linked with IP3R2 signaling in astrocyte.

Disclosures: F. Wang: None. **G. Peringod:** None. **G. Hoang:** None. **J. Sun:** None. **G.R. Gordon:** None.

Presentation Number: NANO24.14

Topic: B.09. Glial Mechanisms

Support: R15MH130900

Title: Sex-dependent astrocyte reactivity: chronic stress-induced changes across brain regions

Authors: *M. T. MANNERS;
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Abstract: Chronic stress is a major precursor to various neuropsychiatric disorders and is closely associated with increased inflammation in the brain. However, the bidirectional association between inflammation and chronic stress has yet to be fully understood. Astrocytes are one of the key inflammatory regulators in the brain. The morphological change of reactive astrocytes serves

as an important indicator of the presence of inflammation in the brain. In this study, we evaluated the sex-specific astrocyte response to chronic stress or systemic inflammation in key brain regions. We conducted the Unpredictable Chronic Mild Stress (UCMS) paradigm to model chronic stress, or lipopolysaccharide (LPS) injection to model systemic inflammation. To evaluate morphological changes in astrocytes due to chronic stress or inflammation, we quantified branch bifurcation and branch terminals. The ramification index and Sholl analysis were utilized to assess astrocyte arborization and complexity. Chronic stress-induced morphological changes in astrocytes, similar to systemic inflammation in all key brain regions. The effects of chronic stress were region and sex specific. Increased astrocyte activation was observed in CA1, CA3, and the hypothalamus in females, while LPS promoted increased astrocyte inflammation in the amygdala in females. These findings indicate that chronic stress induces a proinflammatory environment, resulting in greater astrocyte activation in specific brain regions in females, potentially contributing to sex-dependent mechanisms of disease.

Disclosures: M.T. Manners: None.

Nanosymposium

NANO25: Diagnostics and Biomarkers for Parkinson's Disease

Location: MCP Room N228

Time: Monday, October 7, 2024, 8:00 AM - 9:45 AM

Presentation Number: NANO25.01

Topic: C.03. Parkinson's Disease

Title: The phospho-ubiquitome and biomarkers for neurodegeneration and aging

Authors: S. H. MAJER[1], D. E. PADYKULA[1], J. C. PEDROZA[1], K. W. WALLACE[1], K. J. STINE[1], *M. S. GOLDBERG[2], K. KADIMISETTY[1];
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Abstract: Identification of blood-based biomarkers is the holy grail in the field of neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's diseases (AD), and aging. Currently there are no FDA approved blood biomarkers for PD. Recent research has uncovered a fascinating link between the inability of cells to resolve misfolded proteins or aggregates by ubiquitin proteasome system (UPS) and mitochondrial dysfunction, two major pathological mechanisms in PD and AD. Upon mitochondrial damage by aggregated proteins, oxidative or mutational stress, cells generate copious amounts of phosphorylated ubiquitin (pSer65-Ub) chains on mitochondria which acts as a signal for lysosomal clearance of damaged mitochondria. This process is called mitophagy. While mitochondria localized PINK1 kinase is responsible for phosphorylation of ubiquitin, Parkin E3 ligase amplifies the pSer65-polyubiquitin signal. Mutations to either of the enzymes have been linked to PD. A failure in neuronal mitophagy can reflect as elevated pSer65Ub level in plasma making it an attractive and novel biomarker. We have developed high-affinity, pSer65-ubiquitin selective binding tools that efficiently capture all phospho-ubiquitin chain topologies. Using these novel tools we have developed highly sensitive assays for detecting pSer65Ub in various samples including PD

patient plasma. We have validated the application of this tool in biomarker discovery and demonstrate its superiority compared to previously reported pSer65-Ub antibodies. Further, we identify and propose a panel of phospho-ubiquitinated proteins as potential biomarkers for PD. We believe that the "phospho-ubiquitome" is central to mitochondrial health and understanding neurodegeneration pathologies, aging and its application can change therapeutic and diagnostic landscape.

Disclosures: S.H. Majer: A. Employment/Salary (full or part-time):; LifeSensors Inc. **D.E. Padykula:** A. Employment/Salary (full or part-time):; LifeSensors Inc. **J.C. Pedroza:** A. Employment/Salary (full or part-time):; LifeSensors Inc. **K.W. Wallace:** A. Employment/Salary (full or part-time):; LifeSensors Inc. **K.J. Stine:** A. Employment/Salary (full or part-time):; LifeSensors Inc.. **M.S. Goldberg:** None. **K. kadimisetty:** A. Employment/Salary (full or part-time):; LifeSensors Inc..

Presentation Number: NANO25.02

Topic: C.03. Parkinson's Disease

Support: NINDS RF1NS129567, R01NS100876

Title: Analysis of alpha-synuclein harvested from intra-cranial instruments used in deep brain stimulation surgery for Parkinson's disease

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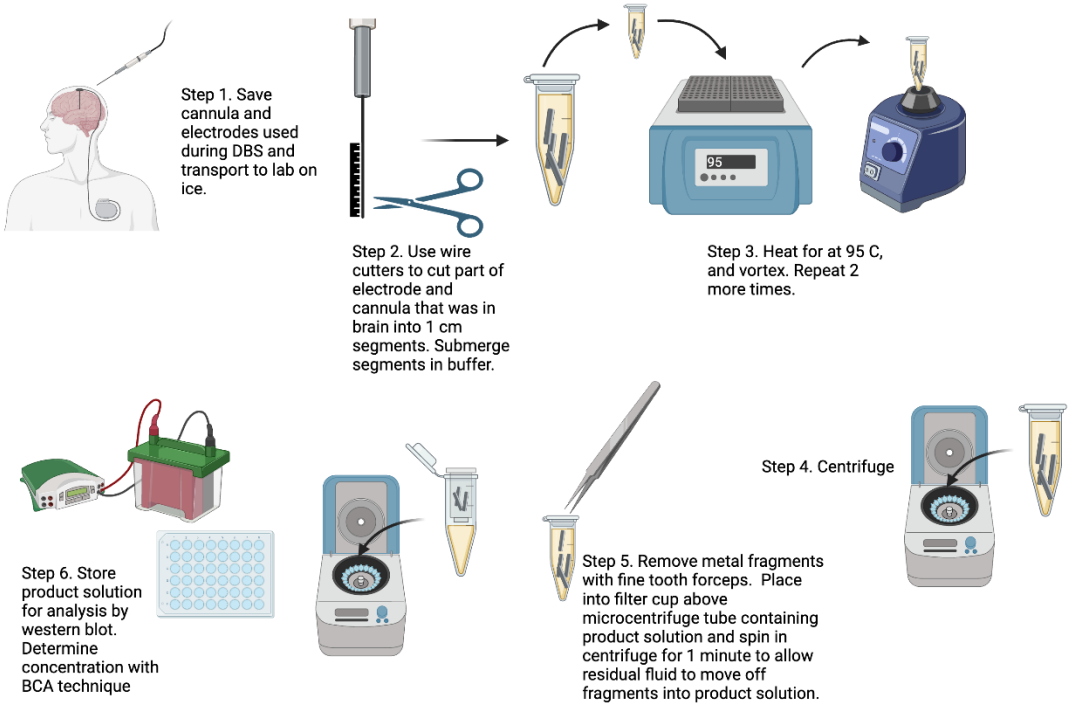
Abstract: Background: The protein alpha-synuclein (αSyn) forms pathologic aggregates in Parkinson's disease (PD) and is implicated in mechanisms underlying neurodegeneration in this disorder. While pathologic forms of αSyn have been extensively studied, there currently exists no method to evaluate αSyn within the brains of living patients with PD. Patients with PD are often treated with deep brain stimulation (DBS) surgery in which surgical instruments are in direct contact with neuronal tissue containing pathologic αSyn; herein, we describe a method by which residual tissue can be purified from DBS surgical instruments in PD and essential tremor (ET) patients and demonstrate that pathologic αSyn is robustly detected. **Results:** 24 patients undergoing DBS surgery for clinically diagnosed PD (17 patients) or ET (7 patients) were enrolled, and two methods were developed for tissue purification. Using an SDS based method, 81.2 ± 44.8 µg protein per sample (n=15) is able to be purified from DBS surgical instruments, with immunoblot assays specific for αSyn reactive in all tested samples. Using a PBS based purification method, light microscopy was used to evaluate the histologic identity of tissue, revealing axons and capillaries as the primary components of purified tissue (n=3). Further analysis of purified αSyn was conducted using western blot, demonstrating that truncated αSyn (1-125 αSyn) was significantly increased in PD (n=5) compared to ET (n=3), in which αSyn misfolding is not expected (signal intensity 0.64 ± 0.25 vs. 0.25 ± 0.12, P = 0.046), thus showing that a known pathologic form of αSyn can be reliably purified from living PD patients with this method. **Conclusions**: We develop a method by which pathologic αSyn can be purified from brain tissue for the first time in living patients; the methods developed herein have diagnostic

importance to confirm the presence of pathologic αSyn and may be useful in identifying therapeutic targets.



Disclosures: Z. Sorrentino: None. **B.I. Giasson:** None. **J. Hilliard:** None.

Presentation Number: NANO25.03

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS Grant NS118146
NIH/NIDNS Grant NS127211

Title: Multi-omic analysis identifies molecular markers associated with gastrointestinal dysfunction in parkinson's disease

Authors: M.-Y. LAI[1], M. ROSENE[1], S. CÁRDENAS ROMERO[1], A. LOPEZ CADAVID[1], S. KULKARNI[2], ***B. A. BENITEZ**[3,1];
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Abstract: Background: Gastrointestinal (GI) symptoms preceding the diagnosis of Parkinson's disease (PD). The underlying molecular changes associated with GI dysfunctions remain unclear. Evidence of extra-CNS and putative gut origin of pathological p-α-synuclein in humans and gut-to-brain transmission of p-α-synuclein have been found in mice models. This study analyzed multi-omic data and self-reported Scales for Outcomes in PD - Autonomic Dysfunction

(SCOPA-AUT) collected from the Parkinson's Progression Markers Initiative cohort to identify molecular signatures linked to GI symptoms in PD patients.
Methods: We first calculated the GI severity for each of the 1260 PD patients using the SCOPA-AUT questionnaire Q1-7. We then utilized longitudinal clustering with k-means design (R-kml) and the CopyMean imputation to identify subgroups with distinct trajectories of GI symptoms over five years. Subsequently, we identified molecular markers associated with GI severity using R-limma, including proteomics in cerebrospinal fluid and urine, transcriptomics in whole blood, and metabolomics in plasma. We also compared the rate of progression between high and low GI severity for UPDRS and MoCA scales using ordinal linear mixed regression and DATscan measurements of caudate, putamen, and striatum volume using linear mixed regression.
Results: We found that PD patients with SNCA mutations exhibited faster GI severity progression ($p = 4.9x10-9$) than sporadic PD or those with LRRK2 or GBA1 mutations. PD patients exhibit two subclusters with distinct trajectories of GI severity. We observed a lower volume of caudate ($p = 0.008$) and striatum ($p = 0.04$) at baseline in high GI severity. The progression rates for UPDRS-I ($p = 8.8x10-5$), II ($p = 4.5x10-6$), III ($p = 0.0012$), and MoCA ($p = 2.8x10-8$) in higher GI severity significantly worsen faster than in lower GI severity. We also found ten metabolites in three categories: PE, galactosylceramide, alpha-tocopherol, and five transcripts (IFI27, CCL2, USP18, EREG, and CCL8) associated with high GI severity, showing high expression of coding gene in the colon, stomach, and small intestine. The predictive model using DATscan and GI as factors can classify PD cases and healthy controls (AUC = 0.98).
Conclusions: We identified novel molecular markers significantly associated with the severity and progression of GI dysfunction in PD patients. We have also established a clear link between GI dysfunction, motor impairment, and cognitive decline. These findings underscore the potential of our multi-omic approach in identifying PD patients who are at a higher risk of developing severe GI disease.

Disclosures: M. Lai: None. **M. Rosene:** None. **S. Cárdenas Romero:** None. **A. Lopez Cadavid:** None. **S. Kulkarni:** None. **B.A. Benitez:** None.

Presentation Number: NANO25.04

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation, Ken Griffin Alpha Synuclein Award

Title: Discovery and First In Human Proof of Concept Studies for MK-7337, A Novel Alpha-Synuclein PET Ligand

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Abstract: Objectives: The accumulation of aggregated alpha-synuclein in the form of Lewy bodies and Lewy Neurites is a pathological hallmark of Parkinson's disease (PD). Currently, alpha-synuclein deposition can only be studied at autopsy or inferred from plasma/CSF levels. Identification of an alpha-synuclein PET ligand would enable *in vivo* quantification of disease

progression and may improve feasibility for long duration disease modification clinical trials in PD. However, high-affinity and selective alpha-synuclein PET ligand candidates do not currently exist. In collaboration with the Michael J. Fox Foundation, and the Ken Griffin Alpha-Synuclein Imaging Award, we sought to advance a viable alpha-synuclein PET tracer candidate towards clinical evaluation in a patient population. Methods: Discovery of MK-7337: Radioligand saturation and competitive binding experiments were conducted in postmortem PD and Alzheimer's disease brain tissue to profile PET candidate affinity and selectivity. Autoradiography experiments in postmortem brain tissue was performed on lead radioligand candidates to validate binding to pathological alpha-synuclein. PET imaging experiments were performed in aged A30P alpha-synuclein overexpressing transgenic mice to assess in vivo brain binding potential. *In Vivo* PET imaging experiments were performed in non-human primates to determine tracer utility for human studies. First in human proof of concept study: PET imaging experiments were conducted to determine if alpha-synuclein pathology could be imaged in idiopathic PD patients using a high-affinity PET tracer. PET imaging studies were conducted in a cohort of idiopathic PD (n=8) and age matched healthy volunteers (n=4) previously enrolled in the MJFF PPMI trial. Results: Collectively, experimental data describe the identification and preclinical in vitro and in vivo profiling of MK-7337, a high affinity alpha-synuclein PET ligand with sub-nanomolar potency and selectivity for alpha-synuclein. First in human trial data will be presented describing the performance of MK-7337 in idiopathic PD patients and age matched healthy volunteers as well as path forward for validation of alpha-synuclein PET ligand tracers.

Disclosures: R. Drolet: A. Employment/Salary (full or part-time):; Merck & Co, Inc. **Z. Zeng:** A. Employment/Salary (full or part-time):; Merck & Co, Inc. **K. Riffel:** A. Employment/Salary (full or part-time):; Merck & Co, Inc. **M.J. Stenslik:** A. Employment/Salary (full or part-time):; Merck & Co, Inc. **L. Ma:** A. Employment/Salary (full or part-time):; Merck & Co, Inc. **J.N. Marcus:** A. Employment/Salary (full or part-time):; Merck & Co, Inc. **S.M. Smith:** A. Employment/Salary (full or part-time):; Merck & Co, Inc. **J.M. Uslaner:** A. Employment/Salary (full or part-time):; Merck & Co, Inc.

Presentation Number: NANO25.05

Topic: C.03. Parkinson's Disease

Support: MJFF 2022 Andrew West

Title: Phosphorylated Rab10 Predicts Inflammation and Disease Severity in Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects millions of people worldwide. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been implicated in both familial and sporadic cases of PD. LRRK2 protein phosphorylates Rab10, a highly-expressed Rab protein involved together with LRRK2 in mediating different types of immunological responses. However, largely due to technical limitations in analyzing phosphorylated-Rab10 in disease relevant cells and tissues, the role of phospho-Rab10 in PD progression, risk, and changes with different disease states has been unclear but could help define the role of LRRK2 in disease risk and progression. In this study, we have developed and

validated an ultra-sensitive single-molecule array assay to assess extracellular LRRK2, Rab10, and phosphorylated Rab10 (pT73-Rab10) levels in low volumes of serum and cerebrospinal fluid (CSF) in mouse and rat models of PD, and in biobanked PD and control samples. Multivariable and weighted correlation network analyses were used to identify genetic, transcriptomic, clinical, and demographic variables that predict the extracellular pT73-Rab10 to total Rab10 ratio. We find that pT73-Rab10 is absent in serum from Lrrk2 knockout mice but elevated by LRRK2 mutations. Bone-marrow transplantation experiments in mice show that serum pT73-Rab10 levels derive primarily from circulating immune cells. Additionally, the ratio of pT73-Rab10 to total Rab10 is elevated in idiopathic Parkinson's Disease (iPD) patients with greater motor dysfunction, irrespective of disease duration, age, sex, or the usage of PD-related or anti-inflammatory medications. pT73-Rab10 to total Rab10 ratios are associated with neutrophil activation, antigenic responses, and the suppression of platelet activation. Overall, the extracellular ratio of pT73-Rab10 to total Rab10 in serum is a novel biomarker for LRRK2-linked innate immune activation associated with disease severity in iPD. We propose that those iPD patients with higher serum pT73-Rab10 levels may benefit from LRRK2-targeting therapeutics to mitigate associated deleterious immunological responses. Overall, this will shed light on the role of Rab10 phosphorylation in PD and inform development of therapeutic strategies targeting the LRRK2 pathway.

Disclosures: H. Li: None. **A.B. West:** None.

Presentation Number: NANO25.06

Topic: E.06. Posture and Gait

Title: Machine learning for the diagnosis of vestibular schwannoma patients - promising results, expected challenges and experiment design recommendations

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Abstract: Proper diagnosis of vestibular schwannoma (VS) patients is an imperfect process, requiring multiple clinical tests with varying degrees of accuracy. To address these limitations, we propose a novel machine learning (ML) approach based on kinematic recordings as a diagnostic tool for VS. We collected kinematic recordings of 32 VS patients (ages 26-81), and 32 healthy controls (ages 25-74) while they performed both an easy (regular walk) and difficult task (tandem walk). Each subject was equipped with inertial measurement units placed on ankles and head, recording both 3-dimensional linear acceleration and angular velocity. The gait kinematics were segmented to produce individual gait cycles, each of which would define a sample as input to the model. We used these cycles to train different ML models, all with the same convolutional neural network backbone, wherein we compared the effect of using different combinations of tasks and sensors as inputs, showing the importance of each parameter on the classification ability of the model. We used the average accuracy by subject over all their cycles as a metric of classifier accuracy (n = 32 iterations of the same model with leave-one-out cross-validation). When using data from the ankle sensor, mean accuracy values went from 72.28% (std = 5.80) to 76.43% (std = 4.48) when using regular vs tandem walk. Interestingly, the regular walk task performed much better at classifying healthy controls than patients (87.14% vs 51.10%

respectively), while the tandem walk had slightly closer values between groups (82.54% vs 58.79%). However, when using data from the head sensor, the regular walk significantly outperformed tandem (69.06%, std = 5.31 vs 60.51%, std = 6.70 respectively). Thus, while ankle sensors may accurately capture more information relating to pathology-related movements with tandem walk compared to regular walk, head sensors seem unable to retain such information during that difficult task. We see promising results for this first stage of development of ML models for this type of clinical assessment, especially when considering the extremely low number of subjects (64) for an ML technique. Our results showcase the highly impactful decision of sensor placement and task selection for the use of kinematic data as input for diagnostic tools. Given the increasing interest in ML tools for diagnostic practices, our study serves as guidance for future endeavors. This proof of concept validates the use of machine learning (ML) for vestibular syndrome (VS) diagnosis, highlighting its potential to enhance current clinical assessments by offering a more objective and precise tool, complementing the expertise of clinicians.

Disclosures: S. Sánchez Manso: None. **L.C. Kohler Voinov:** None. **R. Aryan:** None. **K.E. Cullen:** None.

Presentation Number: NANO25.07

Topic: E.06. Posture and Gait

Title: Cerebellar neuromodulation led to improved standing balance in older adults

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Abstract: The cerebellum (CB) is critical for maintaining upright balance. It undergoes significant age-related structural changes and altered interactions with the primary motor area (M1) that are related to poor standing balance and walking performance in older adults. Prior work on non-invasive brain stimulation to CB has led to improved motor learning, skill acquisition, and locomotor adaptation in healthy young adults. However, these studies did not investigate the implications of CB stimulation on improving balance control in older adults. The purpose of this study was to investigate whether increasing CB excitability led to improved standing balance in older adults. Further, as a secondary objective, we also explored the changes in the neurophysiological mechanisms after CB stimulation by investigating the CB-M1 interactions. We used a sham-controlled study to investigate the effects of intermittent theta burst stimulation using transcranial magnetic stimulation (TMS) on five older adults. We measured standing balance control through the center of pressure (COP) area and CB-M1 interactions using cerebellar brain inhibition (CBI), immediately before and at different follow up points (immediately, 15 min, 30 min) after CB stimulation. The site of stimulation was 3 cm lateral to the inion. For the active CB stimulation, we used a stimulation intensity of 80% of the resting motor threshold (RMT) of the flexor digitorum indices muscle, with three pulse bursts at 50Hz, with a 2 second train repeated every 10 seconds for 200 seconds. Sham CB stimulation consisted of holding the TMS coil perpendicular to the scalp for the same duration. We found that compared to sham, a single session of active neuromodulation of CB reduced 95% ellipse COP area, implying improved balance control in standing. Also, these effects on balance, i.e.

improvements in COP area, persisted for about 20-25 minutes following CB stimulation. Further, following active CB stimulation, we found a decrease in the CBI ratio [(conditioned MEP/test MEP]. Because CB output to M1 is inhibitory in nature, reduction of motor evoked potential (MEP) amplitude from a dual stimulus (conditioning stimulus to CB prior to test stimulus to M1) compared to a test stimulus to M1 alone, implies greater CB output to M1 and in turn, greater CB-M1 interactions. These results indicate that CB neuromodulation can reduce postural sway and induce neurophysiological changes in CB-M1 interactions. Overall, these results support the neuroplastic potential of CB for restoring balance function in older adults and identify CB as a potential target for future interventions for inducing improvements in motor behavior.

Disclosures: A. Sansare: None. **M. Weinrich:** None. **J. Khim:** None. **J. Bernard:** None. **Y. Lei:** None.

Presentation Number: NANO25.08

Topic: E.06. Posture and Gait

Support: International Spinal Research Trust PhD125

Title: Validation of an automated method to predict anatomical joint centres in people with spinal cord injury during reaching tasks.

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Abstract: The functional reach test is a clinical evaluation tool for dynamic sitting balance in people with neurological disorders, such as spinal cord injury (SCI). However, analysing kinematic measurements with manual tracking methods to quantitatively assess performance of the upper extremities and trunk during reaching can be challenging in a clinical setting. In this proof-of-concept study, our primary objective was to validate an automated method for predicting the position of the upper body involved in forward and lateral reaching in people with motor incomplete SCI. Using a single, 2-dimensional camera, we recorded movements of the shoulder, wrist, and trunk during forward and lateral reaching with the less affected arm in ten participants with motor incomplete SCI in a hospital setting. Of those, five participants performed the same tasks twice at 4 and 8 weeks after the initial session, resulting in twenty videos in total. For each task, we manually labelled 500 images (labelled images) with 1030000 epochs acquired from ten participants with SCI in the initial session to train a deep neural network model (DeepLabCut [DLC]). The trained DLC model was then applied to another 500 images acquired from the five participants in the second and third sessions to validate generalisation of the DLC model. Additionally, 250 images from the initial session were labelled by two raters independently to assess inter-rater reliability. For within-sample validation, results suggest that the DLC tracking performance strongly correlated with the traditional manual tracking method, in both forward ($r=1.0$, $p<.001$) and lateral reaching ($r=>.80$, $p<.001$). For the generalisation validation, DLC performance strongly correlated with the manual tracking results in forward reaching ($r=>.70$, $p<.001$) and moderately correlated with the manual tracking method

in lateral reaching (r= .43-.93, p<.001). Furthermore, the inter-rater reliability of the manual tracking method was excellent in both forward and lateral reaching (two-way random intraclass correlation coefficient >.90, p<.001). Our results suggest that performance of deep neural network learning methods can match the traditional manual tracking methods, when using the functional reach test in individuals with motor-incomplete SCI.

Disclosures: M. Hidalgo Mas: None. **C. Chiu:** None. **T. Nightingale:** None. **E. Martinez-Valdes:** None. **Z. Ahmed:** None. **S. Chiou:** None.

Nanosymposium

NANO26: Molecular and Circuit Aspects of Sleep Regulation

Location: MCP Room S106

Time: Monday, October 7, 2024, 8:00 AM - 10:30 AM

Presentation Number: NANO26.01

Topic: F.07. Biological Rhythms and Sleep

Support: Parkinson's Foundation, James R. “Jim Bob” Moffett, Sr. Postdoctoral Fellowship (DS)
HHMI Investigator (YD)
NIH, NINDS, Grant U01NS113358 (YD)

Title: Activation of locus coeruleus noradrenergic neurons rapidly drives homeostatic sleep pressure

Authors: *D. SILVERMAN[1], C. CHEN[1], D. M. DARMOHRAY[1], J. SIMA[1], X. DING[1], B. LI[1], C. MA[1,2], Y. DAN[1,3];
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Abstract: Homeostatic sleep regulation is essential for optimizing the amount and timing of sleep for its revitalizing function. Here we show that optogenetic activation of locus coeruleus (LC) noradrenergic neurons immediately increased sleep propensity following a transient wakefulness, contrasting with many other arousal-promoting neurons whose activation induces sustained wakefulness. Fiber photometry showed that repeated optogenetic or sensory stimulation caused a rapid reduction of calcium activity in LC neurons and steep declines in noradrenaline/norepinephrine (NE) release in both the LC and medial prefrontal cortex (mPFC). Knockdown of α2A adrenergic receptors in LC neurons significantly mitigated the decline of NE release induced by repetitive stimulation and extended wakefulness, demonstrating an important role of α2A receptor-mediated auto-suppression of NE release. Together, these results suggest that functional fatigue of LC noradrenergic neurons, which reduces their wake-promoting capacity, contributes to sleep pressure.

Disclosures: D. Silverman: None. **C. Chen:** None. **D.M. Darmohray:** None. **J. Sima:** None. **X. Ding:** None. **B. Li:** None. **C. Ma:** None. **Y. Dan:** None.

Presentation Number: NANO26.02

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant U01NS113358 (YD)
HHMI Investigator (YD)
Damon Runyon Fellowship DRG-2414-20 (JS)

Title: Restoration of locus coeruleus noradrenergic transmission in sleep

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Abstract: Sleep is indispensable and maintained homeostatically, but its functions remain largely unknown. Here we show that sleep rejuvenates noradrenergic (NE) transmission that diminishes during wakefulness in locus coeruleus (LC) neurons to restore the capacity for arousal. Using optogenetics and fiber-photometry recording of NE biosensors in freely moving mice, we found that three hours of sleep deprivation diminishes laser evoked NE release in LC that gradually recovers in ~30 min in subsequent sleep. This decline of evoked NE release is activity-dependent and accompanied by a reduction in the percentage of LC induced wakefulness. Furthermore, genetic inactivation of the mTOR (mammalian target of rapamycin) pathway specifically in LC decreases the level of tyrosine hydroxylase, the rate-limiting enzyme for NE synthesis, and delays the recovery of evoked NE release from sleep deprivation or prolonged optogenetic stimulation, suggesting a potential role of NE synthesis. In spontaneous sleep-wake cycles, LC evoked NE release also decreases during wakefulness and increases in sleep in a duration-dependent manner. Optogenetic stimulation with longer duration or at higher frequency lengthens the recovery period on the order of minutes. Altogether, these results reveal the activity-dependent functional decline of neurotransmission in wakefulness and the essential role of sleep in its restoration to promote the next cycle of arousal.

Disclosures: J. Sima: None. **D.M. Darmohray:** None. **D. Silverman:** None. **Y. Dan:** None.

Presentation Number: NANO26.03

Topic: F.07. Biological Rhythms and Sleep

Support: SNSF 197827

Title: Comprehensive mapping and functional dissection of sleep deprivation-responsive brain areas

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Abstract: Prolonged wakefulness increases sleep drive and is compensated by increased sleep duration and intensity. The mechanisms that regulate these homeostatic processes remain poorly understood in mammals, despite the identification of broadly distributed sleep regulatory circuits. Here, we identify specific neuronal populations that respond to sleep deprivation and are

required for increased sleep drive during wakefulness. Whole-brain Fos mapping during sleep deprivation, recovery, and unperturbed circadian behavior revealed anatomically restricted correlates for extended wakefulness and recovery sleep. Using genetically targeted manipulations, we identify subsets of deprivation-sensitive cells in the preoptic area and median raphe that promote NREM sleep and increase slow wave activity, a marker for sleep intensity. Strikingly, inhibiting deprivation-sensitive cells reduces baseline NREM sleep, allows animals to sustain longer wake bouts, and nearly abolishes the increased sleep propensity normally observed during sleep deprivation. Sleep deprivation-sensitive cells in the median raphe (MR^{SD} cells) include inhibitory and serotonergic populations that synergistically promote NREM sleep, in contrast to a glutamatergic population that increases wakefulness. MR^{SD} cells project broadly to numerous subcortical targets, and may act in part through known hypothalamic sleep centers. Cumulatively, these results define novel neural circuit elements that promote sleep according to wake duration.

Disclosures: W. Joo: None. **C. Diester:** None.

Presentation Number: NANO26.04

Topic: F.07. Biological Rhythms and Sleep

Support: NIMH R21MH127341
Baszuki Milken for Bipolar Disorder
Harvard Brain Science Initiative Bipolar Disorder Seed Grant

Title: Adaptation to photoperiod via dynamic serotonin-glutamate neurotransmitter segregation

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Abstract: Changes in daylight amount (photoperiod) drive pronounced alterations in physiology and behaviour. Adaptive responses to seasonal photoperiods are vital to all organisms – dysregulation is associated with disease, from affective disorders to metabolic syndromes. Circadian rhythm circuitry has been implicated yet little is known about the precise neural and cellular substrates that underlie phase synchronization to photoperiod change. Here we present a previously unknown brain circuit and novel system of axon branch-specific and reversible neurotransmitter deployment that together prove critical for behavioural and sleep adaptation to photoperiod change. We found that the recently defined neuron type called mr*En1-Pet1* located in the mouse brainstem Median Raphe Nucleus (MRN) segregates serotonin versus VGLUT3 (here proxy for the neurotransmitter glutamate) to different axonal branches innervating specific brain regions involved in circadian rhythm and sleep/wake timing. Whether measured during the light or dark phase of the day this branch-specific neurotransmitter deployment in mr*En1-Pet1* neurons was indistinguishable; however, it strikingly reorganizes on photoperiod change. Specifically, axonal boutons but not cell soma show a shift in neurochemical phenotype upon change away from equinox light/dark conditions that reverses upon return to equinox. When we genetically disabled the deployment of VGLUT3 in mr*En1-Pet1* neurons, we found that sleep/wake periods, voluntary activity, and clock gene expression failed to synchronize to the new photoperiod or were significantly delayed. Combining intersectional rabies virus tracing and projection-specific neuronal silencing *in vivo*, we delineated a Preoptic Area-to-mr*En1Pet1*

connection responsible for decoding the photoperiodic inputs, driving the neurochemical shift and promoting behavioural synchronization. Our results reveal a previously unrecognized brain circuit along with a novel form of periodic, branch-specific neurotransmitter deployment that together regulate organismal adaptation to photoperiod changes.

Disclosures: G. Maddaloni: None. **Y. Chang:** None. **R.A. Senft:** None. **S.M. Dymecki:** None.

Presentation Number: NANO26.05

Topic: F.07. Biological Rhythms and Sleep

Support: Lundbeck Foundation

Title: Astrocytic Ca^{2+} responses are sleep state-dependent

Authors: *M. ANDERSEN[1], S. SIDHU[1], A. TSOPANIDOU[1], P. KUSK[1], H. HIRASE[1], M. NEDERGAARD[2,1], C. KJAERBY[1];
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Abstract: Astrocytes are the most abundant glia cells in CNS and serve to support neuronal function. Astrocytes exhibit somatic Ca^{2+} elevations that are dependent on noradrenergic receptor α1 activation. These global Ca^{2+} events are linked with transitions to states of increased arousal and have been shown to cease during sleep. Until recently this lack of astrocytic Ca^{2+} events during sleep was believed to be due to low tonic locus coeruleus (LC) activity during sleep. However, it has now been shown that LC is periodically active during NREM sleep which creates an oscillatory pattern of norepinephrine (NE) levels. We hypothesize that astrocytic silence during sleep is caused by subthreshold NE oscillations, and that only larger NE changes associated with state-transitions are accompanied by somatic Ca^{2+} elevation in astrocytes. Using fiber photometry alongside electroencephalography (EEG) and electromyography (EMG) recordings from adult (3-4 months old) male and female mice (n=6-7), we characterized somatic Ca^{2+} events implicated in different state transitions. We also probed the dissociation between NE and astrocytic Ca^{2+} during sleep using optogenetic activation of LC in TH-Cre mice (n=4). We found that the brain state is predictive of astrocytic responsiveness to NE elevations and not all awakenings are associated with somatic Ca^{2+} increases. In conclusion, astrocytic Ca^{2+} events are associated with awakenings to a higher degree than micro-arousals and the amplitude of Ca^{2+} events correlate with the degree of arousal response.

Disclosures: M. Andersen: None. **S. Sidhu:** None. **A. Tsopanidou:** None. **P. Kusk:** None. **H. Hirase:** None. **M. Nedergaard:** None. **C. Kjaerby:** None.

Presentation Number: NANO26.06

Topic: F.07. Biological Rhythms and Sleep

Support: National Major Project of China Science and Technology Innovation 2030 for Brain Science and Brain-Inspired Technology (2021ZD0203400)
the innovation grant (Z181100001318004)
Japanese AMED under grant number JP21zf0127005

fellowship from the China Postdoctoral Science Foundation (2023TQ0032)

Title: LKB1-SIK3-HDAC4/TCF4/CREB signalling pathway for transcriptional regulation of sleep amount in mice

Authors: *R. ZHOU[1], G. WANG[2], C. LIU[1], F. MENG[1], C. ZHANG[1], E. ZHANG[1], Q. LI[1], Q. LIU[1];
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Abstract: Sleep amount varies widely across species, from 2 to 5 hoursin giraffes to 18 to 22 hours in koalas. Studies in mice and humans reveal thatsleep quantity is governed by genetic factors. However, the core molecularpathways and effector mechanisms that regulate sleep amount in mammals remain poorlyunderstood. Here, we characterize a major signaling pathway for transcriptionalregulation of sleep quantity in mice by adeno-associated virus-mediated somaticgenetics analysis. Gain-of-function mutation in salt-inducible kinase 3 (*Sik3*)is reported to result in SLEEPY (SLP) protein expression and hypersomniaphenotype in mice. SIK3 is phosphorylated at Thr221 and activated by liverkinase B1 (LKB1). Adult brain chimeric (ABC)-knockout of LKB1 markedly reducesnon-rapid eye movement sleep (NREMS) amount, which is partially restored by ABC-expressionof SIK3/SLP-S^{T221E} in mouse brain neurons. SIK3 phosphorylates histonedeacetylase 4/5 (HDAC4/5) and leads to their sequestration in the cytoplasm. HDAC4/5is dephosphorylated and translocated into the nucleus in the ABC-*Lkb1*^{CKO}mouse brains. Expression of dominant-negative HDAC4/5N^{VP16} in ABC-*Lkb1*^{CKO}mice completely abolishes its insomnia phenotype. Furthermore, gain orloss-of-function of HDAC4/5, whose phosphorylation levels correlate with sleepneed, in mouse brain neurons causes bidirectional changes of NREMS amount.Genetic and transcriptomic studies reveal that HDAC4 cooperates with cAMPresponse element-binding protein (CREB) in both transcriptional and sleepregulation downstream of LKB1-SIK3. Furthermore, our genetic, biochemical, andstructural modeling results demonstrate that transcription factor 4 (TCF4)bridges HDAC4 and CREB to form a transcriptional complex to regulate daily sleepamount. Moreover, brain-derived neurotrophic factor (BDNF)-tropomyosin receptorkinase B (TrkB) signaling regulates NERMS amount downstream of CREB and SIK3. Inconclusion, our study identifies LKB1-SIK3-HDAC4/TCF4/CREB-BDNF/TrkB as thefirst molecular pathway that transcriptionally regulates sleep duration. Thesefindings introduce the concept that signaling pathways target transcriptionmodulators to regulate daily sleep amount and demonstrate the power of somaticgenetics in mouse sleep research.

Disclosures: R. Zhou: None. **G. Wang:** None. **C. Liu:** None. **F. Meng:** None. **C. Zhang:** None. **E. Zhang:** None. **Q. Li:** None. **Q. Liu:** None.

Presentation Number: NANO26.07

Topic: F.07. Biological Rhythms and Sleep

Support: National Major Project of China Science and Technology Innovation 2030 for Brain Science and Brain-Inspired Technology (2021ZD0203400)
the innovation grant (Z181100001318004)

Beijing Municipal Commission of Science and Technology Commission and Chinese Ministry of Science and Technology

Title: Calcineurin is a central regulator of process S and governs homeostatic sleep response in locus coeruleus

Authors: *X. YIN, C. LIU, Z. ZHANG, R. ZHOU, P. ZUO, Q. LI, Q. LIU;
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Abstract: Sleep need accumulates during waking anddissipates during sleep to maintain sleep homeostasis (process S). Besidesregulation of daily sleep amount, a hallmark of process S is the homeostaticsleep regulation: sleep loss causes increased amount and intensity ofsubsequent recovery sleep. The central regulators of process S and specificbrain regions that govern sleep homeostasis in mammals remain unclear. Here, weinvestigate the function of calcineurinin sleep regulation using AAV-mediated somatic genetics analysis, and biochemical/neurosciencemethods. We report that enhanced calcineurin activity in the mouse brainneurons transiently increases non-rapid eye movement sleep (NREMS) to average~17-h/day, while knockout of calcineurin diminishes baseline NREMS to average~4-h/day. In addition, knockout of calcineurin also abolishes recovery NREMS aftersleep deprivation. Lack of sleepis reported to impair performance, while a series of behavioral tests (rotarod,open field, forced swimming, sucrose preference and wheel-running test) indicatethat calcineurin knockout mice show normal behaviors. The sleep need, which isindicated by the strength of delta power (0.5-4 Hz) in NREMS, accumulatesduring wakefulness and dissipates during sleep. Mathematical simulation ofdelta power indicates that calcineurin is critical to the accumulation of sleepneed. At the molecular level, salt-inducible kinase 3 (SIK3) is identified as anew substrate of calcineurin in sleep regulation. Calcineurin antagonizes proteinkinase A (PKA) by dephosphorylating SIK3 Ser551 to regulate sleep time. Sleepis thought to be regulated by specific brain regions and neural clusters. Using$Vgat^{Cre}$;$Rosa26^{LSL\text{-}Cas9}$ and $Vglut2^{Cre}$;$Rosa26^{LSL\text{-}Cas9}$mice, we show that calcineurinpromotes baseline NREMS in both excitatory and inhibitory neurons, but regulates recovery sleepspecifically in excitatory neurons. Moreover, calcineurin isspecifically required in the locus coeruleus-noradrenergic (LC_{NA})neurons for the transcriptomic and homeostatic responses to sleep deprivation.While ablation and inhibition of LC_{NA} neurons diminish recoveryNREMS after sleep loss, chemo-/optogenetic activation of LC_{NA}neurons recapitulates recovery NREMS by increasing the amount and intensity ofNREMS. These results establish calcineurin as a central regulator of process Sand identify LC as a potential sleep need center that governs NREMS homeostasisin mice.

Disclosures: X. Yin: None. **C. Liu:** None. **Z. Zhang:** None. **R. Zhou:** None. **P. Zuo:** None. **Q. Li:** None. **Q. Liu:** None.

Presentation Number: NANO26.08

Topic: F.07. Biological Rhythms and Sleep

Support: R01NS051305
R01NS076980

Title: Breaking free from the clock’s tyranny restores memory to brain damaged flies

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Abstract: The relationship between sleep and memory is an active topic of investigation. In this context, we demonstrate that enhancing sleep restores memory to flies with ablated Mushroom Bodies (MB), a key memory center; this is consistent across several memory assays. Mapping the underlying circuitry reveals circadian modulation of a subset of Dopaminergic neurons (DANs) that modulate aversive learning. Using imaging, we show that MB-ablation disrupts, and sleep restores the time of day these neurons are most responsive. Knocking down the receptor for the clock output signal, *Pigment-dispersing factor* (Pdfr), in this subset of DANs restores memory to MB-ablated flies. Crucially, MB-ablation does not result in memory impairments in the absence of a functioning clock. Our results reveal neuromodulation's key role in cognitive restoration, where sleep aids memory in damaged brains, but a functioning clock unexpectedly hinders this process.

Disclosures: S. Dissel: None. **V. Duong:** None. **P. Morgan:** None. **Z.P. Wakefield:** None. **E.M. Periandri:** None. **B. Holder:** None. **J. Donlea:** None. **L.J. Cao:** None. **Z.J. Li:** None. **S.J. Shetty:** None. **M. Ford:** None. **D.J. Chan:** None. **H.J. Farah:** None. **V.J. Loutrianakis:** None. **L. Streett:** None. **I.J. Huang:** None. **D. Abdalla:** None. **A. Kalra:** None. **B. Van Swinderen:** None. **P. Shaw:** None.

Presentation Number: NANO26.09

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS Grants-in-Aid for JSPS Fellows (22KJ1042)
JSPS (KAKENHI 16H06456)
JSPS (KAKENHI 21H00457)
JSPS (KAKENHI 16H06455)
AMED-CREST (JP21gm1310010)
the Leading Initiative for Excellent Young Researchers (LEADER) from MEXT
JSPS (KAKENHI 22K06309)

Title: Neuropeptidergic control of developmental sleep in Drosophila

Authors: *C. HEMMI[1], K. ISHII[1], K. EMOTO[1,2];
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Abstract: Sleep in the early developmental stages is particularly important for brain development, as supported by epidemiological studies and experimental evidence from mammalian models. Sleep regulation mechanisms in babies likely differ from those in adults, because sleep in the early developmental stages is typically independent of circadian rhythms. Despite its importance, molecular and neuronal mechanisms underlying developmental sleep remain largely unknown. This is partially due to the complexity of the overall neural circuitry in mammalian models and the difficulty of analyzing them at the cellular level. To tackle this issue, we chose fruit fly larvae as an experimental model with simpler neural circuits. We first conducted a genetic screen and found that loss-of-function mutations in the neuropeptide Hugin and its receptor PK2-R1 significantly increased the amount of sleep at the larval stage. Consistently, the suppression of either *Hugin* neurons or *PK2-R1* neurons resulted in prolonged developmental sleep. We further narrowed down the neurons responsible for Hugin-mediated sleep control into Hugin-PC neurons with a characteristic innervation to the protocerebrum. Contrary to developmental sleep in larvae, the same genetic silencing of Hugin-PC neurons failed to promote adult sleep. Based on these data, we will discuss how neuropeptides differentially regulate sleep at the developmental and adult stages in *Drosophila*.

Disclosures: C. Hemmi: None. **K. Ishii:** None. **K. Emoto:** None.

Presentation Number: NANO26.10

Topic: F.07. Biological Rhythms and Sleep

Support: U.S. Department of Defense grant W911NF1910280
NIH grant R01NS131389

Title: Effects of NREM sleep on the cortical synaptic expression of GluA1-containing AMPA receptors

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Abstract: A net increase in synaptic strength accrues in many synapses during wakefulness as the inevitable consequence of ongoing learning. However, to avoid saturation and keep energy costs under control, the wake-related synaptic strengthening needs to be corrected via a broad but selective process of synaptic weakening. Electrophysiological, molecular, and ultrastructural evidence supports the hypothesis that sleep promotes this net decrease in excitatory synaptic strength. Nonetheless, several outstanding questions about sleep-dependent synaptic weakening remain. A major unresolved question relates to whether non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep play specific roles in sleep-dependent synaptic weakening. Another unresolved question is whether a few hours of recovery sleep following acute sleep deprivation are sufficient to bring net synaptic strength back to baseline levels. To address these questions, we used two established molecular markers of synaptic strength, the levels of the AMPA (Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid) receptors containing the GluA1 subunit, and the phosphorylation of GluA1 at serine 845 (p-GluA1(845)). We measured changes of these two markers in synaptosomes of mouse cortex (one hemisphere), using the following 4 experimental conditions (8 C57BL/B6 mice per condition, 14-15 weeks old of both sexes): sleep (5 hours), sleep deprivation (5 hours, with novel objects), recovery sleep (5

hours) after sleep deprivation (5 hours), and REM sleep deprivation (5 hours). Finally, we also tested whether sleep-dependent synaptic weakening is affected by aging. For this experiment we used aged mice (85-86 weeks old) assigned to 3 experimental conditions: sleep, sleep deprivation and recovery sleep after sleep deprivation (4 male mice per condition). We find that relative to after sleep deprivation, GluA1 and p-GluA1(845) are lower in the sleep group and in the REM sleep deprivation group indicating that the expression of these markers decreases after sleep independent of whether the mice enter or not REM sleep. Additionally, 5 hours of recovery sleep following acute sleep deprivation are enough to renormalize GluA1 and p-GluA1(845) expression. Thus, the renormalization of GluA1 and p-GluA1(845) expression seems to crucially rely on NREM sleep and can occur in a few hours of sleep after acute sleep deprivation. Moreover, this process seems to be unaffected by aging.

Disclosures: F. Squarcio: None. **G. Tononi:** None. **C. Cirelli:** None.

Nanosymposium

NANO27: Non-Invasive Neuromodulation

Location: MCP Room S404

Time: Monday, October 7, 2024, 8:00 AM - 10:00 AM

Presentation Number: NANO27.01

Topic: E.05. Brain-Machine Interface

Support: Spark Biomedical, Inc.

Title: Translation from preclinical research to clinical trials: transcutaneous auricular neuromodulation enhances platelet function in humans

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Abstract: Uncontrolled hemorrhage following surgery or trauma can represent a medical emergency that increases patients' risk for blood transfusions, re-operation to control bleeding, hemorrhagic shock, and even death. While surgeons manage bleeding with tourniquets, electrocautery, and hemostatic agents, there is no routine method to prophylactically enhance hemostasis to reduce the risk of bleeding. Previously, we showed that electrical invasive vagus nerve stimulation (VNS) reduces duration of bleeding and total blood loss by >50% in murine and porcine models of soft tissue injury. Characterization of the effects of iVNS on hemostasis revealed the "Neural Tourniquet," a pathway that connects the brainstem to circulating platelets via the vagus nerve. VNS causes splenic T-lymphocytes to release acetylcholine, which binds platelet α7 nicotinic cholinergic receptors to increase calcium influx. These "primed" platelets respond more rapidly to pro-coagulant stimuli, especially thrombin, by increasing α-granule release as indicated by P-selectin expression. Ultimately, these platelets improve systemic

hemostasis by accelerating clot formation at injury sites. To translate our preclinical findings, we conducted the first-in-human single-site, randomized, sham-controlled blinded trial to determine whether the neural tourniquet pathway can be activated by stimulating cranial nerves on and around the ear. Participants (18-65 years, both genders) were consented and randomized to receive either electrical stimulation of the auricular branch of the vagus nerve (ABVN) or transcutaneous auricular neurostimulation (tAN), which targets both the ABVN and the auriculotemporal nerve (ATN). All subjects underwent a 30-minute sham stimulation session followed by a 30-minute active stimulation session. Blood samples were drawn at baseline, post-sham, and post-stim, and assessed for platelet activation by fluorescence-activated cell sorting and viscoelastic testing by thromboelastography. The results demonstrated that tAN, but not ABVN stimulation alone, significantly increases platelet α-granule release by 20% and overall clot strength by 25%, consistent with preclinical studies. These results suggest that tAN is a viable approach to modulate platelet function and improve hemostasis in healthy humans. Further clinical studies are warranted.

Disclosures: C.E. Bravo Iñiguez: A. Employment/Salary (full or part-time):; Northwell Health. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Spark Biomedical Inc. **J. Papoin:** A. Employment/Salary (full or part-time):; Northwell Health. **I. Mirro:** A. Employment/Salary (full or part-time):; Northwell Health. **C.J. Czura:** A. Employment/Salary (full or part-time):; Five Liters, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Five Liters, Inc. and Spark Biomedical, Inc. **C. Benner:** A. Employment/Salary (full or part-time):; Spark Biomedical Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Spark Biomedical Inc. **M. McWade:** A. Employment/Salary (full or part-time):; Spark Biomedical Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Spark Biomedical Inc. **A. Covalin:** A. Employment/Salary (full or part-time):; Spark Biomedical Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Spark Biomedical Inc. **N. Khodaparast:** A. Employment/Salary (full or part-time):; Spark Biomedical Inc., Five Liters Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Spark Biomedical Inc., Five Liters Inc.. **L. Blanc:** None. **J.M. Huston:** A. Employment/Salary (full or part-time):; Northwell Health. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Northwell Health.

Presentation Number: NANO27.02

Topic: E.05. Brain-Machine Interface

Support: NIH R01 GM143362

Title: Regulating inflammation through red-shifted optogenetic activation of cholinergic circuits

Authors: A. C. CHEN[1], K. PARK[2], S. CHAUDHRY[2], T. S. HUERTA[2], ***E. H. CHANG**[2,3]; [1]Zucker Sch. of Med. at Hofstra/Northwell, Hempstead, NY; [2]Northwell Hlth., Manhasset, NY; [3]Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY

Abstract: The nervous and immune systems are engaged in constant crosstalk to maintain health and coordinate host responses. The vagus nerve is an important component of this neuro-immune interface, and vagus nerve stimulation (VNS) is a promising approach in the field of bioelectronic medicine, particularly for managing chronic inflammatory disorders. By activating the vagus nerve-mediated inflammatory reflex, VNS offers potential therapeutic benefits to regulate excess inflammation in patients. However, traditional VNS methods are predominantly invasive and rely on electrical stimulation, which may trigger off-target effects. Our research explores noninvasive alternatives for neuromodulation that can be genetically targeted to neuronal populations or nerve fiber subsets. One such alternative is red-light-shifted optogenetics, which uses longer wavelength red or infrared light that can penetrate biological tissues to reach gene-targeted opsins.
Here, we examined the efficacy of optogenetic VNS using red-light activatable channelrhodopsin (ReaChR) for reducing cytokine production during acute inflammation. We used genetic targeting of ReaChR expressed in choline acetyltransferase-positive (ChAT) neuronal populations (ChAT-ReaChR mice) and another group targeting transient receptor potential vanilloid subfamily member 1 (TRPV1)-ReaChR mice. Acute inflammation was induced via intraperitoneal lipopolysaccharide (LPS) injection. Following LPS injection, we delivered red light (635nm, 10 Hz, 25 ms for 2 min) to the cervical vagus nerve for optogenetic VNS. We find that optogenetic activation of specific cholinergic circuits within brainstem nuclei and the vagus nerve can be controlled with light to regulate inflammatory responses in the body. Specifically, ReaChR activation of ChAT+ vagal fibers led to a significant reduction in splenic TNFα levels ($p < 0.001$) but did not affect cytokine levels systemically ($p = 0.81$). Stimulation of TRPV1-positive vagal fibers did not regulate cytokine production. Notably, ChAT+ fiber activation also induced sustained bradycardia (~23% heart rate decrease), highlighting additional cardiovascular effects of this neuromodulation technique.
These findings highlight the potential of ReaChR-based optogenetic VNS as a non-invasive method for achieving high spatiotemporal control of genetically-targeted neuronal subsets to modulate inflammation. This approach not only reduces the risks associated with surgical interventions but also provides a targeted mechanism to modulate the immune response, opening new avenues for the treatment of various inflammatory conditions.

Disclosures: A.C. Chen: None. **K. Park:** None. **S. Chaudhry:** None. **T.S. Huerta:** None. **E.H. Chang:** None.

Presentation Number: NANO27.03

Topic: E.05. Brain-Machine Interface

Title: Non-invasive focused ultrasound of spleen requires chat^{+} t-lymphocytes and α7 nicotinic acethylcholine receptor to reduce traumatic hemorrhage

Authors: *C. E. BRAVO IÑIGUEZ[1], K. J. TRACEY[2], S. S. CHAVAN[3], J. M. HUSTON[4]; [1]Biomed. Sci. Lab., Feinstein Inst. for Med. Res., New York, NY; [2]Res. Admin., [3]Inst. for Bioelectronic Med., Feinstein Inst. For Med. Res., Manhasset, NY; [4]Surgery, Northwell Hlth., Manhasset, NY

Abstract: Introduction: Trauma is the leading cause of death for Americans 1 to 45 years of age. Uncontrolled hemorrhage is the most common preventable cause of traumatic death. Direct

pressure or tourniquets mitigate extremity bleeding, but surgical management is necessary to control non-compressible truncal hemorrhage. Unfortunately, systemic hemostatic therapies are limited. Electrical vagus nerve stimulation (VNS) accelerates clotting to reduce traumatic bleeding in mice through a pathway requiring $ChAT^{+}$ T-lymphocytes in spleen and α7 nicotinic acetylcholine receptors (α7nAChR) on circulating platelets. Focused ultrasound (FUS) stimulation of spleen decreases traumatic bleeding. Utilizing a murine tail transection hemorrhage model, here we investigate whether $ChAT^{+}$ T lymphocytes or α7nAChR are required for hemostasis following FUS. **Methods:** Adult male wild-type (C57BL6), conditional T-lymphocyte knockout (CD4-$ChAT^{-/-}$), or α7nAChR knockout mice (α7KO) are anesthetized (ketamine/xylazine), placed in the right lateral decubitus position, and the spleen is identified by surface anatomy. The ultrasound probe (Sonic Concepts, H101) is placed on shaved skin with ultrasound gel and aimed at the splenic hilum. The function generator (33120A, Keysight Technologies) and power amplifier (350L RF, Electronics and Innovations) deliver 1 min of stimulation (1.1 MHz, 200 mV per pulse, 150 burst cycles, 500 μs burst period), followed by 30 s of rest, and then 1 min of stimulation. Sham stimulated animals receive FUS over the right quadriceps. Animal tails are warmed in water (37 ± 1°C, 5 min), transected 2 mm from the tip, and bled into water (37 ± 1°C) until hemorrhage stops for at least 10 s. Hemorrhage duration is recorded as bleeding time. **Results:** Compared with sham stimulation, FUS significantly reduces bleeding time in wild-type mice (Sham = 110.5 ± 7.7 s vs. FUS = 72.9 ± 6.6 s, mean ± SEM, n = 10, $p < 0.01$, t-test). Compared with sham stimulation, FUS fails to decrease bleeding time in CD4-$ChAT^{-/-}$ mice (Sham = 117.5 ± 11.3 s vs. FUS = 91.0 ± 14.9 s, n = 5, $p=ns$), or α7KO mice (Sham = 125.7 ± 9.22 s vs. FUS = 127.4 ± 9.54 s, n = 8, $p=ns$). **Conclusions:** FUS stimulation of spleen requires CD4-$ChAT^{+}$ T-lymphocytes and α7nAChR to reduce hemorrhage. FUS warrants further clinical studies in trauma, surgical bleeding, and bleeding disorders.

Disclosures: C.E. Bravo Iñiguez: None. **K.J. Tracey:** None. **S.S. Chavan:** None. **J.M. Huston:** None.

Presentation Number: NANO27.04

Topic: E.05. Brain-Machine Interface

Support: DARPA

Title: Noninvasive ultrasound stimulation to modulate end-organs for treating inflammatory disorders

Authors: *H. LIM;
Univ. of Minnesota, Minneapolis, MN

Abstract: Over the past decade, there has been increasing excitement and research discoveries in using non-invasive ultrasound for modulating the nervous system and end-organs for potentially treating a broad range of health disorders. Particularly for end-organ modulation, multiple preclinical animal studies have demonstrated that noninvasive ultrasound stimulation of the spleen can treat different acute and chronic inflammatory disorders. In my talk, I will provide a brief overview of these previous animal studies and then share the development and translation of a novel wearable ultrasound device for treating patients suffering from rheumatoid arthritis. Ultrasound stimulation of the spleen has been shown to significantly improve clinical outcomes

and reduce joint swelling in an inflammatory arthritis mice model. These encouraging results then led to a pilot clinical trial in which 13 rheumatoid arthritis patients were treated with daily splenic ultrasound stimulation for two months using a wearable device developed by SecondWave Systems (MINI™ device); many of these patients were those who were not sufficiently responding to conventional drugs or advanced biologics. After splenic ultrasound stimulation, the participants exhibited significant improvement in the DAS28-CRP score, which is a widely utilized disease activity metric for rheumatoid arthritis that was the outcome measure for the primary endpoint analysis. More than two-thirds of the participants experienced a clinical benefit to treatment in which all 13 participants indicated the wearable ultrasound device was very comfortable. There were minimal adverse events with no treatment related serious adverse events. These positive clinical results for the use of splenic ultrasound for treating rheumatoid arthritis opens opportunities for leveraging this approach for potentially treating other inflammatory conditions, such as inflammatory bowel disease, pulmonary hypertensions, acute kidney injury, sepsis, and acute infections.

Disclosures: H. Lim: A. Employment/Salary (full or part-time):; SecondWave Systems, Neuromod Devices. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SecondWave Systems, Neuromod Devices.

Presentation Number: NANO27.05

Topic: E.05. Brain-Machine Interface

Support: R01GM128008

Title: Neuromodulation of the celiac-superior mesenteric ganglion complex using non-invasive focused ultrasound stimulation regulates inflammation.

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Abstract: The vagus nerve based *inflammatory reflex* plays a major role in the neural regulation of inflammation (*Nature, 2002*). Recent studies have identified the celiac-superior mesenteric ganglion complex (CSMGC) as an essential component of the efferent arm of the inflammatory reflex. In the CSMGC, both efferent vagus nerve fibers and spinal sympathetic preganglionic fibers interact with the catecholaminergic neurons innervating the spleen, liver and other organs. While the efferent vagus nerve - splenic nerve interaction in the CSMGC is essential for suppressing pro-inflammatory cytokine responses and adverse inflammation, the possibility of direct neuromodulation of the CSMGC to regulate inflammation has been understudied. To provide insight, we used noninvasive focused ultrasound stimulation (FUS) targeting the CSMGC in murine endotoxemia. There is a growing interest in exploring FUS as an anti-inflammatory modality, but whether inflammation can be altered using FUS of the CSMGC remained unknown. We subjected male 10–14 weeks old C57BL6 mice to 5 mins or 2 mins of

FUS (1.1MHz and 200mV per pulse, 150 burst cycles, 500µs burst period) or sham stimulation of the CSMGC followed by intraperitoneal lipopolysaccharide (LPS, 0.25 mg/kg) administration. 90mins post LPS administration mice were euthanized, blood and liver were collected for cytokine analysis. As compared to sham stimulation, FUS of the CSMGC for 5 mins (n=11,12/group) significantly attenuated serum TNF levels (1,875 ± 226.1 pg/ml vs 672.6 ± 96.76 pg/ml, P< 0.0001) and hepatic TNF levels (27.8 ± 2.486 pg/mg vs 16.02 ± 2.061 pg/mg, P= 0.0018), while significantly elevating serum IL-10 levels (6,467 ± 1,027 vs 9,671 ± 875 pg/ml, P= 0.0374) in murine endotoxemia. However, FUS of the CSMGC for 2 mins (n=15/group) did not significantly alter serum TNF levels (1,458 ± 154.9 pg/ml vs 1,120 ± 85.85 pg/ml, P=0.1607). These results demonstrate for the first time the anti-inflammatory effects of direct FUS of the CSMGC and identify the CSMGC as an essential target to suppress systemic and hepatic inflammation with a potential for clinical translation. This work was partially supported by NIGMS.

Disclosures: S. Palandira: None. **A. Falvey:** None. **M. Brines:** None. **S. Chavan:** None. **K.J. Tracey:** None. **V.A. Pavlov:** None.

Presentation Number: NANO27.06

Topic: E.05. Brain-Machine Interface

Support: United Therapeutics Corp. "Bioelectronic treatment of Pulmonary Hypertension"

Title: Autonomic neuromodulation with noninvasive ultrasound of the spleen improves experimental pulmonary hypertension

Authors: S. ZAFEIROPOULOS, U. AHMED, A. BEKIARIDOU, N. JAYAPRAKASH, I. T. MUGHRABI, ***S. ZANOS**;
Bioelectronic Med., Feinstein Inst. For Med. Res., Manhasset, NY

Abstract: BACKGROUND: Inflammation is pathogenically implicated in pulmonary hypertension (PH); however, it has not been adequately targeted therapeutically. We investigated whether neuromodulation of an anti-inflammatory neuroimmune pathway involving the splenic nerve using noninvasive, focused ultrasound stimulation of the spleen (sFUS) can improve experimental PH. METHODS: PH was induced in rats either by Sugen-hypoxia or monocrotaline injection. Animals were randomized to receive either daily, 12-min-long sessions of sFUS or sham stimulation, for 14 days. Catheterizations, echocardiography, indices of autonomic function, lung and heart histology and immunohistochemistry, spleen flow cytometry and lung single-cell-RNA sequencing were performed after treatment to assess effects of sFUS. RESULTS: Splenic denervation right before induction of PH results in a more severe disease phenotype. In both disease models, sFUS treatment reduces right ventricular (RV) systolic pressure by 25-30% compared to sham treatment, without affecting systemic pressure, and improves RV function and autonomic indices. sFUS reduces wall thickness, apoptosis, and proliferation in small pulmonary arterioles, suppresses CD3+ and CD68+ cell infiltration in lungs and RV fibrosis and hypertrophy and lowers brain natriuretic peptide. Beneficial effects persist for weeks after sFUS discontinuation and are more robust with early and longer treatment. Splenic denervation abolishes sFUS therapeutic benefits. sFUS partially normalizes

CD68+ and CD8+ T- cell counts in the spleen and downregulates several inflammatory genes and pathways in nonclassical and classical monocytes, and macrophages in the lung. Differentially expressed genes in those cell types are significantly enriched for human PAH-associated genes. CONCLUSIONS: sFUS causes dose-dependent, sustained improvement of hemodynamic, autonomic, laboratory and pathological manifestations in two models of experimental PH. Mechanistically, sFUS normalizes immune cell populations in the spleen and downregulates inflammatory genes and pathways in the lung, many of which are relevant in human disease.

Disclosures: S. Zafeiropoulos: None. **U. Ahmed:** None. **A. Bekiaridou:** None. **N. Jayaprakash:** None. **I.T. Mughrabi:** None. **S. Zanos:** None.

Presentation Number: NANO27.07

Topic: E.05. Brain-Machine Interface

Support: Lawson Internal Research Fund

Title: Functional Near-Infrared Spectroscopy Based Neurofeedback Training for Imagined Movements of the Upper Limb: Assessing Feasibility in a Healthy Population

Authors: *F. MACRAE[1], K. TSIKRIKIS[2], A. ABDALMALAK[3], A. M. OWEN[4], D. B. DEBICKI[5], S. PETERS[6];
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Abstract: Introduction: Motor imagery (MI) involves the mental rehearsal of an action. MI does not necessitate any movement and may improve motor rehabilitation. Functional Near-Infrared Spectroscopy (fNIRS) is a non-invasive neuroimaging modality that uses light to observe changes in blood oxygenation associated with neural activity. Neurofeedback (NFB) involves processing brain activation data online to relay key features back to the participant in real-time. NFB may have clinical applicability for motor rehabilitation since improving MI may improve clinical outcomes. A support vector machine (SVM) is a machine learning algorithm used for brain computer interfaces (BCI) to extract signal features for task differentiation. fNIRS has not been used to capture brain activation associated with MI for the purposes of task differentiation or NFB. Therefore, we sought to assess the feasibility of NFB training and task differentiation with fNIRS by studying the rate at which MI brain activation can be correctly separated from rest in healthy adults using two session structures. Methods: Participants completed 4 sessions of 60-minute MI sessions with fNIRS based NFB. Participants were randomized to complete MI with minimal rest (group 1), or to receive a ten-minute break halfway through each session (group 2). Participant discomfort, focus, and fatigue were assessed at the end of each session. Intra-participant SVMs were trained with all fNIRS data except a random run for each individual and tested on that random run. One-way ANOVAs were used to assess differences between groups. Results: 17 healthy adults (12 female; median age = 29) were recruited; two withdrew before completing the protocol. An Intra-participant SVM was created for each participant who

completed the protocol. The mean correct classification rate across n = 15 was 68.3% (sd = 20.9). There was no significant difference in task differentiation accuracy according to group (p = 0.8). There were no significant differences between groups for focus level (p = 0.4), discomfort (p = 0.3), or fatigue (p = 0.8) (n = 17). Conclusions: fNIRS is a feasible technique for NFB training with a correct signal classification rate of 68% when trained and tested within an individual. A break halfway through training sessions did not lower the rate of correct classification or decrease participant burden. We recommend completing hour long sessions to reduce set-up time; however, a short rest should be permitted if needed.

Disclosures: F. MacRae: None. **K. Tsikrikis:** None. **A. Abdalmalak:** A. Employment/Salary (full or part-time):; NIRx Medical Technologies, LLC. **A.M. Owen:** None. **D.B. Debicki:** None. **S. Peters:** None.

Presentation Number: NANO27.08

Topic: E.05. Brain-Machine Interface

Support: NIH 5R00AG056636-04
NIH 1R34NS127103-01
NIH R01NS126076-01
NSF CAREER Award 2045120
NSF EAGER Award 2217582
Rita Allen Foundation Scholars Award,
Beckman Technology Development Grant

Title: Brain-wide non-invasive neuromodulation via sono-optogenetics

Authors: *S. JIANG[1,3], N. ROMMELFANGER[4], G. WOODS[4], H. SONTHEIMER[2], G. HONG[3];
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Abstract: Optogenetics has been extensively utilized in neuroscience due to its ability to activate specific neurons by light illumination in a spatiotemporal manner. To achieve light delivery inside the brain, optical fibers and micro light-emitting diodes are commonly employed with craniotomy and invasive implantation. Nonetheless, these implantable platforms require the fixation on the skull, limiting their capability for repositioning for multi-region stimulation. Besides, the invasive implantation will inevitably lead to the perturbation of tissue microenvironment and immune response. To this end, we developed a sono-optogenetic platform with non-invasive deep-tissue light delivery capability that can achieve brain-wide optogenetic screening. Specifically, a circulatory light source of mechanoluminescent nanotransducers (MLNTs) were systematically administrated, generating localized light emission upon tissue-penetrant focused ultrasound (FUS) application. We first characterized the light emission in a tissue-mimicking phantom and an artificial circulatory system, and validated the sono-optogenetic stimulation in Thy1-ChR2-YFP mice via immunohistostaining. Taking advantages of this non-invasive light delivery approach, we have achieved multi-region optogenetic stimulation over a long distance in the same animal by stereotaxically manipulating the applied FUS. Using wearable FUS transducer, we demonstrated rapid optogenetics screening on free-

behaving mice with behavior assays. In conclusion, we developed a generalizable non-invasive, deep-tissue light delivery method, and demonstrated its unique advantages in optogenetic studies. We envision that this method will facilitate new insights for dissecting complex brain functions and dynamics in the near future.

Disclosures: S. Jiang: None. **N. Rommelfanger:** None. **G. Woods:** None. **H. Sontheimer:** None. **G. Hong:** None.

Nanosymposium

NANO28: Alcohol: Motivation and Cognition

Location: MCP Room N427

Time: Monday, October 7, 2024, 8:00 AM - 10:15 AM

Presentation Number: NANO28.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant AA030293
NIH grant AA027768

Title: A Cell-Type-Specific Striatal Engram Encodes Alcohol-Related Memories to Drive Relapse and Is Suppressed by Extinction

Authors: *J. WANG[1], X. XIE[2], R. CHEN[1], Y. HUANG[1], H. GANGAL[3], Z. HUANG[4], V. VIERKANT[1], X. WANG[1], R. J. SMITH[5];
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Abstract: Alcohol use disorder is considered a maladaptive manifestation of learning and memory, potentially encoded by engrams. The role of striatal direct-pathway medium-spiny-neurons (dMSNs) in mediating alcohol consumption is well-established. However, it remains elusive whether alcohol-recruited dMSNs alone shape a functional engram, forming a persistent trace of alcohol memory that promotes relapse. Here, we demonstrate that alcohol-recruited dorsostriatal dMSNs are pivotal for the retrieval of a cue-alcohol memory. These dMSNs exhibit preferential synaptic potentiation from alcohol-activated inputs, and artificially creating engram dMSNs by inducing synaptic strengthening is sufficient for encoding a cue-reinforcer memory. Moreover, extinction training reduces the activity and reactivation of engram dMSNs during cued relapse. Intriguingly, silencing these engram dMSNs via LTD mimics the effects of extinction, persistently suppressing relapse. Collectively, our findings illustrate that alcohol recruits a cell-type-specific striatal engram encoding long-lasting cue-alcohol memories, which can be mitigated by extinction training to curb relapse.

Disclosures: J. Wang: None. **X. Xie:** None. **R. Chen:** None. **Y. Huang:** None. **H. Gangal:** None. **Z. Huang:** None. **V. Vierkant:** None. **X. Wang:** None. **R.J. Smith:** None.

Presentation Number: NANO28.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA026186

Title: Prefrontal cortical-brainstem circuits in the regulation of alcohol withdrawal-associated hyperalgesia in mice.

Authors: *S. QUADIR, M. CONE, D. ZAIDI, S. NASKAR, F. YASMIN, S. PATEL; Psychiatry and Behavioral Sci., Northwestern Univ., Feinberg Sch. of Med., Chicago, IL

Abstract: Alcohol use disorders (AUDs) are chronic relapsing disorders characterized by repeated cycles of binge/intoxication, withdrawal/negative affect, and anticipation/preoccupation. The withdrawal/negative affect stage includes an emergence of heightened pain states, that are thought to drive further drinking and perpetuate the cycle. One important brain area mediating these behaviors is the prelimbic subregion of the prefrontal cortex. The prelimbic cortex (PL) contains a diverse group of neurons, including long-range glutamatergic projection neurons as well as short range GABAergic interneurons. One target of PL neurons is the periaqueductal gray (PAG), an area known for its role in descending pain modulation. In the studies presented here, we investigated the role of PL-PAG neurons in mediating alcohol-withdrawal induced pain states. In the first experiment, we found application of noxious stimuli induced activation of PL-PAG neurons. In the subsequent studies, we investigated the role of PL-PAG neurons in alcohol-withdrawal induced pain by subjecting the mice to a continuous 2-bottle choice drinking paradigm. This model reliably induces both allodynia and hyperalgesia, as evidenced by increased sensitivity to mechanical, thermal, and cold stimuli at 72h withdrawal. We found optogenetic activation of the PL-PAG pathway ameliorated these phenotypes, suggesting this pathway is critical for alcohol withdrawal induced pain states. Current studies are underway to examine changes in excitability of PL-PAG neurons following alcohol withdrawal. Considering projection neurons also receive local inhibitory input from neighboring interneurons, we also examined the effects of alcohol withdrawal on interneuron excitability in the PL using PV:Ai14, VIP:Ai14 and SOM:Ai14 mice. In these experiments, alcohol naïve mice were compared to alcohol-drinking mice in 72h withdrawal, the same timepoint where we saw increased pain sensitivity. In PV+ PL neurons, alcohol withdrawn males exhibited increased neuronal excitability compared to controls, an effect that was largely absent in females. In VIP:Ai14 mice, there was no effect of alcohol withdrawal on VIP+ neuron excitability in males; however, alcohol drinking decreased excitability in females. There was no effect of alcohol drinking on SOM+ neuron excitability. Together, these studies suggest alcohol withdrawal and PL activity are extremely intertwined, as demonstrated by the PL-PAG pain studies as well as interneuron excitability experiments.

Disclosures: S. Quadir: None. **M. Cone:** None. **D. Zaidi:** None. **S. Naskar:** None. **F. Yasmin:** None. **S. Patel:** None.

Presentation Number: NANO28.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Wesleyan University GISOS award

Title: Sex-specific behavioral output following manipulation of GABAergic signaling in the Ventral Tegmental Area: relevance to arousal and alcohol-related behavioral plasticity

Authors: *D. MONTEYS[1], T. HAN[1], I. AHEARN[1], H. KWON[2], Z. RABINOWITZ[1], E. OWEN[1], Z. AWEDA[3], B. BUYUKDEMIRTAS[1], R. NACIF-PIMENTA[1], L. C. MELON[4]; [1]Wesleyan Univ., Middletown, CT; [2]Wesleyan Univ., Suwanee, GA; [3]Wesleyan Univ., Newark, NJ; [4]Biol., Wesleyan Univ., Middletown, CT

Abstract: Sensitivity to alcohol's stimulant effects predicts diagnosis with AUD and symptom severity (King et al., 2021). C57BL/6J mice show sex differences in sensitivity to the stimulant effect of ethanol and in tonic GABAergic signaling in the ventral tegmental area (VTA; Darnieder et al.,2019). We used a chemogenetic approach to dissect a role for GABAergic inhibition in the VTA in regulating sex differences in the stimulant response to ethanol and in the expression of maladaptive reward sensitivity following chronic exposure. Male and female C57BL6/J mice (5-8/group) were administered alcohol (2.0g/kg) for 14 days, with locomotor activity assessed on the first and final days of exposure. Animals were given an opportunity to interact with a sexually-immature juvenile for 5 minutes before (after the last day of exposure) withdrawal or at two time points following abstinence. Brains and bloods were harvested 30 minutes after this social interaction opportunity. Although there was a marginal sex difference in the stimulant response to alcohol, females showed unique sensitization to alcohol's psychomotor effects. Males showed a significant reduction in social preference associated with this sensitization protocol, while females required abstinence to reveal this reduction in reward sensitivity. Neural activity showed significant sex-specific reactivity of VTA-LFP to acute ethanol. To probe the role that GABAergic inhibition in the VTA plays in this sex difference, male and female VGAT-cre mice (7-9/group) received 250 nL of pAAV-hSyn-DIO-hM4D(Gi)-mCherry or the sham mCherry virus (pAAV-hSyn-DIO-mCherry) into the VTA. Animals received a single administration of CNO (1.0mg/kg, ip) and were tested 7 or 14 days later for their reactivity to alcohol (2.0g/kg). Chemogenetic inhibition of VGAT-positive putative GABAergic neurons significantly produced hyperactivity in males only and permitted an increase in ethanol-induced stimulation. This manipulation enhanced a hypolocomotor response for females following ethanol. Immunohistochemistry suggests sex differences in the expression of multiple GABAA receptor subtypes that regulate tonic inhibition on dopaminergic and non-dopaminergic neurons in the VTA. The results support a sex-specific role for VTA GABAergic neurons in the responsivity of the local network to ethanol and in constraining the behavioral changes that follow exposure to the drug.

Disclosures: D. Monteys: None. **T. Han:** None. **I. Ahearn:** None. **H. Kwon:** None. **Z. Rabinowitz:** None. **E. Owen:** None. **Z. Aweda:** None. **B. Buyukdemirtas:** None. **R. Nacif-Pimenta:** None. **L.C. Melon:** None.

Presentation Number: NANO28.04

Topic: G.09. Drugs of Abuse and Addiction

Support: PMRF Fellowship 3702555

Title: Influence of approach and avoidance tendencies on neural dynamics in heavy drinkers: an ERP-based investigation for alcohol group subclassification

Authors: *A. K. VERMA[1], A. D. KUMAR[2], U. CHIVUKULA[1], N. KUMAR[2];
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Abstract: Automatic approach and avoidance tendencies towards alcohol play a major role in maintaining alcohol dependence. However, the approach bias modification interventions are seen to have negligible effects on relapse prevention due to a limited understanding of underlying neural mechanisms and a lack of focus on modulating these mechanisms at the neural level. The current work aims to explore the behavioral and event-related potential (ERP) differences based on these automatic tendencies and further probe into the neurocognitive processes and responsible brain regions. The study was conducted in a laboratory setting on 19 heavy drinkers (Alc) and 20 non-drinkers (NAlc) adults, where participants performed an Alcohol Approach-Avoidance Task (AAT), a task for assessing/categorizing participants with automatic approach (AAppr) or avoidance (AAvoi) tendencies toward alcohol, while electroencephalography (EEG) recording. A 2 (group: Alc, NAlc) x 2 (automatic tendencies: approach, avoidance) ANOVA for behavioral outcome and ANOVA with planned contrasts for Alc and NAlc, AAppr and AAvoi groups, and neutral and alcohol stimuli comparisons were performed on early (100-200 ms), mid (200-300 ms), and late (350-450 ms) ERP amplitudes and latencies. Behavioral results showed AAT scores to be significantly influenced by the group-tendency interaction. For ERP measures, amplitude differences were present between Alc and NAlc groups at the occipital, parietal, and frontal sites, and latency differences between alcohol and neutral stimuli at the occipito-parietal sites. Interestingly, amplitude differences between the AAppr and AAvoi groups were specific to the frontal sites (Fz, F3, F4), showing significantly reduced neural response in the AAppr group, suggesting the recruitment of different neural mechanisms for maintaining alcohol intake behavior. The AAppr and AAvoi differences were prominent for N100-200 and N350-450 amplitudes at the F4 electrode, suggesting the differential modulation of attention, response inhibition, and decision-making processes. The results highlight the role of frontal and right dorsolateral prefrontal cortex (dLPFC; underneath the F4 electrode) areas in driving the differences in approach/avoidance tendency by significantly suppressing the neural responses in the alcoholic population with approach biases. The findings suggest the subclassification of the alcohol-consuming population based on automatic tendencies in future research and clinical practices.

Disclosures: A.K. Verma: None. **A.D. Kumar:** None. **U. Chivukula:** None. **N. Kumar:** None.

Presentation Number: NANO28.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA-K99 AA030828-01A1
NIAAA-R01 AA030710-01
NIAAA-P60AA007611-37

Title: Sex and genetic differences of high and low alcohol-preferring mice in the 5-choice serial reaction time task

Authors: *P. STARSKI[1], A. SIEGLE[2], F. W. HOPF[3];
[1]Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Dept Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alcohol use disorder (AUD) is a result of excessive binge alcohol consumption. Impulsive behavior, a common trait among those who experience frequent binge alcohol episode, may be an identifiable risk factor for potential AUD patients. It is important to understand how impulse behavior and alcohol preference interact as these working in concert may expedite the rate of the development of AUD. Here, we explore the effectiveness of training male and female crossed high alcohol-preferring (cHAP) and low alcohol-preferring (LAP) mice in the 5-Choice Serial Reaction Time Task (5-CSRTT) using a strawberry milk reward. Mice were trained in the 5-CSRTT five days a week to a baseline of 1 second stimulus duration (SD) and 5 second intertrial interval (ITI). Mice were then given impulsivity testing by randomizing the ITI and then attention testing by randomizing the SD. We found several interesting behaviors in these alcohol-preference mouse lines. First, LAP mice were more likely to do less overall behavior when the task became too challenging. Specifically, LAP mice performed less than 30 trials during 1 sec SD, opposed to the >60 trials at a 5 sec SD. Thus, to compare cHAP and LAP mice impulsivity, we performed an impulsivity test with a 5 sec SD. cHAP mice were not more impulsive but performed more trials and with greater accuracy. Further, female cHAP mice were found to have significantly lower correct response latency and less ITI tray entries than their male counterparts. Our study shows that mice genetically bred to prefer alcohol are more engaged with activities around them than the low alcohol-preferring mice as evidenced by the performance in the 5-CSRTT. Additionally, female cHAP mice displayed greater attentiveness and readiness to action than male cHAPs. Together, genetic preference for alcohol may promote an overt fixation on behavioral tasks a potential vulnerability for development of AUD.

Disclosures: P. Starski: None. **A. Siegle:** None. **F.W. Hopf:** None.

Presentation Number: NANO28.06

Topic: G.09. Drugs of Abuse and Addiction

Support: Ministry of Health & Welfare, Republic of Korea (Grant Number HI22C0404)

Title: Interplay between reward and self-referential processing in alcohol craving during naturalistic movie watching

Authors: *M. KWON[1], S. SONG[1], H. LEE[1], J.-S. CHOI[3], Y.-C. JUNG[4], M. KWON[4], M. D. ROSENBERG[5], W.-Y. AHN[1,2];
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Abstract: Real-life cues that induce alcohol cravings are contextually rich, engaging various neurocognitive processes. While neural correlates of these cravings have been identified in laboratory settings, most studies have focused on reward processing. However, recent neuroimaging studies have revealed that aberrant neural activities, suggestive of distinct self-referential processing, also occur when alcohol cues contain contextual elements. Here, we aimed to investigate self-referential and reward processing and their dynamics in understanding cue-induced craving. We conducted functional magnetic resonance imaging as problematic alcohol users watched fifteen videos with alcohol-drinking scenes (20 minutes total) and reported

subjective levels of craving and self-relatedness (i.e., the relevance of the video to oneself) for each video. Outside of the scanner, participants freely spoke about their reasons for drinking alcohol for three minutes. While self-reported craving level, self-relatedness level, and addiction severity were all positively correlated with each other, linear mixed-effects modeling indicated that self-relatedness level significantly explained craving level even after controlling for addiction severity. Furthermore, activations in the default mode network while watching the videos were synchronized when individuals exhibited either similar craving responses, self-relatedness responses, or similar reasons for alcohol drinking. However, higher addiction severity was associated with greater idiosyncratic brain activations, especially in the regions related to reward and self-referential processing. Notably, this neural asynchrony was explained by idiosyncratic self-relatedness responses. Together, our findings suggest that self-referential processing plays a critical role in alcohol cravings when cues contain naturalistic contexts, and craving becomes an idiosyncratic process as addiction becomes severe, potentially due to increasingly idiosyncratic self-referential processing.

Disclosures: M. Kwon: None. **S. Song:** None. **H. Lee:** None. **J. Choi:** None. **Y. Jung:** None. **M. Kwon:** None. **M.D. Rosenberg:** None. **W. Ahn:** None.

Presentation Number: NANO28.07

Topic: G.09. Drugs of Abuse and Addiction

Support: Wesleyan University startup
Ronald E McNair Foundation Scholar

Title: Sex, estrous and parvalbumin interneuron reactivity in the basolateral amygdala and behavioral change to alcohol's anxiolytic and rewarding effects

Authors: L. MERCADO[1], H. KWA[1], A. ROBINSON[2], C. GLICKMAN[1], E. ROSEN[1], *L. MELON[3,1];
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Abstract: Parvalbumin (PV) interneurons play a crucial role in shaping circuit plasticity; defining differences in the capacity for behavioral adaptation noted across development and across factors like sex or social status. In the basolateral amygdala, these PV-interneurons not only constrain fear learning but dynamically respond to neurosteroid tone to facilitate shifts in anxiety state. The current study investigated the impact that factors associated with shifts in endogenous neurosteroid tone, such as sex, estrous status, dominance hierarchy,or isolation, had on the development of rapid tolerance to the anxiolytic effects of alcohol in C57Bl/6J mice. Anxiety-like behavior was evaluated using the elevated plus maze (EPM) in female and male (n=8-13/group) mice following one or two exposures to alcohol (1g/kg, i.p.) and/or saline. Female mice that received their first exposure to ethanol during diestrus developed rapid tolerance, with a 52.9% reduction in alcohol-induced anxiety-like behavior demonstrated by an increase in open arm exploration. This effect was abolished for females who were singly housed. Similarly, males only expressed significant changes in sensitivity to the anxiolytic effects of alcohol when co-housed. Females who were exposed to alcohol for the first time during the estrus phase showed sensitization to the anxiolytic-effects of ethanol when singly housed, and no pharmacodynamic adaptation when co-housed. Brains were harvested following the first or

second exposure to alcohol and changes in cfos immunoreactivity were used as an index of cell activation in PV -positive or -negative interneurons in the basolateral amygdala and ventral hippocampus. Future analysis will evaluate whether BLA PV-interneuron activity predicts the effect that these factors play in the development of rapid tolerance to the anxiolytic effects of ethanol. Taken together these findings suggest an interaction between hormonal status and parvalbumin reactivity in the development of tolerance to alcohol's anxiolytic effect.

Disclosures: L. Mercado: None. **H. Kwa:** None. **A. Robinson:** None. **C. Glickman:** None. **E. Rosen:** None. **L. Melon:** None.

Presentation Number: NANO28.08

Topic: G.09. Drugs of Abuse and Addiction

Support: Regione Autonoma della Sardegna (RAS, CRP2_537-CUP F71J090006200002)
Fondazione Banco di Sardegna, Project ID F74I19000970007 (2018)
NIH Grant AA028982
NIH Grant AA021667
NIH Training Grant T32AA007456

Title: Receptor and metabolic insights on the ability of caffeine to prevent alcohol-induced stimulation of mesolimbic dopamine transmission

Authors: *R. MACCIONI[1], V. BASSAREO[2], G. TALANI[3], I. LORRAI[1], D. LECCA[2], P. ENRICO[4], A. T. PEANA[4], L. DAZZI[2], P. P. SANNA[1], E. SANNA[2], E. ACQUAS[2];
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Abstract: The consumption of alcohol and caffeine affects the lives of billions of individuals worldwide. Although recent evidence indicates that caffeine impairs the reinforcing properties of alcohol, a characterization of its effects on alcohol-stimulated mesolimbic dopamine (DA) function was lacking. Acting as the pro-drug of salsolinol, alcohol excites DA neurons in the posterior ventral tegmental area (pVTA) and increases DA release in the nucleus accumbens shell (AcbSh). Here we show that caffeine, *via* antagonistic activity on A_{2A} adenosine receptors ($A_{2A}R$), prevents alcohol-dependent activation of mesolimbic DA function as assessed, *in-vivo*, by brain microdialysis of AcbSh DA and, *in-vitro*, by electrophysiological recordings of pVTA DA neuronal firing. Accordingly, while the A_1R antagonist DPCPX fails to prevent the effects of alcohol on DA function, both caffeine and the $A_{2A}R$ antagonist SCH 58261 prevent alcohol-dependent pVTA generation of salsolinol and increase in AcbSh DA *in-vivo*, as well as alcohol-dependent excitation of pVTA DA neurons *in-vitro*. However, caffeine also prevents direct salsolinol- and morphine-stimulated DA function, suggesting that it can exert these inhibitory effects also independently from affecting alcohol-induced salsolinol formation or bioavailability. Finally, untargeted metabolomics of pVTA lysates showcases that caffeine antagonizes alcohol-mediated effects on molecules (e.g. phosphatidylcholines, fatty amides, carnitines) involved in lipid signaling and energy metabolism, which could represent an additional salsolinol-independent mechanism of caffeine in impairing alcohol-mediated stimulation of mesolimbic DA transmission. Notably, also one of the few FDA-approved drugs for AUD, naltrexone

(ReVia®; Depade®), prevents the reinforcing effects of alcohol by interfering with its enhancement of the mesolimbic DA transmission. Hence, the outcomes of this study strengthen the potential of caffeine, as well as of $A_{2A}R$ antagonists, for future development of preventive/therapeutic strategies for alcohol use disorder.

Disclosures: R. Maccioni: None. **V. Bassareo:** None. **G. Talani:** None. **I. Lorrai:** None. **D. Lecca:** None. **P. Enrico:** None. **A.T. Peana:** None. **L. Dazzi:** None. **P.P. Sanna:** None. **E. Sanna:** None. **E. Acquas:** None.

Presentation Number: NANO28.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NADIA Consortium

Title: Epigenetic Silencing of Dorsal Raphe Serotonergic Neurons Following Adolescent Intermittent Ethanol is Reversible by Glycyrrhizin Administration

Authors: *S. DE CASTRO[1], F. T. CREWS[2], R. P. VETRENO[3];
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Abstract: Human adolescent binge drinking leads to lifelong social and emotional difficulties, including increased risk for anxiety & affective disorders and substance use disorders. Adolescent intermittent ethanol (AIE; 5.0 g/kg EtOH, i.g., 2-day on/2-day off from P25 to P54), a rodent model of human adolescent binge drinking, produces an array of anxiety-like behaviors that persist into adulthood in the absence of further alcohol consumption. Serotonin (5-HT) is established as a mediator of social and emotional processes, and we previously reported a reduction of serotonergic neurons (i.e., 5-HT+IR) in the raphe nucleus that persists into adulthood. We previously reported that AIE causes a reversible loss of cholinergic neurons through an epigenetic repressive mechanism mediated by neuroimmune activation via high mobility group box 1 (HMGB1). We therefore hypothesized that the AIE-induced loss of serotonergic neurons is mediated by epigenetic silencing of serotonergic genes, and that this silencing would be reversible through an HMGB1-specific anti-inflammatory treatment. Immunohistology for TPH2+IR and 5-HT+IR in the adult dorsal raphe nucleus (DRN) following AIE revealed reductions of both TPH2 and 5-HT relative to age-matched CONs. Loss of 5-HT in the DRN is similar to that seen following injection of lipopolysaccharide, which is consistent with a shared inflammatory mechanism. Chromatin immunoprecipitation assessment of the epigenetic repressive marker H3K9me2 revealed increased occupancy at the serotonin transporter (Sert) and the 5-HT synthesizing enzyme tryptophan hydroxylase 2 (Tph2) in the adult DRN of AIE-treated animals. Administration of glycyrrhizin, a HMBG1-specific anti-inflammatory agent, to adult rats post-AIE reversed the loss of TPH2+IR and 5HT+IR in the DRN. Future work will assess the efficacy of glycyrrhizin in preventing adverse behavioral outcomes thought to be mediated by serotonergic dysfunction following AIE, such as heightened anxiety-like behaviors and social deficits.

Disclosures: S. de Castro: None. **F.T. Crews:** None. **R.P. Vetreno:** None.

Nanosymposium

NANO29: Neurophysiology of the Human Medial Temporal Lobe During Relational and Spatial Learning

Location: MCP Room N227

Time: Monday, October 7, 2024, 8:00 AM - 10:15 AM

Presentation Number: NANO29.01

Topic: H.10. Human Learning and Cognition

Support: KNAW 240-846401 NWA-StartImpuls 2017
NWO Crossover Program 17619 "INTENSE"
Human Brain Project (Agreement No. 945539, "Human Brain Project SGA3")

Title: Pronouns reactivate concept representations in human hippocampal neurons

Authors: *D. DIJKSTERHUIS[1], M. W. SELF[1], J. POSSEL[3], J. PETERS[4], E. C. VAN STRAATEN[5], S. IDEMA[6], S. VAN DER SALM[5], E. J. AARNOUTSE[7], N. VAN KLINK[5], P. VAN EIJSDEN[5], S. HANSLMAYR[8], L. D. KOLIBIUS[9], F. ROUX[10], S. DEHAENE[11], P. R. ROELFSEMA[2];
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Abstract: Language comprehension accumulates over consecutive sentences. During discourse, concepts that are introduced in one sentence often recur in later sentences. To minimize repetition and utterance length, languages use pronouns, like the word ‘he, to refer to nouns that were introduced before (e.g. “John and Mary walked into a bar. He sat down at a table.”). This example illustrates how a narrative activates successive concepts in our brain, including their interrelations, allowing us to incrementally build up a conceptual representation of important aspects of the discourse. It has been suggested that language comprehension requires that pronouns activate the same neuronal representations as the nouns themselves. Previous brain-imaging studies have gained insight into the brain regions that activate during sentence and discourse comprehension. However, the resolution of these non-invasive imaging methods does not suffice for monitoring the activation of singular concept representations in the human brain during reading. In recent years, it has become possible to directly record the activity of single neurons in patients who are implanted with electrodes to locate the source of their epilepsy. These studies demonstrated the existence of ‘concept cells’ in the medial temporal lobe. Concept cells contribute to the representation of meaning by showing a selective response in an invariant and multimodal way. We hypothesized that monitoring the activity of concept cells during reading could provide insight into the dynamics of semantic representations during language

comprehension. Here, we test this hypothesis by recording from individual neurons in the human hippocampus during a reading task. We found that cells that are selective to a particular noun are later reactivated only by pronouns that refer to the cells' preferred noun. These results represent the first measurements of the magnitude, latency and duration of hippocampal single cell responses to nouns and pronouns during reading, while subjects incrementally build up a semantic representation of a narrative. This study uniquely demonstrates on a single cell level how memory and language are linked.

Disclosures: D. Dijksterhuis: None. **M.W. Self:** None. **J. Possel:** None. **J. Peters:** None. **E.C. Van Straaten:** None. **S. Idema:** None. **S. van der Salm:** None. **E.J. Aarnoutse:** None. **N. van Klink:** None. **P. van Eijsden:** None. **S. Hanslmayr:** None. **L.D. Kolibius:** None. **F. Roux:** None. **S. Dehaene:** None. **P.R. Roelfsema:** None.

Presentation Number: NANO29.02

Topic: H.10. Human Learning and Cognition

Support: 2R01NS107357-6A1

Title: Neurophysiological Evidence of Longitudinal Specialization in the Human Hippocampus

Authors: *B. LEGA;
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Abstract: Hippocampal longitudinal specialization is poorly understood in human episodic memory circuits. Several models of longitudinal differences have been proposed, principally based on non—invasive data, but few of the predictions of these models have been tested using direct brain recordings. Such data are critical to establish the specific mechanisms that may mediate functional differences between the anterior and posterior hippocampus. Using a unique dataset of 61 intracranial EEG patients, we compared anterior vs posterior hippocampal neurophysiological activity during successful recollection, novelty detection, and familiarity—based item retrieval. Using mixed effects modeling, we identified greater posterior hippocampal activity during recollection versus elevated anterior activity during identification of novel items. There were no differences during recovery of items via familiarity. We next implemented a mechanism to compare longitudinal differences in hippocampal pattern completion processes via a novel distance metric calculation during associative recollection predicated on a recall to reject framework.. This revealed sustained differences 800 msec after item presentation in the posterior hippocampus. However, there were no differences in pattern separation, quantified during item encoding. We interpret these results in light of proposed models, including gist/detail representation. We suggest how differences in novelty processing may fit within such a framework.

Disclosures: B. Lega: None.

Presentation Number: NANO29.03

Topic: H.10. Human Learning and Cognition

Support: NIH, BRAIN Initiative. U01-NS113198

Title: Spatial information in non-place cells

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Abstract: In this present study we investigated spatial memory encoding in the hippocampus of human refractory epilepsy patients using a virtual navigation task. Participants were asked to remember the locations of six items in a rectangular arena while receiving brain stimulation at either low (3 Hz) or high (8 Hz) theta. Concurrently, we recorded single neuron activity in the hippocampus. Using a Gaussian Process Regression we successfully decoded the spatial position of the patient during the encoding phase of the experiment. Even after excluding previously identified place cells, our decoding analysis remarkably maintained its predictive power. This discovery challenges the notion that spatial information is exclusively sparsely coded in the hippocampus by highly selective place coding cells. Instead, this suggests a gradual decrease of spatial information within other neurons in the hippocampus, not classified as place cells. Although each neuron codes spatial information to a lesser degree, collectively this broad ensemble of neurons contained significant spatial information. Future analyses will include the effect of stimulation on the stability and emergence of place cells, their reinstatement during verbal and spatial retrieval, and the relationship between place cell firing and hippocampal theta events.

Disclosures: L.D. Kolibius: None. **M.S. Hermiller:** None. **C. Holman:** None. **M.F. Khazali:** None. **A. Brandt:** None. **M.J. Kahana:** None. **J. Jacobs:** None.

Presentation Number: NANO29.04

Topic: H.10. Human Learning and Cognition

Support: ZIA NS003144-09

Title: Neural mechanisms across spatial scales during human episodic memory formation

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Abstract: Memory is critical to our everyday experience. We rely upon our memories not only to form our own sense of identity, but also to guide and plan our future actions and behaviors. Understanding the neural mechanisms that underlie human memory formation is therefore critical in order to effectively treat memory disorders which are present in some of the most debilitating yet poorly managed neurological diseases. Our research efforts are focused on investigating the neural correlates of human episodic memory formation by leveraging the opportunities to directly record neural activity across multiple spatial scales from the human brain in patients receiving surgical treatment for drug resistant epilepsy. At larger spatial scales, we find that both specific patterns of localized neural activity and dynamic connections between brain regions emerge as people encode individual items into memory, and similar patterns of activity and connectivity are reinstated when people retrieve those same items from memory. At

the smallest spatial scale, we find that populations of individual neurons in the anterior temporal lobe exhibit temporally organized sequences of spiking activity that are specific to the individual items people are encoding into memory, and that similar sequences are replayed when people retrieve those items from memory. The sequences of spiking activity are ordered based on the semantic category of the individual items, suggesting that sequences of spiking activity may be a fundamental unit of information in the human brain. In addition, these sequences of spiking activity are distributed across spatially contiguous yet distinct functional modules that are approximately the same size as the cortical columns hypothesized to exist throughout the human brain, suggesting a functional organization to how information is encoded across neuronal populations. Together, our results provide novel insights into how information specific to individual memories is represented in the brain, and how this information is accessed as people recall previous experiences from memory.

Disclosures: K.A. Zaghloul: None.

Presentation Number: NANO29.05

Topic: H.10. Human Learning and Cognition

Support: German Research Council (DFG) Grant SFB1089

Title: Attentional modulation of single-unit activity in the human medial temporal lobe

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Abstract: Human medial temporal lobe (MTL) structures are populated by single neurons showing remarkable stimulus selectivity and response invariance, called concept cells. These cells play an important role in conscious perception and working memory, which suggests that they constitute the building blocks of human episodic memory. The brain organizes and guides perception of relevant stimuli through attentional mechanisms. Extensive work in non-human primates has shown modulation of single-unit responses to preferred stimuli by attention, mostly in the visual system. Functional imaging in humans has similarly shown attentional modulation in different brain regions involved in sensory processing. However, the interplay between attention and visual processing on a single-unit level in the human MTL has not yet been addressed. In our study, 20 epilepsy patients with microwire electrodes embedded in four MTL regions performed 35 sessions of a task designed to isolate the effect of attention on single-unit activity. Subjects followed a rapid and pseudo-randomized stream of eight images previously found to specifically activate the recorded neurons. They were tasked with counting the occurrence of a different particular stimulus in each run of one minute duration, allowing us to analyze both attended target runs (TR) and unattended non-target runs (NTR) for each stimulus and unit. We recorded from up to now 189 highly stimulus-selective units in the amygdala (A, 62 units), the hippocampus (H, 35 units), the entorhinal cortex (EC, 13 units), and the parahippocampal cortex (PHC, 79 units). We found that the strength of stimulus-related activity was attention-modulated in 26% of these units in A, 31% of units in EC, 23% in H, and 29% in PHC, with no statistically significant difference between regions ($X^2(3, N = 189) = .62$, $p > .05$). A clear response onset latency was discernible for both TR and NTR in 171 units. We compared

the latencies for TR and NTR within-region using Wilcoxon signed-rank tests. Units in A showed increased response latencies for TR relative to NTR ($z = 2.60$, $p = .009$, median in TR = 352 ms, median in NTR = 340 ms), while units in PHC showed decreased latencies for TR compared to NTR ($z = -2.08$, $p = .038$, median in TR = 316 ms, median in NTR = 354 ms). Our results suggest a functional division among stimulus-selective neurons in the human MTL, with a large minority being sensitive to attentional signals. These subpopulations could play different roles in the transfer of experiences into long-term memory.

Disclosures: I. Vieten: None. **J. Faber:** None. **R. Surges:** None. **V. Borger:** None. **F. Mormann:** None.

Presentation Number: NANO29.06

Topic: H.10. Human Learning and Cognition

Title: Neuronal representations of temporal memory formation and recall in the human medial temporal lobe

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Abstract: Temporal memory reflects the serial order of events in time. Cellular activity in the medial temporal lobe (MTL) contributes to the neural representation of temporal memory. Here, using invasive single and multi-unit recordings in human epilepsy patients (n = 17), we examined whether human MTL neuronal activity represents the serial position of events during memory formation and recall. The patients freely navigated a virtual environment in order to explore and remember the locations and the serial positions of different objects (Kunz et al., Neuron, 2021). During each exploration period, the patients sequentially encountered two or three different objects, placed in different locations. This allowed us to examine single- and multi- unit neuronal firing rates as a function of the serial position in which the objects were presented. Our results indicate that a significant number of units show selectivity to the serial position of objects during the exploration period—for example, by activating most strongly whenever the subject is presented with the first object, independent of object identity. Overall, about 18% (n=109 out of 623 multi units) and about 19% (n=59 out of 312 single units) showed selectivity to serial position. Percentages higher than 10% of both unit types were found in the following regions: amygdala, entorhinal cortex, hippocampus, para-hippocampus and temporal pole. We performed further analyses of the firing rate of selective units during the serial position recall period. The results suggest that most of the selective single-unit activity preserve their serial position preference across memory formation and recall periods in contrast to the multi-unit activity. These results are consistent with a continuous neural code for temporal memory formation and recall.

Disclosures: M. Khazali: None. **A. Brandt:** None. **P.C. Reinacher:** None. **M.J. Kahana:** None. **J. Jacobs:** None. **A. Schulze-Bonhage:** None. **L. Kunz:** None.

Presentation Number: NANO29.07

Topic: H.10. Human Learning and Cognition

Title: Neurophysiological basis of pattern separation and pattern completion in the human brain

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Abstract: The formation and retrieval of episodic memories crucially relies on pattern separation and pattern completion, which are computational mechanisms arising from the hippocampus and hippocampal-neocortical interactions. Using depth electrode intracranial recordings in epilepsy patients, we have uncovered several key findings that bolster our understanding of how these computations operate. We demonstrate that directional hippocampal-cortical interactions, mediated by the theta rhythm, are differentially engaged during pattern separation and pattern completion. We also identified a potentially counterintuitive role for hippocampal pre-stimulus theta in facilitating overgeneralization potentially indicating the importance of state effects and timing on the impact of theta on memory formation and recall. Finally, pattern separation of emotional information seems to rely on bidirectional theta-mediated communication between the hippocampus and the amygdala, while overgeneralization errors are associated with alpha-mediated unidirectional influence of the amygdala over the hippocampus. Altogether, these data point to key functions of theta and alpha rhythms in mediating communication between the hippocampus and other brain regions in the context of pattern separation and pattern completion, and additionally suggest the state, timing and directionality of influence all play important roles.

Disclosures: M.A. Yassa: None.

Presentation Number: NANO29.08

Topic: H.10. Human Learning and Cognition

Title: Single-neuron correlates of attentional working memory states in the human medial temporal lobe

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Abstract: We often need to memorize information to achieve current goals, while simultaneously holding additional information in mind for future use. Currently relevant items in Working Memory (WM) are in the "focus of attention"[1] and their neural representations drive goal-directed behavior. In contrast, task-irrelevant WM items should remain in an "unattended" state with neural representations that minimally affect the task at hand. The debate over the

neural formats associated with these attended and unattended states persists. We directly addressed this question by recording neurons in the medial temporal lobe (MTL) that were tuned to specific concepts, such as celebrities, while they were maintained in different attentional WM states.Across 93 sessions, an interleaved visual search and WM task was performed by 22 epilepsy patients undergoing presurgical monitoring. In each trial, we tracked two distinct concept-specific WM representations - one attended and the other unattended, which reversed states mid-trial following a task switch. Concept-cells (n=98) increased firing rates during cue-delays when their preferred concept (as opposed to an unpreferred one) was maintained in WM, regardless of attentional state. The strength of this preparatory activity predicted behavioral performance on the subsequent subtask. Further population analyses (including 66 broadly tuned "maintenance" cells) suggested orthogonal representations of attended and unattended WM items. Maintaining unattended WM representations in an active yet orthogonal format ensures their rapid accessibility when circumstances change, while mitigating interference with the ongoing task. In concert with extended cortical networks (observed in neuroimaging studies using similar tasks[2-4]), these context-dependent WM representations in MTL may contribute to the effective, flexible execution of goal-driven behaviors.
1.Oberauer (2002), *J. Exp. Psychol. Learn. Mem. Cogn.* **28**, 411–421 2.Peters, Goebel, Roelfsema (2009), *J. Cogn. Neurosci.* **21**, 1081–1091 3.Peters, Roelfsema, Goebel (2012), *J. Neurosci.* **32**, 17003–17011 4.Yu, Teng, Postle (2020), *PLoS Biol.* **18**, 1–21

Disclosures: J.C. Peters: None. **D.E. Dijksterhuis:** None. **M.W. Self:** None. **J. Possel:** None. **E.C. Van Straaten:** None. **S. Idema:** None. **L. Reddy:** None. **P.R. Roelfsema:** None.

Presentation Number: NANO29.09

Topic: H.10. Human Learning and Cognition

Support: SPP 2205 German Research Foundation
SPP 2041 German Research Foundation
SFB 1233 German Research Foundation
Germany's Excellence Strategy (EXC-Number 2064/1)
Volkswagen Foundation: 86 507
SPP 2411 German Research Foundation
German Federal Ministry of Education and Research (BMBF) through the Tübingen AI Center

Title: Temporal phase coding of order memory in human MTL and recurrent neural networks

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Abstract: How do we remember the temporal order of events? A prominent theory proposes that the item order is maintained in memory through the ordered firing of neurons coupled to gamma bursts at different phases of theta oscillations (Lisman, 1998). We probed this theory by directly measuring single neuron activity (1420 neurons) and local field potentials (921 channels) in the

medial temporal lobe of epilepsy patients performing a working memory task for temporal order. During memory maintenance, we observe preferential firing of single neurons to theta phase that varied with item position, but phase order did not reflect item order. We further tested several other hypotheses of the Lisman model taking into account the relationship between single unit firing and gamma oscillations, as well as gamma and theta coupling. Here, our preliminary results suggest enhanced theta-gamma coupling during the memory period, where the phase of gamma power also varied with item order, but again item order did not match phase-order similar to spiking. Finally, we trained recurrent neural networks (RNNs) in an analogous task and observed similar effects as in our neural data. Taken together, in both biological and artificial neural networks we provide validating evidence for the role of phase-of-firing in memory processing while at the same time challenging a long-held theory about the specific relationship between spiking and oscillations during memory.

Disclosures: S. Liebe: None. **J. Niediek:** None. **T.P. Reber:** None. **J.H. Macke:** None. **F. Mormann:** None.

Nanosymposium

NANO30: Modelling Neurodevelopmental Disorders: Mechanisms and Therapeutics

Location: MCP Room S404

Time: Monday, October 7, 2024, 1:00 PM - 3:00 PM

Presentation Number: NANO30.01

Topic: A.07. Developmental Disorders

Support: NSTC 109-2320-B-002-042-MY3
NSTC 111-2320-B-002-045
NSTC 112-2320-B-002-059
NHRI-EX112-11114NI

Title: α6GABA$_A$ receptor-selective positive allosteric modulator as a potential novel therapy for autism: a proof-of-concept study in prenatal valproic acid-exposed juvenile rats

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Abstract: Autism spectrum disorder (ASD), a developmental disorder characterized by social impairment and repetitive behaviors, affects around 1% of children worldwide but remains an unmet medical need. Its underlying mechanisms involve cerebellar transmission anomalies, particularly due to Purkinje cell dysfunction. We've identified a druggable pyrazoloquinolinone compound, DK-I-56-1 (DK), as the first positive allosteric modulator (PAM) for α6 subunit-containing α6GABA$_A$ receptors (α6GABA$_A$Rs), which are almost exclusively expressed in

cerebellar granule cells (GCs). We hypothesize that DK can enhance GABAergic transmission in GCs, thus restoring Purkinje cell activity and ameliorating ASD symptoms. To validated this hypothesis, we used a well-recognized animal model of ASD, the valproic acid (VPA)-exposure juvenile rats. Pregnant rats received a single injection of VPA (500 mg/kg, *i.p.*) or saline on E12.5. Their male and female offspring from P21 were treated daily with DK (10 mg/kg, *i.p.*) or its vehicle (Veh). Social behaviors were quantified by the time spent with different social stimuli in the three-chamber social test. Repetitive behaviors were recorded in an open-field test by a blinded experimenter. The autism composite score was the average of the normalized z-scores of social behaviors and repetitive behaviors. These ASD-like behaviors were compared among four offspring groups: VPA+DK, VPA+Veh, Saline+DK, and Saline+Veh. In the VPA+Veh group, compared with the Saline+Veh group, while female offspring showed comparable ASD-like behaviors, male offspring exhibited impaired social behaviors and increased repetitive behaviors (two-way ANOVA with Tukey post-hoc), and were thus used to evaluate the ASD preventive treatment regimen of DK. Paired comparison revealed that the social impairment of the VPA+DK group significantly improved 30 min after single-dose treatment. Additionally, consecutive DK treatments for five days, but not three, completely restored the social behaviors in the VPA+DK group to Saline+Veh levels (two-way ANOVA with Tukey post-hoc). Following this preventive treatment regimen, the repetitive behaviors and the autism composite score in the VPA+DK group were also restored to the level as in the Saline+Veh group (one-way ANOVA with Tukey post-hoc). Our results suggest that in the VPA-exposed male juvenile rats, single-dose treatment with DK improves social deficits, and daily treatment with DK for five days prevents both ASD-like behaviors. While the precise mechanisms require detailed exploration, these findings highlight the potential therapeutic efficacy of α6GABA$_A$R PAMs to alleviate core features of ASD.

Disclosures: Y. Pan: None. **C. Wu:** None. **M. Chu:** None. **C. Yeh:** None. **D. Sharmin:** None. **J.M. Cook:** None. **H. Lin:** None. **L. Chiou:** None.

Presentation Number: NANO30.02

Topic: A.07. Developmental Disorders

Support: American Association of University Women (AAUW)
University of Wisconsin- Madison Department of Neurological Surgery

Title: A multi-omics approach improves the resolution of the complex molecular etiology of autism spectrum disorder

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Abstract: The complex molecular etiology of autism spectrum disorder (ASD) requires novel approaches to overcome the challenges in characterizing molecular pathways linked to the phenotype. Here we conducted an integrative multi-omic approach in hippocampal tissue from a mouse model of ASD, *Cntnap2* knockout (*Cntnap2* KO), and wild-type (WT) mice to determine if combining multi-omic datasets improves the molecular resolution of the key molecular

mechanisms contributing to the complex ASD phenotype. Genome scale chromatin proximity mapping (Hi-ChIP) revealed significantly more chromatin interactions in *Cntnap2* KO (*N*=5,616) than WT (*N*=4,072) mice (*P*-value < 0.01). Annotation of these chromatin interactions to gene promoters identified 3,690 *Cntnap2* KO-specific and 2,841 WT-specific genes. Pathway analyses only identified terms associated with neuronal processes in the *Cntnap2* KO-specific genes, several of which are considered to have super interactive promoters due to multiple chromatin interactions, such as *Tcf4, Ank3 and Grin2a*. Furthermore, we tested for known transcription factor motifs among the chromatin interactions of both *Cntnap2* KO and WT genes and only found an enrichment of transcription factor binding motifs associated with early development and neuronal processes in the *Cntnap2* KO-specific genes, suggesting a role in gene regulation. Integration of differential RNA sequencing and Hi-ChIP data revealed that approximately 40% of differentially expressed genes comprise promoter-associated chromatin interactions (*N*=541, *P*-value<0.05). Forty-three of these genes also exhibit differential DNA methylation levels in *Cntnap2* KO compared to WT mice, including genes related to ASD (*e.g., Zbtb18, Cttnbp2, Slc29a4*). A pathway analysis of these 43 genes identified highly enriched biologically relevant terms related to ASD, such as synaptic plasticity, dendrite development, and neuron projections. Thus, while each omic dataset identified unique insights into the molecular pathogenesis of ASD the integration of chromatin conformation, transcriptome, and methylome datasets yielded a vastly enriched set of molecular pathways related to neuronal synaptic plasticity and dendrite development. This enrichment is consistent with integrated multi-omic studies of human ASD postmortem brain tissue, suggesting that convergent multi-omic datasets can be used to achieve a greater resolution of the complex ASD molecular etiology.

Disclosures: C.D. Alberca Doto: None. **K. Park:** None. **L.A. Papale:** None. **A. Madrid:** None. **S. Keles:** None. **R.S. Alisch:** None.

Presentation Number: NANO30.03

Topic: A.07. Developmental Disorders

Support: the State Key Program of National Natural Science Foundations of China 81930103

Title: Divergent projections of the prelimbic cortex mediate autism- and anxiety-like behaviors

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Abstract: Aims: Autism spectrum disorder (ASD) is an intractable neuropsychiatric disorder while anxiety disorder is the most common psychiatric disorder in the world. There is increasing evidence that ASD and anxiety are highly comorbid conditions, and understanding the mechanisms of comorbidity is essential for therapeutic strategies for ASD. It is of great significance to take into account both molecular signal and circuit regulation for elucidating the pathological mechanism of autism-anxiety comorbidity. **Methods:** 1) *Tmem74* global knockout mice (*Tmem74*$^{-/-}$ mice) and *Tmem74* specific knockout mice were constructed. 2) Phenotypic changes of mice were observed and analyzed by behavioral methods. 3) Virus tracing technique is used for circuit tracing and neuronal clustering. 4) Neuronal properties were recorded by in

vitro patch clamp technique and in vivo electrophysiological recording method. 5) Chemogenetics, optogenetics, transgenic method and pharmacological regulation were used to induce or reverse the pathological behaviors. **Results:** Bioinformatics data validated the high correlation between *Tmem74* and autism-anxiety comorbidity. Autism and anxiety related behavioral tests and immunofluorescence verified that *Tmem74* knock out led to autism- and anxiety-like behaviors in a prelimbic cortex (PL) dependent manner. The autism- and anxiety-like pathological phenotypes occurs along with the hyperactivity of PL-basal lateral amygdala (BLA) circuit and disturbance of PL-dorsal striatum (dSTR) circuit. Optogenetic manipulation of PL-dSTR circuit or PL-BLA circuit in wildtype/ *Tmem74*$^{-/-}$ mice could induce/rescue autism-like or anxiety-like behaviors respectively. The combination of virus tracing technique and patch clamp recording method validated two anatomically but not electrophysiologically distinct subpopulations of PL pyramidal neurons mediated autism- and anxiety-like behaviors respectively. **Conclusions:** In conclusion, our study reported a new rodent model of autism-anxiety comorbidity and explored the pathogenesis of autism-anxiety comorbidity from the perspective of molecule and circuit, which might shed light on the treatment of comorbidity via targeting TMEM74 signaling in PL-related circuits.

Disclosures: Y. Luo: None. **F. Han:** None.

Presentation Number: NANO30.04

Topic: A.07. Developmental Disorders

Support: Roy J. Carver Charitable Trust to A.M.A (Grant #23-5683)

Title: Influenza A virus infection during pregnancy disrupts fetal brain macrophages and neocortical development in a dose-dependent manner

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[1]Neurosci. Program, [2]Dept. of Animal Sci., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Yolk-sac-derived brain-resident macrophages–microglia and border-associated macrophages (BAMs)–are among the first cells to migrate to the brain and are present at the onset of neurogenesis, placing them at the center of early neuronal support. During fetal brain development, microglia stimulate cortical neural precursor cell (NPC) proliferation and migration and subsequent phagocytosis of excess NPCs in the cortex. Disruption of these microglial-dependent processes has been observed in rodent models that initiate maternal immune activation (MIA) with sterile immunostimulants, and several of these studies directly implicate microglia in MIA-mediated cortical pathologies. Less is known about BAMs, which do not infiltrate the brain parenchyma but rather reside in peripheral regions where they carry out constant immune surveillance. To date, no study has examined how gestational influenza A virus (IAV) infection, which is linked with increased incidence of neurodevelopmental disorders in human offspring, impacts embryonic brain-resident macrophages. We hypothesize that systemic inflammation from live IAV infection during pregnancy redirects fetal brain-resident macrophages from their normal neurotrophic support roles, resulting in altered cortical development. To test our hypothesis, we inoculated pregnant C57BL/6NTac mice on gestational day (GD)9.5 with H3N2 IAV strain X31. Maternal serum, lungs, and fetal brains were collected

on GD16.5, seven days-post-inoculation. Pregnant dams received 10^4 $TCID_{50}$ X31 ($X31_{hi}$; n=10), 10^3 $TCID_{50}$ X31 ($X31_{mod}$; n=9), or a mock inoculation with saline (control; n=10) across three identical replicates per end point. Maternal immune responses, as well as fetal microglia, border-associated macrophages (BAMs), and cortical neurons, were characterized at both time points and complemented with fetal brain transcriptomics. We observed a dose-dependent reduction in upper excitatory neuronal markers and cortical thickness, concomitant with a downregulation in fetal brain transcripts related to neuronal migration. We also found an increase in the number of meningeal BAMs and an overall increase in brain-resident macrophage phagocytic capacity in our high-dose fetal brains only. Ongoing experiments aim to analyze cellular functional capacity *in vitro.* Overall, our data support the existence of an infection severity threshold for IAV-induced fetal cortical abnormalities and altered brain-resident macrophages, confirming the use of live pathogens in MIA modeling to improve translatability to the clinic.

Disclosures: A.M. Otero: None. **M.G. Connolly:** None. **R.J. Gonzalez-Ricon:** None. **I. Chalen:** None. **A.M. Antonson:** None.

Presentation Number: NANO30.05

Topic: A.07. Developmental Disorders

Support: NIH/NIA Grant F31AG077918
JPB

Title: Cic isoforms have differential functions that are critical for survival and brain function

Authors: *H. LEE[1], M. DURHAM[1], E. VILLAVICENCIO GONZALEZ[2], E. CHU[1], M. HASAN[3], A. J. TROSTLE[1], C. STROHLEIN[2], H. CHEN[2], H. Y. ZOGHBI[4];
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Abstract: Capicua (CIC) is a transcription factor that forms a co-repressor complex with either Ataxin1 (ATXN1) or its paralog, Ataxin1-like (ATXN1L), to repress target gene expression. Our lab has demonstrated that the gain of function of the ATXN1-CIC complex drives Purkinje cell degeneration in spinocerebellar ataxia type1. Conversely, complete loss of function of this complex leads to perinatal lethality demonstrating that it is essential for survival. Additionally, heterozygous loss of *CIC* function in humans leads to a neurodevelopmental disorder known as *CIC* haploinsufficiency syndrome (CHS) characterized by intellectual disability, autism, and ADHD. CIC has two major isoforms, CIC-L (long) and CIC-S (short), generated by alternative promoter use. While the known DNA binding domains and ATXN1 binding domain are common in both isoforms, CIC-L has 931 and CIC-S has 22 unique amino acids. Recently, we have identified seven patients with de-novo variants in CIC-L who display symptoms of CHS. Given the importance of CIC in survival, neurodevelopment, and the identification of CIC-L-specific coding variants, we set out to understand the importance of each CIC isoform. We first examined the expression pattern of both isoforms during postnatal development and determined that CIC-S is higher in early development while CIC-L increases as animals mature. This suggests that CIC-L and CIC-S could have different functions throughout development. To investigate the functional consequences of the loss of each CIC isoform, we generated Cic-*L* and *Cic-S* isoform-specific knock-out (KO) mice using CRISPR/Cas9. Interestingly, *Cic-S*-KO mice have reduced

survival at weaning, but *Cic-L*-KO does not lead to premature lethality. Conversely, behavioral analysis in surviving adult mice demonstrates that *Cic-L*-KO mice have impaired learning, memory, and motor coordination, but *Cic-S*-KO does not exhibit these behavioral phenotypes. Transcriptomic analysis using adult mouse brain tissue showed greater gene expression changes in *Cic-L*-KO compared to *Cic-S*-KO. Intriguingly, CIC motif genes were enriched in differentially expressed genes of *the Cic-L*-KO adult brain but not in the P0 brain. These data collectively support the notion that CIC-S is critical for early development while CIC-L is important for adult brain function. We plan to elucidate the mechanism leading to the early lethality of *Cic-S*-KO mice focusing on the lung phenotype that we hypothesize is driving the lethality. Studying the role of the two CIC isoforms will not only help advance our understanding of the biology of CIC but will also provide insights into the contributions of each isoform to various human disorders.

Disclosures: H. Lee: None. **M. Durham:** None. **E. Villavicencio Gonzalez:** None. **E. Chu:** None. **M. Hasan:** None. **A.J. Trostle:** None. **C. Strohlein:** None. **H. Chen:** None. **H.Y. Zoghbi:** None.

Presentation Number: NANO30.06

Topic: A.07. Developmental Disorders

Support: NIH NINDS 1F99NS130926
NIH TL1TR0
NJGOV CAUT22AFP011
NJHF PC 124-22
Autism Research Institute
Busch Biomedical Grant
Lurie Family Foundation

Title: Autism risk genes influence the effects of a perturbed gut microbiome on hippocampal neurogenesis and behavior

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Abstract: Infancy is a critical period of development when the gut microbiome-brain axis is sensitive to both genetic risk and environmental exposures. Such alterations during early life have been linked to adverse health outcomes and neuropsychiatric conditions. We conducted studies in mice using a gene by environment (GxE) model to examine how an altered microbiome affects neurodevelopment and behavior, and if identified alterations were

exacerbated by genetic constitution. Wildtype and 16p11.2 deletion (16pDel) male and female mice were exposed to a brief course of cefdinir, a cephalosporin antibiotic, on postnatal (P) days 5-9, followed by sacrifice at several developmental timepoints. The 16pDel mouse was chosen to model genetic vulnerability due to its significant implications in neurodevelopmental disorders. At P13, we observed perturbations to the microbiome in all cefdinir-exposed mice, with accompanying changes in serum lipids and metabolites. However, there was a unique GxE effect in hippocampal gene expression and stem cell proliferation of cefdinir-exposed 16pDel males. This effect was accompanied by a compromised intestinal barrier in 16pDel males, revealed by increased intestinal permeability *in vivo.* To determine if the microbial and neural alterations following early life cefdinir exposure persisted or recovered, we extended our characterizations to P21. Although the gut microbiome began to recover by P21, there were numerous alterations in hippocampal gene expression in all cefdinir-exposed mice suggesting disorder progression, with the most robust changes in 16pDel males. Lastly, to assess the impact of early life cefdinir exposure on behavior, we conducted a longitudinal behavioral paradigm from P21-P89. Across three cohorts, we observed decreased juvenile sociability, compromised risk-aversion behaviors, heightened social preference, and impaired associative learning in the cefdinir-exposed mice. Taken together, this work highlights the progressive impact of early life microbial alterations on brain development and behavior and provides the novel discovery that genetic constitution influences the subsequent changes in hippocampal gene expression and neurogenesis induced by an antibiotic-perturbed microbiome.

Disclosures: C.R. McDermott: None. **Z. Gao:** None. **A. Mirmajlesi:** None. **K. Kimbark:** None. **C. Ntim:** None. **D. Thomas:** None. **Z. Mughal:** None. **A. Halchenko:** None. **X. Zhang:** None. **X. Zhou:** None. **J.H. Millonig:** None. **B.A. Samuels:** None. **M.J. Blaser:** None. **E.M. DiCicco-Bloom:** None.

Presentation Number: NANO30.07

Topic: A.07. Developmental Disorders

Support: NIH R21MH087829 to U. R.
NIH R5611264201 to U. R.
VETMED Startup Fund to U. R.
The Brain Foundation Grant to M. K.

Title: Evaluation of Novel Treatment Strategies in Genetic Mouse Models of Neurodevelopmental Disorders

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Abstract: Rare genomic copy number variants (CNVs) increase the risk of developing neurodevelopmental disorders (NDD) such as schizophrenia (SCZ) and autism spectrum disorders (ASD). The pathophysiological mechanisms underlying the increased disease risk are

not well understood. On the other hand, there is a need for the development of more effective pharmacological interventions. In order to better understand the neurobiological basis of such CNVs and to evaluate novel treatment strategies, we generated 1q21.1 and 15q13.3 microdeletion and microduplication mouse models, and a *Gldc* (glycine decarboxylase) triplication mouse model. Adult mice carrying these CNVs were used in the seahorse assay to assess mitochondrial bioenergetics, and in behavioral paradigms related to hyperactivity, prepulse inhibition, startle habituation, social interactions and cognitive symptoms such as working memory. One of the pathophysiological phenomena in developmental disorders such as SCZ and ASD are changes in mitochondrial function. Therefore, to determine mitochondrial function, we used the seahorse assay, which measures the oxygen consumption rate (OCR) as a function of mitochondrial bioenergetics in hippocampal subregions. In all the CNV mice we identified changes in mitochondrial bioenergetics in terms of a reduced OCR in CA1 or dentate gyrus of hippocampus. Behavioral studies showed startle habituation deficits, cognitive deficits, and reduced social interactions. Our results provide evidence that any perturbation (either deletion or duplication) in 1q21.1, 15q13.3 chromosomal regions, and triplication of Gldc alters mitochondrial function and induces behavioral changes, consistent with a potential role in the pathophysiology of neurodevelopmental disorders. In preliminary studies, chronic treatment with the partial mitochondrial complex I inhibitor CP2 known for its ability to increase mitochondrial biogenesis, reversed behavioral deficits in mice with 4 copies of *Gldc*. Our long-term plan is to identify neuronal population or ensemble activities in the prefrontal cortex which might be useful as biomarkers to evaluate novel pharmacological treatment strategies with drugs that may be repurposed for NDD treatment, including the PGC1α activator, bezafibrate, which increases mitochondrial biogenesis, and the mitochondrial complex I inhibitor metformin.

Disclosures: M. Kambali: None. **M. Wang:** None. **R. Nagarajan:** None. **J. Lyu:** None. **S. Trushin:** None. **E. Trushina:** None. **U. Rudolph:** None.

Presentation Number: NANO30.08

Topic: A.07. Developmental Disorders

Title: Loss of functional Kmt2c causes abnormal behavior and neuronal development

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Abstract: Neurodevelopmental and neuropsychiatric disorders result from alterations in the formation and function of neural circuits. For the majority of individuals suffering from these disorders, precise etiologies are unknown. In rare cases, highly penetrant loss-of-function mutations in genes critical for brain development are identified. One recently discovered gene in which loss-of-function mutations are tightly associated with these disorders is the Lysine Methyltransferase 2C gene (*KMT2C*). The first germline *KMT2C* mutations were identified in individuals diagnosed with intellectual disability. Subsequent large-scale whole exome sequencing studies of large populations of autistic individuals has confirmed a strong association between autism and autosomal dominant *de novo* loss-of-function mutations in *KMT2C*. Surprisingly, genome-wide sequencing studies in populations of individuals with other neuropsychiatric disorders including schizophrenia and bipolar disorder have also identified deleterious mutations in *KMT2C*. Together, these data demonstrate that haploinsufficiency of

KMT2C is sufficient for development of a spectrum of neuropsychiatric disorders.*KMT2C* encodes a chromatin methyltransferase. The KMT2C protein is a core component of one of the Complex of Proteins Associated with Set1 like (COMPASS-like) complexes. The COMPASS-like complexes methylate Histone H3 on lysine 4 (H3K4 marks) which are associated with chromatin remodeling and typically increased transcriptional activity. KMT2C is one of the catalytic subunits of these complexes with the SET domain being the critical catalytic domain of KMT2C. The target genes of KMT2C in intact neuronal circuits are unknown as are the neuronal abnormalities leading to neuropsychiatric disorders with *KMT2C* mutations.*KMT2C* is richly expressed in the prenatal human brain then its expression decreases following birth although is maintained in adult mammals. In this study, we have discovered that neuronal deficiency of the SET domain of *Kmt2c* in mice results in hyperactivity, reduced despair, abnormal sensory gaiting and impaired motor function. Furthermore, depletion of *Kmt2c* in cultured mouse neurons causes increased complexity of dendritic arborization as well as mature dendritic spines. This data affirms Kmt2c's role in brain development and function. We are now investigating the in vivo morphological and transcriptomic changes associated with complete loss of functional Kmt2c.This work confirms a function for Kmt2c in early neuronal development and that early developmental loss of functional Kmt2c results in functional abnormalities in adult mice.

Disclosures: J. Holder: None.

Nanosymposium

NANO31: Assessment of Stroke Recovery

Location: MCP Room N228

Time: Monday, October 7, 2024, 1:00 PM - 3:30 PM

Presentation Number: NANO31.01

Topic: C.09. Stroke

Support: R01 NS115845

Title: Association of brain aging and focal brain damage in the subacute phase of stroke recovery

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Abstract: Stroke is a leading cause of long-term disability worldwide, underscoring the importance of understanding factors that influence post-stroke recovery. Measures of global brain health, such as brain age, have been associated with stroke outcomes at chronic timepoints. In particular, brain predicted age difference (brain-PAD) is a neuroimaging-derived biomarker that quantifies neurobiological aging relative to chronological age, with higher brain-PAD indicating an older-appearing brain. In the present study, we examined the relationship between initial stroke severity and brain-PAD. We hypothesized that larger focal lesion damage at baseline accelerates brain aging during the subacute phase. Conversely, we also hypothesized that brain age at baseline can explain change in focal stroke damage during the subacute phase. We conducted a longitudinal prospective study examining 38 patients across 2 research sites, collecting high resolution T1-weighted brain structural MRIs at two timepoints: (1) within 21 days post stroke and (2) at 90 days post stroke. Stroke lesions were manually segmented at each timepoint to quantify extent of stroke damage. MRIs were automatically parcellated and segmented according to the Destrieux atlas using FreeSurfer 5.3 to quantify whole brain volume and regional neuroanatomical metrics. These were then used to predict brain age via a pre-trained and publicly available Extra Trees Regression model (PyBrainAge). Brain-PAD was quantified as the patient's predicted brain age minus their chronological age. All structural images and masks were linearly transformed to standard MNI space. Robust linear mixed effects regression models were used to examine the relationships between stroke severity (quantified by lesion volume) and brain aging, with covariates including age, sex, and intracranial volume at baseline, and a random effect of site. Our findings suggest that the greater the stroke damage at baseline, the larger the gap between predicted age and chronological age became over the course of the subacute phase ($b = 1.74$, 95% CI 0.61-2.87, $p = 0.003$). Conversely, the older the brain appeared at baseline (larger brain-PAD), the greater the change in lesion size from baseline to 90 days ($b = 0.02$, 95% CI 0.01-0.04, $p = 0.010$). Through this exploratory analysis, we demonstrate a relationship between brain aging and focal stroke damage during the subacute period. Future studies will be done to investigate how the relationship between brain aging and focal lesion damage relates to functional disability at baseline and recovery during the subacute phase of stroke.

Disclosures: M. Khan: None. **O. Marin-Pardo:** None. **S. Chakraborty:** None. **M.R. Borich:** None. **M. Castillo:** None. **J.H. Cole:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Shareholder and advisor with BrainKey, Claritas HealthTech PTE. **S.C. Cramer:** None. **E.E. Fokas:** None. **N.H. Fullmer:** None. **J. Gumarang:** None. **L.X. Hayes:** None. **H. Kim:** None. **A. Kumar:** None. **E.A. Marks:** None. **E.R. Rosario:** None. **H.M. Schambra:** None. **N. Schweighofer:** None. **G. Song:** None. **M. Taga:** None. **C.J. Winstein:** None. **Z. Zheng:** None. **S. Liew:** None.

Presentation Number: NANO31.02

Topic: C.09. Stroke

Support: NIH Grant R01NS090677
Funding was made available by the Texas Legislature to the Lone Star

Stroke Clinical Trial Network. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Government of the United States
Canadian Institutes of Health Research (PI Boyd: PTJ-148535, MOP-130269, MOP-106651)
NIH R01 NS076348; IIEP-2250-14
Department of Veterans Affairs
P20 GM109040, 1IK6RX003075
European Research Council (ERC NGBMI, 759370), Federal Ministry of Research and Education (BMBF SSMART, 01DR21025A), Deutsche Forschungsgemeinschaft (DFG SO932/7-1)

Title: Cross-sectional Association of Perivascular Spaces with Sensorimotor Outcomes in Stroke: An ENIGMA Analysis

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Abstract: The impact of global brain health measures, such as white matter hyperintensities (WMH) and brain predicted age difference (brain-PAD), have been linked to inter-individual differences in stroke sensorimotor outcomes. Another global brain health measure that could impact sensorimotor outcomes is enlarged perivascular spaces (ePVS). ePVS may be related to

poor metabolic waste clearance and may lead to increased cellular toxicity and blood-brain barrier dysfunction. Furthermore, ePVS have been associated with cerebral small vessel disease and poor quality of life outcomes post-stroke. Here, we hypothesized that 1) higher ePVS volume will be associated with worse sensorimotor outcomes post-stroke, and 2) higher burden of other global brain health measures (WMH, brain-PAD) will be associated with higher ePVS volume. To test these hypotheses, we conducted a cross-sectional analysis of 602 individuals, with a 3D T1-weighted volumetric brain MRI and a manually segmented stroke lesion mask, across 24 sites from a large, multi-site retrospective dataset compiled by the ENIGMA Stroke Recovery Working Group. MRIs were preprocessed and linearly registered to a standard MNI template. ePVS were automatically segmented from the basal ganglia and centrum semiovale of the white matter using a validated algorithm, Frangi filter vesselness probability and optimum threshold. We calculated corticospinal tract lesion loads and extracted WMH and brain-PAD metrics for each subject. Separate robust mixed effects regressions were used to examine a) the effects of ePVS in the basal ganglia and white matter on sensorimotor outcomes, and b) the effects of WMH burden and brain-PAD on ePVS, with age, sex, time since stroke, corticospinal tract lesion load, intracranial volume, and stroke lesion volume as covariates, adding site as a random effect. Worse sensorimotor outcomes were associated with higher ePVS volume in the white matter ($b = -0.06$, $p = 0.047$). Higher burden of deep WMH ($b = 0.17$, $p < 0.001$), periventricular WMH ($b = 0.11$, $p < 0.001$) and higher brain-PAD ($b = 0.01$, $p < 0.001$) were associated with higher ePVS volume in white matter. Future studies may examine the progression of ePVS volume and WMH burden across time to determine trajectories of post-stroke sensorimotor recovery.

Disclosures: S. Chakraborty: None. **O. Marin-Pardo:** None. **M.H. Khan:** None. **J. Choupan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Has a published patent for the PVS segmentation method used in this work.. **G. Barisano:** None. **B.P. Tavenner:** None. **M.R. Donnelly:** None. **J.W. Andrushko:** None. **N. Banaj:** None. **M.R. Borich:** None. **L.A. Boyd:** None. **C.M. Buetefisch:** None. **A.B. Conforto:** F. Consulting Fees (e.g., advisory boards); One consultancy for Boehringer-Ingelheim. **S.C. Cramer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); License Fee Payments, Technology, or Inventions: Telerehabilitation, from UCLA. Stock Options, Medical Equipment & Materials: TRCare, Constant Therapeutics, Panaxium, and Elevian.. F. Consulting Fees (e.g., advisory boards); Dr. Cramer is or has been a consultant Abbvie, Constant Therapeutics, BrainQ, Myomo, MicroTransponder, Neurolutions, Panaxium, NeuExcell, Elevian, Helius, Omniscient, Brainsgate, Nervgen, Battelle, an. **M. Domin:** None. **A. Dula:** None. **B. Hordacre:** None. **S.A. Kautz:** None. **N. Jahanshad:** None. **M. Lotze:** None. **F. piras:** None. **K.P. Revill:** None. **N. Schweighofer:** None. **S.R. Soekadar:** None. **S.I. Thomopoulos:** None. **L.T. Westlye:** None. **G.F. Wittenberg:** F. Consulting Fees (e.g., advisory boards); Scientific Advisory Boards: (1) Myomo, Inc. (2) Neuro-Innovators, LLC. **K.A. Wong:** None. **P.M. Thompson:** None. **S. Liew:** None.

Presentation Number: NANO31.03

Topic: C.09. Stroke

Support: #NRCRSP-EX23010

Title: Outcome Measures and Decision-Making Algorithms for Stroke and Brain Injury in South Korea: A Physical Therapy Perspective

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Abstract: Objective: This study aimed to identify the outcome measures (OMs) most utilized for patients with neurological disorders such as stroke and traumatic brain injury in South Korea and to create a decision-making algorithm to assist physical therapists in selecting appropriate OMs. **Methods:** This study involved twenty highly experienced physical therapy experts from renowned South Korean hospitals, each averaging 18 ± 6 years of experience. Participants responded to a structured questionnaire, and ten were selected for further in-depth interviews. The questionnaires explored the evaluation tools commonly employed, the reasons for their use, and their incorporation into frameworks like the International Classification of Functioning, Disability and Health (ICF) and the Korean Rehabilitation Patient Group (KRPG). Concurrently, a systematic literature review was conducted across PubMed, Embase, EBSCO, and ProQuest to evaluate the OMs based on psychometrics and clinical utility. These findings were then used to formulate five distinct decision-making algorithms. **Results:** On average, physical therapists utilized 9.7 OMs, ranging from 7 to 16. All participating hospitals used at least seven evaluation tools classified under "Integrated Rehabilitation Functional Assessment" for insurance reimbursement. Eighty percent of hospitals adopted the KRPG system, while a smaller number implemented the ICF, with three hospitals using both. The review of 123 journal articles produced a comprehensive list of 140 OMs, which informed the creation of decision-making algorithms structured around the disease type, rehabilitation settings, KRPG classification, disease stage, and patient's functional status. **Conclusions:** These algorithms provide a structured approach to help physical therapists make more informed decisions regarding OM selection, potentially increasing the precision of patient assessments. Further validation and testing of these algorithms are recommended to ensure their effectiveness across various levels of therapist experience.

Disclosures: S. Kim: None. **S. Hwang:** None.

Presentation Number: NANO31.04

Topic: C.09. Stroke

Title: Epigenetic mechanisms mediate sex differences in post-stroke brain injury and recovery

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Abstract: Stroke is a leading cause of death that displays sex disparities in incidence, severity, and outcome. Emerging evidence suggests that epigenetic mechanisms play a crucial role in modulating these sex differences by regulating gene expression in response to stroke-related insults. For example, the ten-eleven translocases (TET1-3) that regulate the DNA hydroxymethylome and DNA methylome have been shown to mediate endogenous protection against stroke-induced brain injury. In the current study we aimed to examine the impact of sexually dimorphic epigenetic regulation in post-stroke recovery in young and middle-aged

transgenic mice. C57Bl6/J mice were subjected to middle cerebral artery occlusion (MCAO) to induce focal cerebral ischemia which induced brain damage in the striatum, hippocampus, and cortical regions of the brain. Spatial transcriptomics of the post-stroke brain between 1 and 7 days following MCAO revealed sex- and age-dependent changes in epigenetic enzymes including TETs and genes associated with neuronal function. Mice were further subjected to a battery of neurobehavioral tests to assess motor function and cognitive function recovery up to 30 days following stroke induction. Both young and middle-aged female mice displayed improved motor function recovery compared to young and middle-aged male mice, respectively. Wild-type, $TET1^{-/-}$, $TET2^{-/-}$, and conditional $TET3^{-/-}$ knockout mice displayed alterations in the DNA hydroxymethylome and showed increased infarct sizes and edema in the brain compared to WT mice. Viral and pharmacological replenishment of TET enzymes showed more robust post-stroke cognitive and motor function recovery in female mice compared to male mice. Collectively, this study indicates that sexual dimorphism in post-stroke epigenetic modulation contributes to differences in brain region-specific gene expression, brain damage, and post-stroke recovery.

Disclosures: H. Huang: None. **K.C. Morris-Blanco:** None.

Presentation Number: NANO31.05

Topic: C.09. Stroke

Support: NIDCD P50DC012283
NIDCD F30DC021092

Title: Properties of neural dynamics influence long-term network changes supporting recovery in post-stroke aphasia

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Abstract: Recovery from post-stroke aphasia (PSA), which refers to difficulties with producing and/or understanding language varies greatly, with factors such as initial severity and lesion characteristics being among the most reliable predictors. However, variation in recovery trajectories remains incompletely explained. Our previous work investigating dynamic functional connectivity (dFC), i.e., time-varying interregional correlations in the functional MRI BOLD signal, in PSA found that greater temporal variability (TV) of dFC predicted greater treatment-induced improvement in picture naming. We proposed the following mechanism for this finding: (1) Transient inter-regional synchronization facilitates synaptic plasticity between regions and (2) greater TV represents a greater diversity of connectivity configurations sampled over time, producing more opportunities for plasticity. This study sought to test this mechanism by investigating the relationships between TV, treatment-induced network changes, and behavioral treatment response. Treatment-induced network connectivity changes were assessed in 30 participants with PSA who received a semantic treatment for aphasia. These changes were quantified using graph metrics of static functional connectivity (sFC, i.e., time-invariant interregional BOLD signal correlations) which were then related to baseline TV of dFC. Additionally, simulations of healthy neural dynamics were performed using a parametric mean

field model to further investigate the influence of short-term dynamics on long-term connectivity changes. Each simulation included one of several alternative Hebbian plasticity rules whereby transient coactivation leads to changes in connection strength.Higher TV was found to be predictive of treatment-induced decreases in node-level sFC strength, which were in turn associated with greater behavioral treatment gains. These decreases in node-level strength were also significantly associated with global increases in small-worldness, a measure of balance between local clustering of nodes and global network efficiency, which was in turn significantly associated with better behavioral treatment response. Simulation results were consistent with these findings showing that only plasticity rules that drove down connection strengths between non-hub nodes produced increases in global graph measures similar to those seen in patients with greater treatment gains. Overall, both the experimental findings and simulations provided support for higher TV facilitating node-level changes that result in global network changes that support recovery.

Disclosures: I. Falconer: None. **M. Varkanitsa:** None. **A. Billot:** None. **S. Kiran:** None.

Presentation Number: NANO31.06

Topic: C.09. Stroke

Support: NIH R01 HD109157
NSF 2401215

Title: Asymmetry of Motor Pathway Tractography in Chronic Hemiparetic Stroke

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Abstract: Stroke is a leading cause of adult long-term disability, frequently resulting in upper extremity motor impairment. The common loss of corticospinal and corticobulbar (corticofugal projections) has been shown to result in an increased use of the contralesional hemisphere. Utilizing neuroimaging data from Diffusion-weighted Magnetic Resonance Imaging (DWI), this study aims to provide imaging biomarkers regarding the changes in the integrity of motor descending motor pathways from each of the two hemispheres and between hemispheres. This early-phase study analyzed five stroke participants (Fugl-Meyer scores: 22-42) and six individuals in a control group, ensuring gender balance (55% male, 45% female) and matching ages between the two groups (p-value: 0.452; non-significant difference). We discovered a smaller fractional anisotropy (FA) value in the ipsilesional corticospinal projections compared to the contralesional side in hemiparetic stroke participants. This indicates fiber loss in the motor descending pathways in the lesioned hemisphere. No such differences between the two hemispheres were observed in the control group. In addition, we are also exploring the integrity

of cortico-reticular projection from the contralesional hemisphere versus controls given the previously reported findings from a much larger stroke sample that reported greater integrity of medial reticulospinal projections at the contralesional side compared to controls. Furthermore, comparing the corpus callosum body projections between stroke and control on a case-by-case age-matched condition, we found there is a smaller FA for stroke subjects. Overall, these preliminary findings contribute to our understanding of post-stroke anatomical changes to the corticofugal and transcallosal pathways and may provide future imaging biomarkers for determining the link between losses in neural circuitry and motor deficits in chronic hemiparetic stroke.

Disclosures: R.H. Peng: None. **P.B. Camacho:** None. **B. Sutton:** None. **J.P. Dewald:** None. **Y. Yang:** None.

Presentation Number: NANO31.07

Topic: C.09. Stroke

Support: NIH Grant R01 AG075727
NIH Grant R01 AG075876
Boston University office of research - Kilachand Fund
Boston University Neurophotonics Center
NIH Grant RF1 AG062831
NIH Grant RF1-AG043640
NIH Grant R01-AG078460

Title: Label-free assessment of the structural integrity of myelin with quantitative birefringence microscopy

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Abstract: Many neurodegenerative diseases and aging processes manifest functional deficits, such as motor and cognitive impairment. While many deficits reflect loss of neurons, as in Alzheimer's Disease, others are due to damage to myelin "disconnecting" critical brain circuits, highlighting the importance of assessing myelin degradation as a biomarker for disease. Electron microscopy (EM) is the gold standard for identifying damaged myelin, but due to complex sample preparation and low imaging throughput (≤100 μm^2 per image), it is almost impossible to use EM for examination of the whole brain in large-brained species such as monkeys or humans. In contrast, birefringence microscopy (BRM) is a widefield, label-free imaging technique with minimal sample preparation that enables large-area (mm^2), high-resolution (≤400 nm), structural imaging of myelin in thin post-mortem brain sections (<50 μm). BRM uses polarized light to extract quantitative parameters (i.e., orientation and myelin density), which can be used to assess myelin structural changes in individual myelinated axons. Here, we present preliminary results using BRM to quantify the extent of myelin damage in a rhesus monkey model of cortical injury by placing a focal lesion in the hand representation of the primary motor cortex of one

hemisphere by transecting pial blood vessels. Cell death occurs at the site of the injury, followed by Wallerian degeneration of the axon and myelin sheath. Using BRM, we examined two age-matched monkeys, with 15 whole-brain coronal sections taken every 300 µm through the extent of the lesion in each monkey. One brain was harvested at 6 weeks post-injury and the other at 12-weeks post injury. In each section, we quantified the frequency of myelin debris in the corpus callosum (CC) and found a lower density of debris in the 12-week case compared to the 6-week case, likely reflecting innate CNS repair and clearance. Furthermore, in the 6-week case, BRM revealed an accumulation of myelin debris concentrated in CC regions at the mid-lesion level, which decreased with increasing distance from the lesion, both anteriorly and posteriorly. These preliminary results confirm BRM's utility in studying myelin alterations in disease models and its potential for assessing treatment response. We plan to use BRM to evaluate myelin changes across the brain in cortical injury monkeys following a novel treatment - mesenchymal stem-cell derived extracellular vesicles - a therapeutic known to facilitate myelin repair and recovery of function. BRM is a highly accessible technique, which can enable novel studies of myelin changes in a wide array of disease models and treatment strategies.

Disclosures: A.J. Gray: None. **R. Robinson:** None. **S.A. Berghol:** None. **T.L. Moore:** None. **D.L. Rosene:** None. **I.J. Bigio:** None.

Presentation Number: NANO31.08

Topic: C.09. Stroke

Support: Sheikh Khalifa Stroke Institute
American Heart Association (24POST1187285, PI: Bellinger)
ReproRehab Program (NICHD/NCMRR R25HD105583)

Title: Assessing post-stroke motor impairment across the continuum of rehabilitation using the Fugl-Meyer Motor Assessment

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Abstract: The Fugl-Meyer Motor Assessment (FMA) is a highly recommended standardized outcome measure for stroke-related motor impairment. Longitudinal assessment of impairment allows for phenotyping of recovery trajectories, which can facilitate precision rehabilitation approaches. In 2021, the Sheikh Khalifa Stroke Institute at Johns Hopkins Hospital implemented a new standard assessment battery for routine clinical administration across the continuum of rehabilitation, which included electronic health record entry of the Upper Extremity FMA for occupational therapists and the Lower Extremity FMA for physical therapists. This involved administration of the FMA at initial evaluation in acute care; initial evaluation in inpatient rehabilitation (if not assessed in acute care); and initial evaluation, 30 days, 90 days, and discharge in the outpatient setting. Here, we aimed to 1) determine the mean change in FMA score across the continuum of rehabilitation and 2) determine the proportion of patients that achieved the FMA's minimal clinically important difference (MCID) throughout rehabilitation. The data repository managed by the Johns Hopkins Rehabilitation Precision Medicine Center of Excellence included 1454 Upper Extremity FMA scores and 1073 Lower Extremity FMA scores

from June 2021 to December 2023. The final analytic set, which required repeated measures for patients, consisted of 549 Upper Extremity scores from 219 patients and 383 Lower Extremity scores from 141 patients. The MCIDs used were 10 points for the upper limb (maximum score of 66) and 5 points for the lower limb (maximum score of 34). The mean ± standard deviation overall change was 5.75 ± 12.96 points for the upper extremity and 1.35 ± 5.33 points for the lower extremity. For patients with multiple scores in settings, mean change during inpatient therapy was higher than mean change during outpatient therapy for both the upper (7.60 vs. 4.70) and lower (1.88 vs. 1.01) limb. Across the continuum of rehabilitation, 26.5% of patients achieved the MCID for the upper extremity and 19.9% achieved it for the lower extremity. For patients with repeated measures in each setting, greater proportions of patients achieved the MCID during inpatient (43.6% for upper, 31.3% for lower) than outpatient (18.5% for upper, 10.5% for lower) rehabilitation. These findings provide new knowledge about the trajectories of the Upper and Lower Extremity FMA across the continuum of care in real-world rehabilitation settings. Future work will explore differences in social determinants of health and clinical characteristics between patients that do and do not achieve the MCID to inform predictive models of motor recovery.

Disclosures: G.C. Bellinger: None. **K.H. McLaughlin:** None. **B. Ye:** None. **K.N. Daley:** None. **P. Raghavan:** None. **R.T. Roemmich:** None.

Presentation Number: NANO31.09

Topic: C.09. Stroke

Support: NIH 1R01NS117606-01A1
NSF 1916894
new faculty start-up funds from UTHealth

Title: Longitudinal MRI to Assess Brain Characteristics in Murine Transient and Permanent Middle Cerebral Artery Occlusion Models

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Abstract: Ischemic stroke poses a significant global health challenge, demanding reliable experimental models for understanding its pathophysiology and developing therapies. Filament middle cerebral artery occlusion (fMCAO) and photothrombotic middle cerebral artery occlusion (pMCAO) are established murine models of ischemic stroke, simulating transient and permanent occlusions, respectively. Despite their widespread use, there remains a need for a comprehensive evaluation of brain characteristics induced by these models. This study aimed to address the gap by employing longitudinal magnetic resonance imaging (MRI) analysis to assess blood-brain barrier (BBB) permeability, infarct volumes, brain edema, and hemorrhagic transition in murine fMCAO and pMCAO models. By evaluation of the real-time efflux rate of gadolinium contrast from blood into the brain underwent dynamic contrast enhanced (DCE) T1-weighted MR

perfusion, we quantified the volume transfer constant (K^{trans}) map and observed a significant increase in BBB permeability in the ischemic hemisphere at 6 hours post-stroke, followed by a subsequent decrease at 24 hours in both models. However, analysis of cerebral infarct volumes using T1-weighted image (T2WI) sequences showed a significant increase at 24 hours compared to 6 hours post-treatment in both models. Interestingly, we found that the volume of brain edema and midline offset distance were aggravated at 24 hours in the fMCAO model, while in the pMCAO mouse model, it was attenuated at 24 hours compared with 6 hours. The discrepancy in brain edema between fMCAO and pMCAO models likely stems from differing pathophysiological mechanisms. Furthermore, T1-star-weighted image (T2*WI) sequences demonstrated invisible hemorrhagic transitions in both models at both time points. The evaluation highlights the importance of timing in stroke assessment and therapeutic development. Understanding their temporal dynamics aids in refining preclinical models and translating findings to clinical settings, enhancing ischemic stroke management.

Disclosures: H. Hu: None. **S. Wu:** None. **C. Payne:** None. **J. Lee:** None. **S. Tabassum:** None. **S. Huang:** None. **C. Lee:** None. **A. Gusdon:** None. **H. Choi:** None. **X.S. Ren:** None.

Presentation Number: NANO31.10

Topic: C.09. Stroke

Support: GRC Grant CRC1451

Title: Exploring the structural reserve: Dynamic motor network integration depends on structural connectivity after stroke

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Abstract: Motor recovery after stroke arises from the functional reorganization of the motor network compensating for lesion-induced deficits. Both structural connectivity of the motor network[1] and changes in dynamic functional network connectivity (DFNC) assessed via fMRI[2] have been linked to motor impairment and recovery. Still, the understanding of network-level mechanisms underlying motor recovery needs to be completed.
We aimed to improve our mechanistic understanding of functional motor network reorganization by providing the first direct comparison of DFNC and structural motor network connectivity. In particular, we assessed whether specific alterations in structure-function relationships may contribute to motor recovery post-stroke.
We assessed DFNC and structural connectivity in 25 chronic stroke patients using resting-state fMRI and diffusion spectrum imaging. We applied a sliding window approach[2] to quantify DFNC between the primary sensorimotor cortex (SM) and a set of regions in the cortical sensorimotor network (SMN), basal ganglia (BG), and cerebellum (CB). Structural connectivity

between these regions was quantified via tract-based anisotropy[2] and compared to DFNC. Patients exhibited DFNC-states representing distinct levels of motor network segregation and integration. Higher levels of patients' structural connectivity between M1 and specific SMN, BG, and CB motor areas were positively correlated with motor recovery after stroke and resting state connectivity in states with higher levels of integration between the same regions. Higher levels of such integration-specific DFNC states and overall time spent in such states positively correlated with motor recovery.
Our results suggest a vicarious use of the motor network's structural reserve, resulting in enhanced resting-state within-network integration that supports motor control. In other words, elevated levels of premorbid structural motor network connectivity may serve as a structural prerequisite for increased functional motor network integration levels, which seem crucial for successful motor recovery after stroke. Our findings align with the notion that higher levels of structural connectivity between ipsilesional M1 and other motor areas may result in a lower propensity of the motor network to assume a state of high functional segregation, previously linked to higher levels of deficit in acute stroke patients.[2]
1. Paul T et al. Interhemispheric Structural Connectivity Underlies Motor Recovery after Stroke. Ann Neurol 2023
2. Bonkhoff AK et al. Acute ischaemic stroke alters the brain's preference for distinct dynamic connectivity states. Brain 2020

Disclosures: J. Günther: None. **T. Paul:** None. **L. Hensel:** None. **V.M. Wiemer:** None. **A.K. Bonkhoff:** None. **M. Cieslak:** None. **C. Tscherpel:** None. **C. Grefkes:** None. **S.T. Grafton:** None. **G.R. Fink:** None. **L.J. Volz:** None.

Nanosymposium

NANO32: New Insights About Visual Cortical Representation

Location: MCP Room S106

Time: Monday, October 7, 2024, 1:00 PM - 4:15 PM

Presentation Number: NANO32.01

Topic: D.06. Vision

Support: China 2018YFA0701400
China U20A20221
China 81961128029
China 2020C03004

Title: New Insights into Cortical Representation of Foveolar Vision in Macaque Monkey

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Abstract: Introduction: A common tenet of sensory representation is that species-specific behaviors are reflected in specialized brain organizations. In humans and nonhuman primates, the central one degree of vision is processed by the retinal foveola, a structure comprising a high density of photoreceptors crucial for primate-specific high acuity vision, color vision, and gaze-directed visual attention. However, the functional organization of foveolar visual cortex is not known. **Methods:** Functional EPI images (7T MRI, 0.6x0.6x1mm^3, custom 16 channel RF coil) were acquired from two macaque monkeys trained to fixate within a 1 deg window. Only trials with precise fixations were used. Visuotopic maps were obtained using fine 0.15º lines and arcs as well as phase encoding using fine iso-eccentricity and iso-polar stimuli. Foveolar representations were mapped using small 0.4º-0.8º centrally presented spot stimuli. **Results:** Visuotopic maps revealed maps and cortical magnification factors consistent with previous studies, adding direct data for the central 1 degree. Distinct foveolar activations to spot stimuli were observed, one each at the borders of dorsal and ventral V1/V2, V2/V3, V3/V4, and V4/TEO, resulting in 8 distinct loci. As observed in both hemispheres of Monkey 1 & 2, these loci did not converge but rather formed a ringed network encircling a substantial area of cortex not within visuotopic cortex (the "foveolar core"). The foveolar core was populated by mm-scale functional domains, sensitive to high spatial frequencies (11-18 cyc/deg), consistent with high spatial acuity vision, and distinct from low spatial frequency domains, as well as domains for motion and color. In Monkey 3, optical imaging of this cortical region supported these results. In Monkey 4, focal infrared neural stimulation of loci in foveolar cortex revealed networks of mesoscale brainwide activations. **Discussion:** Our results show that use of highfield fMRI mapping and foveolar stimuli in well-trained monkeys enable precise mapping of foveolar cortical locations. In contrast to previous models of foveal representation, the *foveolar core* is a distinct cortical region, outside the classical visuotopic areas, marked by distinct functional organization, and functionally linked to brainwide mesoscale networks. We hypothesize the foveolar core is a higher-order cortical specialization in primates that coordinates the multiple foveolar loci for dynamically engaging distinct brainwide cortical circuits, thereby mediating the different roles of foveolar vision.

Disclosures: Q. Meizhen: None. **J. Wang:** None. **Y. Gao:** None. **X. Zhang:** None. **J. Hu:** None. **A.W. Roe:** None.

Presentation Number: NANO32.02

Topic: D.06. Vision

Title: A transient signal in foveal superior colliculus neurons for jumpstarting peripheral saccadic orienting

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Abstract: The superior colliculus (SC) is critical for saccade generation. Recent work has shown that, despite bursting at times other than saccades, SC population activity at the time of saccade motor bursts is more temporally aligned than for visual bursts (Jagadisan & Gandhi, 2022). Similarly, population activity in motor bursts resides in different subspaces to visual bursts (Baumann et al., 2023), and even the sensory signal embedded in SC motor bursts (Baumann et al., 2023) is transformed relative to visual bursts, such that the same individual neurons "prefer"

different visual features during the two bursting epochs. However, how might such a transformation from a visual regime to a motor regime be realized? Here we first show that when a planned, voluntary saccade is finally released with a go signal (removal of a fixation spot in our case), peripheral SC neurons (representing the saccade target location) exhibit a robust, short-latency pause in spiking, before the motor bursts eventually erupt. This pause starts within ~50 ms from the go signal, and it is stimulus-dependent (e.g. having a stronger firing rate dip for a salient peripheral stimulus). Additionally, this pause still occurs, to a weaker extent, with saccades to a small spot or a blank. Interestingly, such a pause does not happen in simultaneously recorded peripheral primary visual cortex (V1) neurons, suggesting that it is not necessarily inherited from V1, and also suggesting that a peripheral activity pause is not merely an obligatory outcome of fixation spot removal somewhere else in the visual field. When we then recorded from foveal SC neurons in similar tasks, we found that these neurons actually burst after the go signal, rather than paused. Remarkably, these foveal bursts occurred (and peaked) several milliseconds earlier than the pauses in the peripheral SC neurons, and they were not fully explained by offset responses to the removal of the fixation spot, which we confirmed by explicitly measuring offset responses during foveal response field mapping. Foveal bursts also occurred when releasing memory-guided saccades (with no peripheral visual targets), and they were not sensitive to peripheral target appearance. In a task with immediate, reflexive saccades to a visual onset, these foveal bursts still occurred, this time together with stimulus-driven peripheral SC neuronal visual bursts. Thus, we found a robust, transient foveal SC signal jumpstarting voluntary peripheral saccadic orienting. This signal likely facilitates a necessary representational transformation needed for saccade motor bursts to occur.

Disclosures: T. Zhang: None. **Z.M. Hafed:** None.

Presentation Number: NANO32.03

Topic: D.06. Vision

Support: Fiona and Sanjay Jha Chair in Neuroscience (J.H.R)
R01-EY028723

Title: A high-entropy columnar code for visual space

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Abstract: The brain must combine parcellated visual input from across the retina to construct the unified representation of visual space that we experience. Neurons within individual cortical columns of the primary and extrastriate visual cortex have overlapping response fields that are organized in a retinotopic manner. This specificity for a visual position within cortical columns can be described as a low entropy code where the entire population only responds to a small region of visual space. Receptive fields increase in size and tuning complexity as one moves up the cortical hierarchy, and visual inputs are highly integrated in prefrontal cortex (PFC). How is visual space encoded within the cortical columns of PFC after such extensive integration? One candidate mechanism is through a maximum-entropy code, where individual neurons encode different regions of space, with each position being encoded across the population, maximizing the number of visual positions the neural population can encode. Using Neuropixels probes, we

recorded simultaneously from hundreds of neurons in PFC of the common marmoset. We leveraged the lissencephaly (smoothness) of the marmoset cortex to record neurons throughout entire columns of the PFC. Flashed stimuli were used to map the spatial response fields of neurons within each column. We find that in layers IV, V, and VI of ventral PFC areas (8aV, 8c, and 6aV) there is a high incidence of individual neurons with spatial receptive fields. Unlike early visual areas, the response fields in individual PFC columns are typically spatially heterogeneous and we found that the population within some columns tiled the entire visual field. For a high fraction of columns, the population response (mean and distribution shape) to each position in visual space was statistically highly similar. This is consistent with PFC utilizing a high-entropy code for visual space. These findings support a model in which low-entropy columns (such as those found in primary visual cortex) compute basic properties of visual stimuli (such as orientation) while high-entropy columns serve as the computational units of high-level visual experience.

Disclosures: N.M. Dotson: None. **J.H. Reynolds:** None.

Presentation Number: NANO32.04

Topic: D.06. Vision

Support: NIH grant R01EY023915
Office of Naval Research grant S5122
Office of Naval Research grant 10802
NSF CAREER grant 1844724
NSF IIS-1822683
NSF IIS-1822929.

Title: A single computational objective drives specialization of streams in visual cortex

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Abstract: Human visual cortex is organized into dorsal, lateral, and ventral streams. A long-standing hypothesis is that the functional organization into streams emerged to support distinct visual behaviors. An alternative hypothesis suggests that a set of physical constraints, such as wiring length, could produce the functional organization of the brain into streams. Here, we use a deep artificial neural network (DANN)-based computational model and a massive fMRI dataset to test how visual streams emerge. We instantiate the multiple visual behaviors hypothesis by training three different models, each using a state-of-the-art DANN that is trained through supervision on the stream-specific visual behavior. We instantiate the spatial constraints hypothesis using a topographic DANN (TDANN, Margalit 2024). In the TDANN, model units in each layer are assigned a position in a 2D simulated cortical sheet, and during training a spatial constraint is balanced together with contrastive self-supervised learning (SimCLR, Chen 2020). To compare models to the brain, we leverage a massive fMRI dataset (NSD, Allen 2022) and develop a new algorithm that estimates an optimal 1-to-1 mapping between model units and voxels using an iterative version of the Kuhn-Munkres algorithm. We find that models trained

for stream-specific visual behaviors both fail to capture the spatial organization into streams and poorly predict neural responses in the NSD. Instead, a self-supervised TDANN, which encourages nearby units to respond similarly, not only captures the spatial organization into streams, but also successfully predicts neural responses in visual cortex of the eight participants in the NSD, with performance approaching the noise ceiling in the ventral and dorsal streams. Strikingly, even though the TDANN is trained with a single task, model units assigned to the ventral stream perform better on a categorization task while units assigned to the dorsal stream perform better on a position task. This suggests that self-supervised training with a local spatial constraint can lead to functional differentiation across streams. Overall, these findings challenge the prevailing view that streams have evolved to separately support different behaviors, and instead suggest that functional organization arises from a single principle: balancing general representation learning with local spatial constraints.

Disclosures: D. Finzi: None. **E. Margalit:** None. **K.N. Kay:** None. **D. Yamins:** None. **K. Grill-Spector:** None.

Presentation Number: NANO32.05

Topic: D.06. Vision

Support: NINDS 1RF1NS127107-01
Simons Collaboration on the Global Brain
NIH CRCNS R01 EY035896

Title: Timescales of neuronal population interactions between and within visual cortical areas V1 and V2

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Abstract: Sensory processing requires the coordination of neuronal population activity across brain areas. To understand this process, it is important to disentangle the concurrent feedforward and feedback signaling between areas, and to distinguish these signals from those local to each area.
We used Neuropixels probes to record hundreds of neurons simultaneously across all layers of V1 and V2 in macaque monkeys, while we presented drifting grating stimuli. We employed a latent variable model, Delayed Latents Across Groups (DLAG), to decompose the measured activity into components (or signals) evident solely within either V1 or V2 as well as components that were shared between these areas. The across-area signals included a delay parameter, allowing us to infer their directionality as feedforward or feedback.
While previous perturbation experiments have suggested a weak influence of feedback signals on

neuronal activity, we found that feedforward and feedback signals were equally important for explaining neuronal activity in V1 and V2, consistent with their equal anatomical prominence. We observed no difference in the timescales of feedforward and feedback signals; they ranged broadly over ~10-180 milliseconds. Within each area, we found that the timescale of a signal was unrelated to its spatial spread. Even fast (<50ms) within-area signals could be shared between groups of neurons many millimeters apart in either V1 or V2. However, within- and across-area signals had distinct timescales: the fastest within-area signals were observed in V1, followed by within-area signals in V2, with the slowest signals being those shared between V1 and V2. One implication of these different timescales is that the temporal resolution of an analysis can affect the estimated strength of functional interactions between areas: the contribution of slower across-area signals may be underestimated during short observation periods.
Our results indicate that activity at different stages of the visual system, known to involve a well-described hierarchy of timescales at each stage, is linked together with equipotent feedforward and feedback signals operating on distinctly slower time scales.

Disclosures: A. Krishna: None. **E. Gokcen:** None. **A.I. Jasper:** None. **B.M. Yu:** None. **C. Machens:** None. **A. Kohn:** None.

Presentation Number: NANO32.06

Topic: D.06. Vision

Support: STI 2030 – Major Projects (2021ZD0200401)
National Natural Science Foundation of China (31872776)
National Key R&D Program of China (2018YFA0701402)

Title: Decoding visual features from neural oscillatory patterns in the macaque visual cortex

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Abstract: In the primate visual cortex, mesoscale functional domains ranging from hundreds of micrometers to several millimeters in width respond selectively to specific visual features such as orientation and color. These domains consist of neuron populations exhibiting similar responses to a given visual feature. Neural oscillations generated by synchronized neural activity at specific frequencies occur in the visual cortex in response to visual stimuli. However, it remains unclear whether the spatial patterns of visually induced neural oscillations reflect the organization of functional domains. In this study, intrinsic signal optical imaging (ISOI) followed by electrocorticography (ECoG) was performed in the visual cortex of anesthetized macaques to investigate whether visual features presented in stimuli can be decoded from the patterns of neural oscillations thought to originate from the functional domains. Macaques underwent surgery under isoflurane anesthesia to expose visual areas V1, V2, and V4, followed by ISOI under propofol anesthesia to identify cortical areas and functional domains. Visual stimuli consisted of achromatic (black and white) or isoluminant chromatic (red and green) drifting gratings, either horizontal or vertical, presented to each eye for 3.5 s. After ISOI, a 64-

channel ECoG electrode array (500μm spacing) was implanted on the imaged cortical surface. ECoG recordings were performed under the same stimulus and anesthesia conditions as for ISOI. Applying multivariate pattern analysis to the spectral power of ECoG signals recorded during stimulus presentation, it was shown that classifiers could significantly discriminate between achromatic and chromatic stimuli with 70-80% accuracy (bootstrap test) based on beta (13-30 Hz) and gamma (30-80 Hz) power patterns. However, classifiers failed to significantly discriminate horizontal and vertical orientation in any frequency band. Classification of stimulus presentation to the right or left eye was significant in the beta and gamma bands with an accuracy of approximately 60%. Searchlight analysis revealed that electrodes over visual area V4 had the highest classification accuracy for achromatic versus chromatic stimuli. The temporal evolution of classification accuracy peaked at the onset and offset of the stimuli. These results indicate that visual information can be extracted from oscillatory oscillation patterns in the beta and gamma bands in visual cortex and provide insight into the role of oscillatory patterns in encoding visual features their relationship to functional domains.

Disclosures: H. Tanigawa: None. **C. Li:** None. **X. Wang:** None. **Z. Pan:** None. **T. Zhou:** None. **D. Hu:** None.

Presentation Number: NANO32.07

Topic: D.06. Vision

Support: NIH Grant 1DP2EY035176-01
NSF CAREER 2143077
David and Lucile Packard Foundation

Title: Relating population coding to neurons' functions via deep generative networks

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Abstract: Primates need to extract various types of information, such as object identity, category, and orientation, from a complex visual environment. Experimentally, this information can be extracted from the activity of neuronal populations in the visual cortex using supervised decoders. However, it is not clear how this population-level information is distributed across individual neurons and neuronal microclusters. One hypothesis is that neurons form an emergent code, where they adopt different functions based on population activity; another is that neurons form a reducible code, where the entire activity can be fully understood by knowing the neurons' tuning. We used an image synthesis algorithm relying on generative adversarial networks (GANs) to test each hypothesis. We recorded from neurons in macaque areas V1, V4, and posterior inferotemporal cortex using chronic arrays. Initially, we measured the tuning of single neurons and microclusters by generating strongly activating images (prototypes). These prototypes were not object-like, so we identified their critical features by selectively scrambling pixels of the parts of the synthetic image and tracking neuronal responses. We found that the similarity between natural images and prototypes could predict neuronal responses, particularly within the localized feature patch. Then, we explored how neurons' tuning related to population

coding using a reconstruction method. We presented photographs ("target images") to monkeys and measured the population code for each image. We then tried to generate an image that would evoke response patterns closely matching those of the target population code. This method proved successful, as the population response pattern of the generated image matched well with that of the target image, even reaching the theoretical noise ceiling. Furthermore, we observed that the similarity between the target and reconstructed images increased during the reconstruction process. We found that improvements in the similarity between reconstructed and target images in local patches were linked to the local features (prototype) of the neurons in the population, indicating that population codes emerge from the combination of local features to which the neurons are tuned.

Disclosures: A. A. Dehaqani: None. **B. Wang:** None. **C.R. Ponce:** None.

Presentation Number: NANO32.08

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

Support: Wellcome Trust 223144 and 219627
UKRI Frontier Award 5953442
Gatsby Charitable Foundation GAT3755
Royal Society NIF\R1\231927

Title: High-dimensional neuronal activity from low-dimensional population dynamics

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Abstract: Introduction. Large-scale recordings of neuronal activity in mouse visual cortex have revealed that spontaneous population activity is high-dimensional, in the sense that neuronal firing rates do not lie in a finite-dimensional linear subspace (Stringer et al. Science 2019; Manley et al. Neuron 2024). These findings are apparently at odds with the view that neural population dynamics approximate low-dimensional dynamical systems implementing computations (Vyas et al. Annu. Rev. Neurosci. 2020). In this work, we reconcile these views by showing that high-dimensional neuronal activity can indeed arise from low-dimensional population dynamics. Methods. We acquired two-photon calcium recordings of spontaneous neuronal activity in the visual cortex of awake, transgenic mice expressing GCamP6s with a Light Beads Microscope (LBM). Mice were head-fixed but free to run on a wheel. The movies were preprocessed with Suite3D (Haydaroglu et al. SfN 2024) to segment and extract activity from individual cells. Results. First, we model neurons as ReLU units receiving low-dimensional inputs from latent variables estimated from the data. We show that this model, despite having a latent space with low linear dimensionality, can produce power-law-decaying PCA eigenspectra, a hallmark of high-dimensional neuronal activity. Second, we estimate the activation functions of single neurons with non-parametric methods. We show that applying the estimated nonlinear activation functions to low-rank approximations of neuronal population activity suffices to produce power-law-decaying eigenspectra. Finally, we use a mean-field RNN model to prove that power-law-decaying eigenspectra can be produced even when population dynamics can be exactly reduced to an effective low-dimensional autonomous dynamical system. Conclusions.

These results show how the nonlinearity of neuronal activation functions can explain the concurrence of high-dimensional neuronal firing rate activity and low-dimensional effective population dynamics, reconciling two seemingly opposing views on dimensionality in systems neuroscience.

Disclosures: V. Schmutz: None. **A. Haydaroglu:** None. **M. Carandini:** None. **K.D. Harris:** None.

Presentation Number: NANO32.09

Topic: D.06. Vision

Support: NIH Grant 1DP2EY035176-01
NSF CAREER 2143077
David and Lucile Packard Foundation

Title: Functional segregation of inputs in artificial neural networks for vision

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Abstract: Vision relies on computations performed by excitatory and inhibitory neurons. However, little is known about the role of inhibitory activity in high-level visual cortex such as inferotemporal cortex. Here, we used artificial neural networks (ANNs) as a testbed for circuit dissection of high-level visual mechanisms. We investigated how learned representations of ANN classification units depended on their positive (excitatory) or negative (inhibitory) inputs. First, we performed input-silencing experiments in ANNs by optimizing synthetic images to visualize the learned representations of each unit. As expected, we found that silencing positive inputs to a given unit decreased its response, while silencing negative inputs increased it slightly. When analyzing the optimized images of intact- vs. input-silenced units, we found that the representation changed more when silencing positive- vs. negative inputs; specifically, object-related features were abolished when silencing positive inputs, while still preserving background textures. To determine if this functional segregation depended on image classification vs. other learning objectives, we trained models to replicate the activity of neurons in monkey visual cortex, using recordings from V1, V4, and posterior inferotemporal cortex (PIT). After fitting each model, we performed silencing experiments and optimized images under control and silenced-input conditions. We found that image optimization produced images containing local features preferred by actual visual cortex neurons, distributed across multiple image locations. Incidental features around these preferred features evoked surround inhibition in visual cortex neurons, a phenomenon not captured by the fitted models. However, consistent with the first task, the learned representations of these neuron models changed more upon silencing positive than negative inputs. We conclude that ANNs learn to segregate object or foreground information into the positive weights, with background or contextual information into the negative weights. These results hint at the relevance of inhibition into shaping feature selectivity in the ventral stream, a hypothesis we are testing in vivo.

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

Presentation Number: NANO32.10

Topic: D.06. Vision

Support: NIH/NINDS (5U01NS103488, 1RF1NS113251, 1RF1NS110501 and 1U01NS126057)
Wellcome Trust (223144 and 219627)
UKRI Frontier Award 5953442
Kavli Foundation
Gatsby Charitable Foundation (GAT3755)

Title: Relating the structure of visual and behavioral activity in the mouse visual cortex

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Abstract: Aims. Neurons in the mouse visual cortex encode both high-dimensional sensory information and lower-dimensional behavioral variables. Here we asked how these signals are organized across the cortical volume, and whether they share a common structure.
Methods. We constructed and used a Light Beads Microscopy (Demas et al, Nature Methods 2021) to functionally image Ca2+ transients at cellular resolution from large volumes of cortex (3 x 3 x 0.5 mm^3) in awake, transgenic mice expressing GCaMP6s. Mice were head-fixed but free to run on a wheel. To segment individual cells, we developed Suite3D, a volumetric cell extraction pipeline that accelerates and extends Suite2P algorithms (Pachitariu et al, bioRxiv 2017) to three dimensions. We analyzed data from periods of spontaneous activity without visual stimulation, as well as responses to drifting gratings or sparse noise.
Results. First, we compared the spatial structure of visual and behavioral activity in the cortex. While visual responses were organized retinotopically, spontaneous activity was not: distant pairs of cells in different visual areas that responded to the same receptive field did not have higher spontaneous correlations. Similarly, we found that pairs of cells with the same orientation tuning did not have similar spontaneous activity when controlling for distance. While the spontaneous activity of cells did not predict their visual tuning, it predicted the timecourse of their average stimulus response. Next, we found that behavioral activity followed a distinct, weak spatial organization at length scales up to ~1mm. We found that the strongest dimensions of spontaneous activity were globally distributed across visual cortex. However, higher dimensions of activity were spatially localized.
Conclusions. Behavioral signals are widely distributed across the visual cortex, and follow a weak spatial structure. This structure is not predicted from the visual tuning of cells, and consists of a few globally distributed dominant dimensions and many weaker, spatially localized dimensions of activity.

Disclosures: A. Haydaroglu: None. **M. Krumin:** None. **V. Schmutz:** None. **L. Xu:** None. **S. Dodgson:** None. **D. Meyer:** None. **J. Guo:** None. **A. Vaziri:** None. **K.D. Harris:** None. **M. Carandini:** None.

Presentation Number: NANO32.11

Topic: D.06. Vision

Support: European Research Council (ERC), grant ID 682117 BIT-ERC-2015-CoG
Italian Ministry of University and Research under the call FARE (project ID: R16X32NALR) and under the call PRIN2017 (project ID: XBJN4F)

Title: The link between space and time along the human cortical hierarchy

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Abstract: In humans, very few studies have directly tested the link between the neural coding of time and space. Here we combined ultra-high field functional magnetic resonance imaging with neuronal-based modelling to investigate how and where the processing and the representation of a visual stimulus duration is linked to that of its spatial location. Results show a transition in the neural response to duration: from monotonic and spatially-dependent in early visual cortex, to unimodal and spatially-invariant in frontal cortex. This transition begins in extrastriate areas V3AB, and it fully displays in the intraparietal sulcus (IPS), where both unimodal and monotonic responses are present and where neuronal populations are selective to either space, time or both. In IPS, space and time topographies show a specific relationship, although along the cortical hierarchy duration maps compared to spatial ones are smaller in size, less clustered and more variable across participants. These results help to identify the mechanisms through which humans perceive the duration of a visual object with a specific spatial location and precisely characterise the functional link between time and space processing, highlighting the importance of space-time interactions in shaping brain responses.

Disclosures: D. Bueti: None. **G. Fortunato:** None. **V. Centanino:** None.

Presentation Number: NANO32.12

Topic: D.06. Vision

Support: NIH Grant R01MH130529
Neukom Institute for Computational Sciences
BBSRC BB/V003917/1

Title: Retinotopically-specific, top-down activity structures perception and memory cortical interactions in the resting brain

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Abstract: Recent evidence suggests that a low-level visual code, retinotopy, structures mutual activity across functionally-linked perceptual and mnemonic areas of the human brain (Steel*, Silson* et al., 2024). Specifically, discrete visual stimulation drives voxels in perceptual areas, but suppresses voxels in memory areas in a retinotopically-specific fashion. Conversely, top-down recall drives voxels in memory-areas, but suppresses voxels in perceptual areas in a retinotopically-specific fashion. Does such a retinotopic push-pull dynamic persist during rest, structuring mutual activity across perceptual and mnemonic areas even when activity is not task-

driven? We used the Natural Scenes Dataset, a densely sampled high-resolution 7T dataset of eight participants who underwent pRF mapping, localizers, and resting-state fMRI to test this. Consistent with our prior work, we observed robust retinotopic coding in the scene-selective occipital place area (OPA) and in mnemonic cortical areas associated with place memory recall (lateral place memory area, LPMA). Notably, LPMA contained a high proportion of pRFs with negative amplitude (-pRFs). When we evaluated these regions' interaction during resting-state fMRI, we found that OPA +pRF and LPMA -pRFs are generally anti-correlated. Critically, we observed the strongest anti-correlation between pRFs representing similar portions of the visual field, suggesting retinotopic structuring even during rest. This opponent interaction was specific to regions within the same functional network. We further characterized this interaction by detecting spontaneous events in individual pRF resting-state time series in a given region (e.g., LPMA) and examining the concurrent activity in pRFs in the opposite region (e.g., OPA). We found that the retinotopically-structured interaction was driven by discrete events detected at individual voxels: positive events detected in LPMA coincided with relative decreases in OPA activity. Intriguingly, events in LPMA influenced activity of OPA more than events in OPA influenced LPMA, suggesting that top-down signaling has a greater relative influence compared to bottom up signaling during rest. Our results show that a low-level code, retinotopy, structures a mutually-suppressive dynamic across functionally-paired perceptual-mnemonic areas in the resting brain.

Disclosures: A. Steel: None. **P.A. Angeli:** None. **E.H. Silson:** None. **C. Robertson:** None.

Presentation Number: NANO32.13

Topic: D.06. Vision

Support: HHMI

Title: Dynamics of representations of conscious perception and physical stimulus across inferotemporal, parietal and prefrontal cortex in binocular rivalry

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Abstract: Consciousness is arguably the most important reason why it matters to us whether we are dead or alive. Yet, the neural mechanisms of conscious perception remain unknown. To reveal the mechanisms of how new conscious percepts are generated and coordinated across different levels of the cortical hierarchy, we used newly developed primate Neuropixels probes to simultaneously record from thousands of neurons across different face patches in macaque IT cortex as well as nodes of parietal and prefrontal cortex during a binocular rivalry paradigm. In binocular rivalry, a constant visual input evokes spontaneous changes of conscious perception, enabling dissociation of neural representations of conscious percept and physical input. We employed a novel no-report binocular rivalry paradigm that allowed us to infer conscious percepts from eye movements. We find that neural activity during spontaneous perceptual switching in rivalry differs dramatically from activity when physically alternating an unambiguous stimulus. First, in contrast to physical alternation, where only the unambiguous consciously perceived stimulus is encoded, during rivalry cells in face patches encoded not only

the consciously perceived but also the suppressed stimulus. Indeed, on time intervals of 1 s, we could decode the suppressed stimulus with 100% accuracy, even though the average perceptual switching time inferred from eye movements was much longer. We further compared neural representation dynamics within different nodes of IT cortex as well as between IT, parietal, and prefrontal cortex. Overall, our results strongly challenge the notion that anterior IT cortex harbors a pure representation of conscious percepts and suggest a much more complex, dynamic, and distributed representation of consciousness.

Disclosures: J.K. Hesse: None. **F. Lanfranchi:** None. **Y. Shi:** None. **D.Y. Tsao:** None.

Nanosymposium

NANO33: Sensory Motor Systems

Location: MCP Room N227

Time: Monday, October 7, 2024, 1:00 PM - 3:00 PM

Presentation Number: NANO33.01

Topic: F.01. Neuroethology

Support: NSF Grant 1457291
NSF Grant 2238071
Alfred P Sloan Foundation

Title: Both heritable and plastic effects are evident in the brains of working Labrador retrievers bred and trained for different skills

Authors: *S. A. BARTON[1], S. GANESHRAM[2], C. OTTO[3], E. E. HECHT[4];
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Abstract: Working dogs are selectively bred to learn and perform cognitively demanding tasks that benefit human society. One of the most popular breeds of working dog is the Labrador retriever, which was historically bred for hunting game birds, but is now also used for scent detection, service work, and other purposes. While the performance of these skills must rely on brain structure and function, virtually nothing is known about the neural basis of skilled working behavior in the dog brain. To address this gap, we examined T2-weighted and diffusion-weighted neuroimaging studies of 56 working line Labrador Retrievers involved in hunting, scent detection, service work, or no work at all. We used voxel-based morphometry, in addition to a tract-based spatial statistics, to investigate differences in neuroanatomy between the four groups. We found widespread cortical and subcortical differences in regional gray matter volume, regional white matter volume, and white matter fractional anisotropy across the comparisons. Specifically, hunting Labrador retrievers had expanded gray matter in the right posterior hippocampus, while service Labrador retrievers had increased gray matter volume in the thalamus. Scent detection Labrador retrievers had expanded gray matter in regions of the occipital cortex and sylvian gyrus, in addition to higher fractional anisotropy in the olfactory

cortex. These results demonstrate that different brain regions are implicated in different working behaviors, and that these regions are plastic in response to training. We discuss the potential relevance of our results to working dog selection and training, as well as to our general understanding of neuroplasticity in response to skill learning.

Disclosures: S.A. Barton: None. **S. Ganeshram:** None. **C. Otto:** None. **E.E. Hecht:** None.

Presentation Number: NANO33.02

Topic: F.01. Neuroethology

Support: HFSP Long-term Fellowship

Title: Sensorimotor nuclei organize flow stimuli relative to direction and sensor position across body axes

Authors: *E. T. LUNSFORD[1], C. WYART[2];
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Abstract: Motor command circuits rely on sensory systems to relay vital information about the environment in order to select optimal motor actions during navigation. The sensorimotor transformation that converts sensory information into motor action selection is not resolved, often due to the difficulty to comprehensively record sensory inputs, interneurons, and motor output. The flow sensitive lateral line (LL) system of fishes that detects changes in the fluid environment to mediate essential behaviors such as navigation is an ideal sensory organ to solve this question. The LL end organs on the head (anterior LL) and body (posterior LL), referred to as neuromasts, are comprised of mechanoreceptive hair cells that convert physical properties of flow into bioelectrical signals. These signals are transmitted to the medial octavolateralis nuclei (MON) in the hindbrain to process the inputs and select motor outputs. Current methods have yet to discern recruitment of MON neurons as a function of different stimulus parameters such as sensor position, flow direction, intensity, and symmetry across body axes. To dissect where integration of different flow stimuli occurs within the MON and how this topography informs motor circuits, we combined microfluidics, functional calcium imaging, and targeted photoactivation. We systematically stimulated individual neuromasts across the body while simultaneously recording hindbrain activity in Tg(*elav3:H2B-GCaMP6f*) zebrafish larvae. We find the organization of flow responsive cells in the hindbrain accurately represents the stimulus site on the left-right and rostral-caudal axes. Anterior MON neurons reliably respond to anterior LL stimulation and anterior-to-posterior directional flow whereas stimulation within posterior LL and posterior-to-anterior flow reliably activate the posterior MON. We also observe a majority of MON activity ipsilateral to the stimulated periphery with some contralateral activity. Input-output connectivity of the MON is key to solve how hindbrain neurons compute flow along body axes and select motor actions from the behavioral repertoire. Our findings suggest a topographical organization of the sensorimotor network that recruits specific populations of MON neurons contingent on different stimulus parameters, which integrates this sensory information and likely projects to command neurons to recruit subsets of command neurons responsible for ipsilateral turning and forward swimming.

Disclosures: E.T. Lunsford: None. **C. Wyart:** None.

Presentation Number: NANO33.03

Topic: F.01. Neuroethology

Support: NIH 1R34DA059513-01

Title: Auditory cortex is required for antiphonal calling in the Mongolian gerbil

Authors: *R. E. PETERSON[1,2], V. J. IVAN[1,2], A. H. WILLIAMS[3,4], D. M. SCHNEIDER[3,4], D. H. SANES[3,4];
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Abstract: Interactive vocal communication is a complex sensorimotor phenomenon that requires coordination between the auditory and motor systems, as well as coordination between animals. Work in Scotinomys teguina, the singing mouse (Okobi et al. 2019, Banerjee et al. 2024), has revealed how motor cortical dynamics subserve vocal production during antiphonal communication (i.e. turn-taking), however less is known about the role of auditory processing in generating appropriate vocal outputs. Here, we report that Mongolian gerbils — a highly social fossorial species that live in large multigenerational families — engage in ultrasonic antiphonal communication that depends on auditory cortex (AC) activity. Male and female gerbils (postnatal days 36-99) were exposed to an ultrasonic vocalization (USV) bout (2 seconds, 20 trials, 30-60 second inter-trial interval) in an isolated testing chamber while both video and audio recordings were obtained. We found that gerbils (n=21) responded to this auditory stimulus with seconds-long sequences of stereotyped USVs. Given the robust vocal response, we used this behavior to test whether AC activity was required for antiphonal responses. Bilateral muscimol infusion into AC through chronically implanted cannulae significantly reduced antiphonal responses and vocalization-induced postural orientation responses, as compared to saline-infused controls (n=5). Unilateral inactivation of either AC reduced antiphonal responses as compared to saline controls, however vocal responses were significantly higher than bilateral inactivation. This suggests that there may be coordination between hemispheres to process conspecific vocalizations critical for antiphonal calling. Finally, preliminary chronic wireless silicon probe recordings showed that vocalization playback activated AC neurons, even on playback trials where the animal did not respond vocally. Therefore, AC signals that encode ongoing social auditory experience are necessary for antiphonal vocalizing, and are likely transmitted to downstream social/motor structures that coordinate vocal turn-taking behavior.

Disclosures: R.E. Peterson: None. **V.J. Ivan:** None. **A.H. Williams:** None. **D.M. Schneider:** None. **D.H. Sanes:** None.

Presentation Number: NANO33.04

Topic: F.01. Neuroethology

Support: ANR-10- LABX-0010/ANR-10-IDEX-0001-02 PSL*
NIH Grant 1RF1NS128865-01
NSF Grant PHY-1607611

European Union's Horizon 2020 Research and Innovation program under the Marie Skłodowska-Curie Grant No. 813457

Title: Multiscale structure, plasticity and individuality in larval zebrafish behavior

Authors: *A. COSTA;
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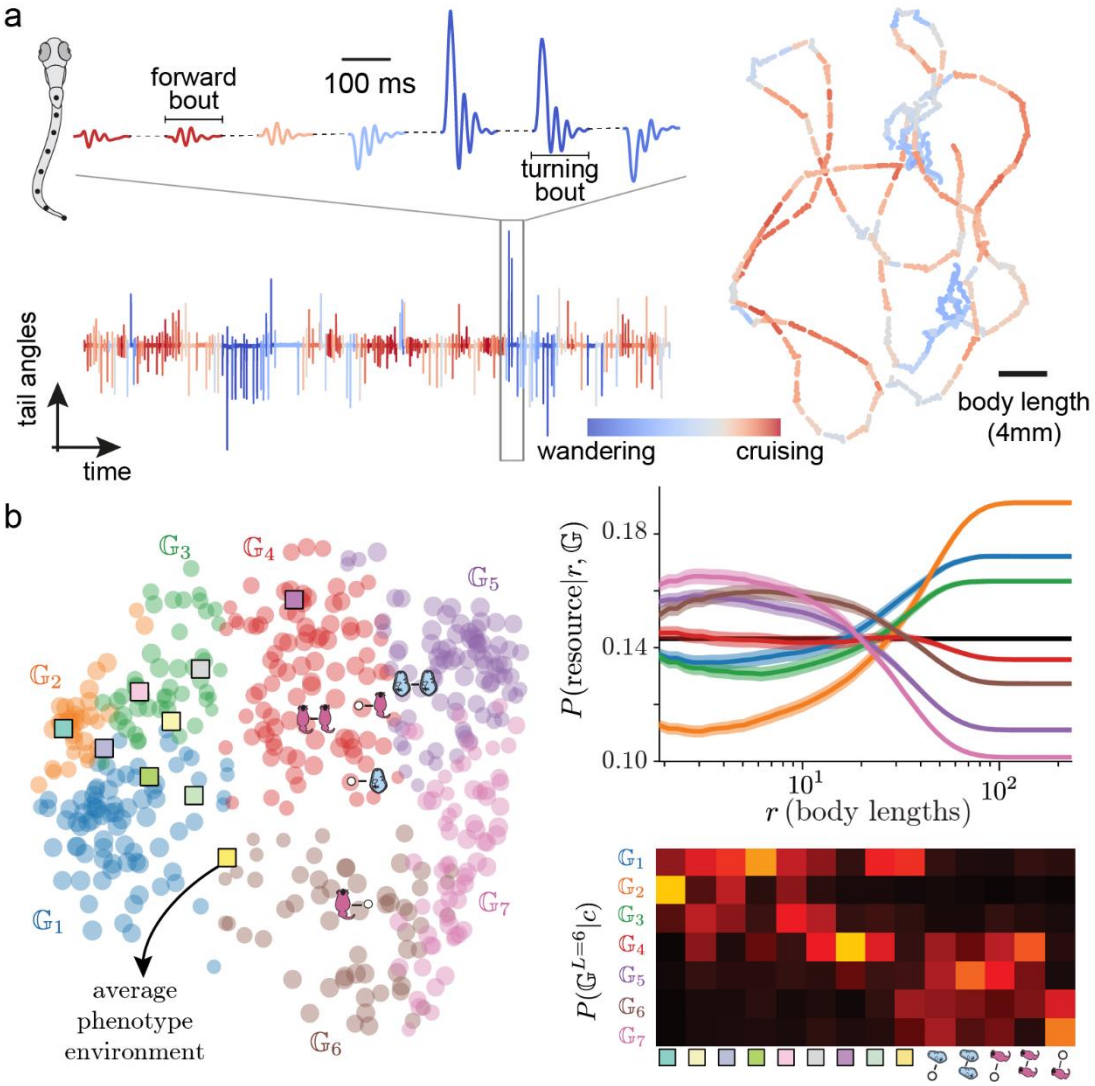
Abstract: Animals navigate and adapt to complex environments by chaining short-time movements into long-lived motor strategies that ultimately also reflect their internal states and individuality. Understanding how these complex patterns in behavior emerge requires a comprehensive approach that embraces variability across scales. We build high-fidelity Markov models from maximally-predictive sequences, bridging across scales from posture dynamics to long-lived navigation states across species. In *C. elegans* and larval zebrafish we find that behavioral plasticity leads to heavy-tailed statistics in behavior, and we uncover a fundamental role of neuropeptide signalling in the emergence of such heavy tails. Additionally, we obtain a parsimonious encoding of larval zebrafish behavioral dynamics, and discover structure to inter-fish variability that points to a trade-off between sensory inputs and internal states. We find a major impact of prey exposure on the multiscale structure of larval zebrafish locomotion, and discover exploration-exploitation biases that point to internal states driving phenotypic variation.



Disclosures: A. Costa: None.

Presentation Number: NANO33.05

Topic: F.01. Neuroethology

Support: NIH R01DK135212
NIH R01DK131446
NIH R01DK136284
NIH R01DK120858
NIH R01DK109934
DOD HT94252310156

Title: Dopaminergic Neurons in Zona Incerta Drives Appetitive Self-Grooming

Authors: *Z. JIANG[1], C. YOUNG[2], J. CAI[3], Y. XU[4], Y. JIANG[5], Q. TONG[6];
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Abstract: Dopaminergic (DA) neurons are known to play a key role in controlling behaviors. While DA neurons in other brain regions have been extensively characterized, those in zone incerta (ZI^{TH} or A13) receive much less attention and their function remains to be defined. Here we showed that optogenetic stimulation of these neurons elicited intensive self-grooming behaviors and promoted place preference, which could be enhanced by training but couldn't be converted into contextual memory. Interestingly, the same stimulation increased DA release to periaqueductal grey (PAG) neurons and local PAG antagonism of DA action reduced the elicited self-grooming. In addition, A13 neurons increased their activity in response to various external stimuli and during natural self-grooming episodes. Finally, monosynaptic retrograde tracing showed that the paraventricular hypothalamus represents one of the major upstream brain regions to A13 neurons. Taking together, these results reveal that A13 neurons are one of the brain sites that promote appetitive self-grooming involving DA release to the PAG.

Disclosures: Z. Jiang: None. **C. Young:** None. **J. Cai:** None. **Y. Xu:** None. **Y. Jiang:** None. **Q. Tong:** None.

Presentation Number: NANO33.06

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF CAREER: Descending neural control of a motor sequence in Drosophila 1943276

Title: An inhibitory circuit for limb coordination during Drosophila grooming

Authors: *D. S. SYED, P. RAVBAR, J. H. SIMPSON;
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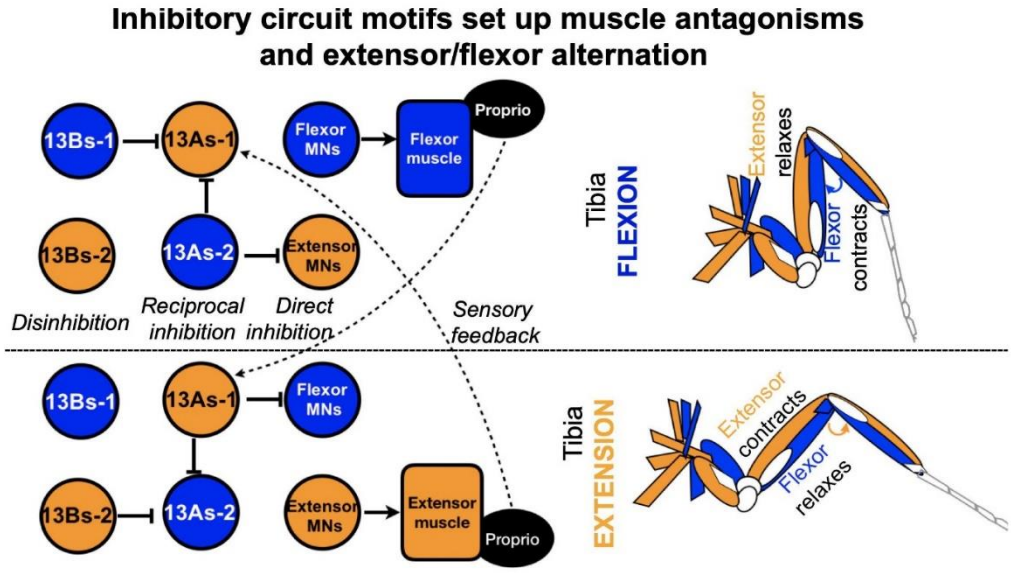
Abstract: Motor programs controlling different muscles within a limb to coordinate diverse grooming actions are not well understood. The basic architecture of motor neurons that activate muscles which articulate joints for antagonistic flexion and extension movements is conserved from flies to vertebrates. While excitatory pre-motor circuits are expected to establish sets of leg motor neurons that work together, our study uncovered an instructive role for inhibitory circuits. Dusted flies removed debris by alternating between rhythmic body sweeps and leg rubbing. We employed high-resolution limb tracking to analyze each joint of the front legs during head sweeps and leg rubs to characterize their coordination during stable oscillations. Within a leg, multiple joints extend or flex together during leg rubbing, while a different set of muscles are co-active during head sweeps. A behavioral screen identified GABAergic neurons from the 13A/B hemilineages in the ventral nerve cord that are normally required for grooming.
Using electron microscopy data, we categorized ~120 inhibitory neurons from the 13A/B hemi-lineages into classes based on similarities in morphology and connectivity. By mapping their synaptic partners, we uncovered redundant pathways for inhibiting specific groups of motor neurons, disinhibiting antagonistic counterparts, or inducing alternation between flexion and extension. Proprioceptive feedback further contributes to this coordination. We tested the function of specific inhibitory neurons through optogenetic activation and silencing, using quantitative leg movement assays for coordination during grooming. Behavior experiments and

modeling demonstrate that inhibition can induce rhythmic motion, highlighting the importance of inhibitory circuits in motor control.



Disclosures: D.S. Syed: None. **P. Ravbar:** None. **J.H. Simpson:** None.

Presentation Number: NANO33.07

Topic: F.01. Neuroethology

Support: NIH Grant 1R01NS116595-01
NIH Grant 1U01NS131438-01
Air Force Office of Scientific Research, Contract: FA9550-23-1-0722

Title: Towards a comprehensive understanding of the neural circuits controlling Drosophila flight

Authors: *I. COHEN;
Physics, Cornell Univ. Col. of Arts and Sci., Ithaca, NY

Abstract: The robust navigation essential for animals' survival relies on the integration of sensory information from various modalities, enabling them to generate precise locomotive responses. With the recent unveiling of Drosophila's brain and ventral nerve cord connectome, alongside the accessibility of genetic toolkits facilitating single-cell manipulation, Drosophila presents an unparalleled opportunity for comprehending the intricate neural circuits responsible for creating a robust internal representation of the external world, which in turn guides their navigation and control. To illustrate our approach in unraveling the fly's navigation program, we harness genetic fly lines that we helped generate to target specific neuronal populations within the flight motor system, shedding light on the neuromuscular underpinning of the fly's rapid flight stabilization reflex (approx. 10ms). Our rigorous quantification of the flight stabilization

reflex in this fast time-scale regime represents a key step of our ongoing effort with multiple labs in understanding how the underlying neural network adapts to incorporate sensory modalities such as visual, gyroscopic and airflow sensors with varying temporal responses to control flight. In this talk, we will showcase paradigms we proposed in manipulating multiple sensors at once to tease out the principle behind sensory processing and integration.

Disclosures: I. Cohen: None.

Presentation Number: NANO33.08

Topic: F.01. Neuroethology

Support: MURI grant N00014-19-1-2373

Title: Reward Learning and Memory in Octopus Peripheral Arm Nervous System

Authors: *J. CUI[1], E. GRIBKOVA[2], E. STINE[3], R. GILLETTE[1,4];
[1]Neurosci. Program, [2]Coordinated Sci. Lab., [3]Mol. and Cell. Biol., [4]Dept. of Mol. & Integrative Physiol., Univ. of Illinois at Urbana Champaign, Urbana, IL

Abstract: Chemotactile reward learning in the octopus typically entails the arms grasping a training stimulus with their suckers, which are exquisitely sensitive to both taste and texture, and bringing it to the mouth for final decision for consummation or rejection. Subsequent to training, the arms/sucker may reject or acquire the stimulus, depending on its learned positive or negative valence. We investigated the potential roles of the peripheral nervous system in the arms in reward learning. We tested the arms' abilities to distinguish different chemicals and tactile cues and associate them with positive and negative reward training. We assayed arm responses to

stimuli in animals placed in a still state by “octopus hypnosis”, where individual arms were isolated from the CNS by local anesthesia at the proximal arm base. We observed that such isolated arms showed the same preferences for handling stimuli as did the intact, freely behaving animals. This study can further explain the interface between the peripheral nervous system of the arm and the CNS and where the memory for different odors is stored. These observations are consistent with the arm/sucker’s roles in mediating stimulus location and incentive and suggest that local memory engrams are important to their computations.

Disclosures: J. Cui: None. **E. Gribkova:** None. **E. Stine:** None. **R. Gillette:** None.

Nanosymposium

NANO34: Neural Representation of Value and Choice During Decision Making

Location: MCP Room N426

Time: Monday, October 7, 2024, 1:00 PM - 4:00 PM

Presentation Number: NANO34.01

Topic: H.03. Decision Making

Support: R01MH128344-01A1

Title: Temporal-orbitofrontal pathway for integrating reward and perceptual novelty

Authors: *T. OGASAWARA[1], K. XU[7], A. Z. SNYDER[2,3], J. S. PERLMUTTER[1,2,3,4], T. MINAMIMOTO[9], K.-I. INOUE[10], M. TAKADA[10], I. E. MONOSOV[1,5,6,7,8];
[1]Dept. of Neurosci., [2]Mallinckrodt Inst. of Radiology, [3]Dept. of Neurol., [4]Dept. of Physical Therapy and Occup. Therapy, [5]Dept. of Neurosurg., [6]Pain Ctr., Washington Univ. Sch. of Med., St. Louis, MO; [7]Dept. of Biomed. Engin., [8]Dept. of Electrical Engin., Washington Univ. in St. Louis, St. Louis, MO; [9]Natl. Inst. Quantum Scinece and Technol., Chiba, ; [10]Syst. Neurosci. Section, Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Japan

Abstract: Humans and other primates interact with the world by observing and exploring visual objects. They especially desire to interact with objects associated with both future rewards and novelty. Despite the importance of object value and novelty, and their behavioral interactions in our daily lives, little is known about how the brain controls decisions in which agents consider both reward and novelty. To study this issue, we focused on the perirhinal cortex (PRH), an area involved in novelty detection that we recently linked to signaling predictions of future novelty to guide novelty seeking behavior (Ogasawara et al., 2022, Nature Neuroscience). Notably, PRH strongly projects to the orbitofrontal cortex (OFC), a core region for processing the value of objects and decision offers. We hypothesized that the PRH–OFC pathway critically contributes to the behavioral interaction of novelty and reward in decision making. To assess this, we trained monkeys with a task consisting of several contexts. In the first context, five offers predicted future novel versus familiar objects with five distinct probabilities (% of novel/familiar objects: 0/100, 25/75, 50/50, 75/25, 100/0). Obtaining novel objects was associated with large rewards, while obtaining familiar objects predicted small rewards. Oppositely, in a second context, five other offers also predicted novel objects with the same five probabilities, but here, novel objects

were associated with small rewards instead of big rewards, while familiar objects were associated with big rewards rather than small rewards. Hence, in the first context, novel objects had a high reward value, while in the second, they had a low reward value. When given an option to choose among these offers, monkeys consistently chose offers more with high expected reward and high expected novelty over offers with high expected reward and low expected novelty, even if their reward probabilities were the same. We next tested whether and how the PRH-OFC pathway contributes to this boosting of reward value by novelty. To do so, we injected an AAV viral vector expressing hM4Di in PRH and performed chemogenetic disruption of the pathway by injecting DCZ into OFC where hM4Di was expressed on PRH axons. This changed the monkeys' preferences, altering the interaction of novelty and reward in their decisions. Our data suggests that the PRH–OFC pathway causally contributes to decisions guided by both reward and novelty, such as those we and other primates often make in our daily lives.

Disclosures: T. Ogasawara: None. **K. Xu:** None. **A.Z. Snyder:** None. **J.S. Perlmutter:** None. **T. Minamimoto:** None. **K. Inoue:** None. **M. Takada:** None. **I.E. Monosov:** None.

Presentation Number: NANO34.02

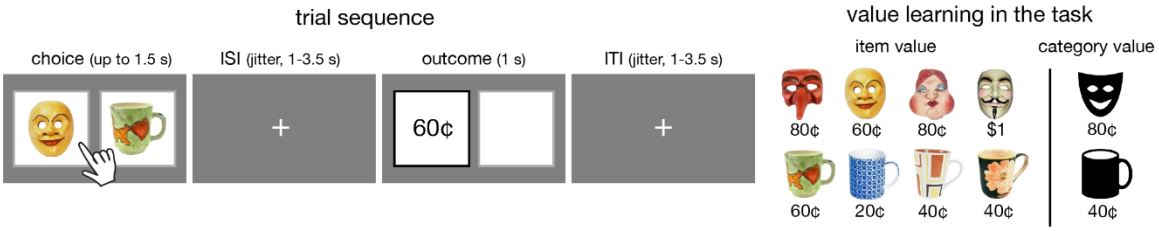
Topic: H.03. Decision Making

Title: The role of conceptual knowledge in value-based decisions

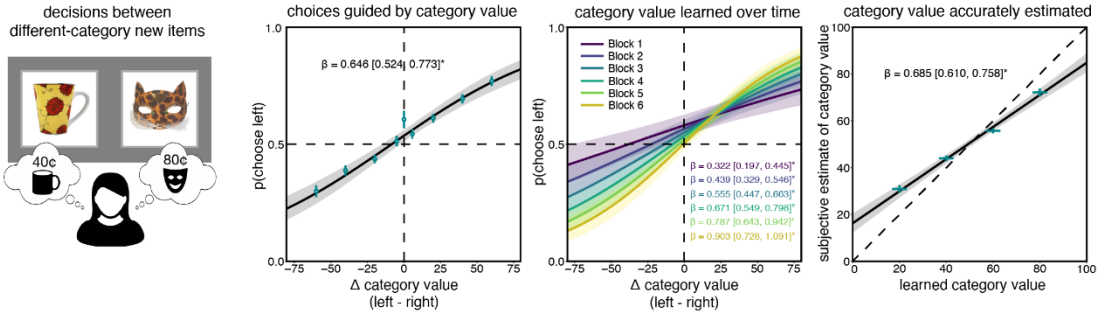
Authors: *N. BIDERMAN[1,2,5], C. INSEL[1,2], D. SHOHAMY[3,1,4];
[1]Psychology, [2]Mortimer B. Zuckerman Mind, Brain, Behavior Inst., [3]Zuckerman Inst., [4]The Kavli Inst. for Brain Sci., Columbia Univ., New York, NY; [5]Psychology, Stanford Univ., Stanford, CA

Abstract: Research on value-based decisions has focused on learning associations between arbitrary cues (e.g., shapes) and their reward values (e.g., monetary outcomes). This kind of reinforcement learning involves the striatum and the ventromedial prefrontal cortex (vmPFC). However, in most real-world situations, reward cues are not arbitrary - they have structure and meaning - and we use knowledge about cues to guide decisions. Here we aimed to understand how category knowledge provides a scaffold for reward learning and to explore the brain circuits involved. Using fMRI we had participants (n=44) perform a task in which they learned from reinforcement about the value of known categories. On each trial, participants made decisions between pairs of items for reward and received immediate feedback. In one condition, the choice was between novel items that were drawn from familiar categories, allowing participants to generalize learned category value to guide decisions ("New" trials). In another condition, the choice was between two previously seen items from the same category ("Old" trials), requiring participants to instead rely on retrieval of memory of the item value. We found that participants learned to update the category values and used them to guide choices between new items. When deciding between old items, participants instead used memory of the specific items' value to guide choices. These two types of learning and decision strategies activated distinct networks of brain regions. Decisions based on category value, compared to item value, involved activation in the vmPFC, in the hippocampus and in visual cortical regions. Furthermore, across participants, performance on "New" trials was correlated with reward-related activity in the vmPFC and choice-related activity in visual cortex, suggesting a specific role for these regions in learning to

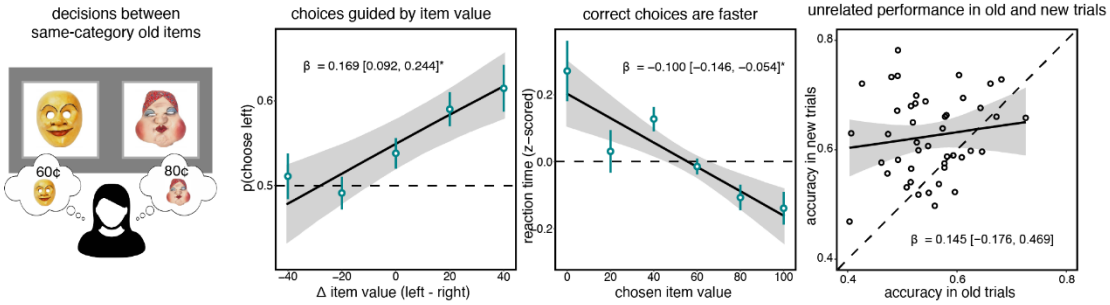
associate categories with value. Our findings reveal the ways in which people learn and use category level knowledge to generalize value to new choices and how this form of learning differs from retrieval of an item-specific value.



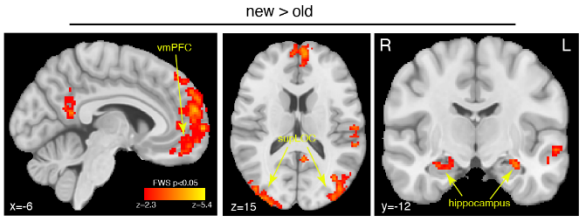
generalization of category value guides choices between novel items



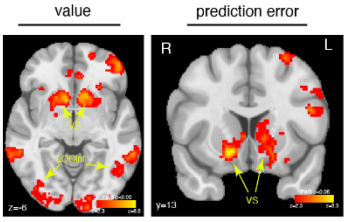
value of specific items is learned and used to guide decisions between old items from the same category



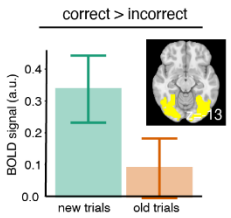
regions active in the choice phase in new and old trials



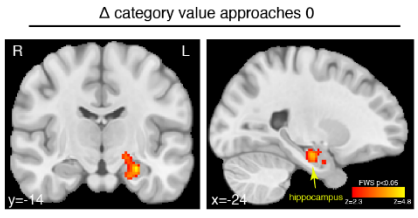
learning of value across all trials activates an established valuation network



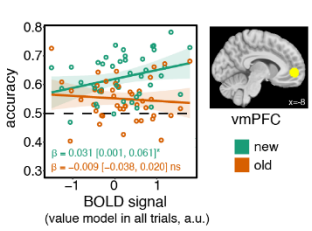
visual regions relate to behavior in new trials



anterior hippocampus tracking difficult decisions in new trials



value-associated activity in the vmPFC relates to behavior in new trials



Disclosures: N. Biderman: None. **C. Insel:** None. **D. Shohamy:** None.

Presentation Number: NANO34.03

Topic: H.03. Decision Making

Support: IBS-R015-D1
1711198566

Title: A derivative-like dopaminergic computation across valences

Authors: *M. PARK[1], J. YUN[2], H. CHOI[3], H. R. KIM[4];
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Abstract: Recognizing the predicted danger and learning how to escape the situation is necessary for an animal's survival. Prior studies in rewarding situations have addressed that phasic activity in midbrain dopaminergic neurons can be explained by temporal difference (TD) reward prediction errors (RPEs). Based on the notion that TD RPE is approximately a derivative of a value function, previous study showed that slowly fluctuating dopamine activity during approach-to-reward can be explained by a moment-by-moment TD error. It is unclear, however, whether dopamine activity facing aversive situations can also be a 'teaching signal' of a similar kind. Here, we examined whether dopamine activity in a situation with negative valence can also be explained by TD errors.

We designed an active avoidance task in a virtual linear track that consists of three zones: beginning, shock, and safe zone. To avoid electric tail shocks (0.8mA, 150ms duration, 3.5s interval), mice should run out of the shock zone. Once mice learn the task, the value of the shock zone would be lower than those of the two other zones. In this case, TD error would predict the gradual inhibition at the entrance of the shock zone and excitation at the exit. While the animals performed the task, we measured dopaminergic activity in the ventral striatum with fiber photometry using a dopamine sensor (DA2m). We observed gradual inhibition (ramp-down) followed by excitation (n =10 mice), consistent with the TD error prediction.

We further examined the nature of the responses by manipulating positions. We varied the speed of the visual scene: slow (x0.5), standard (x1.0), and fast (x2.0). Faster speed gain amplified both the early inhibitory and later excitatory dopamine responses (n = 10 mice), which is consistent with the prediction that the slope of value function increases with speed. Furthermore, we transported animals forward to skip the shock-zone, or backward to repeat the shock-zone. Phasic excitation and inhibition were followed after being teleported forward and backward, respectively (n = 5 mice), assumed to be caused by sudden changes of value. These results confirm the idea that dopamine activity during aversive situations follows a moment-by-moment derivative of value function. Finally, we added the second track in which mice obtained a reward after the exit of the shock zone. Dopamine response right before receiving reward increased greater than the conditions without reward (n = 4 mice), which suggests that dopamine activity in the ventral striatum integrates both positive and negative valences using a unified derivative process.

Disclosures: M. Park: None. **J. Yun:** None. **H. Choi:** None. **H.R. Kim:** None.

Presentation Number: NANO34.04

Topic: H.03. Decision Making

Support: NIH R01MH097061

Title: Mesolimbic dopamine encodes subjective value of behavioral strategy, not reward value

Authors: *A. ARORA[1], T. OTT[2], S. REN[3], T. S. GOUVEA[4], A. KEPECS[5];
[1]Neurosci., Washington Univ. In St. Louis, Saint Louis, MO; [2]Bernstein Ctr. for Computat. Neurosci., Humboldt Univ. of Berlin, Berlin, Germany; [3]Washington Univ. Sch. of Med., Saint Louis, MO; [4]Fundacao Champalimaud PT507131827, Lisbon, Portugal; [5]Neurosci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: From deciding how long to wait for a cupcake to investing years in a degree, the time we commit reveals the subjective value we place on our choices. This 'revealed preference' measure of value, central to behavioral economics, differs from the common neuroscience approach of algorithmically inferring value from reward history. How these two notions of subjective value relate remains unclear.To bridge this gap, we developed a novel rat decision-making task integrating both perspectives. Rats performed a two-armed bandit task, unpredictably ‘baited’ with rewards that varied across trials, and invested time to obtain uncertain, delayed outcomes. Reinforcement models based on reward and choice history predicted both the choice and the time investment in each trial. Crucially, the time rats invested predicted their selection of higher-valued options, providing a trial-by-trial, graded measure of revealed value.Fiber photometry in the ventral striatum using GRAB-DA sensors revealed phasic mesolimbic dopamine release around choice - a phasic response at trial initiation and another at the time of choice. Dopamine at the time of choice robustly predicted rats' upcoming time investment, suggesting it encodes the revealed subjective value guiding these decisions. The phasic response at trial initiation reflected the rats’ commitment to the current strategy. Notably, neither response consistently tracked model-inferred value from reward history. This suggests that dopamine release tracks a computation related to the value of the behavioral strategy, and not the reward value itself. We designed a normative model to predict the trial-to-trial value associated with a particular behavioral strategy (e.g. stay vs. switch), accounting not just for the expected reward of each choice, but also for the expected value of the knowledge gained from exploratory choices. Our study introduces a novel behavioral task that unites reinforcement learning theory with behavioral economics and identifies a role for mesolimbic dopamine in guiding time investment decisions.

Disclosures: A. Arora: None. **T. Ott:** None. **S. Ren:** None. **T.S. Gouvea:** None. **A. Kepecs:** None.

Presentation Number: NANO34.05

Topic: H.03. Decision Making

Support: F30MH136699
R01MH118369

Title: Investigating the role of GABAergic interneurons in the dorsomedial striatum in value-based decision-making

Authors: *E. ILIAKIS[1], A. RAMIREZ[3], C. L. GARCÍA[4], L. VARGAS[2], K. CHOI[2], S. M. FERRIGNO[2], E. DIAZ-HERNANDEZ[2], E. N. HOLLY[5], D. J. MARGOLIS[6], M. V. FUCCILLO[2];
[1]Dept. of Neuroscience, Sch. of Med., [2]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [3]Neurosci., Icahn Sch. of Med. at Mt. Sinai, New York City, NY; [4]Rutgers Univ. Behavioral and Systems Neurosci., Highland Park, NJ; [5]Mol. and Behavioral Neurosci., Rutgers University-Newark, Newark, NJ; [6]Cell Biol. & Neurosci., Rutgers, The State Univ. of New Jersey, Piscataway, NJ

Abstract: Value-based decision-making aims to maximize rewards in changing environments. Abnormalities in value-based decision-making are a feature of a range of neuropsychiatric diseases and are functionally impairing. The dorsomedial striatum (DMS) is critical for the execution of value-based decision-making. Within the DMS, the role of sparse GABAergic interneurons in behavior is of increasing interest. Tyrosine hydroxylase-positive (TH+) interneurons (THINs) are necessary for maintaining goal-directed strategies, while somatostatin-positive (SST+) low-threshold spiking interneurons (LTSIs) exhibit novel reward-related activity that decays throughout operant learning. However, the role of DMS THINs and LTSIs in value-based decision-making is not known. To study their role in value-based decision making, we have developed a head-fixed joystick-based two-alternative forced choice behavioral paradigm in mice that assays the effect of varying relative reward values, reward probabilities, and net reward environment on value-based goal-directed choice and motor vigor. Constitutive silencing of LTSIs using overexpression of the potassium inward rectifier channel Kir2.1 suggests LTSIs play a role in the regulation of motor vigor. Fiber photometry recordings of these interneuron subtypes suggest key roles of their tonic and phasic activity in execution of value-based choice and modulation of motor vigor. These findings contribute to a growing evidence base on the role of striatal microcircuitry in striatal function and goal-directed behavior, with potential translational relevance suggested by evidence implicating striatal interneurons in a range of neuropsychiatric disease presentations.

Disclosures: E. Iliakis: None. **A. Ramirez:** None. **C.L. García:** None. **L. Vargas:** None. **K. Choi:** None. **S.M. Ferrigno:** None. **E. Diaz-Hernandez:** None. **E.N. Holly:** None. **D.J. Margolis:** None. **M.V. Fuccillo:** None.

Presentation Number: NANO34.06

Topic: H.03. Decision Making

Support: F32MH130027
R01MH129439

Title: Examination of human posterior cingulate cortex electrophysiology during reward-based decisions

Authors: *S. R. KOSLOV[1], S. A. SHETH[4], D. YOSHOR[1], H. CHEN[1], K. A. DAVIS[2], J. W. KABLE[3], B. Y. HAYDEN[4], B. L. FOSTER[1];

[1]Dept. of Neurosurg., [2]Dept. of Neurol., [3]Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Neurosurg., Baylor Col. of Med., Houston, TX

Abstract: Human neuroimaging studies of posterior cingulate cortex (PCC) regularly link the region to episodic memory. In contrast, electrophysiological recordings conducted in non-human primates associate the PCC with decision-making. Interestingly, while not often emphasized, many human neuroimaging studies of reward-based decision-making implicate the PCC, suggesting convergence across species. However, these neuroimaging findings report differences in the timing of PCC engagement during choice selection and feedback periods, which are best resolved with temporally precise methods. To bridge the cross-species gap, and to better understand how and when the PCC may support economic decision-making in humans, we obtained intracranial recordings while participants performed a two-option risky choice task that was closely related to one commonly used in non-human primate research. On each trial of the task, participants selected between a guaranteed, fixed low-reward option or a gamble option with a specified probability of a high-reward. Behaviorally, participants selected both the risky and safe options, demonstrated a small though reliable risk-aversion bias, and were more likely to select the risky option as the expected value of the gamble increased. Reaction times increased as a function of the expected value difference between options. Having established that the behavioral patterns observed were consistent with previous work, we next focused on neural data, specifically broadband gamma activity (BBG; 70-150Hz) within PCC. We observed increased BBG during choice selection compared to baseline periods, and furthermore, found preliminary evidence suggesting BBG correlated with the expected value of risky gambles. Additionally, comparisons of the timing of BBG responses in PCC were similar to responses observed in other regions, like the lateral and medial prefrontal cortices, contextualizing the dynamics of PCC participation in decision-making. These results support the involvement of PCC in human choice behavior during decision-making, bridging observations across species. More broadly, these findings suggest both executive and episodic processes are supported by the PCC, advancing our understanding of this region's broader role in cognition and disease.

Disclosures: S.R. Koslov: None. **S.A. Sheth:** None. **D. Yoshor:** None. **H. Chen:** None. **K.A. Davis:** None. **J.W. Kable:** None. **B.Y. Hayden:** None. **B.L. Foster:** None.

Presentation Number: NANO34.07

Topic: H.03. Decision Making

Title: Hypocrisy in value based decision making: investigating the discrepancy between best option assessments and the actual choice

Authors: *P. MODAK, J. W. BROWN;
Indiana Univ., Bloomington, IN

Abstract: In this work, we investigate the phenomenon of apparent hypocrisy in value-based decisions. There is a large body of literature focusing on the discrepancy between value judgments and choices, where the options with higher value judgments are not necessarily chosen. This discrepancy is often ascribed to the difference in the response modes of the choice and the judgment conditions. Some previous research has, however, demonstrated that individuals do not always select what they think is the best option even when response modes are

the same. For instance, Bechara et al. (1997) reported that individuals with damage to the ventromedial prefrontal cortex could accurately evaluate options and determine which were superior, yet their choices did not reflect this knowledge.In our study, we tested the hypothesis that the process of making a choice is not the same as the process of finding the best option with respect to reward maximization. We collected functional MRI data from healthy human participants while they performed a risky decision-making task under three different conditions, all with a choice-based response mode. In the first, the 'best-option' condition, we asked participants to explicitly indicate the option they assess to be the best, with reward maximization externally enforced through the task's reward structure. In the second, the 'choice' condition, participants were asked to choose an option with no specific goal externally enforced. In the third, the control condition, participants were instructed to select a particular option. In all conditions, participants had to choose between a gamble and a sure reward.Results from pilot data (n = 16) reveal important differences in behavior, cognition, and the neural underpinnings of the best-option and choice conditions. Specifically, we observed greater involvement of the mid-cingulum in the 'choice' condition and that of the bilateral caudate in the 'best-option' condition. We also find significant differences in choice behavior, with greater risk-seeking in the 'choice' condition and in the shapes of the RT distributions. The differences in risk-seeking and the shapes of the RT distributions are, in turn, related to differences in BOLD activation of the mid-cingulum during the two conditions. Our results suggest that the apparent hypocrisy in value-based decision-making arises because the conditions—choice versus best-option—target different neural and cognitive processes.

Disclosures: P. Modak: None. **J.W. Brown:** None.

Presentation Number: NANO34.08

Topic: H.03. Decision Making

Support: Grant-in-Aid of JSPS for Research Activity Start-up No. 18H06090
Grant-in-Aid of JSPS for JSPS Fellows No. 19J00964
Grant-in-Aid of JSPS for Early-Career Scientists No. 20K16475
Grant-in-Aid of JSPS for Early-Career Scientists No. 23K14680
the Impulsing Paradigm Change through disruptive Technologies (ImPACT) program in Japan
NIMH Grant R00 MH125021

Title: Mental Effort Learning, unlike Reward Learning, Requires Direct Experience

Authors: *A. M. NAGASE[1], K. ONODA[2], K. MORITA[3], T. KAWAGOE[4], S. YAMAGUCHI[5], R. AKAISHI[6], R. HANAJIMA[7], A. WESTBROOK[8];
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Abstract: We learn to avoid mental labor involved in writing manuscripts and emails, or reading massive documents, as much as possible, even if we know that we need to exert effort to achieve our goals. As a result, we sometimes fail to obtain rewards because of insufficient effort. What

algorithms govern adaptive avoidance of mental effort? Reward-learning systems are well-studied. Evidence suggests that reward-learning involves temporal-difference (TD) learning, or possibly being retrospective causal learning. The learning algorithm of mental effort costs, however, is still unknown. The positive neural correlation that the cost prediction error (CPE) of mental effort in the dorsomedial frontal cortex/dorsal anterior cingulate cortex (dmFC/dACC) was reported previously. Initially, we hypothesized that effort cost learning follows the TD learning algorithm and that expected costs are be updated in response to cues about upcoming effort. We predicted that at the time of a fully informative effort cue, dmFC/dACC activity would positively, and striatal activity negatively correlate with CPE. We conducted two fMRI experiments of demand-selection tasks, using mental division and cube-folding problems (Exp. 1, n=30; Exp. 2, n=28). In a trial, subjects chose between two options that were either associated with hard or easy versions of each task, and the upcoming effort levels of each option changed over time. Between choice options and problems, we deterministically cued upcoming efforts. Most subjects avoided high-demand problems, reflecting multiple past experiences to their current choices. We conducted model-based fMRI analyses with a reinforcement learning model based on effort levels for demand avoiders (Exp. 1, n=25; Exp. 2, n=23) and a conjunction analysis between Exp. 1 and 2. Contrary to our predictions, trial-wise CPEs correlated with neither activity in the dmFC/dACC nor striatum at the time of the effort cue. Therefore, we formulated two alternative hypotheses that expected costs are updated at either effort initiation or effort completion. At effort initiation, the activity of the orbitofrontal cortex/ventromedial prefrontal cortex/ventral ACC correlate positively with CPE. At effort completion, activity in the dmFC/dACC correlates positively, and in the bilateral caudate correlates negatively, with CPE. We infer that the learning of mental effort costs does not follow the traditional TD learning algorithm, nor does it conform to the more recent retrospective causal learning algorithm, and suggest instead that expected effort cost learning requires effort exertion.

Disclosures: A.M. Nagase: A. Employment/Salary (full or part-time):; Rutgers University. **K. Onoda:** A. Employment/Salary (full or part-time):; Otemon Gakuin University. **K. Morita:** A. Employment/Salary (full or part-time):; The University of Tokyo. **T. Kawagoe:** A. Employment/Salary (full or part-time):; Tokai University. **S. Yamaguchi:** A. Employment/Salary (full or part-time):; Shimane Prefectural Central Hospital. **R. Akaishi:** A. Employment/Salary (full or part-time):; RIKEN CBS. **R. Hanajima:** A. Employment/Salary (full or part-time):; Tottori University. **A. Westbrook:** A. Employment/Salary (full or part-time):; Rutgers University.

Presentation Number: NANO34.09

Topic: H.03. Decision Making

Support: NIH Grant DA038615

Title: Change of mind in continuous decisions: cingulate encoding of compositional goal policies

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Abstract: Naturalistic contexts are dynamic, continuous, and interactive. Adaptive behavior in these contexts requires us to switch between and dynamically blend goal-seeking strategies while continuing to act. Regulating multiple goal-seeking behaviors simultaneously therefore requires meta-control processes. To understand the neural bases of meta-control in naturalistic contexts, we recorded from neurons in the dorsal anterior cingulate cortex (dACC) and dorsal premotor cortex (PMd) while macaques performed a continuous pursuit task with two moving prey that actively evaded them. We used a novel control-theoretic decomposition of subjects' moment-to-moment pursuit behavior, allowing us to identify a latent meta-control variable that determined subjects' blend of goal pursuit. We used this model to examine on-line changes of mind between pursuit goals, and found a low-dimensional neural representation of the proposed meta-control signal. These changes-of-mind were supported by neural dynamics that were conserved across switch contexts, pointing to a population mechanism supporting online choice. These results indicate that control of behavioral state reflects the interaction of brain processes found in dorsal prefrontal regions that implement a compositional mixture over low-level control policies.

Disclosures: J. Fine: None. **S.M. Yoo:** None. **B.Y. Hayden:** None.

Presentation Number: NANO34.10

Topic: H.03. Decision Making

Support: NIH F32 MH115416
NIH F32 MH123047

Title: Simultaneous brainwide recordings reveal a cortico-striatal subnetwork mediating perceptual choice

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Abstract: Making a choice based on noisy sensory inputs is thought to depend on neural activity across the brain, and widespread brain activity correlates with the decision process. While interactions between such brain regions are presumably critical for choice formation, the nature of these interactions is obscure. A major obstacle has been the difficulty of observing neural activity simultaneously across many brain regions at behaviorally relevant timescales. Here, we recorded spikes simultaneously from over 3,000 neuronal units bilaterally across 12 brain regions/hemisphere while rats performed a freely moving auditory evidence accumulation task. To measure the decision-related interactions among brain regions, we quantified each region's evolving representation of the internal decision process (the "decision variable"; DV), and examined their correlations. Importantly, we presented repeat trials with identical stimulus sequences, which enabled us to subtract the choice- and stimulus- conditioned mean DV. This allowed us to isolate moment-to-moment co-fluctuations in the decision process not directly driven by the sensory stimulus or the behavioral choice. The structure of the residual correlations revealed three anatomically interconnected frontal cortical and striatal brain regions-dorsomedial frontal cortex, primary motor cortex, and anterior dorsal striatum-whose decision dynamics are highly coupled. Along with findings from causal perturbation, this result suggests that these three

frontal cortical and striatal regions form a subnetwork that mediate choice formation. To test this possibility, we used a recent method to infer from the neural activity and behavioral choice the moment within each trial at which the animal stops accumulating evidence and commits to a choice. We found that the correlations amongst the three regions abruptly diminished after the inferred moment of decision commitment. Our results suggest a frontal cortico-striatal subnetwork mediates the formation of perceptual choices and highlight the promise of large-scale simultaneous neural recording for uncovering brain-wide interactions underlying cognition.

Disclosures: A.G. Bondy: None. **J. Charlton:** None. **T. Luo:** None. **S.C. Venditto:** None. **W. Stagnaro:** None. **C.D. Kopec:** None. **C.D. Brody:** None.

Presentation Number: NANO34.11

Topic: H.03. Decision Making

Support: STI2030-Major Projects 2021ZD0202200

Title: Mouse PPC encodes strategy for visual evidence accumulation and its execution

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Abstract: Evidence accumulation is crucial for decision-making, enabling animals to integrate sensory inputs and make optimal decisions. Significant research has highlighted the role of the posterior parietal cortex (PPC) in visual decision-making. However, it remains unclear whether the PPC encodes the strategy for evidence accumulation and its involvement in executing this process. We addressed these questions by employing an unrestrained two-alternative forced-choice task based on flash evidence accumulation. Mice were trained to associate the rate of temporally biased flashes with directional choices and received water as a reward. We demonstrated that mice accumulate evidence for two choices with different time weights, corresponding to uneven psychophysical kernels.
To test that the PPC is necessary for this task, we inhibited its neural activity by injecting muscimol and found that the mice's performance significantly decreased. We then recorded the PPC neuronal activity in freely moving mice to investigate whether PPC encodes evidence accumulation strategy. Specifically, we first functionally located the PPC through retinotopic mapping, then utilized miniature two-photon fluorescence microscopy to obtain high-throughput single-neuron calcium activity during the task (17 mice). To establish the relationship between neural activity and strategy, we calculated the time derivative of activity for each neuron and the pairwise correlation coefficients among them, obtaining a correlation matrix of activity change rates. The Hopkins statistic indicated a clustering tendency in the matrix, and it could be optimally clustered into two groups. The result showed that the time derivatives of activity for the two neural ensembles were significantly correlated with the psychophysical kernels of the two choices, respectively. Furthermore, we conducted reversal experiments where the association rules of the flash rates and choices were switched. Some PPC neurons dynamically tuned their activity patterns and stabilized once the new rule was learned.
Additionally, we analyzed how flashes influenced neural activity at various time points across the stimulus stage. By calculating activity changes across all time bins, we found that the activity changes in the time bins with flashes significantly differed from those without flashes, and the

magnitudes of change showed a significant linear correlation with the net weight of the left-right choices, indicate that mouse PPC encodes execution of evidence accumulation.
In summary, this study provides evidence that the mouse PPC is responsible for encoding strategy for evidence accumulation and its execution.

Disclosures: J. Han: None. **D. Zhang:** None. **L. Zhang:** None. **H. Cheng:** None.

Presentation Number: NANO34.12

Topic: H.03. Decision Making

Support: HHMI
R01NS113113
T32EY013933
Kavli Institute for Brain Science

Title: A neural signature of covert decision termination in the superior colliculus

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Abstract: Many decisions arise through the integration of noisy evidence to a threshold, or bound. For perceptual decisions about random dot motion, it is known that neurons in the lateral intraparietal area (LIP) represent the accumulation of evidence (i.e., drift-diffusion) accompanying each decision, whereas neurons in the superior colliculus (SC) appear to implement the bound to terminate the decision with an eye movement (Stine et al., 2023). In a free response task (a.k.a. choice-response time), both the choice and the time of the decision are expressed overtly. A similar mechanism is thought to govern covert decisions in which termination is not accompanied by an immediate eye movement but by a commitment to the choice and the cessation of evidence integration (Kiani et al., 2008). It is unknown if neurons in the SC also play a role in terminating such covert decisions. To address this question, we recorded simultaneously from SC and LIP while monkeys performed a variable-duration (VD) motion discrimination task. Motion viewing durations ranged from 120 to 1600 ms and were followed by a random delay before the monkey was allowed to indicate its choice. On ~60% of trials, we observed brief burst-like activity in the SC that was not associated with an eye-movement. These non-saccadic bursts (ns-bursts) were associated with transient increases in LIP activity. Such ns-bursts were also observed in the free response task but with much lower frequency (~10% of trials). Several observations suggest that ns-bursts are associated with covert decision termination in the VD task: *(i)* on longer duration trials, ns-bursts were more likely to occur during motion viewing than in the delay period; *(ii)* ns-bursts occurred earlier during trials with strong motion; *(iii)* ns-bursts often occurred early in the delay period on short trials with weak motion. This pattern is consistent with decision times predicted from bounded evidence accumulation. Additional support for this idea is adduced from a causal intervention using brief motion pulses. As described previously (Kiani et al., 2008), motion pulses in the VD task bias choices when presented early but are ignored if presented later—consistent with covert termination. We replicated and extended this finding by showing that motion pulses bias choices when they are presented before ns-bursts but not after. These results broaden the role of the SC

in decision termination to include covert choices that are unaccompanied by an immediate eye movement.

Disclosures: M.J. Pensack: None. **G.M. Stine:** None. **M.N. Shadlen:** None.

Nanosymposium

NANO35: Neural Mechanisms of Language Production and Comprehension

Location: MCP Room S401

Time: Monday, October 7, 2024, 1:00 PM - 4:15 PM

Presentation Number: NANO35.01

Topic: H.11. Language

Support: NIH Grant R01 MH081990
University of Macau CRG2021-00001-ICI
University of Macau MYRG2022-00200-FAH
University of Macau MYRG2022-00265-ICI

Title: Native and non-native languages are processed at different paces through shared visual, auditory, and speech motor streams

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Abstract: More than half of the Earth's population can use more than one language. However, it remains unclear whether native and non-native languages are processed through shared or distinct neural circuits. Existing functional magnetic resonance imaging (fMRI) studies have identified overlaps in the cortical representations of different languages but have yet to reveal the temporal dynamics of parallel and sequential information processing streams across the language network. Here we used rapid phase-encoded fMRI designs to unravel the spatiotemporal brain dynamics of sentence processing during periodic reading, listening, reading-aloud, and shadowing tasks. Human subjects (Group 1: n = 31, sequential bilinguals; Group 2: n = 30, sequential trilinguals) were scanned using a 32-channel coil in a 3T MRI scanner. Each subject participated in two fMRI sessions: one in native language (L1 = Chinese) and the other in non-native language (Group 1: L2 = English; Group 2: L3 = Portuguese). Fourier-based analyses and surface-based traveling waves revealed overlapping streams of language information flows, which propagate in the same directions through visual, auditory, and speech motor cortices for all three languages. On each stream, dozens of vertices were sequentially selected along a path perpendicular to the wavefront of traveling waves over the cortical surface. The timing of traveling waves was then computed from the phase of a periodic signal (16 cycles per scan) at each vertex on the path. In ventral and dorsal visual streams, hemodynamic traveling waves of non-native language information propagate slower and further away than those of native language. Similarly, traveling waves of non-native languages propagate slower through auditory and speech motor streams. These results suggest that, even with controlled proficiency level,

later-acquired languages are processed less efficiently through the same neural pathways shared by the native language.

Disclosures: R. Huang: None. **T. Leong:** None. **V. Lei:** None. **D. Li:** None.

Presentation Number: NANO35.02

Topic: H.11. Language

Support: UCSF Schwab DCDC Innovation Fund

Title: Individual differences in high-dimensional semantic representations during narrative speech comprehension

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Abstract: Individuals differ in brain function due to natural variations, age, or disease. Investigating these individual differences is necessary for accurately characterizing cognitive functions and dysfunctions in individuals. Here we developed a novel framework to examine individual differences in high-dimensional functional representations recovered with voxelwise encoding models. In an fMRI experiment, 24 participants (aged 21-35, 11 females) listened to ~2.5 hours of narrative stories. A separate voxelwise encoding model was created for each participant to predict brain responses from the semantic content of the stories. For each voxel, the model weights describe the voxel tuning for multiple semantic features. For each participant, the set of all model weights is a distribution in a high-dimensional semantic feature space, and this distribution determines the semantic concepts represented by each participant. Between-participant differences in these high-dimensional distributions correspond to individual differences in semantic representations. To measure these individual differences, we developed a metric based on the optimal transport theory, which we call the Distribution Similarity Measure (DSM). DSM is computed with a leave-one-participant-out scheme to compare each participant to a group, and it is interpreted as the variance in a participant's model weights explained by a group distribution. To determine the semantic dimensions along which each participant differed the most from the group, we examined the variance unique to each participant. For this experiment, the average DSM value (i.e., variance explained) is 79% (min 75%, max 82%), indicating that semantic representations are consistent across participants who are listening to the same narrative stories. Furthermore, 21% of the variance in the model weights is unique to each participant. This unique variance is associated with semantic dimensions idiosyncratic to each participant. For example, the first two semantic dimensions that were recovered separately in each participant explain on average 37% of the unique variance in the same participant (min 31%, max 46%), but only 1% of the unique variance in the other participants (min 1%, max 7%). These idiosyncratic semantic dimensions do not correspond to known dimensions (e.g., abstract-concrete, animate-inanimate, social-nonsocial), but reflect complex combinations of semantic concepts. Because our framework can be used to accurately characterize individual differences in functional representations, it provides a novel and rigorous way to study how brain function is affected by natural variations, age, or disease.

Disclosures: M. Visconti di Oleggio Castello: None. **T. Dupré La Tour:** None. **J.L. Gallant:** None.

Presentation Number: NANO35.03

Topic: H.11. Language

Support: NSF 1941626
NSF 1631563
NSF 2219815
NSF 2219739
Alfred P. Sloan Foundation
NIH NS-092988
NIH AG-067419

Title: Pre-adaptations for language: Individual variation in the chimpanzee arcuate fasciculus is associated with both vocal and gestural communication behavior

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Abstract: The arcuate fasciculus plays a central role in human language, but its link to communication behavior in other great apes is currently untested. We used probabilistic tractography in 67 chimpanzees to assess relationships between the anatomy and lateralization of the arcuate fasciculus (AF) and communication behavior. We found that the chimpanzee arcuate fasciculus is not left-lateralized at the population level, in marked contrast with humans. However, individual variation in the use of communicative sounds under volitional orofacial motor control was associated with leftward asymmetry of the volume of the arcuate fasciculus tract and its frontal gray matter terminations. This association was specific to males, which parallels the more frequent use of these sounds by male chimps, potentially reflecting their adaptive value within behavioral contexts that influence social status and mating opportunities. Furthermore, individual variation in the use of manual communicative gestures was associated with measures of white matter tract structure within both the arcuate fasciculus and superior longitudinal fasciculus. These findings suggest that brain-behavior relationships between communication and these tracts were likely present in the last common ancestor of chimpanzees and humans, indicating that selection pressures unique to the human lineage may have led to the increased robusticity, elaboration, and asymmetry of this tract in modern humans. Alternatively or in combination, these species differences may result from humans' but not chimpanzees' immersion in a language-rich environment from birth.

Disclosures: E.E. Hecht: None. **S. Vijayakumar:** None. **Y. Becker:** None. **W. Hopkins:** None.

Presentation Number: NANO35.04

Topic: H.11. Language

Support: NIH R01DC019653
NIH U01NS123130

Title: Single neuronal dynamics underlying human language production revealed by large language models

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Abstract: Language production relies on transforming a stream of words into complex, grammatical sentences. Despite previous work having identified interconnected regions in frontal and temporal cortices underlying this process, the specific role of individual neurons in supporting sentence construction during language production remains elusive. Here, we utilized rare opportunities for single neuron recordings in human, combined with state-of-the-art large language models (LLMs), to directly investigate on the single neuronal dynamics supporting sentence construction. We demonstrate that each individual neuron preferentially encodes one specific grammatical aspect, and these aspects can be accurately decoded from the collection of neurons. Further, the neuronal population exhibit a hierarchical organization that can be predicted by LLMs, indicating a convergence of processing between the human brain and artificial neural networks. Remarkably, these neuronal codings exhibit highly dynamic ordering. They show intrinsic syntactical planning preceding word utterance, followed by a dramatic decrease in sentence construction computation upon word onset. Together, our findings reveal a detailed single neuronal map of syntactic and compositional computation underlying speech production planning, and begin to identify some of the basic components of single neuronal processing in human.

Disclosures: J. Cai: None. **Y. Kfir:** None. **S.S. Cash:** None. **Z.M. Williams:** None.

Presentation Number: NANO35.05

Topic: H.11. Language

Support: Howard Hughes Medical Institute

Title: Deep neural networks reveal context-sensitive speech encoding in single neurons of human cortex

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Abstract: Speech is a dynamic acoustic signal that requires listeners to continuously extract and integrate information at multiple timescales. Prior studies have shown that local neural populations (Bhaya-Grossman & Chang, 2022; Yi, Leonard, & Chang, 2019) and single neurons in human superior temporal gyrus (STG) (Leonard, Gwilliams et al., 2023) encode various phonological and linguistic speech properties, including acoustic-phonetic features, prosodic cues like pitch and intensity, and word-level surprisal. However, since speech is not simply a concatenated sequence of isolated sounds, we are yet to understand how such features are dynamically computed and integrated with the surrounding context. Here, we used deep-neural-

networks (DNNs) to investigate contextual speech processing across hundreds of STG neurons that were recorded from all layers of the human cortex with high-density Neuropixels probes while participants listened to naturally spoken sentences. Specifically, we built encoding models that could predict the spiking activity of each neuron using the hidden states of speech DNNs (Hsu et al., 2021) that have been shown to capture rich, contextual speech features in their internal states. To investigate the degree of context-sensitivity across neurons, we varied both the amount of prior context available to the DNN (20-1000ms) and the DNN layer from which states were extracted (Jain & Huth, 2018). Overall, STG neurons showed varied sensitivity to prior context— from capturing fast spectrotemporal variations to speech information spread across hundreds of milliseconds. We found substantial diversity in context-sensitivity even across cortical layers within a single STG site and across neurons tuned to the same speech feature, suggesting that context-sensitivity is a distinct dimension of the neural code for speech. Lastly, given the heterogeneity in both tuning and context-sensitivity within and across STG sites, we hypothesised that population-level neural activity could capture an integrated representation of the speech input at the level of perceptually-meaningful units like words and phrases. Through population-level decoding analyses, we found that neurons and columns with long context-sensitivity faithfully represented speech over timescales consistent with higher-order speech information (~1sec). Together, our results suggest that heterogeneity in both linguistic tuning and context-sensitivity enables the STG to track multiple, hierarchical levels of spoken language representations.

Disclosures: S. Jain: None. **R. Gandhi:** None. **M.K. Leonard:** None. **E.F. Chang:** None.

Presentation Number: NANO35.06

Topic: H.11. Language

Support: NIH Grant NINDS 1R01NS109367
NIH Grant NINDS 1R01NS115929
NIH Grant NIDCD R01DC018805

Title: Functional Connectivity Classification in ECoG during Speech Perception and Production

Authors: *Y. ESMAEILI[1], A. KHALILIAN-GOURTANI[2], P. DUGAN[3], D. FRIEDMAN[4], W. K. DOYLE[2], O. DEVINSKY[2], A. FLINKER[5];
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Abstract: Functional Connectivity (FC) is an established technique in neuroimaging used to elucidate brain networks and their dynamics by examining how brain regions communicate during cognitive tasks. A large body of the literature has focused on intrinsic networks during resting-state and cognitive tasks, primarily obtained through fMRI. However, there is still a gap in understanding the FC dynamics during language processing and how these connections relate to perception and production. In this study, we aim to address this gap by leveraging human Electrocorticography (ECoG) data during language perception and production, by investigating the specific connections driving each cognitive state. We acquired recordings from ten neurosurgical patients while they performed a battery of language production tasks (auditory

word repetition, picture naming, and visual word reading). We extracted the analytic amplitude of high gamma broadband neural activity (70-150 Hz), and defined five cognitive states: auditory perception, picture perception, word reading perception, speech production, and baseline. For each participant, we then computed Pearson's Correlation as the measure of functional connectivity between electrodes in each trial of these states. To investigate the predominant connections, we classified the FCs within each state using a linear Support Vector Machine (SVM) multiclass model. Classification revealed high decoding accuracy of cognitive states across participants (mean accuracy %86.09, SEM %1.14). Visualizing the decision boundary hyperplanes on the cortex revealed unique state-specific connections.. Auditory perception exhibited connections from superior temporal gyrus to frontal regions, while visual tasks showed connections between occipital cortex and frontal gyri. Speech production highlighted connections within the speech motor cortex and negative connections with superior temporal gyrus, possibly reflecting the self-produced speech feedback processing circuit. Overall, our study illuminates unique functional connections during language processing, revealing the importance of network interactions above and beyond local neural activity. These insights advance our comprehension of neural mechanisms underlying language processing, showcasing the potential of ECoG-based FC analysis.

Disclosures: Y. Esmaeili: None. **A. Khalilian-Gourtani:** None. **P. Dugan:** None. **D. Friedman:** None. **W.K. Doyle:** None. **O. Devinsky:** None. **A. Flinker:** None.

Presentation Number: NANO35.07

Topic: H.11. Language

Support: NIH Grant NIDCD F32DC019533
NIH Grant NINDS 1R01NS109367
NIH Grant NINDS 1R01NS115929
NIH Grant NIDCD R01DC018805

Title: Low-activity coding of syntax during language production

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Abstract: Syntax, the abstract structure of language, remains poorly understood, in large part due to the literature's heavy reliance on comprehension paradigms and neural measures with limited spatial or temporal resolution. Here, we report several novel findings regarding the neural instantiation of syntax, enabled by the use of electrocorticography (ECoG) and a controlled production experiment with 3 blocks: sentence production, picture naming, and list production - a control task. Sentence production trials additionally contained a manipulation that varied syntax (eliciting either active or passive sentences) while holding constant words and meaning. We contrasted 2 approaches to identifying syntax: (1) Following common practice, we compared sentences (which are syntactically structured) and lists (which are not). This analysis identified 60 electrodes with higher sentence activity. Second, we compared active and passive trials,

identifying 125 electrodes that were sensitive to syntax. Critically, only 6 electrodes were commonly identified by these two analyses, meaning that the sentence-list comparison failed to identify over 95% of electrodes sensitive to syntax. To quantify and separate syntax from event-semantic and (sub)lexical information, we employed Representational Similarity Analysis to quantify the processing of these three representation types in each electrode. This identified three distributed networks which, contrary to claims in the literature, were largely specific - i.e., encoding only one representation type. To better understand the sentence-list comparison's failure to identify syntax, we compared the degree of processing to neural activity for each representation type. This analysis revealed a surprising pattern: syntactic processing was unrelated to the degree of activity in an electrode. This "low-activity coding scheme" contradicts the widespread assumption that information processing is indexed by elevated neural activity, and is problematic for approaches like sentence-list comparisons that rely on this assumption. While event semantics also exhibited low-activity coding, (sub)lexical processing was significantly correlated with activity. Based on these findings, we propose a that the brain uses different coding schemes for different kinds of representations. For sensory and motor processes, which are evolutionarily older and spatially concentrated, the brain relies on the amount of activity. In contrast, higher-order systems like syntax, which are spatially distributed and evolutionarily newer, rely on subtler differences, independent of the overall amount of activity which may remain very low.

Disclosures: A.M. Morgan: None. **O. Devinsky:** None. **W.K. Doyle:** None. **P. Dugan:** None. **D. Friedman:** None. **A. Flinker:** None.

Presentation Number: NANO35.08

Topic: H.11. Language

Title: A motor somatotopy for speech production revealed by large-scale intracranial recordings

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Abstract: A motor somatotopy for speech production revealed by large-scale intracranial recordingsJinlong Li, Tessy Thomas, Nitin Tandon
Speech production requires a coordinated control of articulators that distinguish sub-lexical structures such as phonemes. Previous research has shown the encoding of articulatory kinematics and phoneme identities in the ventral sensorimotor cortex and inferior frontal gyrus during articulation. Using a large cohort of intracranial recordings, we extensively evaluated the differential representation of spatiotemporal profiles of articulatory features across the motor cortex, in premotor, primary motor, and subcentral cortices. We included 118 patients (24 subdural grids, 94 depth electrodes) undergoing intracranial epilepsy monitoring (1401 electrodes from left hemisphere, 1209 from right hemisphere). Patients performed different combinations of single-word speech production tasks. Words with consonant-vowel-consonant structures were selected for analysis. Phonetic identities and timings were transcribed from manually annotated text response and recorded audio using Montreal Force Aligner and proofread. Phonemes were characterized as functions of their articulatory features (e.g. place/manner of articulation). We systematically analyzed the broadband gamma activation

(BGA; 70-150 Hz) traces time-locked to articulation onsets at phoneme level. Using the phoneme selectivity index, we extracted separability metrics for different articulatory classifications for BGA both before and after phoneme onset. Lastly, surface-based models were used to render a dynamic group-level spatially separable organization of articulatory and phonemic features. Preliminary results uncover a sequential BGA pattern, initiating from premotor cortex spreading to M1 and SCG. We delineate a separation of articulatory features both in the pre-articulatory (causal) and articulatory (anti-causal) windows in the ROIs. We present a comprehensive and uniquely dense spatiotemporal profile in the motor cortex for articulatory information at the phoneme level with high density coverage. Our effort will deliver implications for surgical planning and the development of intracranial speech brain-machine interfaces for people with speech deficits.

Disclosures: J. Li: None. **T.M. Thomas:** None. **N. Tandon:** None.

Presentation Number: NANO35.09

Topic: H.11. Language

Support: NIH NINDS U01 NS128921
NIH NINDS NS098981

Title: Shared cortical language networks with convergent hierarchical network dynamics for lexicosemantic processing in comprehension and naming

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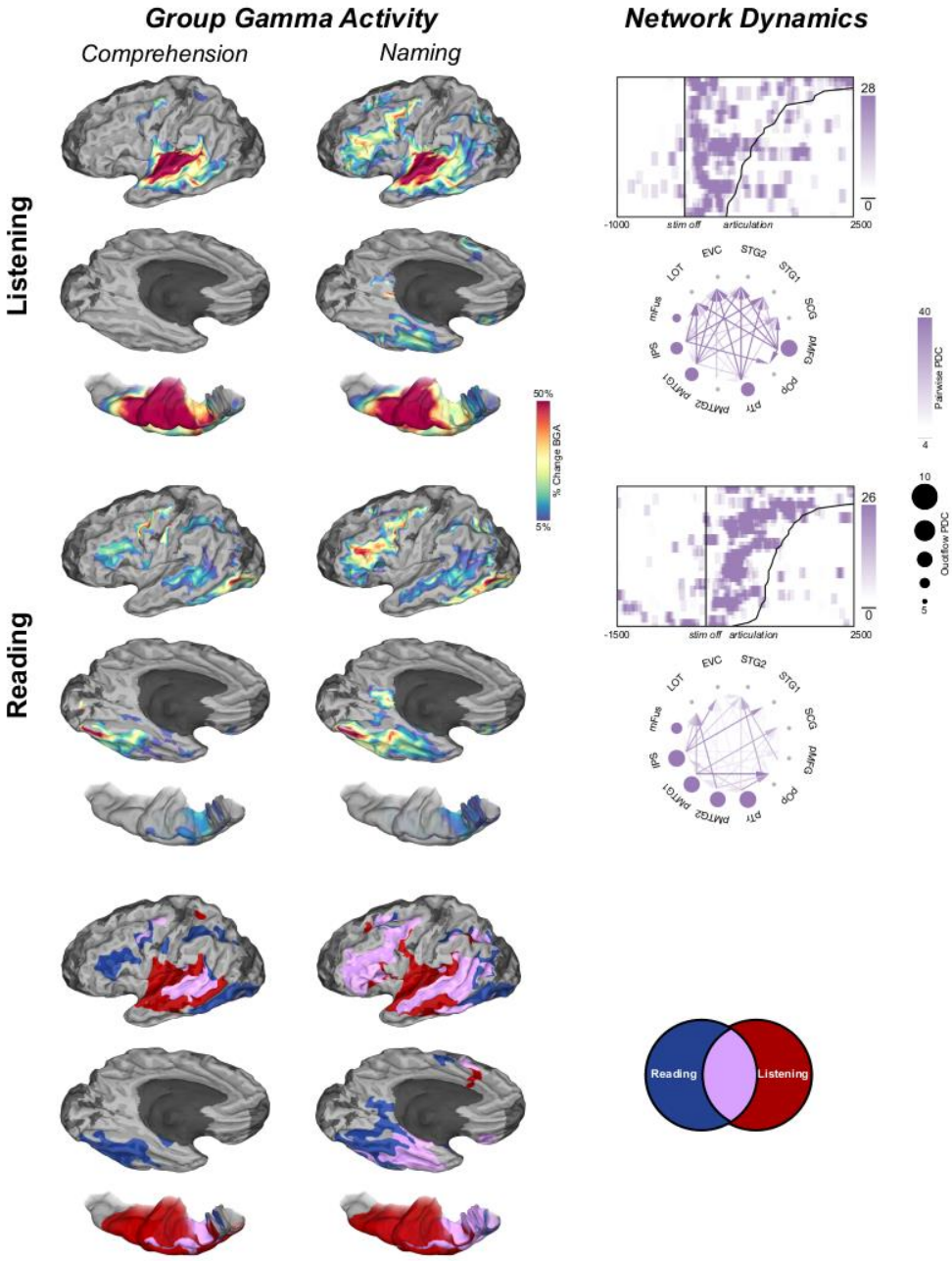
Abstract: Theoretical models suggest that spoken and written language engage a shared lexicosemantic processing network in perception and production, yet convergent neural mechanisms are unclear. We used ECoG to identify spatiotemporal network dynamics of lexicosemantic processing in comprehension and naming.
65 ECoG patients completed auditory (AN) and orthographic (ON) naming. We analyzed gamma activity (70-115Hz) with mixed-effects multilevel analyses to identify the lexicosemantic processing network during comprehension and naming. We mapped network dynamics using autoregressive hidden Markov models (ARHMM). We used direct cortical stimulation (DCS) to attribute causality to critical nodes.
At speech onset, activation of superior temporal gyrus (pSTG) was followed by superior temporal sulcus (pSTS) and middle temporal gyrus (pMTG). For each written word, visual cortex activity was followed by activation of lexical (fusiform gyrus, Fus; pSTS; pMTG) and phonological (intraparietal sulcus, IPS; pSTG) reading routes. Both modalities engaged posterolateral temporal cortex (pLTC) for comprehension, and activity was correlated with phrasal composition ($p<0.01$) implicating it in compositional semantics. The last word activated a shared network (pLTC; Fus; IPS; pars triangularis, pTr) for naming. ARHMM isolated 5 states for AN and 6 for ON with 3 convergent states. The first convergent state occurring at stimulus

offset was characterized by outflow from pLTC, Fus, IPS, and pTr, and state duration was correlated with reaction time ($p<0.001$) implicating it in lexical access. Lastly, during stimulus presentation, DCS of Heschl's gyrus disrupted listening, while DCS of planum temporale and pLTC disrupted listening and reading. At stimulus offset, DCS of pLTC, Fus, IPS, and pTr disrupted AN and ON.

Juxtaposing network dynamics of multimodal lexicosemantic processing in speech perception and production informs our understanding of specialized and shared language networks providing new insights to facilitate designs of neural prosthetics for language disorders.



Disclosures: K. Snyder: None. **K. Forseth:** None. **O. Woolnough:** None. **E. Murphy:** None. **G.S. Hickok:** None. **N. Tandon:** None.

Presentation Number: NANO35.10

Topic: H.11. Language

Support: NIH Grant R21 HD107511

Title: Greater fidelity of neural patterns during reading is associated with lower variability in behavioral concreteness ratings

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Abstract: Semantic word features are represented across a set of language cortical regions. Previous work has used representational similarity analyses (RSA) to determine that specific regions encode semantics in a set of individuals. However, such work does not address whether neural semantic patterns are consistent across individuals. Exploring pattern consistency (fidelity) across individuals provides a novel way to study the nature of representational content across individuals. We hypothesize that there will be a relationship between neural fidelity and semantic task variability (behavioral fidelity) within semantic brain regions. To test this, we used fMRI and an RSA approach to compute representational fidelity (RF), which represents the consistency of representational similarity matrices (RSM) across individuals. 20 participants underwent fMRI scanning while reading 160 words presented 4 times. For each participant, an RSM was generated by cross-correlating the activation patterns within a region of interest (ROI) elicited from each word stimulus. RF was then calculated by correlating each participant's RSM with the group average RSM while leaving that participant out (e.g., the correlation between RSM1 and the mean of RSM2-RSM20). To index the unique contribution of each word to the overall RF of an ROI, we then recomputed the RF in each ROI while leaving out that word. ROIs were selected from the Schaefer 400 parcellation atlas based on their approximate mapping to 8 left-hemisphere brain regions previously associated with reading and semantics. To measure semantic behavioral fidelity, the standard deviation (SD) of concreteness ratings for the same words from a different set of participants was obtained. The SD of z-scored reaction time (RT) measures for the same words was obtained from the English Lexicon Project. Multiple regressions with RF as the dependent variable were performed for each ROI with the concreteness SD as the variable of interest and word length and the RT SD as covariates. There was a negative association between the concreteness SD and RF in regions corresponding to the posterior cingulate cortex/precuneus ($\beta = -.70$, $t(144) = -2.26$, $p = .03$) and the dorsomedial prefrontal cortex ($\beta = -.61$, $t(144) = -1.98$, $p = .05$). That is, lower concreteness SD corresponds to higher RF within these regions previously associated with semantics. Thus, words with lower variance in the concreteness ratings have more consistently represented neural patterns within semantic processing regions. This demonstrates that the fidelity of neural patterns is related to the behavioral fidelity of semantic task performance.

Disclosures: C.M. McCabe: None. **D. Rothlein:** None. **S. Rosenberg:** None. **M. Brooks:** None. **W.W. Graves:** None. **D.J. Bolger:** None. **J.J. Purcell:** None.

Presentation Number: NANO35.11

Topic: H.11. Language

Support: NIHR-UCLH BRC Hearing Health Theme

Title: Of mice, men, and models: a temporal processing model for predicting auditory brain activity in anesthetized mice outperforms standard methods for speech tracking in human EEG

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Abstract: Temporal mechanisms of human speech perception can be explored via "speech tracking": using linear modelling to reconstruct continuous speech from human EEG responses or to predict EEG responses from speech. The aim is to identify temporal features in speech that drive cortical activity. Speech-tracking analyses typically rely on representations of speech derived solely from the audio signal, such as the acoustic envelope, and do not take into account non-linearities in auditory temporal processing that may influence cortical drive. We postulated that human speech tracking might be improved with a simple non-linear model of auditory temporal processing originally developed to predict brain activity in the auditory thalamus of anesthetized mice [1]. An essential part of this model is normalization by recent sound level history, which transforms the acoustic envelope into an “adaptive gain” signal representing stimulus-dependent central auditory responsiveness. Here, we asked if cortical tracking of continuous speech in human EEG recordings, usually measured using the speech envelope, could be improved by using the adaptive gain representation instead. We analyzed two datasets from different laboratories [2,3] consisting of continuous EEG recordings from either British (n=18) or Danish (n=22) participants listening to audiobooks. Cortical speech-tracking performance, quantified by correlating the EEG-decoded speech representation with the actual representation extracted from speech, was significantly improved when linear modelling was performed using the adaptive gain representation instead of the speech envelope (Wilcoxon test $p<0.001$, Cohen's $d=0.87$). This improvement in speech tracking was observed in both datasets ($p<0.001$, $d>0.8$ for both) and in all listeners individually ($p<0.05$ for 87.5% of listeners, similar but non-significant trend for others). Similar results were obtained for an encoding model (better EEG prediction for adaptive gain versus envelope representation; $p<0.01$, $d>0.58$ for both datasets together and each dataset individually; $p<0.05$ for 30% of individual listeners, n.s otherwise). Results indicate that adaptive gain provides a more accurate representation of acoustic events driving cortical speech tracking than the speech envelope. We conclude that fundamental insights into auditory temporal processing gained from anesthetized mice are relevant to understanding the mechanisms of human speech perception.
[1] Anderson LA & Linden JF (2016). J Neurosci 36(6), 1977-1995.
[2] Etard O & Reichenbach T (2022). 10.5281/zenodo.7086208
[3] Simon A, Bech S, Loquet G & Østergaard J (2022). 10.5281/zenodo.7500806

Disclosures: A. Simon: None. **M. Chait:** None. **J.F. Linden:** None.

Presentation Number: NANO35.12

Topic: H.11. Language

Title: Relationship of microstructure and lateralization of Broca's area superficial white matter to individual language performance

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Abstract: The brain's left hemisphere is generally recognized as the primary site for language processing. While lateralization of the brain's deep white matter connections and their relationship to language is better understood, little is known about lateralization of the superficial white matter (SWM) despite its importance for cortico-cortical communication. Accordingly, in this study, we used diffusion MRI tractography and a novel atlas of SWM to investigate the microstructure and lateralization of SWM connections to Broca's area and their relationship to language performance in the Human Connectome Project - Young Adult. We analyzed diffusion MRI data and language performance scores from 1,065 participants (575 females) aged 22-36, with a mean age of 28.75. This study was preregistered on the Open Science Framework. 21 SWM connections from our atlas were identified as connecting to Broca's area, defined as Brodmann areas 44 and 45 using the FreeSurfer parcellation. These 21 SWM connections were subdivided into two categories based on length: medium- and long-range SWM connections. In addition, we analyzed the arcuate fasciculus, a well-studied deep white matter tract for language processing. Furthermore, we used cognitive measures of language performance from the NIH Toolbox - the Toolbox Picture Vocabulary Test (TPVT) and the Toolbox Oral Reading Recognition Test (TORRT) - to examine how language performance may be associated with SWM microstructural measures. The p-values were corrected for multiple comparisons using the false discovery rate. Long-range SWM connections were significantly left-lateralized in fractional anisotropy (FA, $p<0.001$) and the number of streamlines (NoS, $p<0.001$). Medium-range SWM connections were significantly left-lateralized in FA ($p<0.001$) and right-lateralized in NoS ($p<0.001$). Medium-range SWM microstructure (FA and NoS) was significantly associated with language performance (TPVT and TORRT) in both hemispheres (all $p<0.001$). Long-range SWM microstructure (FA) was significantly associated with language performance (TPVT and TORRT) in both hemispheres (all $p<0.05$). Arcuate fasciculus microstructure (FA and NoS) was significantly associated with language performance (TPVT and TORRT) in only the left hemisphere (all $p<0.01$). Overall, we observed mixed lateralization of the superficial white matter connecting to Broca's area. In addition, the relationship between SWM microstructure and language performance exists in both hemispheres, while in the arcuate fasciculus, this relationship is limited to the left hemisphere. Our findings highlight the potential significance of SWM in language processing.

Disclosures: S. Hassanzadeh-Behbahani: None. **Z. Lan:** None. **L.R. Zekelman:** None. **R.J. Rushmore:** None. **T. Xue:** None. **S. Pieper:** None. **S. Cetin-Karayumak:** None. **N. Makris:** None. **F. Zhang:** None. **Y. Rathi:** None. **L.J. O'Donnell:** None.

Presentation Number: NANO35.13

Topic: H.11. Language

Support: CAS Project for Young Scientists in Basic Research YSBR-041
National Natural Science Foundation of China 11905291

Title: A neural network model for embodied-concept extraction and communication

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Abstract: In the human brain conceptual spaces are constructed from the abundant sensorimotor experience, and in turn, regulate the sensorimotor system to allow flexible behavior, facilitate learning, and by mapping with symbols further enable communication between individuals. So far, how these processes are computationally implemented in the brain or artificial neural networks remain elusive. Here, we propose a dual-module neural network model that extracts embodied concepts from the visual experience of performing visual recognition tasks and exploits a formed conceptual embedding space to learn new skills and communicate. In our design, the task-solving (TS) module, e.g., a ResNet, is dedicated to performing visual recognition; the concept-abstraction (C) module, e.g., a multi-layer perceptron, is connected to the TS module to perform hierarchical gating to the latter. The combined network, i.e. a C-TS agent, was trained by performing binary classifications: given a concept represented, e.g., bird, in the C-module, the network judges whether an image presented to the TS module belongs to this category. We show that, through end-to-end training by backpropagation, the C-module successfully learned to extract conceptual representations and used them to gate the TS module to perform corresponding recognitions. Importantly, the agent can learn visual recognitions by 1) manipulating its conceptual space without any change to the TS module, i.e., forming new concepts by itself or 2) transferring concepts between aligned conceptual representations derived by different agents, i.e., forming new concepts by communication. To study how the mechanisms implemented are related to the brain, we compared the conceptual representation learned by C-TS agents to that obtained from human subjects (N = 29) participating in a functional MRI picture naming experiment. Interestingly, ~30% the C-TS agents exhibited conceptual representation correlated significantly with the neural representation in the fusiform gyrus, which is a key area for visual concept representation, and early visual cortices, suggesting that the potential feature gating mechanism in the visual cortex. Taken together, this work proposes an operational network model that exhibits the core functions of embodied-concept extraction and communication, and shows its potential link to the human brain. We expect that it will shed light on the computational mechanism of brain's conceptual learning and representation, and facilitate the design of future embodied artificial intelligence systems.

Disclosures: Y. Chen: None. **L. Guo:** None. **H. Chen:** None. **Y. Bi:** None. **S. Yu:** None.

Nanosymposium

NANO36: Advances in Network Analysis: Theory and Modelling

Location: MCP Room N427

Time: Monday, October 7, 2024, 1:00 PM - 3:45 PM

Presentation Number: NANO36.01

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

Title: Memory capacity of spiking networks with preserved weight distributions

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Abstract: How changes in synaptic connections underlie learning and memory is a central question in Neuroscience. Previous modelling efforts focused on biologically realistic learning rules and dynamics, but these rules, typically Hebbian, usually produce bimodal weight distributions that don't align with experimentally observed lognormal distributions of synaptic weights. Furthermore, measured EPSPs and spine volumes exhibit the same distribution before and after learning. How biological learning rules preserve such unimodal distributions and also retain information, i.e., learn, remains unresolved. In particular, the memory capacity of the network that is limited due to the scarce supply of strong synaptic weights is unknown.
To address these questions, we set up a recurrent spiking neural network with asynchronous and irregular dynamics and a number of fixed input stimuli that produce increased firing rates in their target neurons. Learning is quantified as pattern completion, i.e., network responses to incomplete stimuli. We achieve preserved weight distributions by assuming a fixed set of weights and creating a trade-floor of synaptic weights, where each neuron can re-allocate its own input weights. We find that functional swapping of excitatory weights between existing synapses can achieve robust pattern completion, but overall network stability can become compromised. Expanding the trade-floor scheme to inhibitory weights restores realistic dynamics, but the network relies on a delicate balance that is vulnerable to synaptic perturbations and turnover.
To explore how a network learns multiple, overlapping stimuli, we presented sequential stimuli to networks with trade-floor rules. We calculate the capacity of these spiking networks as a function of the pattern size, network sparsity, and pattern overlap. We find a decreased capacity compared to Hopfield networks, and demonstrate that other ways to preserve the weight distribution, namely, sampling from- and retaining the type but not the first and second moments of the synaptic weight distribution still lead to lower capacity then would be feasibly required for memory storage. Finally, we show that clustered networks are advantageous for learning when correlations in stimulus structure are already embedded in the network, but have decreased capacity for random stimuli. Our results illustrate a major constraint of synaptic weight changes as the sole substrate for long-term memory.

Disclosures: M. Levy: None. **T.P. Vogels:** None.

Presentation Number: NANO36.02

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

Title: Interleaving asynchronous and synchronous activity in balanced cortical networks with short term synaptic depression

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Abstract: Cortical populations are in a broadly asynchronous state that is sporadically interrupted by brief epochs of coordinated population activity. Cortical models are at a loss to explain this combination of states. At one extreme are network models where recurrent inhibition dynamically stabilizes an asynchronous low activity state. While these networks are widely used, they cannot produce the coherent population-wide activity that is reported in a variety of datasets. At the other extreme are models where short-term synaptic depression between excitatory neurons can generate the epochs of population-wide activity. However, in these networks, inhibition plays only a perfunctory role in network stability, which is at odds with many reports across cortex. In this study we analyze spontaneously active *in vitro* preparations of primary auditory cortex that show dynamics that are emblematic of this mixture of states. To capture this complex population activity, we use phenomenological rate-based networks as well as biologically realistic networks of spiking neuron models where large excitation is balanced by recurrent inhibition, yet we include short term synaptic depression dynamics of the excitatory connections. These models give very rich nonlinear behavior that mimics the core features of the *in vitro* data, including the possibility of low frequency (2-12 Hz) rhythmic dynamics within population events. In these networks, synaptic depression enables activity fluctuations to induce a weakening of inhibitory recruitment, which in turn triggers population events. In sum, our study extends balanced network models to account for nonlinear, population-wide correlated activity, thereby providing a critical step in a mechanistic theory of realistic cortical activity.

Disclosures: J. Dunworth: None. **Y. Xu:** None. **M. Graupner:** None. **B. Ermentrout:** None. **A.D. Reyes:** None. **B. Doiron:** None.

Presentation Number: NANO36.03

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

Title: Non-negative synaptic weights are superior to signed weights for knowledge representation in the CNS

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Abstract: The question of how the brain represents concepts has long intrigued scientists. Some suggest that the brain uses a subspace representation involving both positive and negative synaptic weights, though this approach faces challenges in implementing hierarchical structures. Our findings indicate that restricting synaptic weights to non-negative values, a biologically plausible limitation, significantly improves the encoding of concepts. Evidence shows that

representations in the central nervous system (CNS) distribute across multiple neurons in a population, where neighboring neurons are usually uncorrelated. This population coding must preserve an invariant that maintains the concept's meaning through neural processing. This necessity rules out simple encoding methods, like spike rate vectors, because population processing would be analogous to random matrix multiplication, which fails to preserve any meaningful invariant. Subspace representations address this issue effectively, offering the added advantage of allowing intuitive and semantically meaningful operations like intersection and projection.

Unfortunately, subspace representations are inefficient at expressing the crucial property of one concept subsuming another. Here, we have demonstrated that *convex cones* can replace subspaces by leveraging the non-negativity of biological synaptic weights. Unlike subspaces, convex cones can form hierarchies without wasting dimensions (see fig.), which is critical for efficient representation.

This work builds upon a biologically accurate mechanistic model of a generic neuron described in [1-2].

[1] *Channel current fluctuations conclusively explain neuronal encoding of internal potential into spike trains.* Phys. Rev. E, American Physical Society, 2021, 103, 022407 (DOI 10.1103/PhysRevE.103.022407)

[2] *Mechanistic explanation of neuronal plasticity using equivalent circuits.* bioRxiv, Cold Spring Harbor Laboratory, 2023 (DOI 10.1101/2023.05.21.541639)

Disclosures: M.N.P. Nilsson: None.

Presentation Number: NANO36.04

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

Support: JSPS Postdoctoral Fellowship PE20032
ARC DECRA Fellowship DE190100157
JSPS Postdoctoral Fellowship P23383

Title: A computational bottom-up, mesoscale approach for the study of the interaction between claustrum and cortex

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Abstract: The claustrum, a structure having extensive connectivity with the rest of the brain and being involved in many high-cognitive processes, is still one of the least understood parts of the mammalian nervous system. One of the reasons is its complex location and geometry: a folded, thin layer of neurons, sandwiched between other cellular groups and white matter tracts, which creates specific challenges for experimentation. However, in recent years the claustrum has been studied intensely in mice, revealing many details about its cellular composition and dynamics, but still without a satisfactory mechanistic explanation of its function.
This work investigates through computational simulations the dynamics of the interaction between the claustrum and the cortex. To this end, we built a bottom-up, mesoscale in-silico model of the mouse claustrum that we reciprocally connected with a simplified model of the cortex. Specifically, we used NEST and NESTML to create adaptive exponential integrate and fire (AEIF) neurons (Brette and Gerstner, 2005) for the claustrum and Wang-Buzsaki cortical neurons with difference-of-exponentials time-course synaptic conductances (Wang and Buzsaki, 1996; Palmigiano et al, 2017). First, we present how we reached with NEST and Python to the sets of parameters that replicate the responses of claustrum neurons in vitro, their arrangement in space and their measured connectivity (Kim et al, 2016; Jackson et al, 2018; White and Mathur 2018; Graf et al, 2020; Narikiyo et al, 2020; Marriott et al, 2021). Second, replicating the Palmigiano et al, 2017 network allowed the production of a complex, cortical-like signal. Finally, this cortical network was reciprocally connected with a subset of the claustrum neurons to study the effect of the cortical signal on the direct target claustrum neurons and the following dynamics in the rest of the claustrum network. The results show that, as the inter-regional weights increase, the claustrum and the cortex modify each other's dynamics more dramatically, to a point of high predictability of their signals and low transfer of information. Interestingly, only the direct targets from cortex, together with their immediate neighbours are affected, the rest of the claustrum network maintaining a steady activity.

Disclosures: R. Gamanut: None. **C. Gutierrez:** None. **K. Doya:** None.

Presentation Number: NANO36.05

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

Support: NSF (Neuronex award) #2015276
NSERC R0370A01
BrainsCAN at Western University through the Canada First Research Excellence Fund (CFREF)
Compute Ontario (computeontario.ca)
Digital Research Alliance of Canada (alliancecan.ca)
SPIRITS 2020 of Kyoto University
Western Academy for Advanced Research

Title: Predicting traveling waves: a new mathematical technique to link the structure of a network to the specific spatiotemporal dynamics that occur

Authors: *R. BUDZINSKI, L. E. MULLER;
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Abstract: Understanding spatiotemporal dynamics of neural systems, and how these dynamics are related to the structure of the underlying network, presents a significant challenge in computational neuroscience. Recent advances in connectomics now provide detailed maps of neural connections, from microscopic synaptic patterns to long-range fiber tracts between brain regions. At the same time, however, an important critique is that, even if we knew the complete set of connections in a neural system, it remains difficult to say anything about the dynamics that will result. This is due to the general fact that, when a network system is nonlinear, it is a difficult problem in applied mathematics to link the structure of connections to the resulting dynamics. To address this challenge, we develop a new mathematical framework for networks of nonlinear oscillators. We focus on the Kuramoto model, which captures phase interactions between oscillating neural populations [Breakspear et al., Front. Hum. Neurosci, 2010]. We study the emergence of traveling waves in these systems, which have recently been observed to occur at both the mesoscopic scale of single brain regions [Rubino et al., Nature Neuroscience, 2006] and at the macroscopic, whole-brain scale [Muller et al., eLife, 2016]. With our mathematical approach, we can consider an individual pattern of connections - for example, the large-scale fiber tracts estimated by the Human Connectome Project - and mathematically predict the traveling wave patterns that will occur at a specific oscillation frequency. Importantly, our approach generalizes to systems with distance-dependent time delays [Budzinski et al., Phys Rev Res, 2023], which are often neglected when modeling these spatiotemporal dynamics. Because waves often travel at the speeds of the underlying fiber networks, this technique provides a powerful tool to link connectivity and time delays with spatiotemporal dynamics in these systems. Traveling waves are increasingly appreciated to occur in neural systems, and results from this work can provide a critical link in connecting these spatiotemporal dynamics to underlying circuit architectures. These results also provide a key theoretical example for connectomics, where knowing the precise pattern of connections in a neural system can lead to quantitative predictions that can be directly tested in experimental recordings. We will discuss applications of this framework to data recorded from depth-electrode intracranial recordings of human sleep, and potential predictions for how spatiotemporal dynamics of sleep rhythms change across health and disease.

Disclosures: R. Budzinski: None. **L.E. Muller:** None.

Presentation Number: NANO36.06

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

Title: energy subunits substantially enhance the predictive power of spectrotemporal receptive field models in the human auditory cortex

Authors: *G. LIAO[1], D. BOEBINGER[1], C. M. GARCIA[2], K. V. NOURSKI[3], M. A. HOWARD III[4], T. WYCHOWSKI[5], W. H. PILCHER[6], S. V. NORMAN-HAIGNERE[7];
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Abstract: Neural responses in the auditory system have been classically modeled as a weighted sum of a time-frequency image of sound (the "spectrotemporal receptive field" or STRF), analogous to simple-cell receptive fields in the visual cortex. STRFs are interpretable and easy to fit with limited neural data, but have limited predictive power in the auditory cortex, particularly in non-primary regions of the human brain that respond selectively to complex natural sounds such as speech and music. Here, we show that a simple modification of this framework, inspired by complex cells in the visual system, substantially enhances the ability of STRF-based acoustic models to predict human cortical responses to natural sounds, measured using spatiotemporally precise intracranial recordings from neurosurgical patients. Specifically, we model neural responses as the weighted sum of a set of energy subunits, each of which computes a phase-insensitive measure of spectrotemporal energy using a collection of STRFs in quadrature pair. We show that the energy operation substantially and reliably increases the variance explained by this model across a diverse set of natural sounds, with the largest improvements in non-primary regions. Our two-stage subunit model retains the key advantages of standard STRF models, while substantially enhancing their ability to predict neural responses to complex, ecologically relevant sounds such as speech and music.

Disclosures: G. Liao: None. **D. Boebinger:** None. **C.M. Garcia:** None. **K.V. Nourski:** None. **M.A. Howard:** None. **T. Wychowski:** None. **W.H. Pilcher:** None. **S.V. Norman-Haignere:** None.

Presentation Number: NANO36.07

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

Support: NIH Grant U24NS129945
NIH Grant U24EB029005
NSF Grant 1935749
NSF Grant 1935771

Title: Human Neocortical Neurosolver (HNN): An open-source software for cellular and circuit-level interpretation of human MEG/EEG

Authors: *N. TOLLEY[1,2], M. JAS[3], R. THORPE[4,2], G. T. DANG[5], D. DANIELS[6], K. DUECKER[6], C. DIAZ[5], S. R. JONES[4,2];
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Abstract: The Human Neocortical Neurosolver (HNN) is a user-friendly neural modeling software designed to provide a cell and microcircuit level interpretation of macroscale magneto- and electroencephalography (M/EEG) signals (hnn.brown.edu, Neymotin et al 2020). The foundation of HNN is a biophysically-detailed neocortical model, representing a patch of neocortex receiving thalamic and corticocortical drive. The HNN model was designed to simulate the time course of primary current dipoles and enables direct comparison, in nAm units, to source-localized M/EEG data, along with layer-specific cellular activity. HNN workflows are constructed around simulating commonly measured ERPs and low frequency oscillations. The HNN model can be accessed through a user-friendly interactive graphical user interface (GUI) or through a Python scripting interface.
The foundation of HNN, referred to as HNN-core (Jas et al. 2023), is a Python package containing all of the core functionality of HNN, and is implemented with a clear application programming interface (API). A new GUI has recently been implemented using HNN-core functions. Tutorials on how to simulate ERPs and low frequency oscillations in the alpha, beta and gamma bands are distributed for both the HNN-GUI and HNN-core. HNN-core was created with best practices in open-source software to allow the computational and human neuroscience communities to understand and contribute to its development. HNN-core contains additional functionality beyond that accessible through the HNN-GUI, including the ability to modify local network connectivity and to simulate layer specific local field potential signals and current source density. Ongoing work includes the integration of simulation based inference (SBI) to enable the identification of distributions of parameters that fit with experimentally recorded data (Tolley et al. 2024). The package is available to install with a single command on PyPI (pip install hnn_core), is unit tested and extensively documented. HNN is additionally accessible through computing resources offered by the Neuroscience Gateway (NSG) enabling large simulation workloads. Overall, HNN is a one of a kind openly distributed tool designed for a broad user community to develop and test hypotheses on the multiscale origin of human M/EEG.

Disclosures: N. Tolley: None. **M. Jas:** None. **R. Thorpe:** None. **G.T. Dang:** None. **D. Daniels:** None. **K. Duecker:** None. **C. Diaz:** None. **S.R. Jones:** None.

Presentation Number: NANO36.08

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

Support: NIMH Grant 1R21MH132240-01
NINDS Grant R01NS130693

Title: Individualized models connect nontrivial whole-brain dynamics across rest and task conditions

Authors: *R. CHEN, M. SINGH, T. S. BRAVER, S. CHING;
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Abstract: Analysis of whole-brain dynamics is becoming popular in functional neuroimaging and has generated new insights into cognition. However, it remains debated whether such dynamics are best characterized as unimodal stationary processes sampled over time, or instead, in terms of nonlinear dynamical systems that generate non-trivial fluctuations in brain-wide activation and coactivation patterns. In resting state fMRI (rfMRI), the temporal dynamics of whole-brain covariation (time-varying functional connectivity, tvFC) are often characterized as switching between multiple discrete states rather than as a consequence of continuous fluctuations. In several cognitive tasks including the N-back working memory task, single-trial neural and behavioral responses were also found to be best described by more than one metastable state (Ashwood et al., 2022; Nakuci, Covey, et al., 2023; Nakuci, Yeon, et al., 2023). However, the mechanistic underpinnings of these nontrivial metastable states and the connections between observed nontrivial states across resting state and task remain elusive. Here, we address this question by constructing whole-brain dynamical models from individual resting state and N-back task fMRI 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 tvFC. Interestingly, we found here that MINDy models shown a diversity of non-trivial dynamics, 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. In ongoing work, we extend MINDy model to task state by adding a task-related input that couples with the recurrent dynamics through a learnable weight matrix. We investigate the existence of nontrivial attractors in the task state model and analyze whether different attractors explain trial-to-trial variation in neural and behavioral responses. Overall, our results provide new insights into the intrinsic spatiotemporal organization of brain activity and suggest opportunities for single-trial-level brain-behavior association analyses derived from MINDy-based dynamical characterization of fMRI data.

Disclosures: R. Chen: None. **M. Singh:** None. **T.S. Braver:** None. **S. Ching:** None.

Presentation Number: NANO36.09

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

Support: NIH Grant R01NS130693
NIH Grant T32NS126157

Title: Identifying latent neuromodulatory brain states within individualized cortical dynamics

Authors: *A. SCHWAMB[1], S. CHING[2];
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Abstract: Understanding large-scale brain dynamics is a fundamental goal in human neuroscience. Several tools, including our own, now exist for performing data-driven parametric modeling from high-dimensional non-invasive recording modalities such as EEG, MEG or fMRI

(Singh et al., 2020, 2023; Chen et al., 2024; Schwamb et al., 2024). These models produce a *time-invariant* description of the internal dynamical mechanisms that give rise to observed brain activity. In this work, we consider a generalization of these data-driven methodologies that enables the inference of time-varying neuromodulation alongside a nominal basal or rest dynamic architecture. In clinical and task-based settings, such inference may be particularly important since individual brain dynamics may exhibit alterations due to differing stages of neurological dysfunction, or while implementing different cognitive functions during a complex task. To solve this problem, we augment our previous mesoscale individualized neural dynamics (MINDy) modeling approach with parameters corresponding to neuromodulation, which are in turn parameterized by a hidden Markov model (HMM). An HMM provides a method for determining the most likely series of latent states, given a series of observations. By defining each latent state of our HMM as corresponding to a different instance of a modulatory parameter in our models, we can then determine both the current latent state of the HMM and, consequently, the state-specific modulation of the basal MINDy model. The goal of our method is to perform the inference of these states in an unsupervised manner, i.e., without prior knowledge of the timing or modulatory mechanisms of latent states. Thus, in the proposed hidden Markov MINDy model (HMMINDy model) we simultaneously estimate the parameters of the HMM, the modulatory matrices and the base MINDy parameters. For this purpose we use a Baum-Welch algorithm, with a predetermined number of latent states in the HMM. The optimal number of these states can be determined by fitting multiple HMMINDy models with varying numbers of latent states, and using an information criterion to determine the most likely number of hidden states. The modulatory matrices themselves can be fit using common gradient-based optimization. By allowing the dynamics of the MINDy model to be modulated based on which state of the HMM the brain is in at each timepoint, we can identify when the brain is changing state, as well as gain mechanistic insight into *how* such changes are effected at an individual subject level, thus providing a dynamical underpinning of behavioral observations such as neurological dysfunction or changing task strategies.

Disclosures: A. Schwamb: None. **S. Ching:** None.

Presentation Number: NANO36.10

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

Support: Schmidt Futures Foundation SF 857
National Human Genome Research Institute grant 1RM1HG011543
National Science Foundation grant 2134955

Title: Generative Foundation Model for Cortical Organoid Electrophysiology

Authors: *K. HUSSAIN[1], A. ROBBINS[1], D. PARKS[1], H. SCHWEIGER[3], S. HERNANDEZ[4], M. A. MOSTAJO RADJI[2], M. TEODORESCU[1], D. HAUSSLER[1];
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Abstract: Modern electrophysiology methods yield significant improvements in data through both higher spatial resolution and longitudinal recordings. This scale of raw data, coupled with recent application of large generative models to raw data, yield an opportunity to provide insight

into neural activity with predictive capability. We developed a foundation machine learning model for the generative prediction of mouse cortical organoid activity on the scale of a high density multi-electrode array (HD-MEA). We constructed an encoder-decoder architecture to map the input to a latent space, alongside a recurrent network to internally forecast temporal activity, necessarily encoding the semantic structure of neural activity to achieve high accuracy. The encoder and decoder consist of convolutional layers across space and time windows that are forward predicted by a long short term memory (LSTM) recurrent artificial neural network (RNN). The model is trained on a large dataset consisting of 92 ten minute cortical organoid recordings from the Maxwell MaxOne MEA (1024 channels) with 5 contiguous recordings held out for evaluation. We show that our model generalizes cortical organoid data and serves as a baseline model for downstream tasks such as regression, generation, and classification. The model is able to forward predict 3ms of high accuracy electrophysiology of data with temporal roll-outs of up to 300ms having reduced accuracy. The model inherently learns to predict spike signal propagation, showing understanding of the spatio-temporal footprints. This system builds upon the basis of raw neural data processing and acts as an direct pipeline from raw signals to raw signals, providing a foundation for the analysis stack of spike sorting, de-noising, curation, and forward prediction.

Disclosures: K. Hussain: None. **A. Robbins:** None. **D. Parks:** None. **H. Schweiger:** None. **S. hernandez:** None. **M.A. Mostajo Radji:** None. **M. Teodorescu:** None. **D. Haussler:** None.

Presentation Number: NANO36.11

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

Support: W911NF-22-1-0223

Title: Simulation of projection-based connectome reproduces cortical responses to different sensory stimuli

Authors: *G. SUN[1], D. B. FORGER[2];
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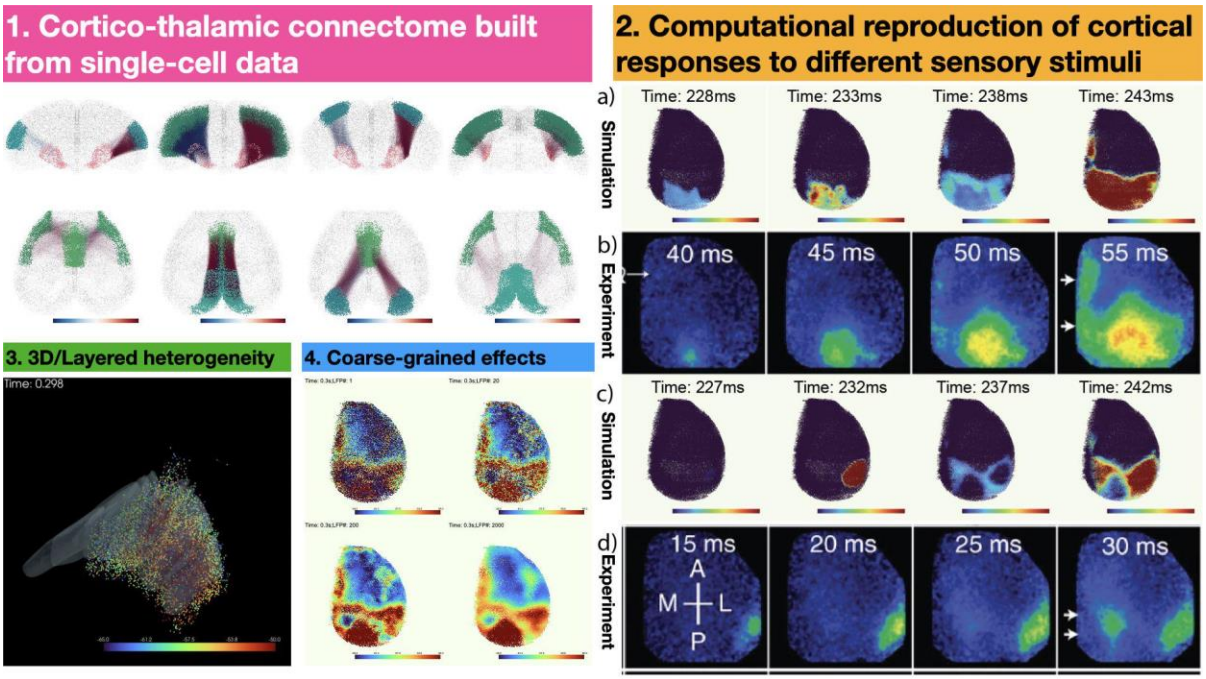
Abstract: With advanced spatial, molecular and projection profiles of the mouse brain provided by the Allen Brain Atlas, we use a simplistic stochastic algorithm to construct a neuron-to-neuron connectome of one mouse hemisphere. We then conduct a series of large-scale simulations with Hodgkin-Huxley type of neurons (N>200,000) on the derived cortico-thalamic connectome to model cortical responses to different sensory stimuli. Amazingly, our computer simulations closely reproduce the experimental recordings[Nat Neurosci 16, 1426-1435]. Our computer simulations further provide insights into the propagation of spatiotemporal patterns generated by different stimuli and identify common sinks and sources on the cortex during those activities. Complimentary to past experimental and computational data that are 2D, our new simulations can reveal the 3D structure of different cortical activities and heterogeneity within cortical layers of different regions. In the end, effects of coarse-grained measurements and up/down-scaling of the network is also investigated. Our model and simulations demonstrate the possibility of state-of-the-art computations in the future with affluent single-cell data and advanced computational power.



Disclosures: G. Sun: None. **D.B. Forger:** None.

Nanosymposium

NANO37: Insights Into Cortical Development: Genetic, Molecular, and Environmental Influences

Location: MCP Room N227

Time: Tuesday, October 8, 2024, 8:00 AM - 10:15 AM

Presentation Number: NANO37.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R00NS111731 (NINDS)
Young Investigator Award from the Brain & Behavior Research Foundation
Alfred P. Sloan Foundation
Rose Hills Foundation
Klingenstein-Simons Fellowship from the Esther A. & Joseph Klingenstein Fund
Simons Foundation
UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Training Program

Title: A meta-atlas of the developing human cortex identifies modules driving cell subtype specification

Authors: *P. R. NANO[1], E. FAZZARI[1], D. J. AZIZAD[1], C. V. NGUYEN[1], R. L. KAN[1], B. WICK[2], M. HAEUSSLER[2], A. BHADURI[1];
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Abstract: Human brain development requires the generation of hundreds of diverse cell types, a process targeted by recent single-cell transcriptomic profiling efforts. Through a meta-analysis of seven of these published datasets, we have generated 225 meta-modules - gene co-expression networks that can describe mechanisms underlying cortical development. Several meta-modules have potential roles in both establishing and refining cortical cell type identities, and we validated their spatiotemporal expression in primary human cortical tissues. These include meta-module 20, associated with FEZF2+ deep layer neurons. Half of meta-module 20 genes are putative FEZF2 targets, including TSHZ3, a transcription factor associated with neurodevelopmental disorders. Human cortical organoid experiments validated that both factors are necessary for deep layer neuron specification. Importantly, subtle manipulations of these factors drive slight changes in meta-module activity that cascade into strong differences in cell fate - demonstrating how of our meta-atlas can engender further mechanistic analyses of cortical fate specification.

Disclosures: P.R. Nano: None. **E. Fazzari:** None. **D.J. Azizad:** None. **C.V. Nguyen:** None. **R.L. Kan:** None. **B. Wick:** None. **M. Haeussler:** None. **A. Bhaduri:** None.

Presentation Number: NANO37.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS 5K08NS112598-02

Title: Mosaic organoids with second hit mutations in TSC2 show altered neuronal differentiation

Authors: *K. WINDEN[1], I. GISSER[1], T. PHAM[1], S. SINGH[2], T. KASAI[2], S. CHELVANAMBI[2], M. SAHIN[1];
[1]Boston Children's Hosp., Boston, MA; [2]Brigham and Women's Hosp., Boston, MA

Abstract: Tuberous Sclerosis Complex (TSC) is a genetic disorder caused by heterozygous variants in either *TSC1* or *TSC2* and is associated with epilepsy, autism, and intellectual disability. Patients display focal cortical dysplasias or cortical tubers in the brain, which are characterized by fewer neurons, astrocytosis, and dysmorphic cells. As opposed to benign tumors in TSC that frequently show second hit mutations in *TSC1* or *TSC2*, only a minority of cortical tubers show these changes, suggesting that either second hit mutations are not necessary for cortical tuber formation or these genetic alterations affect only a small percentage of cells. We previously demonstrated that cortical tubers with mostly heterozygous cells show similarities to stem cell derived neurons that have biallelic mutations in *TSC2* (*TSC2*-/-). Therefore, we hypothesized that second hit *TSC2*-/- cells might be capable of altering surrounding cells, leading to cortical tuber formation. To study this phenomenon, we developed a mosaic organoid model where a majority of the cells are heterozygous for *TSC2*+/-, while 10% of cells are either *TSC2*-/- or have the patient variant corrected using genome editing (*TSC2*+/+). Single cell sequencing of organoids with *TSC2*-/- cells revealed a substantial reduction in developing cortical pyramidal neurons with a concomitant increase in a specific subset of outer radial glia. Further, *TSC2*+/- cells at the neuroprogenitor stage showed reduced TBR2 expression when exposed to *TSC2*-/-

cells, consistent impairment of cortical neuron differentiation pathways. To determine whether this effect was mediated by secreted factors, we performed neuroprogenitor differentiation in transwell plates and observed that *TSC2*+/- cells show decreased *TBR2* mRNA expression when cultured with *TSC2*-/- cells. We then isolated extracellular vesicles from organoids with all three *TSC2* genotypes (*TSC2*+/+, *TSC2*+/-, and *TSC2*-/-) and characterized them with proteomics. Interestingly, there is a significant enrichment of regulators of the actin cytoskeleton, implicating cellular remodeling in aberrant neuronal differentiation induced by *TSC2*-/- cells. Our data suggest that a small percentage of *TSC2*-/- cells with second hit mutations can alter neuronal differentiation in neighboring cells via extracellular vesicles, providing a mechanism for amplifying the effect of second hit mutations and leading to cortical tuber formation.

Disclosures: K. Winden: None. **I. Gisser:** None. **T. Pham:** None. **S. Singh:** None. **T. Kasai:** None. **S. Chelvanambi:** None. **M. Sahin:** None.

Presentation Number: NANO37.03

Topic: A.01. Neurogenesis and Gliogenesis

Title: Mtorc1 activation in astrocytes initiate postnatal cortical neurogenesis in a neurodevelopmental disorder mouse model

Authors: *C. WANG;
Univ. of Cincinnati, COM, Cincinnati, OH

Abstract: Active adult neurogenesis only spatially occurs in two specific “neurogenic” brain regions: the subventricular zone of lateral ventricule and the hippocampus under physiological conditions. The generation of new neurons is generally believed to be void in cortex of mammals after birth. Here, we reported postnatal cortical neurogenesis because of hyperactivated mammalian target of rapamycin complex 1 (mTORC1) in parenchymal astrocytes. We established a model to conditionally knock out (cKO) an upstream negative regulator of mTORC1, Tuberous Sclerosis Complex gene 1 (Tsc1), in mouse brain. Our single cell RNA sequencing revealed an unexpected mTORC1 dependent neurogenesis program in postnatal cortex of Tsc1 cKO mice. Further analysis indicated that a population of mTORC1 activated astrocytes acquired characteristics of neural stem cells to differentiate into neurons in postnatal cortex. Using two astrocyte specific models to activate mTORC1 in postnatal cortex, we identified newly generated new cortical neurons with expected electrophysiological signatures. Interestingly, hyperactivated mTORC1 in cortical astrocytes repressed Notch signaling, which was essential for their postnatal neurogenesis activity. These findings provide a novel model to reveal previously unknown mechanisms of mTORC1 activation in astrocytes for postnatal cortical neurogenesis.

Disclosures: C. Wang: None.

Presentation Number: NANO37.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: SFARI 697827
U01MH114825

R35NS097305
P01NS083513
R01NS123912
K99MH131832

Title: Molecular and cellular dynamics of the developing human neocortex at single-cell resolution

Authors: *L. WANG[1], C. WANG[1], J. LI[2], A. R. KRIEGSTEIN[3];
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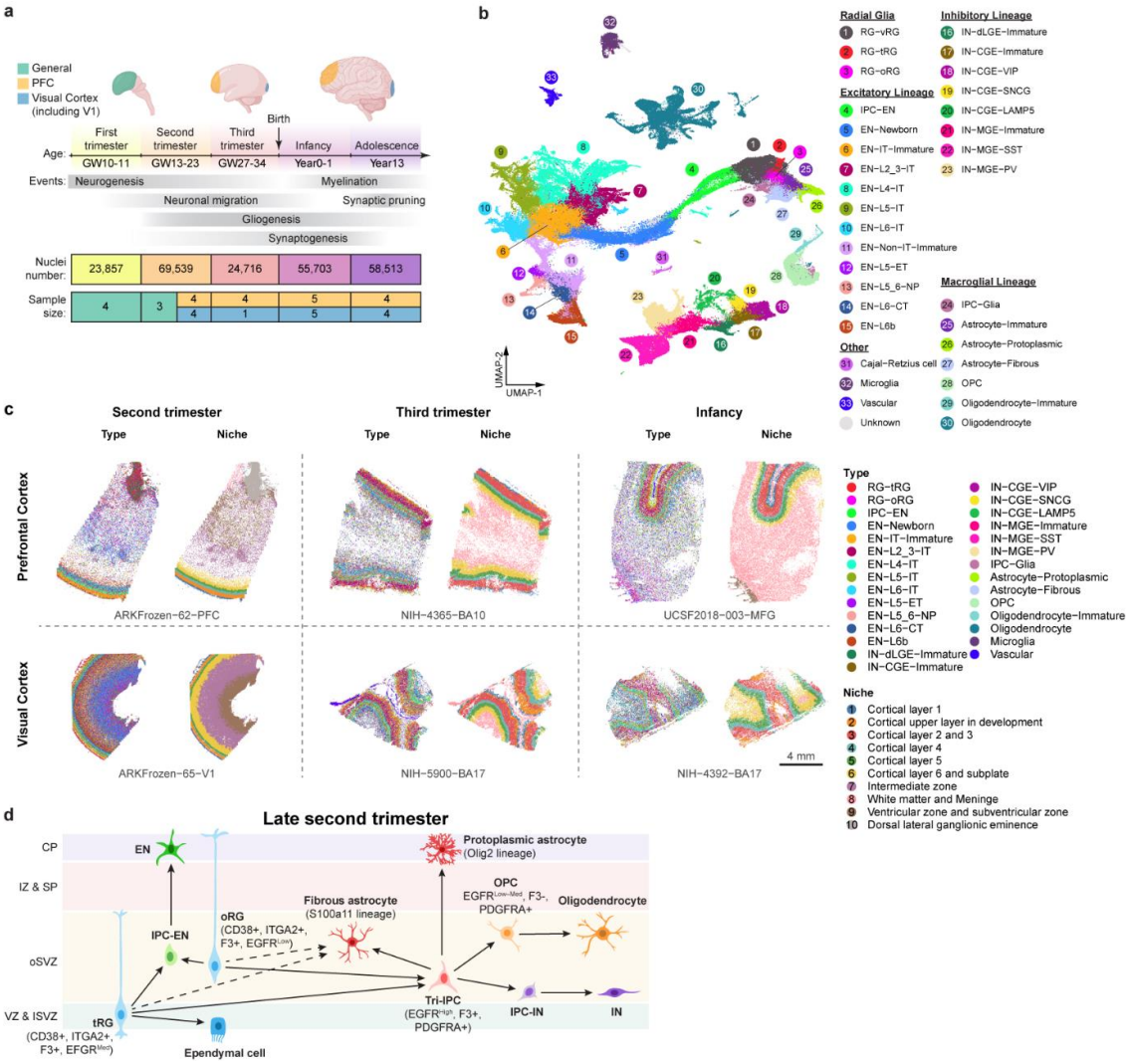
Abstract: The development of the human neocortex is a highly dynamic process and involves complex cellular trajectories controlled by cell-type-specific gene regulation. Here, we collected paired single-nucleus chromatin accessibility and transcriptome data from 38 human neocortical samples encompassing both the prefrontal cortex and primary visual cortex. These samples span five main developmental stages, ranging from the first trimester to adolescence. In parallel, we performed spatial transcriptomic analysis on a subset of the samples to illustrate spatial organization and intercellular communication. This atlas enables us to catalog cell type-, age-, and area-specific gene regulatory networks underlying neural differentiation. Moreover, combining single-cell profiling, progenitor purification, and lineage-tracing experiments, we have untangled the complex lineage relationships among progenitor subtypes during the transition from neurogenesis to gliogenesis in the human neocortex. Specifically, we find a tripotential intermediate progenitor subtype termed Tri-IPC responsible for the local production of GABAergic neurons. Furthermore, by integrating our atlas data with large-scale GWAS data, we created a disease-risk map highlighting enriched ASD risk in second-trimester intratelencephalic projection neurons. Our study sheds light on the gene regulatory landscape and cellular dynamics of the developing human neocortex.



Disclosures: L. Wang: None. **C. Wang:** None. **J. Li:** None. **A.R. Kriegstein:** F. Consulting Fees (e.g., advisory boards); Neurona Therapeutics.

Presentation Number: NANO37.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS K99NS135123
NINDS R01NS035129
NINDS R01NS032457
NHGRI R01HG013185

Title: Spatially Resolved Single-cell Analysis Reveals Early Prenatal Specification of Human Cortical Layers and Areas

Authors: *X. QIAN[1], C. STRINGER[2], M. LI[3], C. A. WALSH[1,4];
[1]Genet. and Genomics, Boston Children's Hosp., Boston, MA; [2]HHMI Janelia Res. Campus,

Ashburn, VA; [3], Dept. of Biostatistics, Univ. of Pennsylvania, Philadelphia, PA; [4]Howard Hughes Med. Inst., Boston, MA

Abstract: The human cerebral cortex, pivotal for advanced cognitive functions, is composed of six distinct layers and dozens of functionally specialized areas. These structures are distinguished both molecularly, by diverse neuronal and glial cell subtypes, and structurally, through intricate spatial organization. While single-cell transcriptomics studies have advanced molecular characterization of human cortical development, a critical gap exists due to the loss of spatial context during cell dissociation. Here, we utilized multiplexed error-robust fluorescence in situ hybridization (MERFISH), augmented with deep-learning based cell segmentation, to examine the molecular, cellular, and cytoarchitectural development of human fetal cortex with spatially resolved single-cell resolution. Our extensive spatial atlas, encompassing 16 million single cells, spans eight cortical areas across four time points in the second and third trimesters. We uncovered the early establishment of the six-layer structure, identifiable through the laminar distribution of excitatory neuronal subtypes by mid-gestation, long before the emergence of cytoarchitectural layers. Notably, while a gradient-like distribution of neuronal subtypes was generally observed along the anterior-posterior axis in most cortical areas, a striking exception was the sharp molecular border between the primary (V1) and secondary visual cortices (V2) at gestational week 20. At this distinct border, we discovered an abrupt binary shift in neuronal subtype specification, challenging the notion that continuous morphogen gradients dictate mid-gestation cortical arealization. Moreover, incorporating single-nuclei RNA-sequencing and in situ whole transcriptomics analyses, we discovered an early upregulation of synaptogenesis in V1-specific Layer 4 neurons, suggesting that activity-induced specification might underlie the discrete border formation. Collectively, our findings underscore the crucial role of spatial organization in determining the molecular specification of cortical layers and areas. This work not only provides a valuable resource for the field, but also establishes a spatially resolved single-cell analysis paradigm that paves the way for building a comprehensive developmental atlas of the human brain.

Disclosures: X. Qian: None. **C. Stringer:** None. **M. Li:** None. **C.A. Walsh:** None.

Presentation Number: NANO37.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: 2024 Irv Boime Graduate Student Fellowship

Title: MicroRNA targeting logic in direct neuronal reprogramming of glioblastoma

Authors: *L. YUAN[1], A. KIM[2], A. YOO[1];
[1]Developmental Biol., [2]Neurosurg., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: microRNA (miR)-124 is one of the main drivers of inducing mitotic exit during neurogenesis, which is also recapitulated in miR-9/9* and -124 (miR-9/9*-124)-mediated direct neuronal reprogramming. In the reprogramming of various somatic cell types, miR-9/9*-124 induce erasure of non-neuronal cell identity before activating the neuronal program. Despite the different transcriptome landscape in the original somatic cell types, there exists a commonly repressed core gene regulatory network (GRN) in various cell types commonly targeted by miR-9/9*-124, with cell cycle progression as the most significant pathway. We reason that miR-9/9*-

124 can induce cell fate erasure in cancerous cells by repressing similar GRNs including inducing cell cycle exit. Glioblastoma multiform (GBM) is a class of highly aggressive and heterogeneous brain tumor with poor prognosis. Mechanistic insights into GRNs repressed by miR-9/9*-124 while inducing the postmitotic state would facilitate the understanding gene networks that govern the tumorigenic identity of GBMs and potentially offer novel therapeutic implications. We found that ectopic expression of miR-9/9*-124 readily induced mitotic exit in patient-derived GBM cell lines, repressed GBM cell fates, and promoted neuronal identity over time as assessed by RNA-seq. In the intracerebral xenograft model, mice receiving GBM with miR-9/9*-124 survived significantly longer than those receiving non-specific control miRNA. Global profiling of miRNA targeting activity by AGO2 eCLIP reveals that miR-124 binds to a panel of transcripts involved in G1/S transition and mitogen response, in concordance with the phenomenon that miR-124 is the primary driver of cell cycle exit. On the contrary, miR-506, a miR-124 homolog, despite of sharing identical seed sequences, has significantly less targeting activity and fails to induce cell cycle exit. We are currently dissecting how the difference in the miRNA 3' sequences between miR-124 and miR-506 gives rise to drastic differential biological outcomes. Preliminary data suggests that the additional one nucleotide in miR-124 alters the efficiency of miRNA loading into AGO2 and consequently affects targeting specificity to cell cycle regulator transcripts. However, different permutations of miRNA 3' sequence, though sufficient to induce mitotic exit, are not competent to achieve neuronal reprogramming, indicating the mitotic exit and neurogenic activities of miR-124 could potentially be decoupled. We hope that understanding the grammar logic-embedded miRNA targeting sites would provide deeper insights into how miRNAs can be leveraged to erase and channel GBM identity.

Disclosures: L. Yuan: None. **A. Kim:** None. **A. Yoo:** None.

Presentation Number: NANO37.07

Topic: A.01. Neurogenesis and Gliogenesis

Support: Simons Foundation
NIH

Title: Investigating the role of transcription factor TCF4 in the development of cortical interneurons

Authors: *C.-Y. WANG, G. FISHELL;
Broad Inst., Cambridge, MA

Abstract: Cortical interneurons constitute a diverse population of inhibitory cells that exhibit distinct morphologies, physiological properties, connectivity, laminar locations, and marker gene expression. Moreover, these inhibitory cells play key roles in regulating cortical circuits, with their dysfunction frequently linked to various neurodevelopmental and neuropsychiatric disorders. How the astonishing diversity of cortical interneurons is generated is poorly understood. Previous studies have identified dozens of transcription factors critical for cortical interneuron development, but our understanding of transcriptional specification of cortical interneurons remains incomplete. Here we investigated the role of basic helix-loop-helix transcription factor TCF4 in interneuron development. TCF4 is expressed in all four major types of cortical interneurons. In addition, mutations of TCF4 cause Pitt-Hopkins syndrome, a severe

neurodevelopmental disorder. We employed mouse genetics to ablate TCF4 in cortical interneurons and then conducted immunohistochemistry to evaluate its loss-of-function effect. Our data showed that TCF4 deletion leads to marked reduction of cortical interneuron numbers. Moreover, the expression of canonical interneuron markers and their laminar distribution are also perturbed. These data indicate that TCF4 is a crucial factor that controls interneuron development. We are currently employing single-cell and bulk transcriptomic/epigenomic analyses to elucidate how TCF4 ablation affects interneuron diversification and the underlying genomic regulatory mechanisms. This research advances our understanding of interneuron development and its implications for neurodevelopmental disorders.

Disclosures: C. Wang: None. **G. Fishell:** None.

Presentation Number: NANO37.08

Topic: A.01. Neurogenesis and Gliogenesis

Support: BWF PDEP
NIH 5K00ES033033-05

Title: The heavy metal lead suppresses gliogenesis in the developing human brain

Authors: *M. SAMPSON[1], E. HILL[1], A. LANE[2], S. N. LANJEWAR[3], C. SOJKA[3], V. FAUNDEZ[4], S. A. SLOAN[3];
[2]Physiol., [3]Human Genet., [4]Cell Biol., [1]Emory Univ., Atlanta, GA

Abstract: Early-life toxicant exposures disrupt nervous system development and can lead to persistent changes in brain function. Modeling developmental toxicity in primary fetal tissue and human induced pluripotent stem cell-derived 3D organoids, we show that prenatal exposure to the heavy metal lead (Pb) affects the generation and maturation of glia in the developing human brain. Previous Pb toxicity studies have largely focused on neuronal toxicity, but here we focus on radial glia, the primary progenitor cells in the developing brain, and astrocytes. Astrocytes are specialized glial cells that protect the brain from toxicants by participating in the blood brain barrier, regulating inflammation, and providing redox and metabolic support to neurons and other cells. We demonstrate that both fetal and organoid-derived cells rapidly take up Pb and establish an exposure paradigm that achieves biologically relevant Pb burdens in organoid tissue. Organoids exposed to Pb upregulated genes for metal buffering proteins and NRF2-mediated antioxidant response element targets including the antioxidant glutathione. Several astrocyte genes were downregulated, suggesting a possible inhibition of glial generation or maturation. To examine cell-specific transcriptional responses to Pb, we performed single-cell RNA-seq on organoids and found that most cell types showed a strong ER-stress response. Chronic Pb increased the ratio of neurons to astrocytes in organoids and altered differentiation genes in radial glia. Primary human radial glia (GW16-20) exposed to Pb generated fewer new-born astrocytes ex vivo. Overall, our data suggest that developmental Pb may influence the cellular balance of neurons and astrocytes in the developing brain, which could contribute to Pb-associated cognitive, affective, and behavioral deficits later in life. Identifying the molecular pathways that are disrupted by developmental Pb exposures is critical for establishing interventions to mitigate toxicity.

Disclosures: M. Sampson: None. **E. Hill:** None. **A. Lane:** None. **S.N. Lanjewar:** None. **C. Sojka:** None. **V. Faundez:** None. **S.A. Sloan:** None.

Presentation Number: NANO37.09

Topic: A.01. Neurogenesis and Gliogenesis

Support: DBT-inStem core fund
DBT/Wellcome Trust India Alliance: Intermediate Career Fellowship- IA/I/19/1/504288
DST-SERB: Start up research grant SRG/2020/001612
DBT- Har Gobind Khorana Innovative Young Biotechnologist Fellowship -BT/13/IYBA/2020/02
Pratiksha Trust grant
M.K. Bhan Young Researcher postdoctoral fellowship- HRD-12/4/2020-AFS-DBT

Title: Elucidating the role of chromatin modifier RBBP4 in regulating neurogenesis in the developing mouse neocortical primordium

Authors: *S. K. DHANYA[1], K. KALIA[1], S. MOHANTY[1], T. AZAM[1], A. CHANNAKKAR[1], L. D'SOUZA[1], S. K S[1], P. REDDY[2], B. MURALIDHARAN[1];
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Abstract: Cerebral cortex consists of diverse neuronal and glial subtypes arranged cytoarchitecturally in six layers. Dynamic interaction between chromatin modifiers drives the generation of neuronal diversity from neural progenitors. Aberrations in epigenetic modifiers result in many neurodevelopmental disorders (Stessman et al., 2017). Putative risk variants in Retinoblastoma binding protein 4 (RBBP4) which is a core subunit of several chromatin modifying complexes have been associated with autism spectrum disorder (ASD; Firth et al., 2009). However, the exact molecular basis by which RBBP4 functions alter the epigenetic regulation of cortical development needs to be explored. To understand the functional role of RBBP4 and to investigate its genome-wide occupancy profile we performed RBBP4 knockdown using the CRISPR/Cas9 approach on neocortical progenitors derived from embryonic age 12.5 embryonic brains, the stage at which deep layer neurogenesis occurs namely the production of Layer VI and layer V neurons. Our study demonstrates that downregulation of RBBP4 in E12.5 neocortical progenitors resulted in the reduction of neuronal output from progenitors, specifically decreasing the CTIP2-expressing layer V neuronal numbers with no significant impact on the TLE4-expressing layer VI neurons. Genome-wide occupancy analysis revealed that RBBP4 primarily binds to distal regulatory elements, and neuron differentiation is a significant GO biological pathway of RBBP4-bound genes. Interestingly, we found that RBBP4 binds to Cdon, a receptor protein in the Shh signaling pathway, and knockdown of Cdon phenocopies RBBP4 knockdown resulting in a significant reduction in neurogenesis, particularly CTIP2-positive layer V neurons. CDON overexpression could rescue the phenotype caused upon loss of RBBP4 in the neocortical progenitors thereby suggesting the functional link between RBBP4 and its target gene CDON. Our results shed light on the cellular role of RBBP4 and identify CDON as a novel regulator of deep-layer neurogenesis in the neocortical progenitors.

Disclosures: S.K. Dhanya: A. Employment/Salary (full or part-time):; Post doctoral fellow, Institute For Stem Cell Science and Regenerative Medicine. **K. Kalia:** None. **S. Mohanty:** A. Employment/Salary (full or part-time):; Project Trainee, Institute For Stem Cell Science and Regenerative Medicine. **T. Azam:** A. Employment/Salary (full or part-time):; Junior research Fellow, Institute For Stem Cell Science and Regenerative Medicine. **A. Channakkar:** A. Employment/Salary (full or part-time):; Junior research Fellow, Institute For Stem Cell Science and Regenerative Medicine. **L. D'Souza:** A. Employment/Salary (full or part-time):; Junior research Fellow, Institute For Stem Cell Science and Regenerative Medicine. **S. K s:** A. Employment/Salary (full or part-time):; Project Trainee, Institute For Stem Cell Science and Regenerative Medicine. **P. Reddy:** A. Employment/Salary (full or part-time):; Assistant Professor, Shiv Nadar University. **B. Muralidharan:** A. Employment/Salary (full or part-time):; Assistant Professor, Institute For Stem Cell Science and Regenerative Medicine.

Nanosymposium

NANO38: mRNA Regulation and Translation in Plasticity

Location: MCP Room S404

Time: Tuesday, October 8, 2024, 8:00 AM - 11:30 AM

Presentation Number: NANO38.01

Topic: B.05. Synaptic Plasticity

Support: Research Grant Council of Hong Kong GRF 17106018
Research Grant Council of Hong Kong GRF 17117720
Research Grant Council of Hong Kong GRF 11102422
Research Grant Council of Hong Kong Collaborative Research Fund C1024-22G
University Grants Committee of Hong Kong Area of Excellence Scheme Grant AoE/M-604/16
University Grants Committee of Hong Kong Theme-based Research Scheme T13-605/18-W

Title: Heterogeneous and specific synaptic targeting of different mRNAs in neuronal dendrites

Authors: *X. WANG, K.-O. LAI;
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Abstract: Neurons are unique cells in the body due to the presence of long axons and elaborate dendrites. To facilitate rapid changes of protein composition in these extensions, neurons can synthesize specific proteins at distal sites. This machinery requires the long-distance transport of specific mRNAs from the cell body. Different mRNAs are packaged into separate granules by RNA-binding proteins and carried by kinesin motor proteins along the neuronal microtubule. mRNAs can be localized near synapses, suggesting that local translation underlies changes in neuronal connectivity during synaptic plasticity. However, whether distinct mRNAs are selectively distributed near synapses and differentially regulated after synaptic stimulation are poorly understood. Here we observed restricted movement and specific docking of *Actb* mRNA

granules at the base of dendritic spines. Furthermore, we found that *Actb* and *Camk2a* mRNAs showed frequent colocalization underneath dendritic spines, suggesting the spine base as the hotspot for mRNA delivery. However, after chemical long-term potentiation (cLTP) the colocalization of *Actb* and *Camk2a* mRNAs beneath dendritic spines was significantly reduced, and they exhibited specific distribution patterns near dendritic spines. Consistent with previous study, cLTP led to the invasion of Microtubule-Associated Protein 2 (MAP2) to some dendritic spines. Our study demonstrated a preferential localization of both *Actb* and *Camk2a* mRNAs to MAP2-containing dendritic spines after cLTP. Taken together, our findings indicate that the base of dendritic spine acts as the hot spot for capturing mRNAs, while different mRNA molecules exhibit heterogenous and specific synaptic targeting in response to synaptic activity.

Disclosures: X. Wang: None. **K. Lai:** None.

Presentation Number: NANO38.02

Topic: B.05. Synaptic Plasticity

Support: Lo Kwee-Seong Biomedical Research Fund (J.I)
Faculty Innovation Award (FIA2020/A/04) from the Faculty of Medicine, CUHK (J.I.)
Hong Kong RGC Early Career Scheme (24117220; J.I.)
Hong Kong RGC General Research Fund (14117221; J.I.)
Brain & Behavior Research Foundation Young Investigator Grant (28049; JI)

Title: Synaptic plasticity and non-coding RNA in dendritic targeting of synaptic mRNAs and proteins

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Abstract: Neuronal circuits in our brain are known to be plastic and are subject to experience-driven changes causing neurons to modify their structure, and functional connectivity and responses. To gain a deeper understanding of the mechanisms underlying plasticity, we utilize monocular deprivation (MD) as an experimental model. MD involves briefly closing one eye, causing an imbalance in input to neurons in the primary visual cortex (V1). This manipulation serves as a valuable tool for studying ocular dominance plasticity, a specific form of visual cortical plasticity. During the critical period of visual development, MD leads to a weakening of functional responses in V1 neurons to the deprived (closed) eye, followed by a strengthening of responses to the non-deprived (open) eye. Previous studies have suggested that MD during the critical period induces dendritic spine remodeling, which contributes to the functional plasticity of V1 neuron responses. However, the precise mechanisms underlying dendritic spine remodeling and the functional characteristics of the eliminated spines in response to MD remain

to be elucidated. We aim to identify novel regulatory mechanisms of plasticity. Circular RNAs (circRNAs) are regulatory noncoding RNAs abundantly found in brain tissue. Their synaptically-enriched, activity-inducible, and developmentally-regulated properties suggest a role in experience-dependent synaptic plasticity. However, functional investigation of circRNAs in neurons is still in its infancy. Relatively little is known about their role in experience-dependent plasticity. Here, we identified unique activity-dependent circRNAs upon plasticity induction. We found that circRNAs were robustly and differentially regulated. We demonstrated the changes of circRNAs in cortical and hippocampal synaptic plasticity. Our study provides evidence of an experience-dependent circRNA that is a crucial regulator of synaptic development and plasticity as well as learning and memory.

Disclosures: P. Ip: None. **Y. Cai:** None. **H.W. Tsang:** None. **N. Mellios:** A. Employment/Salary (full or part-time):; Circular Genomics Inc, Albuquerque, NM. **K. Lai:** None.

Presentation Number: NANO38.03

Topic: B.05. Synaptic Plasticity

Support: NIH Grant F32NS119376

Title: Shedding light on the role of rna regulation in synaptic plasticity

Authors: *R. A. SINGER;
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Abstract: Neurons are one of the most asymmetric cell types in the body, consisting of a cell body or soma, several branched dendrites, and a long axon that can be 1,000-fold the diameter of the cell body. Therefore, how a neuron, with only one cell nucleus and thus a single supply of active genes, obtains such a diverse distribution of proteins is of profound interest in neuroscience. As an additional challenge, to achieve synaptic plasticity underlying learning and memory, neurons must dynamically regulate transcription and translation in response to neural activity. This model has driven molecular neuroscientists to search for factors that localize and regulate synaptic RNAs. We are only beginning to compile a list of these key regulators, such as RNA binding proteins (RBPs), particularly in the synapses of hippocampal neurons that are involved in memory formation, and to date very little is known about how these factors regulate RNA. Our laboratory has generated a platform for Crosslinked Immunoprecipitation (CLIP) of RBPs in the living brain of mice. These ‘cTag’ mice conditionally express GFP-tagged RBPs in vivo in selected cell populations using the Cre/Lox system. While cTag-CLIP has furthered our understanding of the cell-specific regulatory functions of neuronal RBPs, so far this technology has only been used to measure steady state RNA regulation. To elucidate the role of neuronal RBP-mediated RNA regulation in synaptic plasticity, combined the cell-specific resolution afforded by cTag-CLIP with the unprecedented precision of optogenetics to achieve non-invasive optical control of specific neurons. We have used this new methodology, termed opto-CLIP, to identify the specific mRNA sequences bound by several RBPs in excitatory neurons of the mouse hippocampus. Opto-CLIP will further our understanding of RBP-mediated RNA regulation, enhance our knowledge of the role of RNA metabolism in synaptic plasticity, and provide new insight into the pathological mechanisms underlying neurological disorders.

Disclosures: R.A. Singer: None.

Presentation Number: NANO38.04

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01NS083085

Title: Single-molecule imaging of the endogenous Camk2a mRNAs reveals activity-dependent local regulation of the mRNA at dendritic spines

Authors: *D. HWANG[1], S. DAS[2], R. H. SINGER[1];
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Abstract: Calcium/calmodulin-dependent protein kinase type II (CaMKII) integrates a transient Ca^{2+} signal into long-term synaptic plasticity and structural changes at the excitatory postsynaptic compartments - dendritic spines. Among the subunits that make up the CaMKII holoenzyme, CaMKIIα has been shown to be critical for the maintenance of long-lasting synaptic changes at dendritic spines. Notably, CaMKIIα's persistent abundance in an individual spine during the maintenance period renders it a key molecule that facilitates perpetuation of synaptic transmission, triggered by an otherwise fleeting Ca^{2+} signal. However, it has yet to be clearly understood how CaMKIIα becomes persistently available even if proteins in spines are subject to dynamic exchange through diffusion and protein turnover. By tracking a single *Camk2a* mRNA, encoding the α subunit, we find a rapid activity-dependent localization of the fluorescently tagged endogenous mRNAs to stimulated dendritic spines (~1 minute) in dissociated mouse hippocampal neurons. The spine-localized *Camk2a* mRNAs remain in situ and undergo on-site protein synthesis, both of which persist over extended periods (~30 minutes). The interplay between the cis regulatory elements in the 3' UTR of the *Camk2a* mRNA and the cognate RNA-binding Cpeb proteins is required for the spine localization. These findings suggest that Cpeb-mediated activity-dependent spine localization and local protein synthesis of *Camk2a* mRNA may serve to tag an individual dendritic spine by supplying a spine-specific pool of CaMKIIα during the maintenance of long-lasting synaptic changes.

Disclosures: D. Hwang: None. **S. Das:** None. **R.H. Singer:** None.

Presentation Number: NANO38.05

Topic: B.05. Synaptic Plasticity

Support: CIHR Fellowship 295104 (HHW)
CFREF & McGill HBHL Postdoctoral Fellowship (HHW)
FRQS Postdoctoral Fellowship 259572 (HHW)
QBIN Scholarship 35450 (HHW)
NSERC DG2016-05118 (AJW)
CHIR PGs 153150 & 173290 (AJW)
CIHR PG 156223 (PJS)

Title: Local protein synthesis in axons sustains synapse-specific neurotransmission

Authors: *H.-W. WONG, A. J. WATT, P. J. SJOSTROM;
McGill Univ., Montreal, QC, Canada

Abstract: For > 60 years, memory formation has been linked to protein synthesis (PS), with the prevalent view that it originates in the cell body. Yet recent omics studies have found hundreds of mRNAs in axons, suggesting that presynaptic PS controls neural communication. Here we show that local PS in axons of pyramidal cells sustains neurotransmission in mouse visual cortex.

Neurotransmission was suppressed within minutes after PS inhibitor wash-in (62% ± 4%, n = 19, $p < 0.001$). To localize the PS need, we selectively manipulate pre- or postsynaptic neurons with intracellular drug loading. We found synaptic response (excitatory postsynaptic potential; EPSP) deficits and exaggerated paired-pulse ratio (PPR) after presynaptic blockade with M7 cap analog (EPSP: 47% ± 6%, n = 16, $p < 0.001$; $\Delta PPR = 0.13 \pm 0.05$, $p < 0.05$), indicating synaptic release is boosted by presynaptic PS. Using 2-photon laser microsurgery to sever axon from cell body, we showed that axonal PS sustained neurotransmitter release (55% ± 4%, n = 7, $p < 0.001$). To understanding the dynamics of axonal PS, we live imaged RNA localization and translation. Taking advantage of FAM-puromycin incorporation as proxy for PS, we captured the rapid and localized activity-dependent mRNA translation at presynaptic boutons ($dG/R = 0.17 \pm 4$, n = 31, $p < 0.001$). Interestingly, endogenous RNA revealed persistent and discrete docking patterns at individual presynaptic sites, suggesting bouton-specific regulation. In agreement, PS sustained neurotransmission at synapses from pyramidal cells to other pyramidal cells, but not to inhibitory basket balls and Martinotti cells (55% ± 8%, n = 5 vs. 98% ± 2%, n = 12, $p < 0.01$). We furthermore found that axonal PS is required in high- but not low-frequency neurotransmission (98% ± 3%, n = 13, $p = 0.5$), suggesting a role in high-fidelity information transfer and memory formation. Local PS in axons is therefore a previously unappreciated principle for supporting neurotransmission at specific synapse types.

Protein synthesis has emerged as a promising candidate target for treating neuropathology such as autism and Alzheimer's disease, yet the focus has historically been postsynaptic. Our results highlight the potential for disease interventions that rely on synapse-type-specific local translation in axons.

Disclosures: H. Wong: None. **A.J. Watt:** None. **P.J. Sjostrom:** None.

Presentation Number: NANO38.06

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01 MH125772
NIH Grant R01 NS113600
NIH Grant R01 MH116673
NIH Grant R35 GM136296

Title: Presynaptic autocrine BDNF/TrkB signaling is required for long-term plasticity and promotes local protein synthesis

Authors: *E. GRIEGO[1], S. KHAROD[1], P. J. LITUMA[2], R. H. SINGER[1], Y. J. YOON[1], P. E. CASTILLO[1];
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Abstract: Growing evidence indicates that some forms of presynaptic long-term plasticity in the mature mammalian brain rely on axonal protein synthesis, but how this process is regulated remains poorly understood. Brain-derived neurotrophic factor (BDNF) and its cognate receptor TrkB are known to promote local protein synthesis in dendrites and play a key role in postsynaptic plasticity. Much less is known about the role of BDNF/TrkB and local translation in presynaptic plasticity. To address this issue, we examined hippocampal mossy fiber to CA3 pyramidal cell synapse (MF-CA3), which expresses both structural and functional presynaptic plasticity. MFs also express uniquely high levels of BDNF. We recently showed that MF-LTP and the associated structural remodeling of MF giant boutons (MFBs) require axonal protein synthesis, which is negatively regulated by fragile X messenger ribonucleoprotein (FMRP). Here, we investigated whether presynaptic BDNF/TrkB signaling promotes local translation and MF-LTP. We found that *Bdnf* or *Trkb* conditional deletion from GC (*Bdnf* and *TrkB* cKO) by injecting AAV-CaMKII.Cre.mCherry in the dentate gyrus of $Bdnf^{fl/fl}$ or $TrkB^{fl/fl}$ mice, respectively, significantly reduced MF-LTP and LTP-induced presynaptic translation, whereas basal synaptic transmission and short-term plasticity remained unchanged. In addition, brief (1 hour) exposure to an enriched environment increased presynaptic protein synthesis, which was not observed in presynaptic *TrkB* cKO mice. By injecting a virus encoding for a BDNF reporter (AAV-DJ.CaMKII.BDNF-YFP) in the dentate gyrus of wildtype mice, we found that the LTP induction protocol released BDNF from MFBs. Consistent with previous studies, the R-type voltage-gated calcium channel blocker (SNX-482, 400 nM) abolished MF-LTP induction, suggesting that R-type calcium channels could mediate BDNF release from MFBs. Altogether, our findings reveal that autocrine BDNF/TrkB signaling supports presynaptic MF-LTP and promotes local translation in MFBs.

Disclosures: E. Griego: None. **S. Kharod:** None. **P.J. Lituma:** None. **R.H. Singer:** None. **Y.J. Yoon:** None. **P.E. Castillo:** None.

Presentation Number: NANO38.07

Topic: B.05. Synaptic Plasticity

Support: NIH-R01-NS0117821
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
Craig H. Neilsen Foundation
the South Carolina Spinal Cord Injury Research Fund
Merkin Peripheral Neuropathy and Nerve Regeneration Center

Title: Conserved axonal protein synthesis inhibitor impedes cns axon regeneration

Authors: *P. K. SAHOO[1], J. L. TWISS[2];
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Abstract: Inhibition of the stress granule proteins G3BP1 in mammals enhances peripheral nervous system (PNS) axon regeneration. The expression of G3BP1's acidic 'B-domain' not only speeds up axon recovery following nerve damage but also offers a promising method to encourage neural restoration in the PNS. This study explores whether suppressing G3BP1 could also aid in regenerating axons within the mammalian central nervous system (CNS), where such regeneration does not occur naturally. Our findings reveal that expressing the G3BP1 B-domain

does indeed promote axon regrowth in the spinal cord and optic nerve of mammals. Furthermore, a cell-permeable peptide derived from G3BP1 B-domain, facilitates the re-growth of reticulospinal axons through a peripheral nerve graft bridging the gap in a severed spinal cord. These findings suggest that G3BP1 granules are a significant barrier to axon regeneration in the CNS and that dismantling these granules could be an innovative approach to enhance neural repair following CNS injuries.

Disclosures: P.K. Sahoo: None.

Presentation Number: NANO38.08

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01NS109607
NIH Grant R56AG062354
BrightFocus A2021030S

Title: Formation of disease-associated transcription factor heterodimers through local mRNA translation

Authors: *U. HENGST;
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Abstract: mRNA localization and local translation are necessary for many aspects of neuronal development, maintenance, and function. The need for localized protein synthesis in morphologically highly polarized cells such as neurons is frequently explained in terms of logistics, i.e., the ability to modulate protein abundance in a just-in-time/just-in-place manner. However, this model seems to fail to explain the local synthesis of nuclear proteins, including transcription factors, in the neuronal periphery. We have discovered that signal-dependent, synchronized local synthesis of transcription factors can allow the formation of transcription factor heterodimers that are otherwise stoichiometrically disfavored. Specifically, we find that exposure to soluble oligomeric $A\beta_{1-42}$ triggers the local synthesis and subsequent heterodimerization of the bZIP transcription factors ATF4 and CREB3L2. This heterodimer is significantly more abundant in the brain of Alzheimer's disease patients as controlled to age-matched controls, and it promotes the transcriptional dysregulation of several disease-linked pathways such as tau hyperphosphorylation. Transcriptional changes are a feature of many diseases and are explained by epigenetics, mechanisms involving non-coding RNAs, or, most frequently, by altered expression of individual transcription factors. Differently, we identify transcription factor heterodimerization mediated by their local translation of as a driver of pathogenesis in Alzheimer's disease. Our findings identify an important master regulatory event for Alzheimer-associated gene expression changes and more broadly provide a rational explanation for how local synthesis of transcription factors can encode peripheral signals and transmit them to the neuronal nucleus.

Disclosures: U. Hengst: None.

Presentation Number: NANO38.09

Topic: B.05. Synaptic Plasticity

Support: MICIU/AEI/10.13039/501100011033 Grant PID2022-139451OB-I00
MICIU/AEI/10.13039/501100011033 Grant SAF2016-76347-R (with FEDER funds)
Alzhiemer´s Association Grant AARG-19-618303
Alzhiemer´s Association Grant AARG-19-618303-RAPID

Title: Glial control of protein synthesis in neurons

Authors: *J. BALERIOLA;
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Abstract: The neuronal soma is considered the primary origin of axonal, dendritic and synaptic RNAs and local translation is thought to be mainly controlled by the neuron itself. However, increasing hypotheses suggest that surrounding glia might provide an additional layer of translational control in neurons. Indeed, several evidence indicate the existence of active horizontal RNA and even ribosome transfer from Schwann cells to regenerating peripheral nerves. Although a few mechanisms have been proposed for ribosome transfer from glia to neurons, these and other bioactive molecules are likely delivered within EVs. EVs are membrane-enclosed vesicles secreted by all cell types with a recognized role in intercellular communication. Interestingly, Shwann cell-derived EVs improve regeneration of peripheral nerves after injury. In this context, it has been proposed that glial EVs induce regeneration by changing the axonal translatome and hence the proteome. However, there is no published evidence on the regulation of local neuronal translation by glial EVs in the CNS in the context of neurodegenerative diseases. The study of this novel aspect of glia-neuron communication might open new venues for therapies for Alzheimer's disease (AD) and related disorders and is the focus of our research. Our work indicates that application of Aβ, a major driver of AD, to primary astrocytes enhances the release of EVs especially within the range size of ~170-300 nm in diameter as measured by nanoparticle tracking analysis. Additionally, proteomic analyses show that astroglial EVs contain more than 50% of the proteins that compose the ribosomes, whereas this protein cluster is not as significantly enriched in neuronal EVs. Importantly, EVs released by Aβ-treated astrocytes increase local translation in axons of healthy neurons compared to control EVs. Additionally, although we have not seen any effect of glial EVs on cell survival, our data indicate that they do affect synaptic the amount of pre- and post-synaptic markers when released in response to Aβ, as well as the complexity of the tubulin network in vitro. Finally, we have identified ribosomal Rsp6 as one of the proteins that might be transferred from glia axons. We are currently addressing whether vesicular S6 is responsible for mediating local translation in neurons and synaptic integrity.

Disclosures: J. Baleriola: None.

Presentation Number: NANO38.10

Topic: B.05. Synaptic Plasticity

Support: 3P50HD104458-04S1

Title: Proteomic analysis of 3D models of Fragile X syndrome

Authors: *N. RAJ;
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Abstract: The development of the human brain is a dynamic process that requires the precise orchestration of a sequence of complex cellular, molecular, and genetic. Disruptions to this process have profound consequences, and emerging evidence implicates targets and pathways that appear to be commonly disrupted across multiple neurodevelopmental disorders (NDDs), despite distinct genetic etiologies. Current approaches have primarily been directed at identifying high-confidence risk genes and networks through large-scale transcriptomic analyses, which have provided invaluable insight into the biology of disorders like autism. However, very few studies have focused on a proteomic analysis, although proteins are the primary effectors of the transcriptome and are often the direct target of therapeutics. Moreover, since distinct mechanisms regulate the expression and turnover, transcription and translation do not always correlate in a linear fashion. Thus, there is a critical gap to validate transcriptomic studies at the protein level and to develop new approaches to assess the broad consequences of altered proteostasis in the developing human brain. Our work complements existing genetic approaches while providing new biological insight into human developmental proteome and the altered steady-state and nascent disease-relevant proteome in human cellular models of the developing brain. Using human patient induced pluripotent stem cell (iPSC) derived 3D organoids, we have identified RNA and protein modules with strong associations to known pathological and clinical phenotypes, including disrupted modules that are preserved across multiple disorders. Our novel approaches allow us to define cell-type and developmental stage-specific molecular signatures that are drivers of disease and can be targeted for therapeutic intervention.

Disclosures: N. Raj: None.

Presentation Number: NANO38.11

Topic: B.05. Synaptic Plasticity

Support: Marie Skłodowska-Curie Actions METAFRAX 892837

Title: Non-ionotropic nmdar in the pathophysiology of fragile x syndrome

Authors: *A. THOMAZEAU;
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Abstract: Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability (ID), and the leading known genetic cause of autism. This dominant phenotype represents a huge hindrance for clinicians in the treatment of FXS because the underlying neuronal deficits remain unknown. Individual neurons store information as we learn and acquire new information by modifying their connections with nearby neurons, a process called synaptic plasticity. This refers to activity-dependent long-term changes in synaptic strength but also synaptic structure, and is highly regulated by NMDA receptors (NMDAR). It has recently been proposed that NMDARs can signal in an unconventional manner. Here, I will present recent work highlighting how unconventional NMDARs drive synaptic plasticity and control protein synthesis, and how this is deregulated in the FXS mouse model.

Disclosures: A. Thomazeau: None.

Presentation Number: NANO38.12

Topic: B.05. Synaptic Plasticity

Support: CIHR Grant 374967
Azerili Grant 501100005155

Title: Role of FMRP in selecting mRNAs for Stalled Ribosomes in Neurons

Authors: *J.-Y. LI[1], M. AMIRI[2], S. DURAIKANNU KAILASAM[3], W. S. SOSSIN[1];
[1]Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada; [2]Biochem., McGill Univ., Montreal, QC, Canada; [3]Canadian Ctr. for Computat. Genomics, Montreal, QC, Canada

Abstract: Local protein synthesis is a crucial process that maintains essential functions such as synaptic plasticity. Ribosomes stalled at elongation and stored in RNA granules make up a sizeable percentage of mRNAs translated in neurons and have been shown to be critical for some forms of plasticity, such as metabotropic glutamate receptor long term depression (mGLUR-LTD). Previous studies have shown that the Fragile X Mental Retardation Protein (FMRP) is highly enriched in RNA granules containing stalled ribosomes and regulates mGLUR-LTD. Previous examination of ribosome protected fragments (RPFs) from stalled ribosomes in neurons have identified sequence motifs that match motifs previously identified in FMRP-associated mRNAs (Anadolu et al, 2023, Journal of Neuroscience doi: 10.1523/JNEUROSCI.1002-22.2023). To determine if FMRP recognition of these motifs is important for stalling, we examined stalled ribosomes and RPFs from P5 mice lacking the FMRP protein. We found that overall, the loss of FMRP had minor effects on the proteins, structure of the ribosomes and the overall distribution of RPFs but significantly decreased the amount of stalling on mRNAs previously shown to associate with FMRP, suggesting that FMRP regulates stalling of a subset of mRNAs, but without changing the positions that mRNAs are stalled at.

Disclosures: J. Li: None. **M. Amiri:** None. **S. Duraikannu Kailasam:** None. **W.S. Sossin:** None.

Presentation Number: NANO38.13

Topic: B.05. Synaptic Plasticity

Support: NIH KL2TR001432
NIH 1R01NS133441

Title: Time-sensitive degradation of activity-induced nascent proteins mediated by neuronal membrane proteasomes (NMPs) induced by enhanced visual experience in Xenopus laevis tadpoles

Authors: *H. HE;
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Abstract: Proteostasis is critical for the normal function of the nervous system. Recent studies from ours and other labs suggest that a neuronal membrane proteasome (NMP) is specifically involved in timely degradation of activity-induced nascent proteins in neurons, and such NMP function may play critical roles in regulating neuronal activity in both central and peripheral

nervous system. Activity-driven upregulation of protein synthesis is a key signature of activity-dependent plasticity mechanisms. To investigate the recruitment of the proteolytic function of NMPs by plasticity-inducing experience, we employed a well-established visually driven plasticity paradigm in the visual circuit of Xenopus laevis tadpoles. Using an optimized *in vivo* bio-orthogonal non-canonical amino acid tagging (BONCAT) method that allows us to effectively label nascent proteins produced within as short as 30 min for quantitative analysis, we examined the level of acutely synthesized nascent proteins at both gross and individual protein levels in the mid brain (major visual processing center) of tadpoles in response to a brief period (30min) of enhanced visual experience (VE). VE significantly increased the production of nascent proteins in the midbrain. Inhibiting NMP activity induced a further increase in nascent proteins on top of the VE-induced upregulation, suggesting that part of the VE-induced nascent proteome was actively degraded by NMPs. Numerous prior studies predict that functionally distinct proteins are likely synthesized in temporally distinct 'waves' following plasticity-inducing activities. To test if NMP activity is recruited to degrade nascent proteins produced in these different waves, we labeled nascent proteins for a 30-min period at different timepoints following VE to evaluate the transient rate of nascent protein production with or without the NMP inhibitor. Our result showed that distinct transcriptional-independent and dependent peaks of *de novo* protein synthesis were detected in the tadpole midbrain at discrete timepoints following VE. Importantly, NMP inhibition revealed a significant contribution of NMPs at some but not all protein-synthesis peaks, suggesting a selective role of NMP function in regulating the proteostasis of VE-induced nascent proteins. These results shed light on the temporal dynamics of the proteostasis of activity-induced nascent proteins in vivo and provide new evidence for NMP-mediated degradation of nascent proteins as an important regulatory mechanism for activity-dependent proteostasis in the nervous system.

Disclosures: H. He: None.

Presentation Number: NANO38.14

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 5R01NS102272
NIH Grant F32NS105363

Title: Local translation in microglial processes during phagocytosis

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Abstract: Recent studies have illuminated the importance of several key signaling pathways in regulating the dynamic surveillance and phagocytic activity of microglia. Yet little is known about how these signals result in the assembly of phagolysosomal machinery near targets of phagocytosis, especially in processes distal from the microglial soma. Here, we tested whether there is regulated local translation within peripheral microglial processes of mouse brain. We show that microglial processes contain ribosomes that engage in *de novo* protein synthesis, and these are associated with transcripts involved in pathogen defense, motility, and phagocytosis. Using a live slice preparation, we further show that acute translation blockade impairs formation

of phagocytic cups, localization of lysosomal proteins within them, and phagocytosis of apoptotic cells and pathogen-like particles. Finally, microglial processes severed from their somata require *de novo* local protein synthesis to effectively surround pathogen-like particles. Collectively, these data suggest a need for new protein synthesis locally within distal microglial processes to support dynamic microglial functions.

Disclosures: M.J. Vasek: None. **J.D. Dougherty:** None.

Nanosymposium

NANO39: New Insights Into Mechanisms of Synaptic Dysfunction in Alzheimer's Disease

Location: MCP Room N427

Time: Tuesday, October 8, 2024, 8:00 AM - 10:45 AM

Presentation Number: NANO39.01

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

Support: NIAAA Grant R21AA026944
NIH Grant R01AG072896

Title: Spatiotemporal differential regulation of extrasynaptic GluN2B receptor subunits and PSA-NCAM in brain aging and Alzheimer's disease.

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Abstract: *N*-methyl-*D*-aspartate receptors (NMDARs) are tetrameric excitatory receptors pivotal for synaptic transmission but its role in Alzheimer's disease (AD) pathophysiology has not been completely characterized. In particular, GluN2B NMDARs play key roles in synaptic plasticity and are spread across synaptic and extrasynaptic spaces (ES). The ES-GluN2Bs are known to activate long-term depression (LTD) pathways and may promote dementia in AD. Polysialylated neural cell adhesion molecule (PSA-NCAM) inhibits ES-GluN2B activity physiologically, and its dysregulation is associated with loss of synaptic plasticity. However, the spatiotemporal changes of ES-GluN2Bs and PSA-NCAM during brain aging vs. AD are unknown. Our study comprehensively examined the spatiotemporal dynamics of NMDARs (Glun-2A, -2B), ES-GuN2Bs, and PSA-NCAM, in young and old Tg2576 AD mice (AD mice) and age-matched wild-type (WT) mice in the cortex, prefrontal cortex, hippocampus, and midbrain, regions associated with different aspects of neural plasticity. We observed significant decrease in overall GluN2B expression (ranging between 47 - 51%, $n \geq 4$) with aging in both WT and AD mice, while GluN2A expression increased region-wide (up to 85%, $n \geq 4$). We also characterized the expression patterns of ES- vs. synaptic-GluN2B by analyzing pull-down of PSD-95 fragments. We found elevated ES-GluN2B expression with disease progression in AD mice (2 - 3-fold, $n \geq 4$) and no increase in WT mice. The observed decrease in overall GluN2B expression with aging

in both WT and AD mice and the elevated expression of ES-GluN2B subunits in AD mice suggests a potential link between ES-GluN2B-mediated signaling and LTD in AD. To determine whether dysregulation in PSA-NCAM is linked to AD progression and ES-GluN2B disinhibition, we assessed the expression patterns of NCAM and PSA-NCAM in WT and AD mice across ages via immunoblotting. We found evidence of decreased expression of PSA-NCAM in AD mice (43 - 58%, $n \geq 4$), especially in the hippocampus and prefrontal cortex; and elevated levels of PSA-NCAM with normal aging (up to 2-fold, $n \geq 4$), with no significant changes in total NCAM expression. This indicates that deficits in PSA-NCAM correlate with AD progression in hippocampal and cortical regions. Our findings highlight the differential changes in ES-GluN2B and PSA-NCAM expression in AD and brain aging, suggesting an interplay between increased ES-GluN2B and downregulation of PSA-NCAM in loss of synaptic plasticity and AD progression.

Disclosures: O.E. Imiruaye: None. **B. Carson:** None. **J. Garcia:** None. **D. Han:** None. **S. Bhattacharya:** None.

Presentation Number: NANO39.02

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

Support: NIH 1R01DA052271-05S1
NIH R21AI152937-02

Title: Iqck, a novel alzheimer’s disease risk gene, significantly reduces postsynaptic density protein 95 in the mouse brain

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Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disease characterized by progressive memory loss and changes in personality and behavior. Recent genome-wide association studies (GWAS) support the idea that genetically driven synaptic failure is central to AD, a fact also confirmed by recent PET images. Multiple GWASs using stringent protocols have confirmed the IQ motif Containing protein K (IQCK) is a risk factor for AD irrespective of *APOE* ε4 genotype, indicating that IQCK may cause AD independent of lipid metabolism. To understand the molecular basis of IQCK, we screened 6000 proteins in a multiplex antibody-based proteomic study and found a significant reduction in the levels of Amyloid Beta Precursor Like Protein 1 (APLP1), which was confirmed by secondary assays including immunocytochemistry and immunoblots in cell lines. To confirm whether such a reduction occurs *in vivo*, we successfully generated and characterized flag-IQCK-expressing transgenic mice. In the IQCK-Tg mice, we confirmed a significant reduction of not only APLP1 but also its related protein APP and, most importantly, APP-interacting protein PSD-95, all of which are known to play crucial roles in synaptic transmission. PSD-95 is known to regulate dendritic spine density, and its disruption is associated with cognitive and learning deficits in schizophrenia and autism. We previously confirmed IQCK levels are markedly increased in AD patient brains, iPSC-derived neurons as well as mouse models of AD. Therefore, IQCK-

mediated significant reductions in PSD-95 levels may reflect the loss of synapses and thus account for the loss of memory in AD. While our findings provide valuable insights into the role of IQCK in the loss of synaptic integrity in AD, they also highlight the need for further research. Understanding the exact mechanism through which IQCK contributes to the loss of synaptic integrity is crucial for the development of new therapeutic targets.

Disclosures: J. Akkaoui: None. **D. Devadoss:** None. **M. Diaz:** None. **H. Wang:** None. **M.P. Nair:** None. **M.K. Lakshmana:** None.

Presentation Number: NANO39.03

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

Support: NIH Grant R01AG071587
NIH Grant R01AG075820

Title: Proteomic and transcriptomic analyses of perineuronal net composition in the 5xFAD amyloidosis model of Alzheimer’s Disease

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Abstract: Perineuronal nets (PNNs) are condensed extracellular matrix structures commonly associated with Parvalbumin Interneurons (PV-INs)—a class of fast-spiking inhibitory interneurons which exhibit selective vulnerability in early Alzheimer’s Disease (AD) pathology. PNNs are complex structures composed largely of chondroitin-sulfate proteoglycans (CSPGs) and the associated glycosaminoglycans (GAGs). PNN functions including the regulation of synaptic transmission and plasticity, are known to be modulated by their composition. However, the nature in which AD pathology affects PNN composition and the way these changes affect surrounded PV-INs remain unknown. In this study, we used the 5xFAD amyloidosis mouse model to assess how AD pathology affects the presence and composition of PNNs and PV-INs, from 1.8 to 14 months of age. First, we used mass-spectrometry based proteomics to assess protein-level composition of PNNs. Interestingly, brevican and tenascin-R (TnR), a core CSPG and link protein respectively, were selectively and significantly reduced in 5xFAD mice, a finding that began at 6 months and was exacerbated by age. To investigate whether this finding is due to aberrant transcription, and if this phenomenon may be cell-type specific, we conducted analyses on previously published single-cell RNA sequenced data comparing 5xFAD mice to WT controls. At the transcriptional level, we found that brevican, but not other CSPGs, is selectively expressed in astrocytes, but that the expression of brevican does not differ significantly in the 5xFAD mice compared to WT. Further, to analyze the age and pathology dependent PNN composition at a finer scale, we utilize immunofluorescence (IF) to probe for PV and several major components of PNNs, including brevican and TnR. Our preliminary analyses reveal that the GAG side chains (as visualized via WFA lectin) are depleted in a manner non-specific to PV-INs in early amyloidosis (3 to 6 months). Taken together our proteomic and transcriptomic findings suggest that AD pathology affects PNN composition via a post-transcriptional mechanism. Our ongoing histological analyses will reveal the region-specific and PV-IN-specific nature of this phenomenon, and how this correlates with early changes in PV-INs.

Disclosures: R. Nelson: None. **C. Espinosa-Garcia:** None. **S. Rangaraju:** None. **P. Kumar:** None.

Presentation Number: NANO39.04

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

Support: NIH K01 AG068366
VA Merit Awards I01 BX004673
NIH RF1 AG061774
NIH R21 MH125242

Title: Early alteration of sleep oscillations and activity of hippocampal parvalbumin neurons in the APP/PS1 mouse model of Alzheimer's disease.

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Abstract: Sleep abnormalities have been recognized as early characteristics of Alzheimer's disease (AD), including changes in overall sleep pattern and sleep oscillations such as slow (<1 Hz) and delta (1-4 Hz) oscillations. These abnormalities correlate with the severity of amyloid-β deposition and cognitive impairment at later stages of the disease. Neuronal network dysfunction is another early characteristic of AD. Previous in vitro recordings in mouse models of AD suggested that altered activities of hippocampal (HPC) parvalbumin (PV) neurons at an early stage of AD were linked to neuronal network alterations and memory impairment. However, the relationship between the AD-related sleep abnormalities and cortical network dysfunction in vivo is not fully understood. Here, we performed longitudinal recordings from the same mice at different ages to assess time course of changes in activity of HPC PV containing neurons, sleep oscillations, and cognition in a mouse model of AD.We performed sleep recordings from 9 APPPS1(C57BL/6;C3H)/PV-cre mice (AD) and 13 control PV-cre mice (Ctr) using local field potential (LFP) electrodes in medial prefrontal cortex (mPFC) and HPC. Recordings were performed for 24h each month at ages 3-6 months-old (mo). To assess PV neuronal activity, we performed fiber photometry recordings monthly in HPC with GCaMP8f while presenting a 40Hz auditory stimulation (2s every 6s for 150 trials) to the mice (AD: n=5, Ctr: n=8). For each session, photometry signals (ΔF/F) from all trials were aligned at the auditory stimulus onset, then, the area under curve during the stimulation period was computed. The spontaneous Y-maze alternation task was performed to assess cognition.The proportion of NREM sleep during the 12h dark (active) phase was significantly lower in AD mice at 6mo compared to Ctr mice (t-test: $p=0.02$), while wakefulness was significantly higher (t-test: $p=0.03$). Band power in slow-wave and delta ranges recorded in HPC and mPFC during NREM sleep were already lower at 3mo in AD mice compared to Ctr mice (t-test: $p<0.05$). Fiber photometry data showed significantly lower values for area under curve during the auditory stimulation in AD mice at 3mo compared to Ctr mice (t-test: $p=0.04$), indicating lower HPC PV activity in AD mice. The Y-maze performance in AD mice at 3&6mo was not significantly different from Ctr mice.The results suggest that changes in activity of HPC PV neurons and sleep oscillation changes in APP/PS1 mice occur as early as 3mo. Cognitive decline was not as robust at 6mo, suggesting that altered

sleep and PV neuron activity could be characteristics of earlier stages of AD before apparent cognitive impairment.

Disclosures: F. Katsuki: None. **J.M. McNally:** None. **D. Gerashchenko:** None. **R.E. Brown:** None.

Presentation Number: NANO39.05

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

Support: NIH R01NS121533: P.I. B. Harrison
P20GM103642: P.I. I. Meng

Title: Cholinergic neurons of the basal forebrain express CD2AP that may contribute to the pathogenesis of Alzheimer's Disease via RAB5-mediated endosomal transport.

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Abstract: Basal forebrain (BF) cholinergic neurons are critical for learning and memory, and are central to the pathogenesis of Alzheimer's disease (AD). GWAS consistently show that genomic variants at the *CD2AP* gene locus as well as genetic variants in *RAB5A* endocytosis genes significantly increase risk of AD. Our previous work shows that CD2AP is a docking-scaffold/adaptor protein that coordinates neuronal nerve growth factor (NGF) and trophic signaling. We have also demonstrated that CD2AP positively regulates RAB5-mediated mechanisms of endocytosis in primary sensory neurons. The purpose of this study was to perform an *in vivo* characterization of CD2AP expression in cholinergic neurons of the brain regions most relevant to AD pathogenesis and to investigate the colocalization of CD2AP and RAB5 in cholinergic neurons of the murine BF. Brain tissue was perfused, harvested from $ChAT^{BAC}$-eGFP transgenic mice (age 10 mo), where cholinergic neurons (co-) express green fluorescence protein (GFP) in central and peripheral neurons that express choline acetyltransferase (ChAT). Frozen tissue sections were used to assess the specificity of the reporter in mouse brain along with localization of both CD2AP and RAB5 (co-) expression using immunofluorescence (IF) analysis of ChAT-GFP+ neurons and primary antibodies against ChAT, CD2AP and RAB5. Image J software was used to develop and optimize a colocalization assay for CD2AP and RAB5 puncta. Experiments were repeated in a follow-up cohort of 18-mo old mice. IF expression of CD2AP was quantified in BF, vDB and striatum and compared to cortical regions of the adult mouse brain. Colocalization of CD2AP was observed in the cell bodies of ChAT-GFP+ neurons of striatum, vDB and BF, where CD2AP expression intensity and number of cell bodies with positive signal increased incrementally. Colocalization analyses revealed near-complete overlap of CD2AP/RAB5 expression in ChAT-GFP+ cholinergic neurons of BF. We conclude that cholinergic neurons express CD2AP in adult and aged-adult mouse brains. These data provide the first evidence of quantifiable CD2AP protein expression of cholinergic neurons specific to the vDB and BF. Together with previous research from our lab,

these data support a role for CD2AP in the pathogenesis of AD through orchestration of endocytosis and retrograde signaling. Ongoing studies are underway to verify these findings in a novel MODEL-AD mouse that incorporates the humanized variant of *CD2AP*, where we aim to further investigate how *CD2AP* variants may affect mechanistic components of RAB5 endocytosis as well as subsequent survival of cholinergic neurons in the context of known amyloid beta and Tau pathologies.

Disclosures: L.A. Fitzsimons: None. **J.E. Lovely:** None. **M. Mueth:** None. **M. Atif-Sheikh:** None. **K.P. Kotredes:** None. **G.R. Howell:** None. **B.J. Harrison:** None.

Presentation Number: NANO39.06

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

Title: Loss of endothelin-converting enzyme-1 (ECE-1) expression in excitatory neurons causes progressive accumulation of synaptic amyloid-beta in mice

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Abstract: Abnormal β-amyloid (Aβ) accumulation is a main feature of Alzheimer's disease (AD), yet the cellular dysfunctions that trigger amyloidogenesis and the cascade of events leading to neurodegeneration and dementia are still poorly characterized. In brain, Aβ concentration is majorly regulated by metalloproteases, as evidenced by rapid 2-10x increases in intraneuronal and secreted Aβ following treatment with the protease inhibitor phosphoramidon (PA). To investigate the contribution of PA-sensitive Aβ degrading enzyme ECE-1 to neuronal Aβ homeostasis, we generated a conditional *Ece1* knockout (KO) mouse with ablation of *Ece1* in Camk2a-positive excitatory forebrain neurons. The resulting neuron-specific *Ece1* KO mice (Camk2a-Cre^{+};$Ece1^{flox/flox}$) were viable, fertile, and normal sized. Littermates (Cre^{+} or Cre^{-}, 8-10 per genotype, either sex) were aged to 2 or 6 months for analysis of Aβ40 and Aβ42 in synaptosomes and extracellular fluid-enriched extracts prepared from forebrain. The effects of genotype and age on Aβ concentration were analyzed by two-way ANOVA and post hoc pairwise comparisons. Neuronal *Ece1* KO resulted in increased synaptosomal Aβ40 and Aβ42 ($P<0.0001$), extracellular Aβ40 ($P=0.0007$) and extracellular Aβ42 ($P=0.0339$). The increase in synaptosomal Aβ was progressive, with a significant interaction between age and genotype for synaptosomal Aβ40 ($p=0.017$), and similar trend for synaptosomal Aβ42 ($P=0.07$). In 6-month-old *Ece1* KO mice vs. controls, synaptosomal Aβ40 and Aβ42 were increased 2x and 1.5x, respectively. The observed increases approach those induced by PA treatment in brain, suggesting that blockade of excitatory neuronal ECE-1 activity is largely responsible for the PA effect on synaptic Aβ accumulation. To confirm this, we injected *Ece1* KO mice and controls intracerebroventricularly with PA or vehicle (n=5 per genotype, either sex) and measured Aβ 18h later. Results of these experiments indicate that ECE-1 activity in Camk2a+ synapses is responsible for ~50% of the overall effect of PA on synaptic Aβ (in synaptosomes derived from combined neuronal subtypes). In contrast, Camk2a neuronal *Ece1* deficiency resulted in smaller (10-25%) increases in extracellular Aβ and did not alter the large PA-induced effect on Aβ in this fraction, suggesting that ECE-1 activity in other cell types, and/or inhibition of other

metalloproteases, is responsible for the observed effect. This novel mouse model, in parallel with ones being developed to ablate *Ece1* in other cell types, will be valuable for investigating the effects of dysregulated synaptic Aβ on physiological Aβ function and potential downstream pathways related to AD pathology.

Disclosures: J. Pacheco-Quinto: None. **D. Clausen:** None. **D.D.C. Bastien:** None. **H. Peng:** None. **E.A. Eckman:** None.

Presentation Number: NANO39.07

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

Title: Neuid: a novel lncrna exclusively expressed in the brain and controls neuronal function and identity

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Abstract: Non-coding RNAs are transcripts that do not encode proteins and comprise 98.5% of the human transcriptome. Among these, long non-coding RNAs (lncRNAs) have been implicated in various biological functions, including the control of gene expression and translation. The central nervous system (CNS) harbors a diverse array of lncRNA transcripts, yet the functions of many remain largely unexplored. In this study, we employed bulk and single-cell RNA sequencing approaches using human and mouse brain tissue to identify novel lncRNAs in both neuronal and non-neuronal cells, potentially associated with neurodegenerative diseases. We discovered a novel brain-specific lncRNA, termed 'NeuID', specifically expressed in neurons of both mouse and human brains. NeuID expression was found to be reduced in the brains of Alzheimer's disease (AD) patients. Knockdown (KD) of NeuID resulted in the downregulation of synaptic plasticity genes and the upregulation of genes associated with glial cell development. Additionally, NeuID KD led to decreased dendritic spine density and reduced neuronal network activity. Furthermore, we demonstrated that NeuID interacts with EZH2, a component of the PRC2 complex known to play essential roles in cell fate determination. Overall, our findings identify a unique brain and neuron-exclusive lncRNA that regulates neuronal function, is dysregulated in AD, and plays a role in maintaining neuronal identity.

Disclosures: R. Pradhan: None. **A. Fischer:** None. **F. Sananbenesi:** None.

Presentation Number: NANO39.08

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

Title: Age-dependent increases in postsynaptic α5GABA-A receptors are missing in a rat model of Alzheimer's Disease

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Abstract: Targeting amyloid for removal in order to inhibit neurodegeneration and cognitive decline in Alzheimer's Disease (AD) using selective therapeutics has made some progress, but an improved understanding of memory dysfunction on a neural circuitry level will be needed to develop acute nootropics for ongoing memory dysfunction. Here, we focus on the hypothesis that if early onset memory dysfunction can be traced to the hippocampal trisynaptic circuit, then it could serve as a target for therapeutic modulation. Inhibitory GABAergic neurons participate in sharp wave ripple (SPW-R) oscillations in the hippocampal trisynaptic circuit as detected in CA1 during sleep and awake inactivity and are believed to function in memory replay and consolidation. Using the transgenic beta amyloid expressing TgF344-AD rat, which develops frank neurodegeneration and memory impairment with age, we previously discovered that ripple amplitude in 9mo and older TgF344-AD rats are insensitive to the nootropic drug α5IA, a negative allosteric modulator of α5-subunit containing γ-aminobutyric acid type A receptors ($\alpha5GABA_ARs$). Here, we ask whether the loss of α5IA potentiation of ripple amplitude might reflect AD-specific downregulation of the predominant extrasynaptic $\alpha5GABA_AR$ isoform. To our surprise, postsynaptic but not extrasynaptic $\alpha5GABA_AR$ levels decrease with aging in the dentate gyrus (DG) of TgF344-AD while increasing in *wt* littermates. The results strongly suggest a model wherein age-associated beta-amyloid inhibits $\alpha5GABA_AR$ trafficking to postsynaptic but not extrasynaptic sites. We posit that reducing $\alpha5GABA_AR$ trafficking to the postsynaptic compartment in DG, and likely in CA1 neurons, may reduce their ability to shape local field potentials, including ripples, and blunt the pharmacological action of α5IA on ripple amplitude.

Disclosures: J.C. George: None. **A.E. Tipton:** None. **N. Volgarine Scaraboto Bonfa:** None. **D.H. Farb:** None. **S.J. Russek:** None.

Presentation Number: NANO39.09

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

Support: BRIGHTFOCUS A2022036S
BRIGHTFOCUS A20201159S
NIH R01AG053588
NIH R01AG059753
NIH R01AG075108
NIH P30 AG072973
NIH P30AG072973

Title: Atp6v1d-mediated dysregulation of presynaptic v-atpase in alzheimer's disease

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Abstract: Numerous lines of evidence underscore synaptic dysfunction as a critical early pathological hallmark of Alzheimer's disease (AD), intricately linked to the onset and progression of cognitive decline. While dysregulation of both pre- and post-synaptic proteins

significantly contributes to the initiation and exacerbation of synaptic dysfunction in AD, the precise mechanism underlying presynaptic damage remains elusive. Vacuolar-type ATPase (V-ATPases) plays a pivotal role in neurotransmitter loading, storage, and release, by facilitating the acidification of synaptic vesicles. The proper functioning of V-ATPase is critical for maintaining the integrity and efficacy of synaptic transmission. In our study, we identified reduced V-ATPase activity in the temporal poles of AD patients and neocortices of a mouse model representing AD-like Brian amyloidosis (5xFAD mice). Further immunoblotting for the major subunits of V-ATPase, we observed a selective reduction of ATP6V1D in AD brains, particularly within isolated synaptosomes. Concurrently, a diminished number of synaptic vesicles and reduced ATP6V1D localization in synaptic vesicles were evident in AD brains. Additionally, loss of ATP6V1D in primary cultured neurons resulted in diminished V-ATPase activity, decreased synaptic vesicle number and acidification, impaired synapse formation, and neuronal loss, mirroring the pathological changes observed in AD. This study suggests a potential mechanism of presynaptic vulnerability in AD and underscores V-ATPase dysregulation due to ATP6V1D deficiency as a promising therapeutic target for AD treatment.

Disclosures: T. Wang: None. **J. Tian:** None. **H. Du:** None. **L. Guo:** None.

Presentation Number: NANO39.10

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

Title: The effect of Tau protein expression on neuroligin-1-mediated synaptic plasticity in a cell model of Alzheimers disease

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Abstract: Introduction
Recent evidence suggests a potential role for neuroligin-1 (NLGN1), a key player in synaptic plasticity processes, during the initial stages of Alzheimer’s disease (AD) in the hippocampus. Despite overlapping mechanisms, the pathological influence of Tau on NLGN1 has not yet been investigated. The aim of this work is to explore whether there is a direct relationship between the change in Tau and NLGN1 expression.
Materials and methods
Mouse hippocampal neurons (P0-2) were transduced for Tau overexpression (hTau40) and control, respectively. The cells were harvested on day 12 for evaluation of NLGN1, total Tau (tTau), phosphorylated Tau (pTau) Ser262, pTau Ser396 levels by western blot and immunocytochemistry. SH-SY5Y cells were transfected with NLGN1 and hTau40 isoform 2N4R followed by glycine and bicuculine-mediated activation of glutamate receptors for live-cell imaging evaluation. Kruskal-Wallis was used for multiple comparisons followed by Dunn’s post-hoc analysis.
Results
A clear attenuation of NLGN1 signal was detected in hTau40 group (-63%, p less than 0.001). A rise in p/t Tau Ser262 was detected in hTau40 (+92%, p less than 0.01), but without significant changes in p/t Tau Ser396, might indicate a role of calcium/calmodulin-dependent kinase 2 as a potential link between Tau and NLGN1. Stimulation of glutamate receptors did not yield any

signal in either of the triple-transfected SH-SY5Y cells indicating a missing link for adequate NLGN1 activation.
Conclusion
This pilot study implies that the overexpression of Tau protein and phosphorylation at Ser262 is related to disruption in NLGN1 levels in hippocampal neurons, providing valuable insight into their potential roles and interactions in early AD pathogenesis.

Disclosures: K. Horvatovic: None. **S. Wegmann:** None.

Presentation Number: NANO39.11

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

Support: Scully Initiative
Taube Family Foundation
Jean Perkins Foundation
Horngren Family

Title: Restoring neuronal and glial activity-dependent transcriptomic signatures in tauopathy mice by in vivo modulation of $p75^{NTR}$ signaling

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Abstract: Introduction: Synaptic dysfunction occurs early in the progression of Alzheimer's disease and related dementias (ADRD), and is characterized by disruption in the balance between excitatory and inhibitory signaling. Disrupted neuronal/astrocyte interaction is a likely key contributor to synaptic failure in ADRD. We have shown that modulating $p75^{NTR}$ signaling with the small molecule LM11A-31 (C31) prevents tauopathy-related synaptic dysfunction, as assessed by long-term potentiation (LTP). We hypothesize that alterations in GABergic interneurons and astrocytes-specific gene co-expression, in response to synaptic stimulation, can be normalized by C31 in tauopathy mice. **Methodology:** 22 Wildtype (WT) and 24 Tau.P301S (PS19) mice were treated with vehicle or C31 for 3 months starting at 6 months of age, when tau pathology was well established. Theta burst stimulation (TBS) was used to induce LTP. Bulk RNA sequencing with cell-type enrichment analysis was performed on unstimulated and stimulated (TBS-LTP) slices using weighted analysis with a soft-threshold power of 18 to achieve scale-free topology $R^2>0.8$. Colocalization of astrocytes and GABAergic interneurons was determined by immunofluorescence staining (GFAP and VGAT markers, respectively) on the same TBS-LTP hippocampal slices. **Results:** In PS19 compared to WT mice, 16 activity-dependent gene co-expression modules were significantly down-regulated, with 9 exhibiting cell-type enrichment for neurons and interneurons. Many of these modules displayed functional enrichment for GABAergic synapse and LTP processes. Conversely, 8 activity-dependent gene co-expression modules were up-regulated with 3 of them showing significant enrichment for glia and functional association to neuroinflammation and complement pathways. Interestingly, C31 treatment normalized the expression pattern of many of the previously altered transcriptional co-

expression modules in PS19 mice. Comparison of these altered modules in PS19 mice to human AD modules demonstrated that those affected in treated PS19 mice have similar up- and down-regulated pattern of expression in human AD. Finally, GABAergic interneuron/astrocyte colocalization was significantly increased in TBS-LTP slices from PS19 mice and restored to WT levels with C31 treatment. **Conclusions:** We identified neuronal/glial crosstalk as one candidate mechanism by which the p75 NTR-modulator C31 might prevent tauopathy-associated synaptic dysfunction and underlying alterations of human AD-relevant gene co-expression networks.

Disclosures: A. Latif-Hernandez: None. **P. Moran Losada:** None. **R.R. Butler:** None. **T. Yang:** None. **H. White:** None. **T. Wyss-Coray:** None. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX.

Nanosymposium

NANO40: Dementia: Non-Abeta Proteinopathy and Vascular Integrity

Location: MCP Room N426

Time: Tuesday, October 8, 2024, 8:00 AM - 10:00 AM

Presentation Number: NANO40.01

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

Support: R01AG034924
R01AG066165
RF1 AG061729
S10OD02365101A1
S10OD019967
S10OD018034
P30 CA138313

Title: Endolysosomal TMEM106B regulates myelin lipid metabolism by interacting with galactosylceramidase

Authors: *H. TAKAHASHI[1], A. PEREZ[1], C. W. LEE[2], X. HAN[3], S. M. STRITTMATTER[4]; [1]Dept. of Neurosci., Yale Univ. Sch. of Med., New Haven, CT; [2]Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ; [3]Univ. of Texas Hlth. Sci. Ctr. At San A, San Antonio, TX; [4]Dept. of Neurosci., Yale Univ., New Haven, CT

Abstract: TMEM106B is a highly glycosylated type II transmembrane protein localized at the endolysosome. Although TMEM106B has been implicated in a great variety of human brain disorders including frontotemporal lobar degeneration (FTLD), Alzheimer’s disease, chronic traumatic encephalopathy, and hypomyelinating leukodystrophy, so far little is known about physiological functions of TMEM106B at the endolysosome and how TMEM106B is involved in a wide range of human conditions at the molecular level. Recent studies using cryogenic electron microscopy have identified amyloid fibrils composed of truncated C-terminal

TMEM106B in human brains. However, the TMEM106B fibrils have been found not only in the brains of people who had neurodegenerative disorders but also in neurologically normal ageing brains. In addition, a disease-protective variant of TMEM106B is also reported to form the amyloid fibrils. Therefore, it remains unclear whether TMEM106B fibrils are responsible for the progression of TMEM106B-associated neurological diseases.
Dysregulation of lipid metabolism has been reported in the brains of FTLD patients as well as several mouse models of FTLD. Given the role of TMEM106B in FTLD, these findings raise a possibility that TMEM106B also regulates lipid metabolism under physiological and pathological conditions. In the present study, we therefore performed lipidomic analysis using the brains of 12 months old TMEM106B-deficient mice and their wild-type littermates. We found that levels of two major classes of myelin lipids galactosylceramide (GalCer) and sulfatide are specifically and significantly decreased in TMEM106B-deficient brains. Interestingly, subsequent unbiased proteomic analysis and co-immunoprecipitation assay revealed that TMEM106B physically interacts with galactosylceramidase (GALC), a lysosomal enzyme that hydrolyzes GalCer. We also found that TMEM106B deficiency significantly increases GALC activity in several brain regions in mice, while having no significant effects on protein levels of GALC. Together, these results suggest that endolysosomal TMEM106B interacts with GALC to regulate myelin lipid metabolism, particularly GalCer and sulfatide levels. GALC inhibition may be a therapeutic target for TMEM106B-associated diseases.

Disclosures: H. Takahashi: None. **A. Perez:** None. **C.W. Lee:** None. **X. Han:** None. **S.M. Strittmatter:** None.

Presentation Number: NANO40.02

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

Support: NIH Grant RF1 NS085070
NIH Grant U54 NS110435
Alzheimer's Association AARF-22-973152

Title: Synergistic effects of α-synuclein, tau, and amyloid pathology on mitophagy in dementia with Lewy bodies

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Abstract: The enzyme pair PINK1 and PRKN together directs a cytoprotective pathway that selectively tags damaged mitochondria with phospho-ubiquitin (pS65-Ub) and facilitate their lysosomal degradation (termed mitophagy). The dynamic pS65-Ub signal accumulates with enhanced activation from increased mitochondrial damage and/or upon reduced autophagic-lysosomal flux. Previous studies including ours showed altered mitophagy and elevated pS65-Ub levels in Parkinson and Alzheimer disease brains that also independently associated with α-synuclein, tau, or amyloid pathology. However, their combined impact on mitophagy and organelle function remains unclear. We here explored this in a large cohort of dementia with Lewy bodies (DLB) cases where all three pathologies coexist. Hippocampus and amygdala from 371 DLB and 30 control autopsy brains were immunostained for the mitophagy marker pS65-

Ub. Associations of pS65-Ub levels with clinico-neuropathological measures from the same regions were evaluated. Immunofluorescence co-staining of pS65-Ub, phospho-α-synuclein, and phospho-tau was performed to study their interactions at single-cell level via super-resolution imaging and AI-driven tools. pS65-Ub signals strongly accumulated in the hippocampus and amygdala of DLB cases compared to controls. Significant associations were observed between pS65-Ub levels with age, brain weight, APOE4 genotype, Braak stage, Thal phase as well as counts of Lewy body (LB), neurofibrillary tangle (NFT), and senile plaque (SP) in both regions. Strong synergistic effects of LB and NFT pathologies on pS65-Ub accumulation were found in the amygdala, while only additive effects of LB and SP pathologies were observed. Single-cell analysis in the amygdala showed that most affected cells contained either LB or NFT inclusion rather than both co-residing within the same cell. Notably, cells harboring LB pathology exhibited mainly smaller, granular pS65-Ub deposits, possibly associated with increased mitochondrial dysfunction. Conversely, those containing NFT showed larger, vacuolar pS65-Ub deposits, especially in APOE4 carriers, likely resulting from reduced lysosomal degradation. Our study revealed complex interactions of α-synuclein, tau, and amyloid pathologies on mitophagy alteration in DLB brains, highlighting different molecular mechanisms underlying α-synuclein- and tau-associated mitophagy regulation.

Disclosures: X. Hou: None. **T. Richardson:** None. **F.C. Fiesel:** None. **M. Heckman:** None. **T. Yan:** None. **S. Koga:** None. **D.W. Dickson:** None. **W. Springer:** None.

Presentation Number: NANO40.03

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

Support: NS085770
AG072977

Title: Patterns of Microglia Activation in a Conditional Transgenic Mouse Model of Human TDP-43 Proteinopathy

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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 hTDP-43, placed downstream of the tetracycline response element (TRE). We have shown appearance of cortical intraneuronal punctate phosphorylated TDP-43 positive inclusions after 14 days of TDP-43 expression following weaning in transgenic mice. After 8 weeks of expression, the number of inclusions was significantly reduced and by 24 weeks, none were visible while concurrently reduced

neuronal density and cortical thickness were observed. In human participants with FTLD-TDP, we have shown that cortical activated microglia display concordance with disease phenotype and patterns of atrophy. The purpose of the present study was to investigate patterns of cortical microglia activation in the conditional hTDP-43 transgenic mice. Fixed, serial brain sections from wild-type or transgenic mice were stained immunohistochemically using an antibody to ionized calcium-binding adapter molecule 1 (Iba1), a marker of microglia. In both wild-type and transgenic mice, Iba1 immunoreactivity was present in a large population of ramified (quiescent) microglia evenly distributed across all cortical areas, with small cell bodies and long thin processes. In transgenic mice, an additional population of activated microglia, characterized by enlarged, ameboid cell bodies and short, thick, and stubby processes was present. Activated microglia were present after 14 days of hTDP-43 expression at which time high density of TDP-43 inclusions are present, were increased in density at 8 weeks when neuronal loss is observed and were slightly reduced in density at 24 weeks. Importantly, activated microglia were present in highest densities in the frontal and temporal cortices, which are areas that harbor the highest densities of TDP-43 inclusions. Immunoreactivity for HLA-DR, a marker of activated microglia, confirmed the pattern observed with Iba1 immunoreactivity and visualized microglia with highest densities in the frontal and temporal cortices. These results suggest that activation of microglia is seen first when TDP-43 inclusions form in neurons and continues during the process of neuronal degeneration. Determination of specific molecular responses of microglia to hTDP-43 must await further investigation.

Disclosures: Y. Antezana: None. **E. Adebanjo:** None. **A. Chiodini:** None. **I.A. Ayala:** None. **A. Bahrami:** None. **K.R. Sadleir:** None. **R.J. Vassar:** None. **M. Mesulam:** None. **C. Geula:** None.

Presentation Number: NANO40.04

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

Support: NIH/NIA 1R01AG084670-01

Title: Tdp-43 citrullination as a structure-altering mechanism for alternate pathological aggregates

Authors: *C. SAUNDERS[1], P. ROCHA-RANGEL[6], J. CRAMER[2], C. B. ROGERS[2], J. HUNT[3], P. T. NELSON[4], D. C. LEE[5], M.-L. B. SELENICA[1];
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Abstract: Transactive response DNA-binding protein 43 kDa (TDP-43) is a nuclear DNA/RNA binding protein linked to the neuropathology of a spectrum of disease—notably by mislocalized cytoplasmic inclusions. These inclusions are classically seen as stress granules, which undergo liquid-liquid phase separation (LLPS) and hyperphosphorylation. Different post-translational modifications, like phosphorylation, are proposed to alter protein structure and function. Our laboratory has recently discovered a previously unknown but significant post-translational modification (PTM) of TDP-43; citrullination by peptidyl arginine deiminase (PAD4), changing

the amino acid charge from a positive arginine to neutral citrulline. This study aims to investigate the impact of PAD4 on TDP-43 pathology within Alzheimer's Disease and Related Dementias (AD/ADRD) such as Limbic-predominant age related TDP-43 encephalopathy (LATE).We performed in vitro PAD4 mediated citrullination on both full length recombinant TDP-43 (citR TDP-43) and C-terminal domain of TDP-43 (TDP-43^{LCD}/citR TDP-43^{LCD}). Citrullinated sites were confirmed by Western blot utilizing our novel site-specific citR TDP-43 antibodies. To investigate the effects of citrullination on TDP-43 aggregate morphologies, TDP-43 and citR TDP-43 were incubated with yeast total RNA to determine differences within TDP-43 RNA binding motifs, which are known to have a direct effect on stress granule assembly and LLPS. We performed thioflavin-T (ThT) and turbidity kinetics where we found a significant delay in citR TDP-43 interaction with RNA compared to unmodified TDP-43. After analyzing fluorescent and bright field morphologies, we found that neither TDP-43^{LCD} or citR TDP-43^{LCD} were particularly influenced by RNA. Interestingly, citR TDP-43^{LCD} alone showed droplet-like formations under higher protein concentrations, suggesting a self-crowding phase transition separate from the RNA-binding motifs. To further look at the structural changes of citrullination on TDP-43, the resulting aggregates were analyzed by transmission electron microscopy (TEM) tomography, showing a drastic reduction of size from a normal globular state (full-length TDP-43) or fibril (TDP-43^{LCD}) to a much smaller peppering formation.We propose that citrullination serves as a molecular switch for LLPS and liquid-solid phase separation (LSPS) transitions, possibly through altered RNA-protein interactions, a known enhancer for normal stress granule assembly. These alternate accumulates can stray from the canonical LLPS aggregates found in stress granules, and may serve as a separate inclusion pathway for TDP-43 pathology.

Disclosures: C. Saunders: None. **J. Hunt:** None. **D.C. Lee:** None. **M.B. Selenica:** None.

Presentation Number: NANO40.05

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

Support: NIH Grant NS124673
NIH Grant NS131169

Title: Single-cell RNA sequencing analyses revealed integrated vascular reactions in promoting angiogenesis after chronic cerebral hypoperfusion

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Abstract: Vascular dementia (VD) is the second most common type of dementia, accounting for 20% of dementia cases world-wide. Pathological alternations in neurovascular unit (NVU) are observed in VD. The NVU encompasses neural structures (e.g. neurons and glial cells) and vascular components (e.g. endothelial cells (ECs), pericytes (PCs) and smooth muscle cells (SMCs)) that highly collaborate to regulate brain homeostasis. Abnormalities in vascular structure contribute to dysfunction of neuronal networks and subsequent cognitive deficits. Therefore, elucidating the mechanism for vascular injury and repair may guide the development of preventive or therapeutic strategies against VD. In this study, we employed single cell RNA sequencing to map the cell populations and explore the vascular responses in mouse brains collected 6 weeks after an asymmetric common carotid artery stenosis (ACAS) model of VD or

sham operation . A distinct tip cell cluster with specific gene expression patterns was identified among 9 EC clusters and validated by double immunostaining of a tip cell marker isolectin B4 and a EC marker CD31. Immunostaining of CD93, another tip cell marker, and CD31 demonstrated that the number of tip cells increased significantly in the ACAS brains compared to sham 6 weeks after ACAS. Further gene ontology (GO) analysis based on differentially expressed genes (DEGs) between tip cells vs. other EC revealed enrichment in biological processes involving angiogenesis and suggested the involvement of Apln/Aplnr signalling in these biological terms. Western blot confirmed an increase in Apln protein expression after ACAS. To further elucidate the function of Apln/Aplnr signaling in VD, Apelin 13 (apln13), an Apln agonist, or same volume of vehicle control was administered (i.p) 1 to 21 days after ACAS. Apln13 treatment improved cognitive functions compared to vehicle-treated ACAS mice as assessed by Y-maze, novel object recognition and passive avoidance tests. Apln13 treatment promoted post-ACAS angiogenesis and increased tube formation in EC cultures. Cell-cell interaction analyses between tip cells and other vascular components highlight a cross talk between astrocyte and tip cells through VEGF-VEGFr interaction. Collectively, our studies provide insights into the molecular basis of angiogenesis and vascular repair in VD brains. Apln13 may represent a therapeutic strategy to improve neurovascular function in VD.

Disclosures: J. Shan: None. **R. Shi:** None. **X. Hu:** None. **F. Xu:** None.

Presentation Number: NANO40.06

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

Support: R01 NS5103212
RF1 NS122174

Title: Endothelial cell MHC class I molecule restricted antigen presentation to CD8 T cells influences brain infiltration and establishment of T resident memory cell populations

Authors: *J. THELWELL[1], A. J. JOHNSON[2], M. MAYNES[2], M. PEDRA SEADY[2], A. HASSANI[2], F. JIN[2], M. HANSEN[2], C. OWENS[3];
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Abstract: Title: Endothelial cell MHC class I molecule restricted antigen presentation to CD8 T cells influences brain infiltration and establishment of T resident memory cell populations

Javonte Thelwell, Mark A. Maynes, Carley Owens, Marina Seady, Asma Hassani, Fang Jin, Michael J. Hanson, and Aaron J. Johnson

Blood Brain Barrier (BBB) disruption plays a role in various neurodegenerative diseases such as Alzheimer’s Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Stroke, Huntington’s Disease (HD), Cerebral Malaria (CM), as well as COVID-19. In our laboratory, we aim to interrogate the immunological underpinnings associated with CD8T cells and barrier leakage. In clinical studies, aging results in a decline in T cell production and a gradual shift to resident memory T cells, attributed to reduced T cell repertoire diversity and weak activation. To model these clinical observations, we sought to define the effect of MHC class I restricted antigen presentation on endothelial cells of brain vasculature on CD8 T cell infiltration of the CNS. We conditionally

ablated H-2K^b and H-2D^b molecules on endothelial cells using Cdh5-Cre mice crossed with our novel K^b LoxP and D^b LoxP mice generated by our research program. In these animals we studied CD8 T cell accumulation in the brain using Theiler's Murine Encephalomyelitis Virus (TMEV) infection of young mice compared to old mice. Our results indicate that the loss of endothelial cell K^b induces a defect in acute and resident memory CD8 T cell populations and further dysregulates the engagement of various immune cell types. These results provide insight into the role that endothelial cell MHC Class I molecules play in inflammaging and sets the foundation to further study the molecular mechanisms that interplay in neuropathology.

Disclosures: J. Thelwell: None. **A.J. Johnson:** None. **M. Maynes:** None. **M. Pedra Seady:** None. **A. Hassani:** None. **F. Jin:** None. **M. Hansen:** None. **C. Owens:** None.

Presentation Number: NANO40.07

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

Support: NIH NIA P20AG068077
Harvey Family Endowment

Title: Examining Vascular Integrity, Inflammation and Cognitive Decline

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Abstract: How vascular factors contribute to both Alzheimer's (AD) and lead to cerebrovascular dementia is unknown. Our UNM Brain Bank contains over 270 consented post-mortem brains from New Mexicans, an ethnically diverse population with 61% Hispanics. Hispanics have a high incidence of mixed dementias (AD plus vascular) as well as pure vascular cognitive impairment and dementia (VCID). White matter injury from vascular pathology alone may present clinically with cognitive impairment. MMP1, MMP3 and MMP10 in CSF and plasma distinguish vascular from AD and mixed dementias. We hypothesized that leakage of these enzymes together with infiltration of macrophages within white matter in the context of dysfunctional autophagy underlie cognitive impairment. We are developing new MRI-pathology correlations to identify abnormalities in ante-mortem MR in post-mortem specimens, focusing on MR hyperintensities, vascular pathology and scores for cognition. We scored cases for AD or VCID by classical histopathology (Bielschowsky-Hirano silver for Amyloid/Braak/CERAD (ABC)) on medial frontal (MFG), inferior parietal lobule (IPL), and hippocampus with p-tau by immunohistochemistry. Cerebral vasculature was examined in H&E-stained slides throughout the brain for evidence of vascular pathology and/or microhemorrhages. As a first step to test our hypothesis, we selected biomarkers for autophagy (ATG) and for small vessel brain injury (µVBI) and investigated their expression and distribution by histopathology in specimens in our UNM Brain Bank. Alterations in ATG expression and localizations (LC3 and p62) corresponded to AD scores. For vascular markers, three matrix-metalloproteinases, MMP1, 3 and 10, that appear in CSF of subjects with mixed but not pure AD dementias, and CD31 for endothelial cells. We performed immune-histochemistry for MMP1, MMP3 and MMP10 on 9 cases with and without AD, VCID and mixed dementias and on one new case of suspected Binswanger's disease. Brain sections were also stained for CD68 (macrophages),TMEM119 (microglia), CD31

(endothelium), Luxol-Fast Blue & GFAP (myelin), and colloidal iron (heme). We further developed serial sectioning and double chromogen staining to correlate locations of p-tau, MMPs, and inflammatory markers**.** MMP1 stained arterial smooth muscle and capillaries part-way along, but not venous structures. MMP10+ perivascular macrophages were more prevalent in cases with evidence of VCID. Loss of myelin corresponded to regions of hyperintensity on ante-mortem MR, where groups of CD68+ cells and loss of TMEM119+ were found. A complex pattern emerges from the expression patterns and distribution of these biomarkers.

Disclosures: E.L. Bearer: None. **K. SantaCruz:** None. **G. Rosenberg:** None.

Presentation Number: NANO40.08

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

Support: NIH/NIA Grant 7R01AG081874-02

Title: Photobiomodulation modulates PRMT4 to improve cerebral blood flow in Alzheimer's Disease

Authors: *M. S. B. UDO[1], C. T. CITADIN[2], J. ZACCARELLI MAGALHÃES[2], L. H. MATUGUMA[2], D. D. J. SMITH[2], J. LANGMAN[2], Q.-G. ZHANG[3], H. LIN[2];
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Abstract: Background: Alzheimer’s disease (AD) is one of the most common form of dementia in elderly population reaching nearly seven million people in US. Women are more prone to develop it than men. Chronic cerebral hypoperfusion have been observed alongside neurofibrillary tangles and β-amyloid plaques. Focal and global cerebral ischemia can cause massive neuronal death, thus, improving cerebral perfusion could prevent the development or at least slow down the progression of the cerebral injury in AD. Overexpression of protein arginine methyltransferase 4 (PRMT4) can be related to the hypoperfusion observed in the brain of aged female 3xTg-AD mice (AD mouse model), by reducing NO release. Photobiomodulation (PBM) is a non-invasive alternative treatment for stroke and traumatic brain injury to reduce neuronal apoptosis by increasing regional cerebral blood flow. Thus, our aim is to investigate PBM as an alternative treatment for AD, through modulation of PRMT4, using female 3xTg-AD mice (AD mouse model). **Methods:** Aged (12-month-old) female 3xTg-AD and C57BL/6J mice were exposed to a continuous-wave low-level laser (808 nm, 25 mW/cm2) placed 35 cm above the animal’s scalp in order to generate an area of 1.5 cm2, for 4 min sessions/day, for 14 days. After the treatment, we evaluated the animal 1) microvascular perfusion using laser doppler and confocal microscopy with RECA1 (endothelial marker) and FITC-dextran; 2) Blood Brain Barrier (BBB) integrity using Evans blue dye (IV), RECA1 and PDGFRb (endothelial and pericyte markers respectively); and 3) animal spatial memory via Barnes maze. **Results:** Our preliminary results show that PBM reverted the hypoperfusion in aged 3xTg-AD mice, decreased the protein expression of PRMT4 ($p<0.05$) and ADMA (PRMT4 metabolic product - $p<0.05$), and increased Notch1 expression. Moreover, PBM preserved the amount of pericytes around the brain vasculature ($p<0.05$), and decreased leakage of Evans blue, suggesting that BBB integrity was restored in the aged 3xTg-AD brain. Further, mice treated with PBM presented reduced latency to find the escape box and spend more time in the target quadrant in the Barnes maze,

suggesting better cognition than mice not treated ($p<0.05$). **Conclusion:** Our preliminary results suggest that PBM for 14 days decreased PRMT4 expression and activity (decreased ADMA), improving the cerebral blood flow by increasing Notch1 expression, and preventing BBB leakage. Together with improved cerebral perfusion we observed improved cognition in the AD mice, suggesting that PBM has potential to be used as an alternative/ adjunctive treatment for AD.**Acknowledgements**: Funding and support from the NIH/NIA 7R01AG081874-02.

Disclosures: M.S.B. Udo: None. **C.T. Citadin:** None. **J. Zaccarelli Magalhães:** None. **L.H. Matuguma:** None. **D.D.J. Smith:** None. **J. Langman:** None. **Q. Zhang:** None. **H. Lin:** None.

Nanosymposium

NANO41: Auditory Processing and Perception

Location: MCP Room S106

Time: Tuesday, October 8, 2024, 8:00 AM - 10:15 AM

Presentation Number: NANO41.01

Topic: D.05. Auditory and Vestibular Systems

Support: ANR-20-CE28-0007

Title: Spatiotemporal Processing of Speech and Melody in the Human Brain

Authors: *A. GUPTA, B. MORILLON;
INS, INSERM, Aix-Marseille Univ., Marseille, France

Abstract: The human brain processes speech and melody differently, with the left hemisphere predominantly processing temporal features and the right hemisphere processing spectral features. This asymmetric processing raises questions about the underlying neural dynamics of acoustic feature encoding for speech and music. To further investigate this, we recorded intracranial EEG data from seventeen epileptic patients with implants in primary and associative auditory cortical regions. We utilized a stimulus set comprising one hundred a cappella songs, each presented in three versions: temporally degraded, spectrally degraded, and original. The study involved two phases: a binary choice task where participants identified whether pairs of song excerpts were identical or different, followed by a passive listening phase while watching a silent documentary. We used Riemannian-space features to train a non-linear classifier to distinguish between sentences and melodies while examining the encoding of temporal and spectral modulations in all patients. Behavioral results indicated a decrease in sentence recognition under temporally degraded conditions and a decrease in melody recognition under spectrally degraded conditions. Decoding accuracies corroborated these findings, showing that speech processing depends predominantly on temporal modulations, whereas melody processing relies on spectral modulations. These patterns persisted consistently across different times and channels, suggesting a spatiotemporal code within the auditory system. Additionally, time-frequency analysis across all patients highlighted the distinct roles of theta and delta frequency bands in encoding these temporal and spectral cues, respectively. In future research, we aim to explore the specific neural networks involved in processing speech and melody.

Disclosures: A. Gupta: None. **B. Morillon:** None.

Presentation Number: NANO41.02

Topic: D.05. Auditory and Vestibular Systems

Support: 2022ZD0204804
2022ZD0204802

Title: Representations of music and speech in human auditory cortex and the deep convolutional neural network

Authors: *R. YANG[1], G. CHEN[1], L. LUO[2], N. XU[3], F. FANG[1], Q. WANG[1];
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Abstract: Music and speech are two acoustic information carriers, essential for human communication in daily life. However, how music and speech are processed from the feature level to the category level in human auditory cortex is unclear. Also, it is unknown whether a deep neural network (DNN), capable of performing auditory tasks at human level, represents music and speech in a manner akin to human auditory cortex.
Here, we recorded intracranial electroencephalography (iEEG) signals in epileptic patients (n = 55) when they listened to music (instrumental melodies) and speech (two-syllable words). A subset of patients (n = 14) were also presented with synthetic music and speech with frequency features matched with each natural sound. We compared the high-gamma activity (HGA, 60-150 Hz) in response to music and speech and identified 173 music-selective contacts and 133 speech-selective contacts in auditory cortex.
Further, we extracted the time-frequency features of HGA and computed the cosine distance between the neural responses to each pair of sounds. In the selective contacts, we found the distances computed for sounds within the preferred category are smaller compared to those within the non-preferred category. In contrast, the distances computed for synthetic sounds within the preferred category and non-preferred category showed no difference. This result indicated that the selective neuronal populations represent sounds within the preferred category more similarly, which could not be explained by tuning to frequency features.
To explore the representation of music and speech in DNNs, we passed the same stimuli through a DNN trained to discriminate music and speech separately. We analyzed unit activations elicited by music and speech and identified music- and speech-selective units. For each layer, we defined the collective activations of all music-selective units as the music-selective response and those of all speech-selective units as the speech-selective response. Then, we computed the cosine distance for music- and speech-selective responses, respectively. Notably, in the fc6 layer, we found the distances computed for sounds within the preferred category are smaller compared to those within the non-preferred category, aligning with findings in neural responses.
In summary, our research reveals the category-selective neuronal populations in the human auditory cortex exhibit representations akin to those found in the late stages of a DNN trained on auditory tasks, suggesting a parallel in the high-level feature processing between auditory cortex and the DNN and offering insights into the neural mechanisms underlying auditory perception.

Disclosures: R. Yang: None. **G. Chen:** None. **L. Luo:** None. **N. Xu:** None. **F. Fang:** None. **Q. Wang:** None.

Presentation Number: NANO41.03

Topic: D.05. Auditory and Vestibular Systems

Support: NSTC grants #111-2423-H002-002

Title: Speech encoding acuity relies on phase-locking in human auditory periphery

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Abstract: In human auditory pathways, the Auditory Periphery (AP) system encodes sound from the ear to the cochlear nuclei. AP system bidirectionally interacts with the higher auditory system, including Medial OlivoCochlear (MOC), inferior colliculus, etc., and goes toward the auditory cortices through Auditory Nerve Fibers (AN-Fs).
ANFs have shown phase-locking to the temporal structure acuity of complex sound stimuli below 2k-5kHz. However, it is unclear how AN phase-locking determines speech perception. Simulation studies were conducted utilizing 1) a simulated human AP model and 2) our decoding model to simulate human speech perception. The decoding model was deep artificial neural networks that reconstruct high-fidelity sounds based on ANFs' spiking activities.
To simulate the human AP model under the Normal Hearing (NH) condition, Ray Meddis' Matlab Auditory Periphery (MAP) model was used. The Acoustic Reflex and MOC Reflex in the efferent pathways were simulated by MAP. The representations of simulated ANFs' spiking activities were called auditory neurograms.
In phase-locking elimination experiments, there were NH and two Limited Hearing (LH) conditions. To create LH conditions, low-pass filters with cutoff frequencies at 50 and 1k Hz were applied to the NH auditory neurograms. Thus, the filtered neurograms had limited information of speech stimuli.
To simulate human speech perception, the LJSpeech dataset was used for training models under NH and LH conditions using our decoding model, and its first 20 wav files were in the test set. The NH model was constructed using the decoding model from our previous study. To test the speech reconstruction performance of these models, Structural Similarity Index Measure (SSIM) was computed to examine the similarity between the spectrograms of original and reconstructed sound stimuli. The NH model achieved mean SSIM scores of 0.9212. On the other hand, the LH models with eliminated AN phase-locking were trained on the filtered neurograms using our decoding model. The results showed mean SSIM scores of 0.654 and 0.823 for the 50 and 1k Hz LH models, respectively. A two-sample *t*-test showed significant differences between each model pair among NH and two LH conditions ($p<0.0001$). Therefore, the LH models performed worse on sound reconstruction, compared to the NH model.
In conclusion, these results suggest that AN phase-locking plays a crucial role in human speech perception in our simulation experiments.
This study implies that our decoding model could be used for accurate human hearing simulations under various conditions, such as impaired hearing and hearing loss.

Disclosures: P.B. Liu: None.

Presentation Number: NANO41.04

Topic: D.05. Auditory and Vestibular Systems

Support: NSF Grant BCS-2242080

Title: A comparative analysis of vocal vs manual synchronization to auditory rhythm

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Abstract: Engaging in rhythmic movements, such as grooving or dancing, in response to music is a ubiquitous human behavior. Particularly noteworthy are behaviors involving motor synchronization to auditory rhythms, which are distinctively observed in humans and parrots. From the speech perspective, rhythm synchronization is hypothesized to be a prerequisite for speech coordination between the dorsolateral system involving pitch-related functions and the ventrolateral system involving phonetic articulation functions. This study aims to explore the cognitive rhythmic mechanisms underpinning these phenomena, particularly focusing on how different motor effectors may exhibit preferences for specific rhythm rates. We selected to compare the supralaryngeal vocal tract and finger as representative motor effectors for speech/singing and body movement, respectively. Given that speech typically occurs at a syllabic rate of approximately 4 Hz, while spontaneous finger-tapping or walking tends to occur around 2 Hz, we investigated motor synchronization to auditory rhythms ranging from 2 Hz to 4 Hz. Participants were instructed to either tap their fingers or vocalize along with auditory rhythms presented at varying rhythms. Preliminary results indicate that both motor effectors exhibit a preference for higher frequencies over lower ones, with accuracies improving from 2 Hz to 4 Hz. Interestingly, we observed individual differences in preferred motor effector, which may be due to participants' musical training backgrounds. These findings shed light on the intricate interplay between motor coordination and rhythmic processing, offering insights into how humans synchronize their movements with auditory rhythms. Further analysis of individual differences and the influence of musical training may provide deeper understanding into the cognitive mechanisms governing rhythmic behavior.

Disclosures: J. Wan: None. **J. Venezia:** None. **G.S. Hickok:** None.

Presentation Number: NANO41.05

Topic: D.05. Auditory and Vestibular Systems

Support: MRC Grant MR/T032553/1
NIH Grant R01-DC04290

Title: Neural bases of illusory acoustic texture perception

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Abstract: Many of the sound scenes we encounter consist of a large number of stochastically timed similar events that together form acoustic textures, such as the sound of a rain shower. These can typically be characterised by a relatively sparse set of summary statistics, such as correlations between modulation envelopes across frequency bands (McDermott & Simoncelli, Neuron 2011). Listeners are thought to automatically extract such statistics when perceiving and remembering textures (McDermott et al., Nat Neurosci 2013). Like other sounds, textures can be perceived to continue through sufficiently loud interrupting white noise, even when the texture is physically absent (McWalter & McDermott, Nat Comms 2019). In the case of textures with stable summary statistics, this illusion can last for several seconds. We tested the hypothesis that such persistence draws on neural circuits beyond auditory cortex, including hippocampus. We presented many exemplars of two different textures to six neurosurgical participants. In each trial, two seconds of texture were followed by two seconds of white noise and then a final second of texture. In a control condition, 200-ms silent gaps were inserted either side of the noise. All participants reported perceiving the illusion in the continuous case, with a significant drop in such reports when gaps were present. Only when the texture was physically present could its identity be decoded from single-neuron firing and high-gamma power in auditory cortex. In contrast, a decoder using theta power across multiple electrodes was able to identify not only the physically presented texture, but also the texture perceived during the white noise. Channels in regions beyond primary auditory cortex, including planum polare and hippocampus, carried the greatest weight in these decoders. In the substantial majority of participants, this decodability based on theta power dropped to chance level when silent gaps buttressed the noise to suppress the illusion. We continue to study the extent to which the extraction and persistence of summary statistics through several seconds of interruption draws on brain areas with longer intrinsic timescales than primary auditory cortex, and interactions between these regions.

Disclosures: A. Billig: None. **J.I. Berger:** None. **R. McWalter:** None. **T. Teichert:** None. **C.M. Garcia:** None. **C.K. Kovach:** None. **C.I. Petkov:** None. **H. Kawasaki:** None. **M.A. Howard:** None. **J. McDermott:** None. **T.D. Griffiths:** None.

Presentation Number: NANO41.06

Topic: D.05. Auditory and Vestibular Systems

Support: Simons Society of Fellows Junior Fellowship

Title: Identifying Neural Dynamics that Aid and Impede Cochlear Implant Speech Processing Using a Time-dependent Deep Neural Network Model of the Auditory Stream

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Abstract: Cochlear implants(CIs) are arguably the most successful neural implants in clinical use. They use sparse encoding and simple pulsatile stimulation patterns yet provide sufficient

information to the auditory stream for CI users to comprehend speech. Still, CI users have deficits including poor speech-in-noise comprehension. The spatial and temporal differences at the cochlear level suggest that much of the success of CI users comes from the ability of the auditory stream to incorporate context and extract information from the degraded signal. Standard clinical scenarios and recording technologies cannot be used to probe how auditory system solves this problem in fine detail, so we create a deep neural network(DNN) model of the auditory stream to use to probe information about auditory processing over time. We also use biophysical modeling approaches to simulate CI-generate cochlear implant responses to use as inputs to the model to compare to naturally generated inputs. We then investigate differences in processing spoken sentences over time and outputting the phonemes heard over time when the model experiences simulated CI inputs and natural inputs. We use a version of DeepSpeech2 comprised of causal LSTMs and linear layers that takes spectrogram inputs and produces phoneme predictions over time. We compare processing of standard 64-channel spectrograms (natural) to CI-generated spectrograms, made using the Advanced Bionics Python Toolbox, from 1000 hours of English sentences from LibriSpeech. We compare DNN performance to human studies from normal hearing and CI subjects and find that our model shows a similar pattern of phonetic confusion in both cases in quiet and with increasing noise levels. We also see increases in time to phoneme prediction when our model processes CI inputs, which mimics increased reaction times observed in humans, and increasing rates of phoneme substitution, omission, and addition that correlate to those observed in human studies. We then investigate how and where in the hierarchical processing of the neural network errors and correct phoneme comprehension occur. We use decoding methods to identify the source of increased phoneme confusion in earlier layers of the DNN and show how it leads to biases in phoneme prediction, especially in the presence of noise. We also find that the dynamics for all phonemes occur with a consistently timed trajectory in which phoneme identity is processed with increasingly delayed time scales deeper in the auditory network. These results point to a shared method for phoneme processing at the network level that can be targeted for future intervention when improving cochlear implant algorithms.

Disclosures: C. Steinhardt: None. **K. Stachenfeld:** None. **N. Mesgarani:** None.

Presentation Number: NANO41.07

Topic: D.05. Auditory and Vestibular Systems

Support: MRC MR-Y014693-1

Title: Distinct auditory cortical signatures of hearing impairment and genetic risk for psychiatric disease in a mouse model of 22q11.2 Deletion Syndrome

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Abstract: People with 22q11.2 Deletion Syndrome (22q11.2DS) have high risk of psychiatric disorders such as schizophrenia and of hearing impairment from middle ear problems. Both hearing impairment and genetic risk for schizophrenia are thought to shift "cortical excitation-inhibition balance" towards excitation, but the definition, nature and impact of this shift are

debated. Here, we used the *Df1/+* mouse model of 22q11.2DS to ask whether hearing impairment and the 22q11.2 deletion have similar or different effects on evoked activity of neuronal populations in the auditory cortex. To mimic middle ear problems affecting a subset of the *Df1/+* mice (n=6 hearing impaired, n=4 normal hearing), we performed ear surgery on WT mice at P11, removing the malleus bone (n=5) or creating sham controls (n=6). Auditory brainstem response threshold measurements at ages 4, 6 and 8 weeks confirmed that malleus removal produced hearing impairment in WT mice comparable to that observed in affected *Df1/+* mice. Then, we recorded spiking activity of auditory cortical neurons (*Df1/+*: 1122 neurons; WT: 1772 neurons) using Neuropixels probes in awake, head-fixed mice listening passively to 16kHz tones at sound levels adjusted relative to hearing threshold. To test cortical adaptation and excitability, we measured changes in tone-evoked firing rates as we increased inter-stimulus interval (ISI=200-1000 ms) and relative sound level (level=threshold+0-30dB). The ISI dependence of evoked firing was abnormally low in *Df1/+* mice compared to WT mice with similar hearing thresholds, and there was no interaction between genotype and hearing ability (ANOVA $p_{genotype}<0.001$, $p_{hearing}=0.13$, $p_{genotype\times hearing}=0.42$). This result was robust across different time intervals for measuring evoked firing rate, different normalizations of evoked activity, and different selection criteria for neurons included in analysis. In contrast, level dependence of evoked firing did not vary with genotype or hearing ability, but phase coherence of the evoked LFP was robustly elevated in mice with hearing impairment regardless of their genetic status. We conclude that hearing impairment and the 22q11.2 deletion have distinct effects on neuronal population activity in auditory cortex.

Disclosures: C. Lu: None. **J.F. Linden:** None.

Presentation Number: NANO41.08

Topic: D.05. Auditory and Vestibular Systems

Support: Wellcome Trust [WT108369/Z/2015/Z].

Title: Contribution of descending auditory corticofugal projections to the spectral and spatial response properties of neurons in the inferior colliculus

Authors: F. R. NODAL[1], I. SOOD[1], A. J. KING[1], ***V. BAJO-LORENZANA**[2]; [1]Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [2]Dept. of Physiol., Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: The descending projection from the auditory cortex to the inferior colliculus (IC) has been implicated in auditory spatial learning, the passive processing of speech-in-noise, innate defensive fear behavior, and processing of behaviorally-salient stimuli. However, stimulation of auditory cortical inputs has both excitatory and inhibitory effects on the activity of IC neurons and some neural properties, such as the spectrotemporal tuning properties of these neurons and their contrast gain control, seem to be independent of cortical inputs. How auditory cortex influences the response properties of IC neurons is therefore far from being understood. We optogenetically activated corticocollicular neurons in anesthetized ferrets and recorded IC activity using Neuropixels probes to explore the effect of these descending inputs on the frequency selectivity of IC neurons and their spatial sensitivity. Channelrhodopsin (ChR2) was expressed in corticocollicular neurons by injecting retrograde virus encoding Cre recombinase

(Retro2 AAV.Cre.GFP) at multiple sites in the IC and a viral construct encoding ChR2 in reversed fashion under the FLEX cassette (AAV.Flex.ChR2.mCherry) at multiple locations in the primary auditory cortex (A1) 4-6 weeks before the recordings. Activation of ChR2 expressed in corticocollicular neurons was achieved by blue laser light illumination delivered by an optic fibre with an intensity of 10 mW at the tip placed in the center of the middle ectosylvian gyrus, where A1 is located. Activation of corticocollicular inputs increased the magnitude of the driven responses to pure tones of most neurons recorded in the central nucleus (CNIC) and the dorsal (DCIC) and external cortex (ECIC) of the IC, while decreasing their frequency selectivity in DCIC and ECIC but not in the CNIC. By contrast, other neurons (24%) showed the opposite effect. In addition, activation of the corticocollicular pathway shifted the frequency tuning of IC neurons, increasing the representation of the most sensitive mid-frequency region (around 10 kHz) in the ferret audiogram. The responses of IC neurons to broadband noise presented in virtual acoustic space also became stronger and more broadly tuned following optogenetic activation of corticocollicular inputs. Our results demonstrate pronounced effects of cortical activation on the spectral and spatial response properties of IC neurons, paving the way for future experiments in behaving animals to explore the role of this descending circuit in active listening.

Disclosures: F.R. Nodal: None. **I. Sood:** None. **A.J. King:** None. **V. Bajo-Lorenzana:** None.

Presentation Number: NANO41.09

Topic: D.05. Auditory and Vestibular Systems

Support: MSCA PROOPI340-USAL4EXCELLENCE
PID2019-104570RB-I00

Title: Medial prefrontal cortex outputs contribute to prediction error generation in the auditory cortex

Authors: *A. HOCKLEY, L. HERNÁNDEZ BOHÓRQUEZ, M. S. MALMIERCA;
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Abstract: Under the Bayesian brain hypothesis, the brain continuously generates a model of the environment based on predictions gained from previous experiences. Incoming sensory information is compared to this model and either confirms the prediction, or if significantly different enough, prediction errors update the generative model. Using the auditory “oddball” paradigm, neural correlates of prediction errors are observed as early as the auditory midbrain, with the strength of error increasing up the auditory hierarchy to the auditory cortex (AC). The medial prefrontal cortex (mPFC), which is involved in planning complex behaviour and decision making, exhibits strong and long-lasting responses solely to auditory deviants, consistent with coding of prediction error. This raises the hypothesis that mPFC exerts a top-down control of deviance detection in sensory cortices, by transmitting prediction signals. To test this, we injected Female Long-Evans rats with 1 µl AAV5-hSyn-eNpHR3.0-EYFP to the mPFC to allow optogenetic suppression of mPFC neurons. After 7-11 days of recovery, 64-channel neural recordings were conducted in the AC under urethane anesthesia. An auditory “oddball” paradigm composed of standard (STD) repeating stimuli, deviants (DEV) and no-repetition controls (CTR) were presented monaurally. This allowed decomposition of the neural mismatch effect into two components: repetition suppression and prediction error, which were measured during

suppression of mPFC neurons. Rats showed expression of EYFP throughout the mPFC, demonstrating successful optogenetic virus transfection. Further, neural recordings using an optrode in the mPFC showed that local LED illumination reduced activity of mPFC neurons during spontaneous and auditory-evoked activity. LFP and single-unit recordings in the AC during the auditory "oddball" paradigm showed robust neural mismatch, with responses to DEV stimuli greater than CTR stimuli, and limited responses to STD stimuli. Inhibition of the mPFC had no effect on neural responses during STD or CTR stimuli, but reduced LFP amplitudes and single-unit responses to DEV stimuli, providing evidence for top-down predictive transmission from mPFC which enhances AC responses to unpredicted stimuli. The reduced deviant response during mPFC inhibition was accompanied by reduced neural synchrony in the auditory cortex, suggesting weakening of the previously described cortical deviant-detector ensembles.

Disclosures: A. Hockley: None. **L. Hernández Bohórquez:** None. **M.S. Malmierca:** None.

Nanosymposium

NANO42: Circuit Dynamics Across Brain Regions During Navigation

Location: MCP Room N228

Time: Tuesday, October 8, 2024, 8:00 AM - 11:30 AM

Presentation Number: NANO42.01

Topic: H.09. Spatial Navigation

Support: Wellcome Trust (223144)
European Union's Horizon 2020 (101022757)

Title: Spatial modulation of sensory activity in multiple brain regions

Authors: *E. H. VAN BEEST, B. TERRY, K. D. HARRIS, M. CARANDINI;
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Abstract: Introduction. Navigation is a complex goal-directed behavior that relies heavily on sensory perception. A key center is the hippocampal formation, where well-known 'place cells' and 'grid-cells' encode the animal's position. Space-related information coding has also been found in other brain regions, including the anterior thalamic nucleus and cortical regions including parietal, visual, and even olfactory cortex. Here we ask whether spatial coding is even more widely distributed across the brain. Additionally, we ask whether spatial and sensory coding occur in separate populations, or integrate within single neurons. Methods. We used Neuropixels probes to record in mice navigating a virtual linear corridor, recording >2000 neurons from regions including visual, somatosensory, retrosplenial, and motor cortex, the hippocampal formation, the dorsal thalamus, striatum, and midbrain. On each trial mice traversed a corridor consisting of two visually-identical halves, containing landmarks whose contrast varied across trials. In a subset of trials auditory cues also indicated landmark locations. The gain of the running wheel varied across trials, to decouple physical and virtual position. We predicted each neuron's activity by summing a "place field" (generic function of position) with temporal kernels for sensory stimuli, reward, and running speed. Results. Neurons whose activity was

modulated by position in the corridor were found in every recorded region. The fraction of position-modulated neurons varied from 8% in medial geniculate nucleus to 56% in the somatosensory cortex, relative to a 24% in CA1 of the hippocampus. However, position-modulation in the somatosensory cortex was coarse, and neurons did not exhibit a sequence of 'place fields' as we know from neurons in CA1. Similar results were found for the encoding of sensory intensity (4-43% of neurons, depending on the region) or reward (10-62% of neurons), and running speed (34-95% of neurons). There was a positive correlation between the number of neurons modulated by virtual position or sensory intensity and the number of neurons that were modulated by both. Conclusions. These results indicate that spatial coding is widely distributed across the brain, and that the proportion of neurons integrating both sensory and spatial information is highest in regions with prominent encoding of those signals.

Disclosures: E.H. van Beest: None. **B. Terry:** None. **K.D. Harris:** None. **M. Carandini:** None.

Presentation Number: NANO42.02

Topic: H.09. Spatial Navigation

Support: NIH R01 NS121413
N00014-23-1-2086
HFSP RGP0045/2022

Title: Contributions of vision, echolocation and memory to Egyptian fruit bat navigation

Authors: N. FINGER[1], K. EVELAND[1], X. YIN[1], S. CHITNIS[2], G. CAPSHAW[1], A. KAPLANOGLU[1], W. CHEN[1], A. KRISHNAN[3], ***C. MOSS**[1];
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Abstract: Egyptian fruit bats, *Rousettus aegyptiacus*, have access to multimodal sensory information and spatial memory to navigate complex three-dimensional environments. Employing both scotopic vision and tongue click echolocation, they can detect and localize objects with little or no light. Tracking of bats in the wild demonstrates their use of cognitive maps to navigate across large and complex foraging routes. Here we report on the relative contributions of vision, echolocation, and spatial memory to Egyptian fruit bat navigation. In a laboratory flight room, bats were trained to land on a perch, during which their echolocation was recorded with a 25-channel microphone array, and their head orientation and flight paths with 16 motion capture cameras. We first investigated the bat's use of spatial memory, vision, and echolocation to navigate under light and dark conditions. In baseline trials, bats navigated to a landing perch at a fixed location over 6 - 8 successive trials. In experimental trials, the perch location was shifted by either 15 or 30 centimeters. In the light, bats modified their flight paths to successfully land on a perch displaced by 15 cm but failed when the perch was displaced by 30 cm. Surprisingly, in the dark, bats consistently failed to land on the perch when it was displaced by only 15 cm. In a second experiment, we introduced conflict between vision and echolocation. Bats wore goggles fit with prism lenses that shifted visual images horizontally by 23 degrees to the left or right. Control trials were run with goggles fit with clear or light-blocking lenses. In experimental trials, bats initially flew in the direction of the prism shift and missed the perch, but

gradually adjusted their flight trajectories to land on the perch. The role echolocation played in this adjustment was explored by disrupting the bat's use of echolocation with sound attenuating ear molds. Bats wearing prisms were unable to land when echolocation was disrupted. These findings suggest that Egyptian fruit bats rely on a combination of vision, echolocation, and spatial memory for navigation. Primarily, this bat species relies on visual cues to navigate, with echolocation serving as a robust auxiliary mechanism under conditions when visual information is unreliable or absent. Furthermore, when confronted with discrepancies between established internal representations and sensory information, bats preferentially rely on spatial memory, especially when sensory data is sparse or ambiguous.

Disclosures: N. Finger: None. **K. Eveland:** None. **X. Yin:** None. **S. Chitnis:** None. **G. Capshaw:** None. **A. Kaplanoglu:** None. **W. Chen:** None. **C. Moss:** None.

Presentation Number: NANO42.03

Topic: H.09. Spatial Navigation

Support: NIH NINDS NRSA F32NS116023
NIH 2R01MH101297

Title: Entorhinal Cortical Subregions Differentially Encode Visual and Olfactory Spatial Information

Authors: *H. DAVOUDI, J. R. CLIMER, J. B. ISSA, D. A. DOMBECK;
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Abstract: As rodents navigate their habitat, they can use multiple spatially-tuned sensory modalities, such as visual or olfactory, 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 modalities (e.g., visual or olfactory) can be difficult to control or measure independently and precisely. We therefore built a multisensory virtual reality environment with independently controlled visual and olfactory cues. We studied the cognitive map in this environment 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 whether each of them preferentially encodes one sensory-spatial modality. To address this, we imaged neural populations in MEC and LEC in separate mice receiving rewards at visual or olfactory targets in our multi-sensory spatial environment. We classified the neurons by the sensory coordinate they best coded, and we found that both MEC and LEC represent visual-spatial and olfactory-spatial information, yet in different proportions. While MEC primarily encoded visual-spatial information, LEC primarily encoded olfactory-spatial information. Moreover, the level of encoding of visual and olfactory spaces depended on the context of different tasks that mice

performed. These findings shed further light on the neural mechanisms of multisensory spatial representations in the cortico-hippocampal network.

Disclosures: H. Davoudi: None. **J.R. Climer:** None. **J.B. Issa:** None. **D.A. Dombeck:** None.

Presentation Number: NANO42.04

Topic: H.09. Spatial Navigation

Support: NIH grant R01DC004260

Title: Head-fixation alters neural circuit dynamics of head-direction system during immobility

Authors: *A. PAK[1], J. AARSE[3], H. ZHU[2], S. CARRILLO SEGURA[2], J.-P. NOEL[2], A. A. FENTON[2], D. E. ANGELAKI[2];
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Abstract: Head-fixation in rodent models has become a prominent method for studying neural circuits, yet its impact on neural dynamics remains unclear. Here, we specifically explored the influence of head-fixation on the dynamics of the mouse thalamic head direction system, revealing impaired population coordination during stationary (immobile) periods. Using a 2D virtual reality (VR) setup, which enables mice to rotate their heads in the horizontal plane, and chronic Neuropixel recordings of head direction cells (HDC) in anterior thalamus, we discovered that stationary periods in head-fixed animals represent a distinct brain state. This was evident through an altered pairwise correlation structure, off-ring manifold activity, and lack of modulation of theta frequency by speed. To isolate contribution of head-fixation from VR, we developed a custom head-fixation apparatus that allows mice to freely explore an open-field arena while maintaining an immobile head relative to their body. Interestingly, despite the mice being exposed to all sensory cues, the HDC exhibited similar alterations during stationary periods as observed in VR. Crucially, we show that in both conditions, the fundamental property of HDC, firing exclusively at their preferred direction, is compromised when the head is restrained. During periods of immobility, HDC with preferred directions close to the current orientation reduce their activity, whereas other non-preferred HDC increase their firing, leading to off-ring manifold activity. Simulations of ring-attractor models indicate that disruption of recurrent connectivity of the HDC attractor, resulting in non-preferred head direction firing, leads to off-ring manifold dynamics, as observed in experimental data. Collectively, these findings suggest that stationary periods in head-fixed mice signify a distinct brain state of HD system, potentially due to the lack of gravity cues. Given the extensive use of head-fixation preparation in systems neuroscience, future studies should consider its potential impact on the specific system under investigation.

Disclosures: A. Pak: None. **J. Aarse:** None. **H. Zhu:** None. **S. Carrillo Segura:** None. **J. Noel:** None. **A.A. Fenton:** None. **D.E. Angelaki:** None.

Presentation Number: NANO42.05

Topic: H.09. Spatial Navigation

Support: NIH (R01 MH117777)
McKnight Endowment Fund for Neuroscience
NIH (P51 OD010425)
Simons Foundation (542955)

Title: Task Events Govern Neuronal Activity in the Monkey Hippocampus

Authors: *J. RUECKEMANN[1], Y. BROWNING[2], A. MALLORY[3], B. KIM[4], A. L. FAIRHALL[5], E. A. BUFFALO[6];
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Abstract: Hippocampal responses have been demonstrated in a variety of tasks without an explicit spatial component, invigorating debate about what drives hippocampal neurons. One hypothesis is that the structure of the task and its defining salient events drive reliable hippocampal activity.
To address this hypothesis, we examined peri-event temporal coding in the hippocampus of monkeys performing two virtual tasks. Neurons (n=3570) were recorded from the full anatomical extent of the hippocampus in three rhesus macaques using chronic drives housing 124 independently movable electrodes. To facilitate comparisons across species, the monkeys performed virtual versions of standard behavioral tasks used in rats: delayed spatial alternation on a Y-maze and open-field foraging. In the Y-maze, 30% of neurons demonstrated task-related activity that was reliable across trials. Notably, these responses were not randomly distributed across space, as would be expected with canonical place cells. Instead, we found that the salient events of the task (e.g. Start, Choice, Reward, Delay) demarcate the temporal boundaries of spiking. Analysis of the aggregate population revealed that boundaries between salient events lead to discontinuities in the population activity, creating distinct representations in the population code for each task phase. In addition, 84% of task-responsive neurons did not remap their responses across visually distinct environments, indicating that the activity of most cells reflects an abstract representation of the well-learned task rather than the specific objects visualized.
Because task phase is inextricably tied to allocentric space in the Y-maze, these results do not fully resolve the question of whether space is a necessary feature of the hippocampal code. Accordingly, we examined activity in an open-field foraging task, where the arbitrary routes to randomized rewards decouple allocentric position from the time course of the foraging behavioral sequence (Orientation, Pursuit, Reward). Strikingly, no neuron in the foraging task demonstrated spatial selectivity, but 17% of neurons exhibited consistent peri-event coding for the behavioral events. Together, the results across experiments indicate that representing task structure is foundational to the hippocampal code and suggest that the generalized function of the hippocampus may be to build temporally-ordered structural representations linking the key events guiding behavior.

Disclosures: J. Rueckemann: None. **Y. Browning:** None. **A. Mallory:** None. **B. Kim:** None. **A.L. Fairhall:** None. **E.A. Buffalo:** None.

Presentation Number: NANO42.06

Topic: H.09. Spatial Navigation

Support: NSF NCS-FO 1734744
N00014-17-1-2736
NIH NINDS R01 NS121413

Title: Hippocampal encoding of object distance in the echolocating big brown bat,Eptesicus fuscus

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Abstract: Recent work has shown that the CA1 region of the hippocampus encodes the location of a moving conspecific, in addition to representing the animal's self-position within an environment. These experiments involved a paradigm in which a passive observer attended to the location of a conspecific to solve a spatial task. It remains unclear whether actively tracking a behaviorally relevant non-social object also results in a representation of the object's location in the hippocampus. Elucidation of these neuronal signatures requires quantifying sensory processing in animals engaged in spatial tasks. The echolocating bat emits high-frequency sounds and processes the time delay between calls and echoes to estimate object distance. Since bats adjust the duration and rate of sonar calls in relation to object distance, this acoustic behavior offers a robust metric for object tracking. We recorded neural responses from the hippocampal CA1 region of the big brown bat, *Eptesicus fuscus*, as it performed a sonar target-tracking task in the dark. Bats were trained to perch on a platform and track a moving target (cluster of mealworms) approaching them from 3m. An array of ultrasonic microphones recorded the bat's echolocation calls, and a high-speed motion capture system measured its head direction and the target position. Multichannel neural recordings were taken from the hippocampal CA1 of three behaving bats, synchronized with audio and video data. We found that a population of hippocampal CA1 neurons encode the call-echo time delay/ distance to an object. We show that these representations exist in multiple coordinate frames, with a subset of cells encoding the egocentric distance of the object and others encoding its allocentric location. Population analyses revealed accurate decoding of the object distance with a relatively small number of neurons (n=15-20). During trials when the bat ceased echolocating, the distance code degraded, suggesting that these object representations depend on the bat's active tracking of the object. These results indicate that spatial attention to a non-social object produces a representation of the object's location in the hippocampus, which may be critical for scene representation during spatially guided behaviors in complex environments.

Disclosures: A. Krishna: None. **X. Yin:** None. **C. Yu:** None. **D.A. Skandalis:** None. **H. Lee:** None. **C.F. Moss:** None.

Presentation Number: NANO42.07

Topic: H.09. Spatial Navigation

Support: NIH 2R01MH101297
NIH F32NS116023

Title: Hippocampal Representational Drift Persists in a Stable Multisensory Virtual Environment

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Abstract: Place cell ensembles in the hippocampus had long been thought of as the stable substrate for spatial long-term memories. Experiments tracking place cells in mice navigating the same environment over days challenged this idea with evidence of significant changes in the representations, a phenomenon termed "representational drift". Drift could simply be a read-out of subtle differences in the sensory environment or mouse behavior that are difficult to measure and control, yet highly notable or perceptible to the animal. This idea is supported by data from the hippocampus CA1 of Egyptian fruit bats showing little drift over days when the behavior was highly stereotyped[1]. We sought to test if representational drift in mice can be explained by sensory and behavioral variability using the control possible under multisensory virtual reality. We first exploited the behavioral control achieved by head-fixed mice using a linear treadmill to determine if behavior impacts the rate of drift in hippocampal CA1 of mice. When we compared stereotyped behavior to more variable behavior, we found no detectable change in the rate of drift, suggesting that behavioral variability does not explain representational drift in mice. We then used the precise sensory control afforded by a multisensory virtual reality system[2] to test if sensory variability can drive drift. We controlled the odor environment by either presenting the same odorant over days or by providing different odors and odor concentrations across days. When we compared these conditions, we again found no detectable change in the rate of drift compared to when the odor environment was uncontrolled. This indicates that sensory changes, when not relevant to the task, are not a substantial driver of representational drift. Finally, when we divided place cells into stable and unstable populations, we found that features associated with the excitability of neurons could predict if a cell was going to be stable over days. This suggests that the intrinsic properties of neurons explain the difference in drift rates between different populations of neurons in CA1 of mice.
1.Liberti, W. A., Schmid, T. A., Forli, A., Snyder, M. & Yartsev, M. M. Nature 604, (2022).
2.Radvansky, B. A. & Dombeck, D. A. Nat. Commun. 9, (2018).

Disclosures: J.R. Climer: None. **H. Davoudi:** None. **J.Y. Oh:** None. **D.A. Dombeck:** None.

Presentation Number: NANO42.08

Topic: H.09. Spatial Navigation

Support: NIH Grant F32MH126643
NIH Grant DP2NS111657

Title: Rules of synaptic plasticity driving the shifting dynamics of hippocampal representations

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Abstract: Synaptic plasticity is widely thought to support memory storage in the brain, but the rules determining impactful synaptic changes in-vivo are not known. We considered the trial-by-trial shifting dynamics of hippocampal place fields (PFs) as an indicator of ongoing plasticity during memory formation. By implementing different plasticity rules in computational models of

spiking place cells and comparing to experimentally measured PFs from mice navigating familiar and novel environments, we found that Behavioral Timescale Synaptic Plasticity (BTSP), rather than Hebbian Spike Timing Dependent Plasticity, is the principal mechanism governing PF shifting dynamics. BTSP-triggering events are rare, but more frequent during novel experiences. During exploration, their probability is dynamic: it exponentially decays after PF onset, but continually drives a population-level representational drift. The probability of BTSP-induction controls the amount of shifting, whereas the shape of the learning rule controls the proportions of forward vs backward shifts. Finally, our results show that BTSP occurs in CA3 but is less frequent and phenomenologically different than in CA1. Overall, our study provides a new framework to understand how synaptic plasticity shapes hippocampal representations during learning.

Disclosures: A.D. Madar: None. **M. Sheffield:** None.

Presentation Number: NANO42.09

Topic: H.09. Spatial Navigation

Support: NIH Grant 1K99EY034700
Helen Hay Whitney Fellowship
New York Stem Cell Foundation – Robertson Neuroscience Investigator Award
Beckman Young Investigator Award
NIH New Innovator Award

Title: Place cells are activated by remote gaze in a highly visual animal

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Abstract: The spatial component of episodic memory is thought to depend on hippocampal place cells. It is still unclear, however, how the brain retrieves remote spatial memories without physically visiting the remembered location to activate corresponding place cells. Both primates and birds primarily experience their spatial context actively through foveal vision. Can remote viewing reinstate spatial representations of distant locations?
It is typically difficult to track gaze in freely moving animals. We found that black-capped chickadees (Poecile atricapillus), which have robust hippocampal place codes, mainly direct their gaze using head saccades rather than eye saccades. We took advantage of this behavior by developing a system to estimate gaze by tracking only the head, and designed a simple foraging task to behaviorally dissociate place and gaze locations.
Surprisingly, most place cells were active not only when the bird visited a particular location, but also when it viewed that same location from elsewhere. Thus place and gaze codes for each location coexist in the same neural population in this highly visual animal.
A striking difference between physical and visual exploration is the discrete nature of visual input, which is structured by head saccades occurring 3-4/s in chickadees. We therefore asked how gaze coding evolves over time during each saccade and subsequent fixation. The target of the current saccade was most strongly represented during two distinct time windows: during the saccade itself, and shortly after the saccade during the fixation. The gaze code during the first

window was affected by the animal's expectation of the state of a visual reward cue, whereas the second peak was affected by the actual state of the viewed cue. These temporal dynamics suggest that gaze saccades may organize alternating predictions and updates of visual location coding in the avian hippocampus.
Although continuous theta oscillations were not observed in the chickadee hippocampus, we wondered whether gaze saccades might similarly structure the activity of distinct populations of neurons. We examined the temporal dynamics of different classes of neurons during saccades and found three primary patterns of activation: one pattern in putative excitatory neurons and two patterns in putative inhibitory neurons. Overall, our results suggest that in a highly visual avian species, viewed location and actual location are represented by a shared neural code, and computation using this code is coordinated by active vision.

Disclosures: H.L. Payne: None. **D. Aronov:** None.

Presentation Number: NANO42.10

Topic: H.09. Spatial Navigation

Support: NIH Grant 2R01NS102537-06A1
NIH Grant 5U01NS131438-02
NIH Grant 1R01MH118926-01
ARO-MURI Grant 72929-EG-MUR-01

Title: Dynamic adaptation of CA1 place field activity in response to conflict between path-integration and landmark cues

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Abstract: The hippocampus forms a cognitive map of an environment within which an animal's location is encoded. This code is maintained by external landmarks, providing positional feedback once they become associated with the cognitive map, and by path integration, an internal computation that integrates self-movement velocities over time. In the absence of landmarks, path integration may accumulate errors, causing misalignments between the hippocampal representation and the animal's actual location. By contrast, in the presence of landmarks, such errors are not only corrected but also harnessed for fine-tuning of path integration through recalibration of its gain factor. The neural mechanisms of this recalibration remain unknown despite its critical role in maintaining the alignment of the hippocampal representation with the animal's actual location.
Using a continuous attractor network (CAN) model, we previously showed that gain recalibration requires some neurons to change their firing rates with error in the network's representation of self-location relative to landmarks (Secer et al., 2024). These changes serve as

a rate code of error that guides gain recalibration in the model. To test this prediction, we analyzed CA1 place cell data from 5 rats navigating a circular VR environment where landmarks were moved as a function of the rat's speed to create persistent conflict with path integration, thereby recalibrating its gain factor (Jayakumar et al., 2019). Under these conditions, place fields ($n = 224$) drifted relative to landmarks by up to ±60° over many laps. The drift was correlated with the rat's speed times the extent of gain recalibration ($r = 0.71$, $p < 0.001$), consistent with the error in a CAN model. In 30% of place fields, drift patterns across laps correlated with the changes in their peak firing rates, consistent with a rate code of error in a CAN model. In another 30%, the drift was explained by a CAN model that combines a rate code of error and associative plasticity between feedback from landmarks and self-location representations. Changes in firing rates of place fields with drift patterns explained by the CAN models were associated with mid-gamma band fluctuations in LFP ($r = -0.2$, $p < 0.001$). Moreover, these place fields clustered around the landmarks ($p < 0.01$), unlike other fields distributed randomly around the track ($p = 0.37$). Our findings show that CA1 place-field activity dynamically adapts to conflicting navigational cues, likely moderated by entorhinal inputs linked to mid-gamma LFP. These adaptations, aligning with theoretical predictions, may serve as a neural code for error in hippocampal representations of self-location relative to landmarks.

Disclosures: G. Secer: None. **R.P. Jayakumar:** None. **M.S. Madhav:** None. **J.J. Knierim:** None. **N.J. Cowan:** None.

Presentation Number: NANO42.11

Topic: H.09. Spatial Navigation

Support: U01 NS111695
R01 NS102537

Title: Effects of Visual Landmark Misalignment on Head Direction Cells in Retrosplenial Cortex

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Abstract: The head direction (HD) network serves as an internal compass to orient the animal in space. Stable HD representations are commonly modeled by ring attractor networks in which neurons are conceptualized as forming a recurrently connected ring architecture that can sustain a stable, bump-like activity pattern that represents the HD. Angular head velocity information updates the bump location in real-time, and inputs to the attractor network from visual landmarks prevent the accumulation of errors inherent in such an inertia-based integrator system. Despite predictions from classic HD models that strong landmark inputs should reset the bump location regardless of the degree of error between the bump and the landmark inputs, experimental

observations indicate a failure in control by landmark inputs when the angular distance between the landmark and the current HD bump exceeds 45-90°. However, the interaction between HD representations of external landmarks and ring attractor dynamics remains underexplored. Here, we manipulated visual cues under dynamic feedback control when the rat moved freely in a virtual reality apparatus (called "the Dome"). In one condition, we gradually and continuously introduced conflicts between the HD cells and the landmarks by rotating the visual cues in the Dome by a continuously increasing gain factor that determined the rate of rotation as a function of the rat's speed. As expected, based on our earlier studies, HD cells were strongly controlled by the landmarks under these conditions. In a second condition, we moved the landmarks in discrete jumps of increasing magnitude that followed the overall profile of the continuous gain manipulation. We recorded signals from 124 cells in the retrosplenial cortex from 3 Long-Evans rats, identifying 11 HD cells, 4 with bilobed tuning curves. When the discrete jump was < 45°, the landmarks reset the preferred firing direction (PFD) in all HD cells so that the PFD aligned with the new location of the landmarks (correlation between landmark jump and PFD reset, $r = 0.95$, $p < 0.01$). In contrast, discrete jumps > 45°resulted in 86% of the tuning curves either failing to maintain any HD tuning or shifting their PFD relative to the landmarks ($r = 0.2$, $p = 0.04$). In a third condition, the HD tuning curves drifted when the landmarks were turned off, but when the landmarks reappeared, all HD cells could realign their tuning curves with the landmarks, even with angular discrepancies > 90°. Our study provides crucial insights into the error tolerance limits between external landmarks and internal dynamics, quantifying a threshold beyond which external cues lose their direct ability to reset the HD dynamics.

Disclosures: Y. Zhou: None. **M. Zhang:** None. **N.J. Cowan:** None. **K. Zhang:** None. **J.J. Knierim:** None.

Presentation Number: NANO42.12

Topic: H.09. Spatial Navigation

Support: CIHR
NSERC
Healthy brains for healthy lives
BrainsCAN
Autism research chair

Title: Resetting of theta oscillations by head movements and jumps in the freely moving marmoset hippocampus translate into different population codes

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Abstract: The hippocampus (HPC) plays an important role in memory and spatial navigation. Previous studies conducted mainly in rodents have shown that local field potential (LFP) oscillations in the theta band (4-12 Hz) play an important role in encoding and retrieving memories (Buzsáki, 2002). However, in primates, theta oscillations are not as sustained as they are in rodents, and their phase is reset to saccades (Doucet et al., 2020; Katz et al., 2022) . Theta

phase-reset could be mediated by internally generated motor efference copies (EfC) targeting interneurons, facilitating synchronization in the HPC (Martinez-Trujillo, 2022). Given that studies in primate HPC physiology have been largely conducted using virtual reality, the role of theta oscillations in freely moving primates is still subject of debate. In this study we investigate hippocampal theta phase-reset in the freely moving marmoset (*Callithrix jacchus*) during multiple behaviors like eye/head movement and vertical jumps. ResultsWe measured eye position in 2 marmosets sitting on a primate chair during a free viewing task and 3D head and body position during a foraging task. We recorded single neuron activity and LFP in the CA1/CA3 region. We found a significant amplitude modulation of the LFP as well as a significant power modulation of the average time frequency response that peaked within the theta band, mean = 8.7Hz, 7.8Hz and 7.1Hz for saccades, head movements and jumps respectively. We found that 36.3% and 12.5% of pyramidal cells and 87.4% and 36.2% of interneurons were significantly modulated by head movements and jumps respectively (ANOVA, $p<0.01$). 95% of jump-responsive cells were also modulated by head movements; however, 17.2% of these cells had significantly different sign of firing rate modulation (e.g., increases/decreases in firing rate relative to event onset) between both types of movements. The latter allowed a population code to distinguish head movement and jump parameters. The magnitude of the LFP and firing rate modulation was correlated with the amplitude of the movement. Lastly, we found that the peak modulation latency for interneurons was shorter than for pyramidal cells (mean difference 49.8ms, rank-sum test $p<0.01$) suggesting phase resetting may be mediated by EfC signals activating inhibitory interneurons.ConclusionTheta phase resetting in the marmoset HPC is coupled to movements of the eyes, head, and body. However, the population codes associated to different movement types differ, and carry information about the movement parameters. This modulation has the signature of EfC signals that shape the activity of neuronal populations to incoming sensory signals.

Disclosures: D.B. Piza: None. **B.W. Corrigan:** None. **J.C. Martinez-Trujillo:** None.

Presentation Number: NANO42.13

Topic: H.09. Spatial Navigation

Support: Helen Hay Whitney Foundation Fellowship
NIH BRAIN Initiative K99MH135993
NIH R01MH126904
NIH R01MH130452
The Vallee Foundation
NIH BRAIN Initiative U19NS118284
P50 DA042012

Title: Hippocampal sequences span experience relative to rewards

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Abstract: Positive experiences must be strongly remembered to promote rewarding behaviors, and these memories must be able to update as reward expectations change. How does the brain

encode strong, flexible memories of rewarding experiences while ensuring a consistent representation of the external world? The hippocampus offers a potential neural circuit for this process. Hippocampal place cells fire in sequences spanning spatial and non-spatial episodes, suggesting that hippocampal activity can anchor to the most behaviorally salient aspects of experience. Reward is a highly salient event, and previous research identified a subset of hippocampal place cells firing precisely at reward locations. However, is it unclear whether hippocampal activity encodes entire sequences of events relative to reward. We hypothesized that sequential hippocampal activity can indeed anchor to rewards across the environment in a subpopulation of cells, thus maintaining a representation of distance to reward even when the animal is far away. To test this hypothesis, we performed two-photon imaging of hippocampal CA1 neurons as mice navigated virtual environments with changing hidden reward locations. When the reward moved, the firing fields of a subpopulation of cells moved to the same relative position with respect to reward. These reward-relative cells constructed sequences at the behavioral timescale that included spatial positions far from the reward, tiling the entire task structure from reward to reward over hundreds of centimeters. The reward-relative sequences became more robust as mice gained familiarity with the task, recruiting new neurons into the sequence as the animals improved their behavioral performance. Conversely, a largely separate subpopulation maintained a spatially-anchored place code. These results demonstrate that the hippocampus simultaneously represents the spatial environment and the animal's distance from reward in flexible, dissociable population codes, with an increased allocation of resources over learning to the reward-centric code.

Disclosures: M. Sosa: None. **M.H. Plitt:** None. **L.M. Giocomo:** None.

Presentation Number: NANO42.14

Topic: H.09. Spatial Navigation

Support: CIHR 390930
NSERC 40352
AARPG PAZ15010
AARPG PAZ17010
ASC 43674

Title: Modulation of Sensory Event-Related Distance and Time Tuning in Hippocampal CA1 Cells

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Abstract: Within the hippocampal CA1 region, neuronal firing patterns are intricately linked to both distance and time relative to specific sensory or behavioral reference points. This study investigates how extrinsic task-related inputs modulate sensory event-related distance and time tuning in individual CA1 cells and explores their influence on the relationships among cells responsive to sensory stimuli, voluntary movements, or variations in running speed. Using calcium imaging techniques, we recorded CA1 neuronal activity as mice navigated

predetermined distances in response to air stream stimuli and paused for designated intervals allowing spontaneous movement. Our findings highlight that peak neuronal firing predominantly occurs around the initiation or cessation of the air stream. We observe distinct patterns of dominance in cells tuned to distance and time during phases of air flow compared to non-flow phases, forming exclusive neuronal populations. Some of these cells, termed conjunctive cells, anticipated the start or end of air flow. Furthermore, these groups of cells were distinct and separate from those cells that responded directly to sensory inputs or voluntary movements. The data suggest the presence of distinct neural networks within the hippocampus that are specialized for encoding sensory event-related spatial and temporal aspects. This study underscores the interplay between time and distance in the encoding of sensory events within the hippocampal CA1 region.

Disclosures: S. Inayat: None. **B.B. McAllister:** None. **I.Q. Whishaw:** None. **M.H. Mohajerani:** None.

Nanosymposium

NANO43: Neurodevelopmental Disorders

Location: MCP Room S404

Time: Tuesday, October 8, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO43.01

Topic: A.07. Developmental Disorders

Support: JSPS Grant 19H03535
JSPS Grant 21H05679
JSPS Grant 23H04217
JST Grant JPMJMS2021

Title: Causal links between neural rigidity and diverse autistic behaviours in human adults

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Abstract: In prior human neuroimaging studies, we reported correlations between reduction in the specific brain-state transition and autistic symptoms in both adults (Watanabe and Rees, 2017) and children (Watanabe and Watanabe, 2023). However, the causality between the rigidity of the collective brain dynamics and autistic behaviours remains unknown, and it has not been tested whether the modification of the neural rigidity would induce the mitigation of the autistic traits. Here, to answer these questions, we conducted multiple longitudinal experiments, in which 50 high-functioning autistic adults underwent weekly non-invasive neural stimulation, and tracked the changes in the brain state dynamics and social-/non-social autistic behaviours. In particular, we adopted a brain-state-driven neural stimulation (BDNS) system to control the autistic brain dynamics because the system can monitor the brain state dynamics of each participant in an almost real-time manner and trigger transcranial magnetic neural stimulation (TMS) only when the participant's brain is dwelling in a specific state (Watanabe, 2021). As a

result, we found that the BDNS over the parietal cortex enhanced the frequency of the specific brain state transition, which was originally reduced in the autistic group compared to the sex-/age-/IQ-matched typically developing cohort. Along with the mitigation of the neural rigidity, the cognitive rigidity of the autistic adults was immediately alleviated; in contrast, the perceptual over-stability and atypical nonverbal communication style seen in the autistic participants were mitigated more slowly. We also identified neural processes behind these slow behavioural responses: the perceptual over-stability was weakened only after the BDNS-triggered neural flexibility successfully enhanced the functional coupling between the frontoparietal network (FPN) and visual network; the atypical non-verbal communication style in autism was changed after the BDNS-induced neural flexibility strengthened the coordination between the FPN, default mode network and salience network. These results indicate the direct/indirect causal relationships between the neural rigidity and multiple autistic behaviours, at least, in high-functioning adults with autism spectrum disorder.

Disclosures: T. Watanabe: None.

Presentation Number: NANO43.02

Topic: A.07. Developmental Disorders

Support: Alcyone Therapeutics

Title: The miREX Platform Technology Allows for Highly Efficacious X-Reactivation for Treatment of X-linked Disorders

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Abstract: X chromosome inactivation is a developmentally regulated process in females, wherein one X chromosome per cell is inactivated through a complex stepwise process involving the Xist RNA. This process allows for dosage regulation in females for X-linked gene expression. As a consequence, female tissue affected by X-linked genetic disorders, such as Rett Syndrome, will be a mixture of cells expressing the mutated version of the gene or the healthy copy depending on which X chromosome is active in any given cell. This mosaicism complicates gene replacement strategies significantly as the healthy cells are at risk of being overdosed. Notably, all cells that express the loss of function mutation contain a healthy copy on the silenced chromosome which, upon re-expression, could ameliorate the disease phenotype. Thus, mechanisms that regulate X-inactivation may be an effective therapeutic strategy for Rett Syndrome and other X-linked disorders. miR106a is a natural regulator of X-inactivation that binds to Xist. Here, we show that sequestration of miR106a via miREX technology (miRNAsponge for Endogenous Gene Expression by X-Reactivation) allows X chromosome

reactivation and expression of target genes such asMeCP2 . Furthermore, viral vector-based delivery of miREX showed efficacy in both Rett patient derived astrocytes and neurons, with a broad spectrum of MeCP2 mutations, as well as in a severe female Rett mouse model. Moreover, the technology was also efficacious in several other X-linked disorders when tested in various patient cell lines. This demonstrates that miREX may serve as a platform therapy for multiple diseases. To ensure optimal therapeutic benefit, effective targeting of the central nervous system is critical for success in the treatment of neurological disorders including Rett Syndrome. For this purpose, Alcyone Therapeutics has developed a novel intra-CSF drug delivery platform (Falcon^{TM}) allowing to optimize drug delivery to the brain. The Falcon technology allows studying and optimizing drug biodistribution taking into consideration disease related changes in CNS anatomy and CSF flow dynamics. Thus, the Falcon technology will ensure optimal delivery of miREX in Rett Syndrome patients further improving the therapeutic potential of our novel treatment for X-linked disorders.

Disclosures: C. Dennys: A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **S. Powers:** A. Employment/Salary (full or part-time):; Forge Biologics. **M. Khani:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **S. Lou:** None. **S.B. Likhite:** None. **L.R. Sass:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **J.A. Sierra Delgado:** None. **S. Sater:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **O. Arters:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **S. Bhatnagar:** None. **B.A. Martin:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **D. Singh:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **P. Anand:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics. **K.C. Meyer:** A. Employment/Salary (full or part-time):; Alcyone Therapeutics.

Presentation Number: NANO43.03

Topic: A.07. Developmental Disorders

Support: 5R01NS057819(HZ)

Title: Profiling neuronal activity-dependent molecular changes in a mouse model of Rett Syndrome

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Abstract: Rett syndrome (RTT) is a neurological disorder caused by loss-of-function mutations in the X-linked gene Methyl-CpG-binding protein 2. RTT girls develop normally for 6-18 months then experience motor and cognitive decline. RTT is characterized by neuronal hypoactivity and broadly altered neuronal gene expression. Activating RTT neurons through deep brain stimulation enhanced neuronal activity and hippocampal plasticity and improved learning and memory by normalizing expression of synaptic protein genes. Recently, we showed that behavioral training in RTT mice during the pre-symptomatic but not post-symptomatic stage rescue certain behavioral abnormalities and improve electrophysiological properties of neurons involved in those behavioral tasks (task-specific neurons). Therefore, we hypothesize pre-symptomatic training elicits transcriptional responses in task-specific neurons which contribute

to behavioral improvement. We isolate task-specific neurons during the behavioral training in naïve, pre- and post-symptomatic WT and RTT mice. Single-nucleus RNAseq of these neurons captured identical cell types but revealed different cell type proportions. Specifically, we observed a shift in inhibitory to excitatory neuron ratio in RTT mice undergoing training. We detected differentially expressed gene (DEGs) in all groups, but the numbers of DEGs are different amongst the various groups. We found that DEGs that are uniquely changed in pre-symptomatic trained RTT mice encompass genes involved in neuronal projection. Next, we will perform network analysis to investigate the expression changes to further dissect the molecular alterations. Overall, this project will allow us to characterize the transcriptional signature of both WT and RTT neurons in response to activity and compare the transcriptomic effect of different training.

Disclosures: Y. Li: None. **J. Revelli:** None. **A. Anderson:** None. **H.Y. Zoghbi:** None.

Presentation Number: NANO43.04

Topic: A.07. Developmental Disorders

Support: NIH Grant HD104558

Title: Coordinated regulation of translation initiation during neuronal development in Fragile X Syndrome

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Abstract: Dysregulation of translation forms the basis of the molecular pathogenesis of Fragile X Syndrome (FXS), one of the most well characterized, X-inherited neurodevelopmental disorders, with individuals presenting intellectual disabilities and autistic spectrum disorder (ASD). FXS is caused by loss of the protein FMRP (Fragile X Messenger Ribonucleoprotein), which results in alterations in synaptic plasticity, neuronal development and growth, and an overall increase in global translation and proteostasis. An RNA binding protein, FMRP has a wide array of functions, including regulation of localization and translation status of specific mRNAs, that are required for proper differentiation and development of neurons. Although FMRP is known to regulate translation and developmental abnormalities in FXS models have been attributed to this dysregulated translation, the precise molecular mechanisms that are responsible for phenotypes in FXS have been elusive. Here we explore the role of FMRP in translation initiation during the course of neuronal development, using excitatory neurons derived and differentiated from patient-derived induced pluripotent stem cells (iPSCs). We observed changes in levels of various translation initiation factors (IFs) over the course of neuronal development in FXS patients compared to normal individuals. Human neuronal development requires multiple stages of translational programming resulting in a reduced translational load, evident in changes in levels of various translation IFs over the course of neuronal development. In FXS however, levels of certain non-canonical IFs, such as eIF4G2, eIF4G1 and eIF3d continue to remain elevated, as does the mTORC1 pathway. FXS patient derived neurons are also more sensitive to knock down of eIF4G2 (Dap5), and less sensitive to inhibition of eIF4E-based cap-dependent translation, indicating that a compensatory, non-

canonical translational control mechanism is in place for these neurons. We predict that neuronal development utilizes cap-dependent but eIF4E-independent methods of translation instead of the canonical eIF4E-based translation initiation, a shift that is mediated directly or indirectly by FMRP. Moreover, in the FXS condition, eIF4G2 plays a critical, compensatory role in modulating the translation of specific mRNAs required for neuronal development and growth. As a consequence, our results indicate that in FXS, the tightly regulated shift between canonical to non-canonical translation initiation methods is lost resulting in aberrant protein synthesis and subsequent neurodevelopmental defects. Funding source: NIH grant HD104558 (G.B. and E.K.).

Disclosures: V.G. Shankar: None. **N. Raj:** None. **G.J. Bassell:** None. **E. Klann:** None.

Presentation Number: NANO43.05

Topic: A.07. Developmental Disorders

Support: R01 NS116418
R21 NS127051
Children's National Hospital Recruitment Fund

Title: Disrupted Neurogenesis From Basal Intermediate Precursor Cells Leads to Altered Development of the Postnatal Neocortex in the TcMAC21 Mouse Model of Down Syndrome

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Abstract: The neocortex subserves cognition, social behavior, speech, and motor skills, higher order capabilities known to be impacted in trisomy 21/Down's syndrome (DS). Prior research using mouse models of DS suggests that alterations in cortical neurogenesis might account for the behavioral phenotypes observed during adulthood. Here we characterized prenatal brain development in a novel mouse model of DS, TcMAC21, in which each cell harbors an extra 93% of the protein-coding genes present on the HSA21q chromosome. Neocortical excitatory neuron diversification occurs through different routes of neurogenesis, whereby precursors of different origins contribute to morphologically and physiologically distinct neurons. Understanding the genetic contribution of the triplicated human chromosome 21 to cortical microstructure abnormalities encountered in patients, as well as whether specific cell lineages are altered in their developmental trajectory, are key lines of research in DS. To investigate neocortical neuron diversification in the TcMAC21 mouse model, acute EdU labeling was used to capture cycling cortical neural precursor cells in the S phase of the cell cycle to determine precursor proliferative capacity and positioning in the embryonic neocortex. Furthermore, we performed in utero electroporation (IUE) and Cre/Lox recombination to fate-map neurons originating from different precursor lineages, allowing for long-term tracking of their contributions to neocortical neuron composition.Interestingly, in striking contrast to prior DS mouse models, we did not detect significant differences in gross cortical morphometry between euploid and TcMAC21 brains. While we did not observe overall differences in levels of proliferation between euploid and trisomic cortices, we detected altered positioning of proliferative intermediate progenitor cells

(IPCs) in the trisomic cortex, suggestive of a lineage-specific delay in neurogenesis. Importantly, the positional phenotype was most pronounced at E15.5, the stage at which superficial neocortical neurons are actively generated. Long-term lineage fate mapping demonstrated a shift towards more superficial positions of IPC-derived neurons as well as an overall reduction in IPC contribution to the excitatory neuronal pool. These data indicate that smaller, cell type-specific alterations may influence overall cortical circuitry without resulting in major anatomical defects. Future work will examine how these lineage-specific changes influence the connectivity and physiological properties of trisomic neocortical excitatory neurons, and consequently their effects on behavior.

Disclosures: Z. Atak: None. **A.K. Srivastava:** None. **S. Gandhi:** None. **K.A. Pasion:** None. **J. Williams:** None. **T.F. Haydar:** None.

Presentation Number: NANO43.06

Topic: A.07. Developmental Disorders

Support: NIH AG079002-01
NIH T32AG057468-06

Title: Early deficits in corticogenesis in Down syndrome cortical organoids

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Abstract: Down Syndrome (DS) due to trisomy 21 (T21) results in significant developmental cortical malformation and invariably accumulation of Alzheimer's Disease (AD) pathology with aging. It is theorized that deficits in corticogenesis lead to abnormal neuronal networks vulnerable to the early, pervasive AD pathology in DS. However, given the current limitations of human tissue and mouse models, the earliest deficits in corticogenesis and AD in DS are yet to be fully elucidated. Induced pluripotent stem cells (iPSCs) derived from two DS patients and their respective isogenic controls were differentiated into dorsal forebrain organoids across multiple rounds. Organoids were characterized across time points via immunocytochemistry (ICC), western blotting, and enzyme-linked immunosorbent assay (ELISA). DS organoids exhibited an early decrease in ki67 and EdU positive cells within proliferative zones via ICC and overall protein expression of DCX and β-III TUB via western blotting. With the appearance of lower layer mature cortical neurons, DS organoids demonstrated deficits in migration and expansion of cortical layers quantified via ICC analysis of PAX6, TBR2, and CTIP2 expression. At this and later time points, ELISA experiments revealed an increase in the production of Aβ40 and Aβ42 in DS organoids. Experiments revealed deficits in early proliferation, expression of markers of early differentiation, expansion of cortical layers, and amyloid precursor protein (APP) processing in DS organoids. Future experiments aim to characterize transcriptomes via single cell RNA sequencing, cortical expansion upon differentiation of upper layer cortical neurons, and amyloid and tau pathology. Together, these experiments will lead to improved understanding of the earliest deficits in DS and better inform the development of future therapeutics.

Disclosures: K. Rakowiecki: None. **O. Lazarov:** None.

Presentation Number: NANO43.07

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 NS086933-01
NIH Grant R01 AG064465
NIH Grant R01 AG083268
NIH Grant T32 MH016880
NIH Grant R21/R33 DA055781
NIH Grant T32 AG052371
Funding from Linda Crnic Institute

Title: Sleep impairment rescue by AAP gene dose correction in Dp16 Down syndrome model mice

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Abstract: Down syndrome (DS) occurs in 1 of 700 to 1000 births and is the most common cause of intellectual disability. Sixty percent of the DS population also experience sleep abnormalities. Although some sleep disturbances are caused by anatomically derived sleep apnea, many are not. The triplication of genes on human chromosome 21 (Hsa21) may also contribute to sleep disturbances in DS. These abnormalities include increased latency to non-rapid eye movement (NREM) sleep, sleep fragmentation, and reduced rapid eye movement (REM) sleep. Dp(16)1Yey mice (Dp16) are trisomic for ~115 genes orthologous to Hsa21 syntenic regions (Mmu16). Among the genes triplicated in DS is the gene for *Amyloid Precursor Protein or App,* which is chronically overexpressed in the brains of DS individuals and known to impact learning, memory, affective behavior, and synaptic plasticity. By age 40, DS individuals characteristically display brain pathology like that seen in Alzheimer's disease (AD) patients, followed by dementia by age 60 (Chen et al., 2021). Previously, we described sleep defects in Dp16 mice (Levenga et al., 2018) and were able to partially rescue sleep architecture by restoring disomic levels of RCAN1 in Dp16 mice (Cain et al., 2024). Because disruption of circadian rhythms has been observed in AD patients and AD mouse models, we hypothesized that we might mitigate the disruption of sleep architecture and EEG characteristics by restoring disomic levels of APP in Dp16 mice. Experimenters blind to genotype examined sleep architecture and EEG patterns in male and female, aged (10-12 mos.) WT, Dp16, and Dp16 *App*2^{n} mice. We observed significant differences in sleep architecture and EEG characteristics among WT, Dp16, and Dp16 *App*2^{n} mice. Restoring APP to disomic levels resulted in marked adjustments in sleep architecture and EEG patterns to those more closely aligned with those seen in WT.

Disclosures: P. Cain: None. **R.A. Milstead:** None. **C. Borski:** None. **K. Kastengren:** None. **M. Griffioen:** None. **S. Cotto:** None. **J. Hanson:** None. **C.D. Link:** None. **C.A. Hoeffer:** None.

Nanosymposium

NANO44: Structural Plasticity: Compartmentalization of Synaptic Function and Plasticity

Location: MCP Room N228

Time: Tuesday, October 8, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO44.01

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01NS091010
NIH Grant R01NS125298
NIH Grant R01DC018545
NIH Grant R01MH128746
NIH Grant T32NS007220
Eric and Wendy Schmit AI in Science Fellowship

Title: Compartment-specific functional synaptic organization and plasticity rules during motor learning

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Abstract: The computational capacity of individual neurons arises, in part, from their extensive dendritic arbors that are capable of nonlinearly integrating and transforming synaptic inputs. Importantly, dendrites are not uniform and can be categorized into discrete compartments that have distinct anatomical and biophysical properties that influence how synaptic inputs are integrated to regulate somatic activity. As a result, different dendritic compartments may favor distinct synaptic organization motifs that confers them with unique computational properties. However, our understanding of the functional organization of synapses along different compartments of the dendritic arbor in the intact brain remains limited. Here, using *in vivo* two-photon imaging to simultaneously image synaptic activity and neuronal output, we examined the functional organization of individual synapses along the apical and basal dendrites of layer 2/3 pyramidal neurons in primary motor cortex during motor learning. We found that synapses in apical dendrites display a greater degree of functional clustering of movement-related information compared to those on basal dendrites. In addition, we found this compartment-specific functional synaptic organization is associated with divergent plasticity rules. Specifically, apical synapses that will undergo structural long-term potentiation (sLTP), as well as their neighbors, display greater levels of local coactivity with nearby synapses, suggesting dendritic areas with high local coactivity promote sLTP and may favor functional clustering. Indeed, we find synapses undergoing sLTP in apical dendrites are spatially clustered, while those undergoing structural long-term depression (sLTD) are not associated these clusters. In striking contrast, synaptic plasticity in basal dendrites is not associated with local synaptic coactivity. Rather, basal but not apical plasticity is associated with activity coincident with somatic activity, such that those with high coincident activity undergo sLTP and those with low coincident activity undergo sLTD, akin to traditional Hebbian plasticity. Supporting the notion that apical

and basal plasticity is associated with local coactivity and output coincidence, respectively, blocking somatic activity selectively disrupted plasticity in basal, but not apical, dendrites. Collectively, these findings indicate that synapses in the apical and basal dendritic compartments display distinct functional organization, which is enforced by divergent plasticity rules.

Disclosures: W. Wright: None. **N.G. Hedrick:** None. **T. Komiyama:** None.

Presentation Number: NANO44.02

Topic: B.05. Synaptic Plasticity

Support: Brain Foundation Grant G00005772

Title: Addressing an imbalance of excitation and inhibition in Kabuki syndrome Type 1

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Abstract: Kabuki syndrome (KS) is a rare multisystem disorder most commonly associated with developmental delay and intellectual disability. The disease is caused by heterozygous mutations in either of the genes KMT2D or UTX (KDM6A) which confer production of a methyltransferase and demethylase, respectively. KMT2D mutations account for a majority of KS cases (Type 1, 70-80%), while UTX mutations account for a much smaller proportion of cases (Type 2, 5-6%). Both enzymes work together to form the ASC-2 binding complex (ASCOM), which is known to be important for regulation of chromatin states and ultimately gene expression. However, the relationship between ASCOM and neurological development and how its disruption can lead to intellectual disability in patients remains poorly understood. In this study, we use a series of mouse and human neuronal models to investigate and characterize distinct neurological phenotypes in KS Type 1. Here, we provide evidence that mouse neurons possessing constitutive heterozygous mutation of Kmt2d as well as human induced neurons possessing conditional heterozygous mutation of KMT2D both exhibit similar imbalances in the ratio of excitation to inhibition (E/I): Mouse primary hippocampal neurons with constitutive Kmt2d mutation display increases in inhibitory pre-synaptic marker vesicular GABA transporter (vGAT) and active zone marker Bassoon (BSN) but a decrease in excitatory post-synaptic density 95 (PSD95), when assessed via immunocytochemistry. Using patch-clamp electrophysiology, we found that neurons from mutant mice exhibit increases in inhibitory post-synaptic currents (IPSCs) and decreases in excitatory post-synaptic currents (EPSCs) when recorded from mouse brain slices, demonstrating a decrease in E/I ratio. We observed a similar increase in vGAT and decrease in PSD95 when viewed in mutant human ASCL1/DLX2-induced inhibitory or NGN2-induced excitatory neuronal cultures, respectively. In summary, the histone modifier KMT2D likely suppresses inhibitory synapse formation and facilitates excitatory synapses formation, accounting for neurological symptoms of KS Type 1.

Disclosures: J.M. Knopp: None. **A.D. Weaver:** None. **S.H. Miller:** None. **I. Wiebelt-Smith:** None. **J. Lopez:** None. **S. Nam:** None. **J. Bergqvist-Patzke:** None. **T. Nomura:** None. **A. Contractor:** None. **C. Patzke:** None.

Presentation Number: NANO44.03

Topic: B.05. Synaptic Plasticity

Support: DBI-1707356
DBI-2014862

Title: Differential entropy of synaptic weight distribution from rat hippocampal area CA1

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Abstract: Synaptic strength is the known unit of storage of information in the brain. Spine head volumes (SHV) are known to have a high correlation with synaptic strength. Quantifying the high variability of synaptic strength is a prominent step toward investigating mechanisms underlying learning and memory. In this research, we have analyzed the distribution of spine head volumes and estimated the differential entropy of synaptic weights distribution in hippocampal area CA1 in adult rat. Differential entropy measures the amount of information carried by continuous distributions and is a notion of entropy in the setting of continuous random variables. Results show that 4.4 bits of information are carried in the fitted distribution of spine head volumes in area CA1. A value that falls between lower bound (4.1 bits) and upper bound (4.6 bits) of synaptic information capacity estimated by Samavat et al, Neural Computation, 2024 for discrete setting in CA1. Estimating differential entropy of distribution of spine head volumes provides a new analytical measure that can be applied to the other correlates of synaptic strength and can be generalized to probe synaptic strengths and capacity for plasticity in different brain regions of different model organisms and among animals raised in different environments or during learning. Finally, any system with continuous synaptic weight distribution may potentially benefit from our method including neuromorphic computing applications.

Disclosures: M. Samavat: None. **K.M. Harris:** None. **T.J. Sejnowski:** None.

Presentation Number: NANO44.04

Topic: B.05. Synaptic Plasticity

Support: P30 AG072977
2R56MH071316-16
R01MH107182
P30 AG13854

Title: Tunneling nanotubes in microglia-synapse crosstalk and inflammation

Authors: *C. R. ZACCARD[1], E. L. PARNELL[1], M. DOS SANTOS[1], I. GIPPO[1], S. ZUR[1], C. GEULA[2], P. PENZES[1];
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Abstract: Brain-resident immune cells termed microglia can mediate synapse formation and plasticity, but become dysregulated in Alzheimer's disease (AD), contributing to chronic neuroinflammation and synapse loss. Yet, the mechanisms underlying direct microglia-neuron

interactions are poorly defined. Pro-inflammatory signals can induce long, thin, membranous connections, i.e., tunneling nanotubes (TNTs), in peripheral immune cells. TNTs have been observed in various cell types and can support trafficking of diverse cargoes. We established a co-culture model of human induced excitatory neurons (iENs) and postmortem adult human microglia and utilized adeno-associated virus vectors to label microglia and emerging TNTs. We treated co-cultures with pro-inflammatory factors, i.e., oligomeric amyloid β (Aβ) and interferon-gamma (IFN-γ), or anti-inflammatory factors for 24 hours (h), and utilized enhanced resolution confocal microscopy to assess TNTs. For *in vivo* studies, we intraventricularly injected adult CX3CR1-GFP microglia reporter mice with IFN-γ or saline, followed 24 h later by transcardial ultrafast fixation. Immunohistochemistry was performed on brain sections, followed by enhanced resolution confocal microscopy of the CA1 hippocampus to assess number of TNTs in relation to synapses. The number of TNT-positive microglia and TNTs per microglia increased in live human iEN-microglia co-cultures under AD-relevant pro-inflammatory conditions compared to anti-inflammatory or control conditions. TNT length and the percentage of TNTs contacting axons, dendrites, or dendritic protrusions increased under pro-inflammatory conditions. We used time-lapse enhanced resolution microscopy to capture microglial TNTs interacting with spine-like dendritic protrusions, which extended dynamic spinules. Additionally, we detected TNTs, which often contacted spines and were most prominent after pro-inflammatory IFN-γ activation, in the adult microglia reporter mouse hippocampus. Our results in human iEN-microglia co-cultures and mouse brain sections indicate that pro-inflammatory signals increase microglial TNT formation and TNT-driven interactions with neuronal elements. TNTs can provide additional contact points and extend the reach of microglia, facilitating communication with distant target microglia or synapses. During chronic neuroinflammation in aging and AD, TNT over-expression likely increases microglia-synapse interactions and supports rapid, cell-cell spread of Aβ. Hence, TNTs may contribute to AD progression and represent a target for therapeutic intervention in neurodegenerative disorders.

Disclosures: C.R. Zaccard: None.

Presentation Number: NANO44.05

Topic: B.05. Synaptic Plasticity

Title: Neurobiological Underpinnings of Social Dysfunction: Exploring the Influence of Neuronal IL-1R1 in Early Life Stress

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Abstract: The signaling between IL-1 and its receptor interleukin-1 receptor-1 (IL-1R1), plays critical roles in maintaining CNS homeostasis, neuroinflammation, and neural circuit functions. Our recent studies highlight the importance of neuronal IL-1R1 (nIL-1R1) for cognition, social interaction, and social cognition in adult mice, yet its role in neurodevelopment remains unclear. Our recent investigation during neurodevelopment reveals dynamic changes in nIL-1R1 expression, especially in brain regions crucial for memory, sensory processing, and social cognition. Our investigation showed that nIL-1R1 expression is dynamic during brain development, particularly in the dentate gyrus of the hippocampus (DG), ventral posteromedial

(VPM), and posterolateral thalamic nuclei (VPL), between postnatal days 3-21. Early life stress (ELS) has profound and enduring effects on neurodevelopment and social functioning, contributing to the risk of neurodevelopmental disorders. Moreover, IL-1R1 in the hippocampus plays a critical role in mediating the impact of stress on social behavior. However, the specific involvement of neuronal IL-1R1 (nIL-1R1) in ELS-induced social deficits remains poorly understood. This study aims to address this gap by utilizing a maternal separation (MS) paradigm and innovative IL-1R1 mouse lines to elucidate the role of nIL-1R1 in MS-induced long-lasting social interaction deficits. Repeated MS heightens nIL-1R1 and Δ-FosB expression in DG and induces social interaction abnormalities in adulthood. Furthermore, the blockade of IL-1R1 with IL-1R antagonist rescued MS-induced social interaction abnormalities. These preliminary data suggest nIL-1R1 has a pivotal role in stress-induced neuronal and behavioral alterations. We aim to evaluate whether nIL-1R1 is necessary or sufficient for MS-induced social deficits and decipher nIL-1R1-dependent synaptic changes in the hippocampus. Through this project, we seek to gain insights into the molecular mechanisms underlying ELS-induced social behavior difficulties. Unraveling nIL-1R1's role in shaping social behavior deficits holds promise for informing therapeutic interventions, ultimately improving mental health outcomes for individuals affected by ELS and neurodevelopmental disorders.

Disclosures: M.C. Monet: None. **M.I. Smirnova:** None. **D. Nemeth:** None. **N. Quan:** None.

Presentation Number: NANO44.06

Topic: B.05. Synaptic Plasticity

Support: Academia Sinica AS-GC-112-L01
NSTC 112-2311-B-001-036-MY3

Title: The stochastic wiring of olfactory local interneurons

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Abstract: The types of neurons are defined by their morphologies, molecular profiles, neurotransmitters, electrophysiological properties and connections. However, neuronal variability and stochasticity largely complicate the definition of neural types and the circuit activities the neurons involve. We recently demonstrated that a single identified olfactory interneuron, TC-LN, exists as many as 849 functionally distinct innervation patterns across individuals. Such variations originate from developmental stochasticity and experience-dependent plasticity, which change their connections. To explore different forms of neuronal variability, a large-scale GAL4 screen was conducted to identify drivers that label identifiable single or the same types of olfactory interneurons. The screen ended with a GAL4 line labeling patchy LNs. Remarkably, patchy LNs have stochastic innervation patterns, while all patchy LNs collectively tile the *Drosophila* antennal lobe. Certain biological constrains limit the stochasticity of patchy LNs: the sphericity and size of glomeruli are the external (environmental) constrains and neural activity is the intrinsic constraint. How patchy LNs may collaboratively integrate the olfactory information across glomeruli will be discussed. In addition, different types of interneuron variability will also be further discussed.

Disclosures: Y. Chou: None.

Presentation Number: NANO44.07

Topic: B.05. Synaptic Plasticity

Support: NSF NeuroNex
NSF GRFP

Title: Synaptic rearrangement in adult dentate gyrus

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Abstract: While most synaptic reorganization is believed to occur early in life, certain brain regions, such as the dentate gyrus (DG) of the hippocampus, maintain structural plasticity into adulthood. This plasticity is thought to be crucial for processing information related to pattern separation, learning, and memory. However, the nature and extent of this plasticity is not fully understood. Addressing this gap, we employ large volume serial-section electron microscopy 'connectomics' to investigate age-dependent changes in dendritic circuitry within the DG of adult mice. On a gross morphological level we find a notable drop (~ 1.7 fold reduction) in DG neuron density from p56 to p115. This prompts the question: what effect does decreased cell density have on circuits? We find that from p30 to p56 the density of spines, boutons and synapses increases but we see little change in those parameters as the number of neurons drops. However, despite the similarity in synapse numbers, we see changes in network properties of DG neurons as neuronal number drops. We find that granule cell dendrites in p56 exhibit a greater degree of shared input from multi-synaptic boutons among neighboring dendrites compared to p115. Additionally, at p56, individual axons are more likely to innervate the same "cohort" of dendrites, while at p115, axons display a more diverse pattern of innervation. Importantly, these changes cannot be attributed solely to geometric factors, suggesting a specificity to these rearrangements.In summary, our study uncovers age-dependent alterations in synaptic circuitry within the DG, underscoring the dynamic nature of adult neural connectivity. These insights yield valuable information about the formation and refinement of adult neuronal networks in the DG, with significant implications for understanding learning and memory.

Disclosures: A. Sorokina: None. **V. Sampathkumar:** None. **N.D. Medina:** None. **A. harshaw:** None. **N.B. Kasthuri:** None.

Nanosymposium

NANO45: Glial Regulation of Neurodegeneration: Focus on Alzheimer’s Disease

Location: MCP Room N427

Time: Tuesday, October 8, 2024, 1:00 PM - 4:15 PM

Presentation Number: NANO45.01

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

Title: Utilizing next generation mouse models to uncover mechanisms of complement-mediated synapse loss in Alzheimer's disease

Authors: *G. HOWELL[1], S. E. HEUER[1], A. UYAR[2], E. PIZZI[1], A. HEWES[1], M. MACLEAN[3], M. SASNER[1], E. BLOSS[1];
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Abstract: The complement cascade has emerged as a central pathway mediating synapse loss in Alzheimer's disease (AD), but critical knowledge gaps remain. Microglia are the major producers of C1Q - the initiating factor in the classical arm of the complement cascade. While significant work has shown that microglia are transcriptionally heterogenous, whether specific microglia state(s) initiate complement-mediated synapses loss is untested. Complement-mediated synapse loss is purported to be mediated through C3-C3R axis and/or the terminal membrane attack complex (MAC). Although C3, the central protein in the complement cascade, is produced at least by astrocytes, the primary producers of other key complement components, such as C2, C4, C5, and the MAC proteins (C6, C7, C8, and C9) are not known. Finally, neural circuits appear differentially susceptible to synapse loss in AD, yet whether complement shapes such circuit-specific vulnerability remains unclear. Our work aims to determine the genetic and cellular processes responsible for circuit-specific synapse loss in AD. Our efforts are focused on testing the specific role for complement in mediating this process. Rather than relying on a single transgenic model that poorly captures the genetic heterogeneity of human AD, we are leveraging genetically diverse mouse models. We have created a panel of wild-derived AD models that show variation in AD-relevant phenotypes including cognitive decline, amyloid deposition, neuronal cell loss and microglia states. We have used state-of-art viral approaches to label specific neural circuits that are differentially vulnerable to age- and AD-related synapse loss. We have found that in contrast to the ubiquitous C57BL/6J (B6) mouse strain, relationships between microglia, amyloid deposition, and synapses appear fundamentally different in PWK/PhJ mice. Our data show that in B6, but not PWK, synaptic density on CA1 neurons is modulated by microglia contact, strengthening the notion that mechanisms mediating AD progression depend strongly on genetic factors. Single nucleus sequencing of hippocampi from B6 and PWK mice with and without amyloid revealed different proportions of cell type-specific clusters in PWK compared to B6, identifying candidate genes and cell-specific processes driving PWK resilience to synapse loss. Spatial transcriptomics and proteomics are being used to assign complement components and putative PWK resilience factors to specific cell types or states that will be tested using gene knockouts. Our long-term goal is to use these data to develop more precise therapeutic approaches to prevent or slow synapse loss in AD.

Disclosures: G. Howell: None. **S.E. Heuer:** None. **A. Uyar:** None. **E. Pizzi:** None. **A. Hewes:** None. **M. MacLean:** None. **M. Sasner:** None. **E. Bloss:** None.

Presentation Number: NANO45.02

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

Support: Alz Assoc AARG-16-441560
Bright Focus Foundation A2022032S

Title: Structural and functional analysis of TREM2 interactions with amyloid beta reveal molecular mechanisms that drive phagocytosis of oligomeric amyloid beta

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[1]Med., Washington Univ. in St. Louis, SAINT LOUIS, MO; [2]Pulmonary, Washington Univ. Sch. of Med., Saint Louis, MO; [3]Med., Washington Univ. in St Louis, Saint Louis, MO

Abstract: The development of new innovative treatments to prevent and ameliorate Alzheimer's disease (AD) requires knowledge of molecular mechanisms that are critical to neuronal health. The receptor TREM2 is part of a signaling complex that modulates inflammatory responses, phagocytosis and cell survival in microglia- resident immune cells in the brain that play a critical role in clearing misfolded aggregates such as amyloid beta (Aβ). In recent years, TREM2 has emerged as a promising drug target for AD. Understanding the molecular mechanisms underlying TREM2 signaling in microglia will facilitate the development of specific, safe and efficacious therapies for AD that target TREM2. With this in mind, we set out to determine the structural mechanism for TREM2 phagocytosis of oligomeric Aβ (oAβ). We utilized comprehensive structural, biophysical, and functional analysis to achieve this goal. We used biolayer interferometry (BLI) analysis to investigate TREM2 interactions with oAβ42 WT and familial variants. We then used X-ray crystallography to determine the structure of TREM2 in complex with an Aβ peptide. Next, we used BLI with structure-guided TREM2 variants to validate the Aβ binding site on TREM2. Finally, we used Aβ phagocytosis assays in HMC3 microglia cells to investigate functional ramifications. We found that N-terminal variants in Aβ did not bind TREM2. Using this information, we co-crystallized a short peptide (Aβ 1-8) in complex with TREM2 and determined the structure. We found that it bound at CDR1 near the hydrophobic site. This was validated by BLI, as mutations to the TREM2 hydrophobic site ablated binding to oAβ42. Finally, we found that the interactions between the N-terminal region of Aβ and TREM2 were critical to drive phagocytosis in HMC3 cells. Our results indicate that TREM2 uses the hydrophobic site (consisting of the CDR1, CDR2, and CDR3 loops) to engage the N-terminal region of the Aβ42 peptide to drive phagocytosis of oAβ42. They also suggest that therapeutics that either directly bind or allosterically alter the hydrophobic site on TREM2 should modulate TREM2 signaling as potential AD treatments.

Disclosures: J.A. Greven: None. **J.M. Alexander-Brett:** None. **T.J. Brett:** None.

Presentation Number: NANO45.03

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

Title: Microglial cellular stress response and type-I interferon axis aggravate neurotoxic amyloid-beta pathology in a mouse model of Alzheimer's disease

Authors: H. PARAISO[1], H. HUANG[4], G. MAAG[2], B. SCOFIELD[3], J. YEN[3], ***I.-C. I. YU**[1,5];
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Abstract: Neuroinflammation is increasingly recognized as a key contributor to neuronal damage and neurodegeneration in Alzheimer's disease (AD). Microglia (MG), as brain-resident immune cells, dynamically regulate the brain's inflammatory responses. Pathological activation

of MG can lead to significant neuronal damage. We have previously established that deficiency in nuclear factor (erythroid-derived 2)-like 2 (Nrf2)—a crucial regulator of the cellular stress response pathway—intensifies the immune training of MG following systemic inflammation. In this study, we demonstrate that knockdown of Nrf2 in human microglial-like cells leads to upregulating type-I interferon (IFN-I) effector genes, including *Isg15*, *Ifi27*, and *Cxcl10*. Conversely, pharmacological activation of Nrf2 reduces the expression of interferon regulatory factor 7 in lipopolysaccharide-stimulated MG, suggesting an interaction between the Nrf2 and IFN-I pathways in MG. To investigate how the Nrf2/IFN-I axis influences microglial responses to amyloid-beta (Aβ) in AD, we crossbred $Nrf2^{-/-}$ mice with 5xFAD mice and examined the offspring at six months of age. These $Nrf2^{-/-}$: 5xFAD mice showed elevated IFN-I responses and an accumulation of diffusely distributed Thioflavin S (ThioS)-labeled fibrillar Aβ plaques. $Iba1^{+}$ MG in $Nrf2^{-/-}$: 5xFAD brains displayed impaired engagement with $ThioS^{+}$ Aβ plaques, indicating a diminished capacity of Nrf2-null MG to process Aβ. Considering phagocytosis as a critical function of MG in limiting Aβ-associated neuropathology, we evaluated whether this capability was affected. Our results revealed that approximately 28% of MG in 5xFAD mice had ingested Aβ labeled with methoxy-X04, whereas only 10% of MG in $Nrf2^{-/-}$: 5xFAD mice showed similar activity, indicating a reduced phagocytic capacity in Nrf2-null MG. Further, we sorted $CD45^{+}$ immune cells from $Nrf2^{-/-}$: 5xFAD brains for single-cell RNA sequencing analysis. While $Nrf2^{-/-}$: 5xFAD mice retained previously identified microglial clusters, they exhibited reduced $Trem2^{+}$ disease-associated MG (DAM) populations and a subset enriched in Rho GTPase pathways. The IFN-response MG (IRM) subsets remained similar; however, Nrf2 deletion led to upregulation of chemokine *Ccl5* expression in IRM, correlating with the increased T-cell infiltration observed in $Nrf2^{-/-}$: 5xFAD mouse brains. Overall, our findings highlight the significant role of Nrf2-IFN-I pathway interactions in modulating microglial functions and the pathology associated with Aβ, providing new insights into the complex interactions of these pathways in neuroinflammation and AD.

Disclosures: H. Paraiso: None. **H. Huang:** None. **G. Maag:** None. **B. Scofield:** None. **J. Yen:** None. **I.I. Yu:** None.

Presentation Number: NANO45.04

Topic: B.09. Glial Mechanisms

Support: NIA R01AG066429
NIA RF1AG054199
NIA R01AG079859-01
NIA P30AG013846
Cure Alzheimer's Fund

Title: Selenoprotein P regulates biogenesis and secretion of extracellular vesicles in microglia in vitro and in Alzheimer's mouse model in vivo

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[1]Mayo Clin. Florida, Jacksonville, FL; [2]Boston Univ. Sch. of Med., Boston, MA; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: We recently identified selenoprotein P (*Sepp1*), a secreted heparin-binding glycoprotein, as a potential regulator of biogenesis and secretion of extracellular vesicles (EVs) in microglia by genome-wide shRNA-based gene screening. Here we evaluated the effect of *Sepp1* silencing on EV secretion from BV-2 microglia by Nanoimager and Nanoflow analysis *in vitro* and by microglial specific CD9 gene reporter virus system in $APP^{NL\text{-}G\text{-}F}$ knock-in Alzheimer's disease mouse model for the validation *in vivo*. We further performed BV-2 transcriptomics under *Sepp1* silencing for the molecular understanding of inhibition of EV secretion. First, we generated BV-2 cells, a murine microglial cell line, stably expressing tdTomato-CD63 EV reporter molecule. They were transduced with lentiviral vector expressing short hairpin RNA (shRNA) targeting *Sepp1* or scramble as a control and monitored for the EV secretion using super resolution microscope (dSTORM), and Flow Nanoanalzer. The cellular RNA samples were submitted for the transcriptomic analysis. To track EVs secretion from microglia *in vivo*, we co-injected lentiviruses which overexpress mEmerald-CD9 (mEm-CD9) reporter molecule in microglia specific manner and mCherry-*conjugated Sepp1* or scramble shRNA into the hippocampus of $APP^{N\text{-}L\text{-}GF}$ mice at 6 months of age. Mice were euthanized at 2 weeks post injection and immunostained for galectin-3 (Mac2, MGnD marker), RFP (mCherry), GFP (mEm-CD9) and fluorostylybenzene (FSB, amyloid plaque). The images of mEm-$CD9^+$ voxels (EV particles) in the proximity of $Mac2^+RFP^+GFP^+$ microglia were captured by super-resolution confocal microscopy and the number of EV particles were quantified after 3D surface rendering of EV particles using IMARIS software. Silencing of *Sepp1* significantly reduced EV secretion from microglia and CD63 loading to EVs. The number of mEm-$CD9^+$ particles secreted from $Mac2^+$ microglia surrounding amyloid plaque was reduced when $Mac2^+$ microglia expressed mCherry-*Sepp1* shRNA compared to scramble shRNA in $APP^{N\text{-}L\text{-}GF}$ mouse brains *in vivo,* validating the results obtained *in vitro*. Transcriptomic profiling of microglia identified that *Sepp1* silencing downregulates EV synthetic machinery, accompanied with enriched pathways of lysosomal degradation and lipid metabolism. Taken together, our results demonstrated that *Sepp1* silencing effectively reduces EV secretion from microglia both *in vitro* and *in vivo* and alters not only EV biogenesis but also lysosomal and lipid metabolism pathways, further suggesting the impact of EV biogenesis on cellular metabolism.

Disclosures: V. Bodart-Santos: None. **Z. Ruan:** None. **B. Melvin:** None. **I. Pandey:** None. **S. Ikezu:** None. **T. Ikezu:** None.

Presentation Number: NANO45.05

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

Support: NIH NIA R01AG05467
NIH NIA R01AG066429-02
NIH NIA RF1AG054199-02
NIH NIA R01AG067763-01
NIH NIA R01AG072719-01
NIH NIA R01AG079859-01
NIH NIA RF1AG082704-01

Title: Microglial-specific Tsg101 deletion restricts microglia activation and ameliorates extracellular vesicle -mediated tau propagation in Alzheimer's disease

Authors: *Z. ZHANG[1], Z. RUAN[1,2], Y. YOU[1], S. HERRON[2], B. MELVIN[1], T. C. IKEZU[1], N. DELIVANOGLOU[1], J. WANG[2], W. E. JOHNSON[3], S. DA MESQUITA[1], H. DAVTYAN[4], M. BLURTON-JONES[4], O. BUTOVSKY[5], E. THOMPSON[1], D. W. DICKSON[1], T. IKEZU[1,2], S. IKEZU[1,2];
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Abstract: Background: Extracellular vesicles (EVs) carry pathogenic molecules and contribute to disease pathology, including the spread of aggregated tau proteins. TSG101, a component of endosomal-sorting complexes required for transport (ESCRT) I, plays a crucial role in endosomal trafficking and ESCRT-dependent EV biogenesis. We hypothesize that targeting Tsg101 specifically in microglia suppresses EV-mediated tau propagation, ameliorating disease progression. To test this, we examined the role of Tsg101 on tau pathology using a conditional *Tsg101* knockout PS19 mouse model, human brain tauopathy cohorts, and *TSG101* deficient human microglia. **Methods:** We crossed PS19 mice with *Cx3cr1*^{CreERT2}:*Tsg101*$^{f/f}$ lines to induce microglia-specific Tsg101 deletion (Tsg101 cKO). The mice received tamoxifen at 2 months of age and underwent comprehensive assessments, including behavioral, neuropathological, and molecular analyses by hippocampal bulk RNA and spatial transcriptomics (GeoMx) at 7 months old. Next, we examined *TSG101* mRNA level of microglia in hippocampal regions from individuals diagnosed with AD, Pick's disease, PSP, FTDP-17, and control cases using RNAscope technique. Finally, we employed CRISPR-Cas9 editing to generate *TSG101* knockout iPSCs to validate the roles of TSG101 on human microglial function. **Results:** PS19:Tsg101cKO mice exhibit decreased tau aggregates (Alz50, MC1) in the hippocampus and a reversal of cognitive impairment as assessed by Y-maze, novel object recognition and fear conditioning compared with PS19 mice. The hippocampal bulk RNA-seq showed suppression of disease associated microglia, neuroinflammation and complement pathway, suggesting that Tsg101cKO mitigated the microglial disease phenotype in PS19 mice. Consistently, spatial transcriptomic data in Iba1$^+$ microglia confirmed reduced synaptic engulfment in CA1 and inhibited phagocytosis in the CA3 and DG regions, as reflected by decreased C3aR1 level. RNAscope analysis with human brain revealed increased *TSG101* RNA level in IBA1$^+$ microglia in AD hippocampus compared to other pathological conditions and controls, particularly accumulating around amyloid beta plaques. Interestingly, elevated *TSG101* levels in microglia significantly correlated with Braak scores in male patients. Furthermore, TSG101 deficient human microglia showed reduction in phagocytosis of extracellular substrates, validating the transcriptomic results obtained from Tsg101 cKO:PS19 mice. **Conclusion:** These data suggested microglia-specific targeting of Tsg101 is a promising approach to attenuate tauopathy progression by regulating their phagocytic activity and inflammation.

Disclosures: Z. Zhang: None. **Z. Ruan:** None. **Y. You:** None. **S. Herron:** None. **B. Melvin:** None. **T.C. Ikezu:** None. **N. Delivanoglou:** None. **J. Wang:** None. **W.E. Johnson:** None. **S. Da Mesquita:** None. **H. Davtyan:** None. **M. Blurton-Jones:** None. **O. Butovsky:** None. **E. Thompson:** None. **D.W. Dickson:** None. **T. Ikezu:** None. **S. Ikezu:** None.

Presentation Number: NANO45.06

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

Support: NIH R01AG064234
NIH RF1AG078338
NIH R21AG078601
VA I01BX003643

Title: Targeting the pathogenic synergy of ceramide and S1P in Alzheimer's disease

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Abstract: The classical ceramide-sphingosine-1-phosphate (S1P) rheostat, which predicts opposite roles for pro-apoptotic ceramide and pro-survival S1P in cancer cells, takes on a distinct function in neurodegenerative disease. Research in our laboratory shows that ceramide and S1P mutually amplify their pathogenic function in Alzheimer's disease (AD). We found that Aβ binds to ceramide-rich extracellular vesicles (CREVs) that are secreted by astrocytes. The Aβ-associated CREVs are taken up by neurons and transported to mitochondria, ultimately leading to mitochondrial damage and neuronal cell death. We also found that Aβ induces the interaction of microglial S1P receptor 1 (S1PR1) with toll like receptor 4 (TLR4), which leads to the activation of microglia that secrete neuroinflammatory cytokines and fail to clear Aβ. In turn, S1P induced suppression of Aβ clearance in microglia and astrocytes and ceramide-mediated Aβ mitotoxicity in neurons synergistically drive AD pathogenesis. We tested several pharmacological approaches to disrupt this pathogenic synergy of ceramide and S1P and mitigate AD pathology. Using the 5XFAD familial AD mouse model, we inhibited Aβ-induced CREV secretion by astrocytes with the neutral sphingomyelinase 2 inhibitor GW4869 and restored Aβ clearance by microglia with the S1PR1 antagonist Ponesimod. These interventions significantly improved AD pathology and memory function in 5XFAD mice. Currently, we are testing additional drugs to target the synergy of ceramide and S1P, aiming to develop new approaches for AD therapy.

Disclosures: Z. Zhu: None. **X. Ren:** None. **Z. Quadri:** None. **L. Zhang:** None. **S. Crivelli:** None. **A. Elsherbini:** None. **S. Spassieva:** None. **E. Bieberich:** None.

Presentation Number: NANO45.07

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

Support: United States Department of Defense USAMRAA award W81XWH2010665 through the Peer Reviewed Alzheimer's Research Program
NIH National Center for Advancing Translational Sciences U18TR004146 and ASPIRE Challenge and Reduction-to-Practice awards
National Institutes of Health (NIH) award, R01MH128866 by National Institute of Mental Health

Title: Lipid droplets: a dynamic cell-organelle to combat Alzheimer's disease and aging.

Authors: *J. RAJPOOT, V. WENDT, Y. CHEN, P. SAKLANI, A. HOSSAIN, P. MANCHANDA, S. VIRANI, G. CHOPRA;
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Abstract: Alzheimer's disease (AD) is characterized by the accumulation of Amyloid-beta (Aβ) plaques where dysfunctional microglia with impaired phagocytosis play a role in disease pathogenesis. Our previous work revealed a key finding that microglial exposure to Aβ results in the formation of lipid droplets (LDs), or dynamic "fat" containing organelles in cells, with higher LD load near Aβ plaques from postmortem brains of human AD patients and in AD mouse model (5xFAD). These LD-laden microglia exhibit compromised phagocytic activity, with distinctive lipid composition marked by reduced free fatty acids (FFAs) and elevated triacylglycerol (TAG). Specifically, we identified that diglyceride acyltransferase 2 (DGAT2) is the key enzyme to catalyze FFA to TAG conversion. The DGAT2 protein is highly expressed in postmortem brains of human AD patients and 5xFAD mice and colocalized with microglial LDs. Inhibiting DGAT2 in microglia *ex vivo* from 5xFAD mice enhanced phagocytosis and reduced the LD burden in microglia suggesting a role of DGAT2 pathway in LD accumulation and microglial phagocytic impairment. However, commercially available DGAT2 inhibitors were unable to reduce LD load in older 5xFAD mice *in vivo* suggesting a role of DGAT2 in stabilizing LDs in late-stage disease. **To address this gap**, we designed, synthesized, and optimized DGAT2-specific protein degraders as bifunctional small molecules for targeted protein degradation. We conducted several *in vitro* and *in vivo* studies in late-stage AD and aging. Specifically, we used primary microglial cells from 11-15 month-old 5xFAD and cell lines expressing DGAT2 and quantified reduction in LDs and DGAT2 protein. To test the efficacy of DGAT2 degraders to reduce LDs *in vivo*, we first used aged-WT mice (18-24 months old) that contain large LDs. We found a significant decrease in LDs along with a reduction in DGAT2 protein *in vivo*, highlighting target engagement and the potential of this degrader in aging. Next, we tested the DGAT2 degrader *in vivo* in aged 5xFAD mice (18-24 months old) to assess its ability to reduce LDs and enhance Aβ phagocytosis. Remarkably, the degrader-treated 5xFAD mice showed a significant decrease in total LDs and DGAT2 along with a reduction in dystrophic neurites. Excitingly, the DGAT2 degrader showed a reduction in LDs in microglial cells with changes in morphology from a reactive amoeboid to ramified morphology along with a significant reduction in Aβ plaques upon treatment in the subiculum and cortex regions. These findings suggest that DGAT2-degrader can become a drug candidate to slow down late-stage AD pathology where the clinical markers are very well defined for patient selection.

Disclosures: J. Rajpoot: None. **V. Wendt:** None. **Y. Chen:** None. **P. Saklani:** None. **A. Hossain:** None. **P. Manchanda:** None. **S. Virani:** None. **G. Chopra:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Agilent Instrument In kind. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meditati Inc., BrainGnosis Inc..

Presentation Number: NANO45.08

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

Support: United States Department of Defense USAMRAA award W81XWH2010665 through the Peer Reviewed Alzheimer's Research Program

NIH National Center for Advancing Translational Sciences U18TR004146 and ASPIRE Challenge and Reduction-to-Practice awards
National Institutes of Health (NIH) award, R01MH128866 by National Institute of Mental Health

Title: Time to face the fats: Can we target lipid droplets for neuroimmunotherapy?

Authors: *G. CHOPRA[1], A. HOSSAIN[1], C. RANDOLPH[1], J. RAJPOOT[1], V. WENDT[1], C. BEVERIDGE[1], Y. CHEN[1], P. SAKLANI[1], P. MANCHANDA[2];
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Abstract: Lipid droplets (LDs) are emerging as crucial players in the pathogenesis and potential treatment of Alzheimer's disease (AD), yet their detailed composition remains largely unexplored. Our previous work revealed that exposure to amyloid-beta prompts microglia to form LDs, fatty deposits within cells, with LD accumulation heightened near amyloid plaques in both human AD brains and the 5xFAD mouse model (PMCID: PMC10274698). Building upon this foundation, our study offers the first comprehensive lipidomic profile of isolated LDs derived from aged and AD model (5xFAD) mouse brains. Leveraging multiple reaction monitoring (MRM) profiling, we incorporated an innovative online liquid chromatography-tandem mass spectrometry (LC-MS/MS) platform with ozone electrospray ionization (OzESI). This novel approach facilitates the rapid resolution of unsaturated, isomeric lipid structures within LDs, providing unprecedented insights into their composition. Our findings unveil significant variations in LD composition between aged and AD mice, indicating distinct inflammatory signals that impact LD composition. Moreover, region-specific lipidomic profiles highlight the unique effects of AD pathology within different brain regions, with notable differences observed between sexes. Additionally, we observed a correlation between LD formation and both age and disease progression, particularly in the hippocampus. Despite variations in LD load, microglia laden with LDs exhibited compromised amyloid-beta phagocytosis. Through unbiased lipidomic analysis, we identified a metabolic shift characterized by increased triacylglycerols (TAGs) and decreased free fatty acids (FFAs) underlying LD formation. Interestingly, these alterations in fatty acid metabolism may be linked to our prior research identifying neurotoxic long-chain saturated fatty acids secreted by reactive astrocytes (*Nature*, 2021, 599, pp102-107, PubMed PMID: 34616039) thereby relating microglia's neuroprotective role in chronic inflammation at the cost of its loss in function. Furthermore, we elucidate the role of DGAT2, a key enzyme in TAG synthesis, in promoting microglial LD accumulation, which is elevated in both 5xFAD and human AD brains. By developing small molecule degraders of DGAT2, we were able to enhance microglial uptake of amyloid-beta and substantially reduce plaque load, along with mitigating dystrophic neurites in the hippocampus of 5xFAD mice. These findings unveil a novel lipid-mediated mechanism underlying microglial and astrocytic dysfunction, offering promising avenues for the development of therapeutic strategies for AD.

Disclosures: G. Chopra: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Agilent Instrument In-Kind. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meditati Inc., BrainGnosis Inc.. **A. Hossain:** None. **C. Randolph:** None. **J. Rajpoot:** None. **V. Wendt:** None. **C. Beveridge:** None. **Y. Chen:** None. **P. Saklani:** None. **P. Manchanda:** None.

Presentation Number: NANO45.09

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

Support: ICON-VLAIO HBC.2019.2523

Title: Enhancing Microglial Targeting in Preclinical Drug Discovery via Human Microglia Chimeric Mice

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reMYND, Leuven, Belgium

Abstract: Microglia, the resident immune cells of the central nervous system, play a pivotal role in neuroinflammation and neurodegenerative diseases, notably Alzheimer's disease. Several disease-associated risk factors are almost exclusively expressed in human microglia, emphasizing the necessity for models that accurately replicate human biology. Targeting microglial pathways offers significant promise for therapeutic intervention in these conditions. Previous studies have demonstrated that humanized mice exhibit distinct transcriptomic profiles compared to conventional mouse models, highlighting the importance of human microglial engraftment for precise disease modeling. In this study, we utilized a previously characterized APP knock-in model (APPSAA) to generate chimeric mice with engrafted human microglia, thereby enhancing the fidelity of human neuroinflammatory responses. Our investigation evaluates the utility of this model for preclinical efficacy and proof-of-concept studies. Immunodeficient (RAG2-/-) newborn mice expressing human CSF1 (to enhance human microglia survival) and three familial APP mutations (APPSAA) were depleted of endogenous microglia using BLZ945 treatment and subsequently engrafted with human microglial precursors. Initial experiments demonstrated robust grafting efficiency, with flow cytometry indicating approximately 80% of microglia is from human origin three months post-engraftment. By three months of age, these mice start to develop amyloid pathology as in APPSAA mice. Notably, activated human microglia surrounding amyloid plaques were observed, as evidenced by human-CD9 staining. Furthermore, widespread distribution of human microglia across the brain was confirmed through hCD45 staining. These findings underscore the value of this model in elucidating microglial pathways and exploring potential therapeutic targets for neurodegenerative diseases. Currently efforts are being made to refine the process of generating chimeric pups, aiming to enhance adherence to study protocols. This innovative platform will represent a valuable tool for expediting the development of novel therapeutics aimed at modulating microglial function and mitigating disease progression.

Disclosures: S. Carmans: A. Employment/Salary (full or part-time):; reMYND. **W. Dejonckheere:** A. Employment/Salary (full or part-time):; reMYND. **T. Cornelissen:** A. Employment/Salary (full or part-time):; reMYND.

Presentation Number: NANO45.10

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

Support: NRF Grant 2022R1A2C3013138
KDRC Grant HU23C0217
NIH Grant R01NS109537

KIST Grant 2E32211
IBS Grant R001-D2

Title: Astrocytic autophagy plasticity modulates the neuropathology in Alzheimer's disease

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Abstract: Astrocytes, one of the most resilient cells in the brain, transform into reactive astrocytes in response to toxic proteins such as amyloid beta (Aβ) in Alzheimer's disease (AD). However, reactive astrocyte-mediated non-cell autonomous neuropathological mechanism is not fully understood yet. Herein, we show that astrocytes, unlike neurons, undergo plastic changes in autophagic processes to remove Aβ. Aβ transiently induces expression of *MAP1LC3B/LC3B* gene and turns on a prolonged transcription of *SQSTM1* gene. The Aβ-induced astrocytic autophagy accelerates urea cycle and putrescine degradation pathway. Pharmacological inhibition of autophagy exacerbates mitochondrial dysfunction and oxidative stress in astrocytes. Astrocyte-specific knockdown of *LC3B* and *SQSTM1* significantly increases Aβ plaque formation and hypertrophic reactive astrocytes in APP/PS1 mice, along with a significant reduction of neuronal marker and cognitive function. An increase of LC3B and SQSTM1 protein in astrocytes is found in the hippocampus of AD patients. Taken together, our data indicates that astrocytic autophagic plasticity is an important cellular event to modulate Aβ clearance and maintain cognitive function.

Disclosures: S.H. Kim: None. **H. Chun:** None. **Y. Kim:** None. **J. Chu:** None. **U. Park:** None. **S. Kim:** None. **A. Yousefian-Jazi:** None. **S.C. Kim:** None. **S. Hyeon:** None. **K. Lee:** None. **S. Oh:** None. **J. Lee:** None. **C. Lee:** None. **H. Ryu:** None.

Presentation Number: NANO45.11

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

Support: BrightFocus Foundation A2023021F

Title: Astroglial oxidative stress in vivo caused by soluble Abeta and Abeta deposition in a mouse model of Alzheimer's disease

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Abstract: One of the key hallmarks of Alzheimer's disease (AD) is the accumulation of Amyloid-beta (Aβ) plaques which can lead to astroglial dysfunction, contributing to the disease's progression. We recently reported evidence of calcium dysregulation in astrocytes *in vivo* from

an Aβ plaque-depositing AD transgenic mouse model (APPswe/PS1dE9; PMID: 19251629), and after direct application of naturally secreted soluble Aβ oligomers (Aβo) to the healthy brain (PMID: 37236808), by using multiphoton microscopy and a genetically encoded calcium sensor targeted to astrocytes. Calcium dyshomeostasis is closely associated with the increase of reactive oxygen species (ROS) in AD and other neurodegenerative diseases, which is responsible for promoting oxidative damage to DNA, RNA, proteins and lipids, triggering cell death, a key hallmark of AD pathology. In this work, we aimed to specifically investigate how Aβ plaques and soluble Aβo contribute to the ROS insult in astrocytes *in vivo*. We characterized the intracellular oxidative stress of astrocytes by intravital microscopy, and using a genetically encoded indicator (*roGFP*, PMID: 38238819) that selectively reports the reduced/oxidized glutathione (GSH/GSSG) ratio specifically targeted to astrocytes (GFA2.roGFP). We evaluated astroglial oxidative stress before and after the deposition of Aβ plaques in APPswe/PS1dE9 mice compared to aged-matched non-transgenic mice, as well as before and after acute application of naturally secreted soluble Aβo (conditioned medium from cultured transgenic primary neurons) onto the living brain of 4-6-mo-old non-transgenic mice. Our data show an increase in oxidative stress in all astroglial compartments (somas, processes, and end feet) in APPswe/PS1dE9 mice compared to non-transgenic mice only after Aβ deposition, as well as upon topical soluble Aβo application. Astroglial oxidative stress levels were similar in transgenic and non-transgenic young mice before Abeta deposition. Likewise, Aβ-immunodepleted transgenic conditioned media and wild-type conditioned media did not alter astrocyte cytosolic oxidative stress, supporting the specificity of the observed effects. These results support a detrimental role of Aβ, which leads to astrocyte oxidative stress *in vivo*, implying that Aβ accumulation is involved in the astrocytic dysfunction observed in AD. Future studies will address whether this oxidative stress is cause or consequence of the pathology, the specific pathways affected, and the implications for neuronal activity, survival and synaptic functionality.

Disclosures: M. Sanchez-Mico: None. **E. Kironde:** None. **R. Paredes:** None. **S.S. Hou:** None. **M. Algamal:** None. **M. Calvo-Rodriguez:** None. **B.J. Bacskai:** None.

Presentation Number: NANO45.12

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

Support: NIH Grant AA027108
NIH Grant AG041274

Title: Inhibition of astrocytic unfolded protein response enhances glymphatic function and reduces tau aggregation

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Abstract: The glymphatic (glia-lymphatic) system is a brain-wide fluid transport pathway crucial for clearing interstitial waste products such as β-amyloid and tau proteins. Despite extensive documentation of glymphatic dysfunction in neurodegenerative conditions, including Alzheimer’s disease (AD), the potential for enhancing glymphatic flow to reduce pathological protein deposition remains unclear. In this study, we demonstrate that glymphatic impairment in AD could be alleviated by restoring protein synthesis in astrocytes. Through analysis of publicly

available single-cell RNA sequencing data from human AD patients and animal models, we identified a robust activation of the PERK-eIF2α signaling pathway of the unfolded protein response in astrocytes. In P301S transgenic mice expressing mutant human tau, PERK activation results in the suppression of protein synthesis in astrocytes. Conditional knockout of astrocytic PERK restores protein translation and enhances glymphatic flow. Furthermore, genetic deletion of PERK reduces tau pathology in both the cortex and hippocampus and improves cognitive function in mice. Together, these results suggest that improving astrocytic protein synthesis by inhibiting the unfolded protein response may be a promising target for protecting against glymphatic failure in AD.

Disclosures: K. Chen: None. **M. Li:** None. **G. Yang:** None.

Presentation Number: NANO45.13

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

Title: Exercise hormone irisin reduces inflammation and amyloid-β in 3D Alzheimer's disease culture model

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Abstract: Irisin is a myokine that is generated by cleavage of the membrane protein FNDC5 (fibronectin type III domain-containing protein 5) in response to physical exercise. Studies reveal that irisin/FNDC5 has neuroprotective functions against Alzheimer's disease (AD) by improving cognitive function and reducing amyloid-β (Aβ) and tau pathologies as well as neuroinflammation in cell culture or animal models of AD. Further mechanistic studies are required to clarify its potential as a therapeutic target for alleviating AD. Using a three-dimensional (3D) human neural cell culture model of AD which displays robust Aβ generation followed by tau pathology (3D-AD cultures), we recently found that irisin treatment reduces Aβ pathology by increasing the activity/levels of Aβ-degrading enzyme neprilysin (NEP) secreted from astrocytes. We identified integrin αV/β5 as the irisin receptor on astrocytes to induce NEP secretion. We also found that irisin reduces astrocyte reactivity and that irisin-induced NEP secretion is mediated by downregulating extracellular signal-regulated kinase (ERK)-signal transducer and activator of transcription 3 (STAT3) signaling, known to be chronically activated in reactive astrocytes. Our recent study showed that neurotoxic A1 reactive astrocyte inducers (IL-1α, TNF-α, and C1q) increased Aβ levels and that ERK activation precedes complement C3 activation in the 3D-AD cultures. Our work not only provides a potential explanation for the mechanisms by which exercise could prevent AD, but also highlights the significant involvement of astrocytes in the AD brain during physical activity.

Disclosures: E. Kim: None. **R.E. Tanzi:** None. **S. Choi:** None.

Nanosymposium

NANO46: Stroke Recovery: Non-Pharmacological Approaches

Location: MCP Room N227

Time: Tuesday, October 8, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO46.01

Topic: C.09. Stroke

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
JSPS KAKENHI Grant 18KK0276
Uehara Memorial Foundation

Title: Parvalbumin interneurons regulate rehabilitation-induced functional recovery after stroke through functional connectivity and gamma oscillation

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Abstract: The trajectory of stroke recovery varies depending on the experience during the recovery period. Rehabilitation is a therapy that promotes functional recovery by enhancing pro-recovery experiences, such as repetitive motor training and environmental enrichment. Previous work showed that stroke recovery relies on biological mechanisms underlying the memory/learning system that involve excitatory and inhibitory circuits. However, the distributed brain circuits that mediate neurorehabilitation-induced recovery after stroke have not been defined. Here, we demonstrate that rehabilitation after stroke selectively enhances synapse formation in presynaptic parvalbumin interneurons and postsynaptic neurons with axonal projection to the stroke site (stroke-projecting neuron). Rehabilitation improves motor performance and neuronal functional connectivity, while inhibition of stroke-projecting neurons diminishes motor recovery. Parvalbumin interneurons regulate neuronal functional connectivity, and their activation during training is necessary for recovery. Furthermore, gamma oscillation, a parvalbumin-regulated rhythm, is increased with rehabilitation-induced recovery in animals after stroke and stroke patients. Pharmacological enhancement of parvalbumin interneuron function improves motor recovery after stroke, reproducing rehabilitation recovery. These findings identify brain circuits that mediate rehabilitation recovery and the possibility of rational selection of pharmacological agents to deliver the first molecular rehabilitation therapeutic.

Disclosures: N. Okabe: None. **S. Carmichael:** None.

Presentation Number: NANO46.02

Topic: C.09. Stroke

Support: AHA 932980

Title: High-definition transcranial direct current stimulation guided by delta-alpha ratio topography for precision stroke rehabilitation: a hypothetical study

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Abstract: Transcranial direct current stimulation (tDCS) shows potential as a therapeutic intervention for motor recovery post brain injuries including stroke. However, traditional tDCS methods encounter challenges of the absence of objective biomarkers to assess their effectiveness and provide online neurofeedback. Our recent studies showed that quantitative EEG metric Delta-Alpha Ratio (DAR) reflects brain function changes post-stroke and indicate reduced motor impairment after tDCS. The current hypothetical study proposes a novel approach utilizing high-definition tDCS (HD-tDCS) guided by individualized DAR topography from quantitative electroencephalogram (qEEG) metrics. The primary objective is to investigate the efficacy of targeted HD-tDCS in stroke rehabilitation by precisely stimulating specific brain regions identified through DAR topography. This individualized treatment approach aims to optimize stimulation targets based on the unique neural dynamics of each stroke patient. DAR values are obtained during resting state before HD-tDCS stimulation to guide the selection of stimulation targets tailored to each patient's neurophysiological profile. The study adopts a double-blind randomized crossover trial design to compare the effects of 1) anodal HD-tDCS guided by DAR topography, 2) anodal tDCS fixed over C3 or C4 without DAR guidance, and 3) sham stimulation. Outcome measures include DAR values before and after HD-tDCS stimulation, assessed at the site of stimulation. Hypothetical results suggest that anodal HD-tDCS guided by DAR topography induces a more significant decrease in DAR, indicating enhanced modulation of cortical activity compared to non-guided HD-tDCS. Additionally, targeted HD-tDCS is expected to yield greater improvements in upper extremity function, as measured by the Fugl-Meyer Upper Extremity score. In conclusion, this hypothetical study underscores the potential of targeted HD-tDCS guided by DAR topography as a precise and effective intervention for stroke rehabilitation. DAR topography emerges as a promising objective biomarker for optimizing tDCS protocols and evaluating neuroplastic changes in stroke rehabilitation.

Disclosures: B. Mulyana: None. **J. Williamson:** None. **Y. Yang:** None.

Presentation Number: NANO46.03

Topic: C.09. Stroke

Title: Remote ischemic conditioning affects extracellular vesicle profiling under conditions of ischemic stroke

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Abstract: Remote ischemic conditioning (RIC) is an interesting non-invasive albeit experimental concept, which yields both reduction of brain injury and enhanced neurological recovery. Although some recent clinical trials suggest a therapeutic potential of RIC in stroke

patients, fundamental questions as to the optimal timing and the duration of RIC cycles are still open for debate, hindering successful translation from bench to bedside even further. Using a preclinical stroke model, mice were exposed to different protocols of hind limb RIC followed by a characterization of small extracellular vesicle (sEV) patterns derived from both the blood and the brain. When mice were exposed to transient middle cerebral artery occlusion (MCAO), induction of RIC starting on day five poststroke for eleven consecutive days (three cycles per day with a duration of 10 minutes per cycle) yielded increased neurological recovery during the observation period of three months. Such animals significantly performed better than controls in the platform arrival test, the foot fault test, the rotarod test, and the corner turn test. Reducing the RIC protocol towards six consecutive days with only one cycle per day also significantly reduced brain injury, whereas three days of RIC were insufficient. Hence, the remainder of the study used a six-day RIC protocol with only one cycle per day. Flow cytometry analyses revealed reduced leukocyte infiltration into the brain due to RIC treatment. The latter also diminished microglia activation, changed the extracellular milieu as indicated by growth factor concentrations, and inhibited stroke-induced proteasome activation. sEV enriched from the frontal lobe of RIC mice did not differ from controls in terms of nanoparticle tracking analysis (NTA) or expression patterns of sEV markers. On the contrary, blood samples from RIC mice displayed a spectrum change in the NTA analysis without affecting western blot analyses. As a matter of fact, sEV derived from blood samples of RIC mice were significantly enriched with miR-1906, which has previously been described by us to induce poststroke neuroprotection. Indeed, when such miR-1906 enriched sEV were intravenously given to non-RIC stroke mice, a profound and sustaining neuroprotection was observed. Interestingly, sEV derived from RIC mice showed a higher therapeutic potential than sEV derived from sham mice or stroke mice that were not exposed to RIC. Our data demonstrate that RIC provides a long-term neuroprotective impact with increased neurological recovery that is mediated by primarily changing the sEV secretome in the periphery but not in the brain itself.

Disclosures: S. Gerner: None. **M. Juenemann:** None. **M. Baehr:** None. **E. Kiliç:** None. **T.R. Doeppner:** None.

Presentation Number: NANO46.04

Topic: C.09. Stroke

Title: Endothelial KCNN4 knockout ameliorates neurological deficits by regulating cerebrovascular function following ischemia stroke

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Abstract: Background: Cerebrovascular activation, encompassing endothelial cells responses and alterations in cerebral blood flow, represents a primary event in ischemic stroke. In this cascade of events, KCa3.1, encoded by the gene KCNN4, exhibits elevated expression and engages in modulating ion dynamics, potentially contributing to vascular tone. Research findings further indicate that KCNN4 inhibition markedly attenuates edema, sodium retention and immune responses, underscoring the profound effects of endothelial KCa3.1 in vascular ion dysregulation. However, it remains elusive how endothelial KCa3.1 orchestrates cerebrovascular function and, consequently, what its specific impact is on ion level.**Aim:**The roles of endothelial

KCNN4 and its underlying mechanisms in ischemia stroke.**Methods:**Transient middle cerebral artery occlusion (tMCAO) was induced in Tek-Cre(-/-)KCNN4 flox/flox (WT) and Tek-Cre (+/-)KCNN4 flox/flox (Kcnn4 eKO) mice. Laser speckle contrast imaging (LSCI) serves to capture cerebral cortical blood flow alterations pre- and post-tMCAO. A battery of behaviours was used to assess neurofunction. qPCR and Flow cytometry was used to detect the knockout effects. Immunostaining were employed to examine white matter injury and cerebrovascular structural or functional integrity after stroke. **Results:**Deletion of endothelial KCNN4 ameliorated behavior deficits and histological long-term outcomes following stroke, with significant benefits observed in the white matter. These improvements occurred without impacting cerebral perfusion, as evidenced by LSCI. Immunostaining on the seventh day post-stroke revealed a substantial reduction in apoptosis among vessel-associated cells and ERG+ endothelial cells in the KO group. A significant decrease in collagen IV coverage was observed within white matter regions, alongside an increase in regressive vessels, pointing to an enhanced stabilization of the vasculature in KO mice. Additionly, KO group exhibited elevated endpoints and diminished average vessel length, suggestive of a denser and more intricate vascular network.**Conclusions:**In ischemic stroke, deletion of KCNN4 attenuates disruptions in cerebrovascular structure and function, thereby providing long-term protection to cerebral white matter. This protective effect is likely mediated through the optimization of vascular ion regulation after the stroke, rather than through modulation of blood flow.

Disclosures: S. Chen: None.

Presentation Number: NANO46.05

Topic: C.09. Stroke

Support: Health Research Council of New Zealand 20/190

Title: Enhancing spontaneous recovery after stroke study (ESPRESSo): Does early, high-dose, high-intensity therapy improve upper limb motor recovery and outcome?

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Abstract: ESPRESSo is a registered, single-site randomised, assessor-blind, controlled Phase IIa clinical trial, that aimed to determine whether a 3-week programme of high-intensity, high-dose neuroanimation therapy (NAT) can improve upper limb recovery and outcomes early after stroke. Eligible patients were recruited on admission if baseline upper extremity Fugl-Meyer (UE-FM) score <51 and positive motor-evoked potential (MEP) status. Exclusion criteria included cerebellar stroke and inability to sit to perform upper limb therapy. All participants began therapy within 2 weeks of stroke. Sixty-four participants were randomised with minimisation into NAT or conventional therapy (COT) groups (n=31,33). In addition to standard care upper limb therapy, both groups received 90 minutes of therapist time per weekday for three

weeks to complete high-dose upper limb therapy. The NAT group used the Mindpod Dolphin platform under therapist supervision, to engage in high-intensity, high-dose spontaneous exploratory arm and hand movements, focused on movement quality in a game-based virtual environment. The COT group received task-orientated training. Measures of active time on task, motivation, fatigue and cognitive efficiency were obtained in all therapy sessions. The main hypothesis was that upper limb motor capacity, measured as Action Research Arm Test (ARAT) score at 12 weeks post-stroke, will be better for the NAT group than the COT group. Secondary outcomes explored effects on motor impairment measured by the UE-FM at 3 months post-stroke, as well as all measures obtained immediately post-intervention and at 6 months post-stroke. Measures of hand dexterity during pinch versus grasp and release tasks were obtained from the paretic and non-paretic sides at all time points. Transcranial magnetic stimulation was used to obtain MEPs for four upper limb muscles, and arm kinematics were obtained during reaching at all post-intervention time points from both sides. The primary and secondary outcomes will be known in June and September respectively such that the trial outcome will be reported for the first time at the Neuroscience 2024 meeting. Outcomes for the two experimental groups will be compared to a case-controlled cohort who underwent usual care (physiotherapy, occupational therapy) at the same hospital. These patients were involved in observational research such that baseline and 3 month UE-FM and ARAT scores were obtained. The findings will reveal the efficacy of NAT relative to time-matched COT, as well as the efficacy of early intensive upper limb therapy relative to standard and usual care.

Disclosures: W.D. Byblow: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment for this trial was supplied in-kind by Mindmaze SA. **J.W. Krakauer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor of MindPod Dolphin and has equity in MindMaze. **C.M. Stinear:** None. **P. Barber:** None. **A. Lee:** None. **T. Kitago:** F. Consulting Fees (e.g., advisory boards); Received consulting fees from MindMaze. **M. Shanks:** None. **L. Duval:** None. **B. Scrivener:** None. **P. Colle:** None. **A. Ren:** None. **J. Cirillo:** None. **N. Ejaz:** A. Employment/Salary (full or part-time):; is a Full-time employee of MindMaze. **G. Garipelli:** A. Employment/Salary (full or part-time):; Full-time employee and has equity in MindMaze.

Presentation Number: NANO46.06

Topic: C.09. Stroke

Support: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID 431549029 – SFB 1451

Title: The effect of reward and punishment on motor adaptation early after stroke

Authors: *T. PAUL[1], V. WIEMER[1,2], J. GÜNTHER[1], F. M. LEHNBERG[1], S. T. GRAFTON[3], G. R. FINK[1,2], L. J. VOLZ[1];
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Abstract: Motor impairment is one of the most common symptoms after stroke and represents a leading cause of permanent disability worldwide. Notably, the motor system can partially

recover lost motor functions during the first weeks and months after stroke by adapting its motor control policies to compensate for the stroke-induced lesion. This re-learning of motor skills during the recovery process is assumed to rely on mechanisms similar to motor learning in the healthy human brain (Krakauer, 2015). Thus, motor adaptation paradigms in which the motor system has to compensate for an experimental perturbation seem particularly well-suited to probe motor learning after stroke, yet corresponding studies remain scarce. While it has been shown that chronic stroke patients can perform error-based adaptation learning in a reinforcement-dependent fashion (Quattrocchi et al., 2017), it remains unknown whether acute stroke patients can perform motor adaptation learning with their paretic hand and, if so, whether reinforcement feedback can modulate such adaptation processes. We therefore assessed twenty-four acute stroke patients (time since stroke: mean=5.3 days, std=5.0 days; age: mean=62 years, std=11 years; 12 male, 12 female) using a joystick-based visuomotor adaptation paradigm. Besides showing for the first time that stroke patients possess the capacity for motor adaptation learning with their paretic hand, we also replicated established phenomena known from healthy participants such as savings between blocks and retention effects. Of note, reward and punishment differentially impacted motor adaptation. Unlike previous reports of a faster learning rate in response to punishment in healthy subjects (Galea et al., 2015), punishment led to poorer performance than reward during the initial adaptation phase in acute stroke patients. Moreover, initial adaptation learning coupled with rewarding feedback enhanced the subsequent retention of the new motor control policy. These findings hold important implications for neurorehabilitation by highlighting the potential of reward feedback on the (re-)learning of motor function during increased plasticity early after stroke.

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Galea, J. M. et al. (2015). The dissociable effects of punishment and reward on motor learning. *Nature Neuroscience, 18*(4), 597-602.
Krakauer, J. W. (2015). The applicability of motor learning to neurorehabilitation. In V. Dietz & N. Ward (Eds.), *Oxford Textbook of Neurorehabilitation* (Vol. 1). Oxford University Press.
Quattrocchi, G. et al. (2017). Reward and punishment enhance motor adaptation in stroke. *Journal of Neurology, Neurosurgery and Psychiatry, 88*(9), 730-736.

Disclosures: T. Paul: None. **V. Wiemer:** None. **J. Günther:** None. **F.M. Lehnberg:** None. **S.T. Grafton:** None. **G.R. Fink:** None. **L.J. Volz:** None.

Presentation Number: NANO46.07

Topic: C.09. Stroke

Support: NIH grant R01NS099210
NIH grant R01NS112942

Title: Improving motor recovery after stroke by combining myoelectric interface for neurorehabilitation conditioning with targeted memory reactivation during sleep

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Abstract: After stroke, individuals with arm impairment often experience abnormal muscle co-activation that limits movement range and coordination. Our prior research on chronic stroke survivors showed a reduction of abnormal co-activation after training with a myoelectric interface that required survivors to learn to decouple electromyogram signals from the co-activating arm muscles to control cursor movement in customized games. At-home training for six weeks with a wearable version, called myoelectric interface for neurorehabilitation (MINT), demonstrated significant improvement in arm function compared to stroke survivors receiving sham therapy (traditional biofeedback). Here, we introduce a novel approach to enhance motor-learning consolidation and amplify the benefits of MINT. Learning includes both training and subsequent consolidation, particularly during sleep. We employed a method known as targeted memory reactivation (TMR), that reinforces specific memories by presenting related audio cues during sleep, leading to enhanced recall of those memories upon awakening. Our previous work revealed TMR's effectiveness during slow-wave sleep (SWS) in improving learning of motor execution in neurotypical subjects performing a myoelectric interface task. Here, we present interim results combining MINT with home-based TMR in stroke survivors, indicating potential improvements in MINT performance compared to those undergoing MINT with sham sounds. Chronic stroke survivors with moderate-to-severe impairment were enrolled and randomized into two groups: MINT+shamTMR (hearing unrelated sounds) and MINT+TMR. Participants engaged in 90-minute daily MINT sessions, at least 6 days per week, over a 6-week period, with muscle pairs changed every 2 weeks. Additionally, stroke participants used a portable TMR system comprising a wrist-worn activity and heart-rate sensor and a smartphone emitting auditory cues. A machine-learning algorithm triggered TMR sounds when it detected SWS epochs. MINT performance, including time to target and cursor path length, was recorded for each 10-minute run. Preliminary findings in the initial 18 participants across all muscle pairs trained showed a trend toward improved performance in MINT+TMR versus MINT+shamTMR. These results suggest that TMR holds promise for enhancing motor learning and rehabilitation in stroke survivors.

Disclosures: A. Khorasani: None. **P. Prakash:** None. **C. Gorski:** None. **N. Whitmore:** None. **K.A. Paller:** None. **M.W. Slutzky:** None.

Presentation Number: NANO46.08

Topic: C.09. Stroke

Support: Fondecyt 11221226

Title: Recovery of upper extremity motor control by alternating transcranial stimulation and selective motor training in chronic stroke patients

Authors: *J. J. MARIMAN RIVERO[1,3], T. BRUNA-MELO[4,2], P. FIGUEROA[2], E. VERDI[2], R. FLORES[2], P. ULLOA[2], G. ÁLVAREZ[2], J. M. BECERRA CAROCA[2], J. ALVAREZ-RUF[2,5]; [1]Physical Therapy, [2]Univ. Metropolitana de Ciencias de la Educación, Santiago, Chile; [3]Physical Therapy, Univ. de Chile, Santiago, Chile; [4]Univ. de Valencia, Valencia, Spain; [5]Facultad de Medicina Clinica Alemana de Santiago Carrera de Kinesiología, Univ. del Desarrollo, Santiago, Chile

Abstract: Stroke patients exhibit a partial recovery of the upper limb motor control that is associated with permanent functional disability in the chronic stage. Transcranial alternating current stimulation (tACS) is a strategy that allows modulating oscillatory brain activity, affecting the development of strength in healthy subjects. In this research, we sought to evaluate the immediate and long-term effect of tACS associated with a training protocol for kinetic and kinematic control of the upper limb (UL). This research corresponds to an RCT. Patients receive a 10-session training in selective control for proximal and distal paretic UL movements, guided by biofeedback in a video game environment. Patients are stimulated by one of three tACS protocols (70 Hz, 169 Hz, or sham), applied for 20 minutes. The intervention is evaluated using clinical scales and instrumentalized motor tasks.23 patients have been enrolled until now (58 years old (33-75 range), 39 months of evolution from stroke (6-151 range)). After training, patients exhibited a significant increase in the upper extremity Fugl-Meyer assessment (FMA ~4 points). Patients who received tACS-70 Hz showed a tendency to higher increment in FMA. In all patients, motor training improved kinematic wrist extensor control, exhibiting lower variability, peak, and mean velocity in the isometric phase of a task. In contrast, the concentric and eccentric phases showed no changes. Our results suggest that selective training of the UL improves kinematic performance, associated with positive changes in motor impairments. This situation can be optimized with the application of neuromodulatory strategies, such as tACS, to enhance remnant neuroplastic capacity in this population. These preliminary results support intervention for intensive training to improve UL control even during the chronic stage of stroke.

Disclosures: J.J. Mariman Rivero: None. **T. Bruna-Melo:** None. **P. Figueroa:** None. **E. Verdi:** None. **R. Flores:** None. **P. Ulloa:** None. **G. Álvarez:** None. **J.M. Becerra Caroca:** None. **J. Alvarez-Ruf:** None.

Presentation Number: NANO46.09

Topic: C.09. Stroke

Support: American Heart Association Predoctoral Fellowship (900190)

Title: Post-stroke bimanual experience reflects bilateral neural plasticity in mice

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Abstract: The majority of strokes are ischemic resulting in blood flow reduction and irreversible cell damage in the core of the stroke. Areas physically surrounding the core of a stroke are 'peri-lesion' areas and may also experience disruptions in blood flow during stroke induction. Neural plastic changes in peri-lesion areas in relation to paretic limb use are well-characterized. In our mouse model using photothrombotic stroke induction, infarcts are centered over the motor cortical representation of the preferred for reaching forelimb. Given the numerous strong transcallosal connections between homotopic cortical areas, it is likely that activity and plasticity in one hemisphere impacts the other. However, interactions between peri-lesion and contra-lesion motor cortices in relation to experience-dependent plasticity are not well understood. This study was focused on post-stroke experience-dependent changes in dendritic spine

dynamics across hemispheres of the brain. We use Thy1-GFP mice, a transgenic strain which expresses green fluorescent protein in a sparse subset of their layer 5 cortical pyramidal neurons. Bilateral cranial window implantation was used to repeatedly measure the same fluorescing dendritic spines across time and track their stability in relation to behavioral experiences. After photothrombotic motor cortical ischemic stroke, mice underwent one of three rehabilitative experiences: unimnaual or bimanual skilled reach training or no training (Controls).
In this study, we compared bimanual and unimanual post-stroke experiences and their impact on dendritic spine dynamics in the peri-lesion and contra-lesion cortices. We've found that mice that experienced bimanual training showed greater dendritic spine stability and persistence in the contra-lesion cortex compared to unimanually trained mice and non-training controls. These data suggest greater functional integration of new spines into cortical circuits. Since these changes did not support improvements in impaired limb function in these mice, they instead likely underpin bimanual movements specifically. Additionally, given the lack of improvements in impaired limb function and indications of plasticity in the contra-lesion cortex, the bimanual manipulation used likely prompted compensatory reliance on the non-impaired limb in order to complete the task. These data highlight unique patterns of plasticity associated with bimanual movement, unique from unimanual movement, which has important implications for understanding the mechanisms underlying recovery of function after stroke.

Disclosures: V. Nemchek: None. **C. Hoang:** None. **V. Mallampaty:** None. **M. McCrea:** None. **N. Potdar:** None. **D. Sundararaman:** None. **T.A. Jones:** None.

Nanosymposium

NANO47: Olfaction: From Physiology to Behavior

Location: MCP Room S106

Time: Tuesday, October 8, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO47.01

Topic: D.03. The Chemical Senses

Support: NIH R01NS113119-01
NSF POLS PHY-1806818

Title: Finding neural representations of navigation strategies using whole-brain imaging of freely moving C. elegans in an odor gradient

Authors: *H. CASADEMUNT[1], C. PARK[1], W. FREDENBERG[1], S. KIM[1], J. VAN DER VEN[1], R. JACOBSEN[1], A. LIN[2], H. ZHANG[3], J. LAVER[4], M. ZHEN[5], J. A. CALARCO[4], A. D. SAMUEL[1];
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Abstract: Many small animals use their olfactory sense as their primary source of information to navigate their environment. We study the neural basis of olfaction in *Caenorhabditis elegans*, a

small nematode whose compact nervous system allows it to detect a wide range of odors and change its behavior to move towards favorable conditions using probabilistic chemotaxis strategies. To detect chemicals in the environment, these worms use large ensembles of sensory neurons and respond with distributed neuron activity in the brain, but how the brain generates chemotaxis strategies is poorly understood. We record whole-brain activity of animals freely navigating attractive odorant gradients to understand how neural activity encodes chemosensory perception and shapes motor decisions. We simultaneously measure whole-brain activity with neuron identities, behavior, and time-varying sensory input as the animal moves along the spatial gradient. We compare brain-wide dynamics generated by navigation towards different attractive odorants. By interrelating sensory, neural, and behavioral variables, we seek algorithms that shape the chemotactic navigational strategy.

Disclosures: H. Casademunt: None. **C. Park:** None. **W. Fredenberg:** None. **S. Kim:** None. **J. van der Ven:** None. **R. Jacobsen:** None. **A. Lin:** None. **H. Zhang:** None. **J. Laver:** None. **M. Zhen:** None. **J.A. Calarco:** None. **A.D. Samuel:** None.

Presentation Number: NANO47.02

Topic: D.03. The Chemical Senses

Support: National Science Foundation CAREER Grant 1749772
R01NS135763
Schmitt Foundation
University of Rochester Research Award

Title: Dissecting odor plume encoding using olfactory illusions

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Abstract: Plumes are complex, dynamic, rapidly fluctuating features of odors that are dispersed spatially by turbulent airflow. For rodents, which rely on using the information carried in these odor plumes to identify food, navigate their environment, avoid predators, and find mates, dissecting what is relevant within an odor plume is critical. The task is made more complicated by the fact that an animal's behavior, including its locomotion, sniffing, and head direction, all impact the ways in which odor identity and intensity is extracted from the odor plumes. Studying the neural code for plumes has proved challenging because plumes are dynamic, difficult to control, difficult to reproduce, and difficult to dissect into components based on temporal structure, contrasting other sensory systems where such deconstruction has been possible (frequencies of sound in auditory research, or Gabor patterns of specific spatial and temporal frequency in vision research). To address these challenges, we generated illusionary odor plumes by optogenetically stimulating ChannelRhodopsin labeled glomeruli in an OMP-ChR transgenic animal (N=4) in patterns that match the statistics of odor plumes while animals were awake and behaving. Light pulses of glomeruli allowed up to reproducibly generate "odor plumes" across multiple trials, and to decompose the complex plumes in elements. In parallel, we recorded neural activity from large populations of Mitral and Tufted (M/T) cells in the main olfactory bulb (MOB) of male and female mice (3-6 month of age). By deconstructing these illusionary odor

plumes into component frequencies, we found that M/T cells differentially encoded information (as measured by the mutual information between the illusionary plume and the spike train) about odor identity (the pattern of glomeruli stimulated), intensity (the magnitude of optogenetic stimulation), and spectrum (the temporal structure of the patterns). Using a Generalized Linear Model (GLM), we fit each neuron's spiking response to the pattern of optogenetic illumination corresponding to the odor plume to both identify what filters determine each M/T cell's response. The results provide a model for how odor plumes may be encoded for in the activity of neurons in the early olfactory system.

Disclosures: K. Padmanabhan: None.

Presentation Number: NANO47.03

Topic: D.03. The Chemical Senses

Support: NIH Grant 1RF1NS128865-01
Grant from NTT Research (no number)

Title: Neural basis of odor guided trail following in mice

Authors: *S. JAYAKUMAR[1], M. RAHMAN[1], A. MATHIS[1,2], V. N. MURTHY[1];
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Abstract: Animals actively sense the environment to acquire features of interest. An everyday example of active sensing behavior is our use of repeated visual saccades for scene recognition. Many behaviors in rodents are guided by odor cues, and active modulation of respiration and orofacial orientations are likely to play important roles. Navigating surface-bound odor trails is thought to involve bilateral comparison of signals from both nostrils across multiple sniffs, followed by subsequent motor actions. Sensory neurons convey olfactory information from the nose to the olfactory bulb (OB), where projection neurons relay it to higher brain regions that include olfactory cortical areas. While much is known about odor coding in these brain regions, the neural basis of such flexible yet precise odor-guided behavior remains poorly understood. To capture the behavioral repertoire during trail following we continuously challenged mice with dynamic odor trails using an "infinite" paper treadmill. By combining high-speed videography with quantitative behavioral analyses, we find that mice learn to quickly follow odor trails with high precision (>70% rewards collected within just 4 sessions as compared to ~30% without odor trail cues). Mice use short term memory to follow trails as evidenced by overshoots upon encountering abrupt turns in the odor trail. We corroborated previous findings that blocking one nostril causes lateralized biases in trail following. Mice use bilateral inputs to accurately follow trails since transecting the anterior commissure leads to impairments in trail following. To investigate the neural basis of trail following, we focused on the anterior olfactory nucleus (AON), an early olfactory cortical area that has privileged access to information coming from both nostrils and broadcasts this information to a wide range of cortical regions, as well as back to the OB. Targeted bilateral chemogenetic perturbations of AON activity leads to severe deficits in trail following whereas unilateral perturbations resulted in lateralized trail following deficits. Lastly, we measured neural activity in the AON using head-mounted miniaturized microscopes in mice following odor trails. In combination with respiratory measurements, these ongoing

experiments will relate olfactory cortical activity to behavioral strategies adopted by mice during trail following.

Disclosures: S. Jayakumar: None. **M. Rahman:** None. **A. Mathis:** None. **V.N. Murthy:** None.

Presentation Number: NANO47.04

Topic: D.03. The Chemical Senses

Support: NIH Grant R21DC021736 (NIDCD)
NIH Grant R01DC021213 (NIDCD)
NIH Grant R01DC017985 (NIDCD)

Title: Chemosensory detection of reptilian kairomones by the mouse accessory olfactory system

Authors: *J. WANG, V. HARAN, J. P. MEEKS;
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Abstract: Chemosensory detection of reptilian kairomones by the mouse accessory olfactory system Mammalian social interaction relies heavily on chemosensory information processed by olfactory pathways. In terrestrial mammals, the accessory olfactory system (AOS) is specialized for detecting non-volatile cues, including kairomones (interspecific social cues) and pheromones (conspecific social cues). We still lack knowledge about the majority of natural AOS ligands, the receptors that detect them, and associated behaviors. Here, we describe studies investigating the chemosensory mechanisms associated with detecting and responding to threatening kairomones in predator snake feces. We performed liquid chromatography-mass spectrometry (LC-MS) on the feces of reptilian mouse predators, insect-fed lizards, and vegetable-fed turtles. We identified several molecules that were enriched in mouse predator feces compared to other groups, including bile acids, metabolic byproducts, dipeptides, and fatty acids. We performed volumetric Ca^{2+} imaging of vomeronasal sensory neurons (VSNs), finding that feces extracts from each class of reptiles elicited distinct activity patterns. To understand the behavioral impacts of mouse predator cues, we used a machine learning (ML)-based analytic workflow. We quantified behavior in high resolution from 3D videos of mice interacting with reptilian fecal chemosignals. The results indicate that mice display distinct behavioral patterns to the mouse predator (snake) feces compared to feces from non-mouse-predators. Taken together, these results suggest that a novel population of VSNs detects reptilian mouse predator chemosignals, and that their activation elicits distinct threat assessment responses. This research provides a basis for identifying kairomone ligands and corresponding receptors that drive behavioral responses to environmental threats.

Disclosures: J. Wang: None. **V. haran:** None. **J.P. Meeks:** None.

Presentation Number: NANO47.05

Topic: D.03. The Chemical Senses

Support: 1R01DC02084201

Title: Sensorimotor prediction errors in the mouse olfactory cortex

Authors: *P. GUPTA, M. A. DUSSAUZE, U. LIVNEH, D. ALBEANU;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Sensation and action operate in closed-loop: movements shape sensory input and sensory inputs guide motor commands. Through experience, the brain learns the reciprocal relationship between inputs and movements to build internal models that predict the sensory consequences of upcoming actions (sensorimotor predictions). In rodents, olfaction is intrinsically linked to motor action through sniffing and body movements. However, to date, most studies have probed olfactory processing during passive odor sampling and 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 olfactory expectations get compared with current odor input within the olfactory cortex to represent olfactomotor prediction errors. To test these hypotheses, we developed a novel behavioral task (Smellocator) where head-fixed mice learn to steer the left-right location of an odor source by controlling a light 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 feedback perturbations in expert animals.
Strikingly, mice readily counter brief sensorimotor perturbations, by making precise corrective movements that provide a read-out of their individually learned sensorimotor predictions. Concurrent single unit recordings from the olfactory cortex show that olfactomotor expectations reshape odor-driven responses of individual neurons, with transient perturbations often evoking strong responses. One challenge in assessing whether these responses represent error signals is that mice also change their sniffing patterns when faced with unexpected perturbations. To untangle potential sensorimotor mismatch responses from sniff-related modulation, we built a linear model aimed at predicting the responses of individual neurons as a function of both sniffing and odor stimulus state (identity, location). While the model explained well neuronal spiking under closed-loop conditions, responses during periods of sensorimotor mismatch could not simply be explained as changes in sniffing.
Our results suggest that the olfactory cortex computes sensorimotor prediction errors by integrating odor 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.

Disclosures: P. Gupta: None. **M.A. Dussauze:** None. **U. Livneh:** None. **D. Albeanu:** None.

Presentation Number: NANO47.06

Topic: D.03. The Chemical Senses

Support: NIH Grant 5R01DC014487
Swartz Foundation
Simons Foundation

Title: A glomerular hierarchy for olfactory discriminations

Authors: *W. G. BAST[1], C. AGHAMOHAMMADI[2], P. GUPTA[1], T. A. ENGEL[2], D. F. ALBEANU[1];
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Abstract: Can we predict the perceptual similarity of two odorants by knowing which odorant receptors (ORs) they activate? This seemingly simple question remains unsolved as difficulties in controlling stimuli at the level of receptor types preclude disentangling their individual contribution in shaping olfactory perception. To overcome this limitation, we exploited the anatomical clustering of ORs to individual glomeruli and identified them in transgenic mice using multiphoton and widefield imaging. After determining their responses to 121 monomolecular odorants, we created synthetic olfactory stimuli by optogenetically activating combinations of glomeruli with sub-glomerular resolution. To determine perceptual distances between glomerular sets, we asked mice to report differences in stimulus identity and quantified the contribution of each glomerulus in shaping stimulus perception. Our psychophysical model revealed a striking glomerular perceptual hierarchy: some glomeruli were up to six times more potent than others in creating a reference percept. We further investigated whether this hierarchy is rooted in the glomerular (ORs) odor response spectra, and found a significant correlation between the perceptual weight of each glomerulus and the similarity of its odor responses to those of other glomeruli in the reference pattern. Alternatively stated, the more a glomerulus odor response spectrum resembles those of other glomeruli in the pattern, the lower its perceptual importance. As such, the perceptual relevance of a glomerulus appears to be an emerging property of the pattern of co-activated glomeruli rather than solely an intrinsic feature of its OR identity. Using an unsupervised method optimized for identifying latent factors in glomerular odor response patterns, we generated testable behavioral response predictions for arbitrary patterns of optically addressable glomeruli. We used this method to guide our search for patterns that generated percepts at specific perceptual distances from the reference percept, and compared the model predictions against the experimental results. Our work contributes to elucidating how the brain maps differences in odorant receptor activation patterns to distinct olfactory percepts. By further combining behavioral analysis and circuit dissection, we aim to bridge the gap between the biophysical features of OR activation, the structure of the perceptual space, and the underlying neural circuits.

Disclosures: W.G. Bast: None. **C. Aghamohammadi:** None. **P. Gupta:** None. **T.A. Engel:** None. **D.F. Albeanu:** None.

Presentation Number: NANO47.07

Topic: D.03. The Chemical Senses

Title: Adaptive modulation of olfactory representations by cholinergic feedback based on behavioral demands

Authors: *B. YU[1], J. YUE[2], T. KOMIYAMA[2];
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Abstract: Adaptive behaviors rely on the ability of sensory systems to change the way they process sensory information depending on the ongoing behavioral demands. Cholinergic modulation is a crucial mechanism underlying such adaptive behaviors, with long-range

cholinergic projections from the basal forebrain modulating response gain and tuning in various sensory regions. However, how behavioral contexts dynamically shape these modulations remains unclear. In this study, we employed two-photon calcium imaging to examine the response of long-range cholinergic projections in the olfactory bulb (OB) across different behavioral contexts. We found during passive exposure to odorants, cholinergic activity strongly correlated with animals' orofacial movements and did not respond to odorant stimuli. However, when the animals engaged in an odor discrimination task, cholinergic axonal activities in the OB rapidly shifted, demonstrating strong phasic responses to odors with weaker responses to orofacial movements. This functional shift of cholinergic response was specific to the OB and not observed in the motor, somatosensory, or visual cortex during the same olfactory task. Furthermore, the phasic odor response of cholinergic axons during the task did not encode odorant identity, and was weaker in the trials when the mice became disengaged within a behavioral session. Local inactivation of cholinergic projection in OB attenuated odor-driven neural activities during tasks and orofacial movement-associated activities during passive odor exposure in OB neurons. This highlights the functional specificity of these projections in modulating local circuit responses in different behavioral contexts. Moreover, the bilateral inactivation of these cholinergic projections to OB using chemogenetics hindered the mice's ability to actively learn odor discrimination tasks, highlighting their significance in olfactory learning. Collateral tracing revealed that OB-projecting cholinergic neurons predominantly target olfactory regions and avoid most dorsal cortical regions. Meanwhile, rabies virus tracing elucidated that these OB-projecting cholinergic neurons receive presynaptic inputs distinct from those projecting to the visual cortex. Our findings reveal a mechanism whereby cholinergic projections modulate sensory processing in a manner that is modality-specific and dependent on behavioral demands.

Disclosures: B. Yu: None. **J. Yue:** None. **T. Komiyama:** None.

Presentation Number: NANO47.08

Topic: D.03. The Chemical Senses

Support: NIH Grant DC010915

Title: Distinct activation dynamics of cholinergic and GABAergic projections from Basal Forebrain to the Olfactory Bulb during odor-guided tasks.

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Abstract: The Basal Forebrain (BF) is an important source of modulation of early olfactory processing due to direct projections of multiple neuronal subpopulations to the olfactory bulb (OB). Dynamic cholinergic signaling and GABAergic activity within the BF have been correlated with discrete components of conditioned odor-guided behavior including detection, reward expectation, and reinforcement delivery, and cholinergic and GABAergic projections from BF to the OB modulate OB circuits, odor response patterns and odor perception. However, the dynamics of these BF-to-OB projections have not been characterized in behaving animals. To address this, we imaged acetylcholine (ACh) signaling in the OB using a genetically-encoded acetylcholine sensor (GRAB-ACh4h), and recorded OB-projecting GABAergic activity from BF

using retrograde-GCaMP8m injected into the OB of VGAT-Cre mice. Head-fixed mice were trained on a Go/No-Go task where one odorant was paired with reward delivery at the end of a 2-sec odor pulse. OB ACh signaling decreased below baseline at the onset of anticipatory licking and was further suppressed upon reward delivery. Two-photon imaging from specific layers of the OB revealed an increase in ACh signaling at odor onset that was sustained throughout the odor presentation and which also included reward-driven suppression. We saw minimal changes in ACh signaling during unrewarded trials. In contrast, using fiber photometry from OB-projecting GABAergic BF neurons, we observed brief bursts of GABAergic activity immediately following onset of both rewarded and unrewarded odors. There were small but significant differences in response amplitudes to CS+ and CS- odors, but no other changes in activity relating to phases of the task, i.e., during anticipatory licking or reward consumption. Our data suggests that BF cholinergic and GABAergic signaling to the OB show activation profiles timed to distinct phases of a reinforcement-based odor-guided task. Bidirectional changes in ACh signaling may modulate the initial stages of olfactory processing as a function of cognitive processes including task engagement and reinforcement, while transient increases in GABAergic activity may modulate olfactory circuits to enhance stimulus detection and impact odor identity coding.

Disclosures: K. Grams: None. **M. Wachowiak:** None.

Presentation Number: NANO47.09

Topic: D.03. The Chemical Senses

Support: National Science Foundation CAREER Grant 1749772
NIH R01MH11392
NIH R01NS135763
The Schmitt Foundation
University of Rochester Research Award
NIH T32 GM007356

Title: Variability and stability of olfactory coding in ventral CA1 region of the hippocampus

Authors: *A. C. KOLSTAD[1,2], K. SZYMULA[1,2], K. PADMANABHAN[3];
[1]Biomed. Engin., [2]Med. Scientist Training Program, [3]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: While the ventral CA1 region of the hippocampus (vCA1) has historically been associated with memory and anxiety, increasing evidence suggests that chemosensory stimuli may influence vCA1 neural responses. The olfactory cortex sends projections to the entorhinal cortex which connects to vCA1, and vCA1 sends monosynaptic feedback projections to the granule cell layer of the main olfactory bulb. Despite this reciprocal connectivity and the increasing evidence for use of olfaction in hippocampal related behaviors, the role of vCA1 in encoding and processing olfactory stimuli remains poorly understood. To answer this question, a necessary first step to is to identify what features (e.g. odor identity, concentration) of olfactory stimuli are encoded for in the firing of neurons in vCA1. To do this, we performed high-density extracellular electrophysiology recordings in vCA1 in awake head fixed 2-3-month-old male and female mice as they ran on a wheel while presented with volatile monomolecular odorants.

While delivering odorants, we simultaneously measured features of the animals' behavior, which allowed us to explore the complex relationships between behavioral state—as measured by locomotion and sniffing—and neural responses to odorants. We found that mice respond to odorant stimuli by increasing their running velocity in a manner dependent on odorant identity. For a panel of 11 odorants, we found that limonene, octanol, and isopentylamine caused increased running. Interestingly, these molecules do not share common physiochemical characteristics. Additionally, we found that running and sniffing were strongly related (median sniff rate of 3.1 Hz (IQR 2.9-3.5 Hz) when stationary vs. 5.4 Hz (IQR 4.3-6.7 Hz) during running), suggesting that locomotion influences respiration, which in turn affects the way odor sampling occurs. Indeed, when we examined neural activity in the vCA1 region, we found a plurality of responding units that differentially encoded features of odors and behavior. Instead of finding simple rules relating modulation of neural activity to single experimental parameters, we found that neural activity was multi-dimensional, reflecting the multi-parametric nature of representations thought to reside in vCA1. Our findings thus provide insight into the stability and variability of neural representations of odors in vCA1 across behavioral states and biological sex.

Disclosures: A.C. Kolstad: None. **K. Szymula:** None. **K. Padmanabhan:** None.

Nanosymposium

NANO48: Neuroprosthetic Strategies for Upper Limb Recovery

Location: MCP Room S401

Time: Tuesday, October 8, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO48.01

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A4820R, A2295R, A3803R)
NIH NINDS (U01NS123101)
NIH NIDCD (K23DC021297)
AHA (19CSLOI34780000)
Cullen Education and Research Fund (CERF) Medical Engineering Prize for ALS
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government.
CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Human intracortical brain-computer interface control of a soft robotic glove

Authors: *J. GUSMAN[1,2,4], D. DE LUCENA[5], T. HOSMAN[1,2,4], K. RAO[3], C. NICOLAS[6], A. KAPITONAVA[6], A. ACOSTA[6], D. WAGNER[5], N. HAHN[7], D. B. RUBIN[6,10], J. M. HENDERSON[7,8,9], J. D. SIMERAL[4,1,2], C. E. VARGAS-IRWIN[3,2,4], C. J. WALSH[5], L. R. HOCHBERG[4,1,6,10,2];

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Abstract: Intracortical brain-computer interfaces (iBCIs) use signals recorded directly from the brain to allow individuals with paralysis to control assistive devices such as computer cursors or robotic arms. For people with tetraplegia, an opportunity for iBCI systems is to provide for the intuitive reanimation of one's own limb and incorporating, for some users, residual proprioceptive feedback. In addition to ongoing work with functional electrical stimulation systems, the recent emergence of soft robotic technology has facilitated the development of soft, wearable exoskeletons that, when paired with a reliable control signal, could be used to restore movement in people with severe motor impairment. In this study, we demonstrated the restoration of basic hand movement through iBCI control of a soft robotic glove (SRG). Furthermore, we investigated neural representations of sustained grasps in human motor cortex and the effects of SRG-induced proprioceptive feedback iBCI grasp decoding.Experimental sessions were performed by two participants with spinal cord injury (T11, C4 AIS-B; T5, C4 AIS-C) and one participant with advanced ALS (T17). Participants T11 and T5 had two 96-electrode arrays and participant T17 had six 64-electrode arrays implanted in the left precentral gyrus (PCG) as part of the BrainGate clinical trial. A fabric-based glove and pneumatic control system was developed to provide 4 functionally relevant grip states: power grip, pinch grip, open hand, and relax. Participants performed an array of "open-loop" and "closed-loop" tasks wherein they were instructed to attempt and hold hand postures for varying durations (0-30s) while the SRG moved either their own hand or a mannequin hand. Recorded neural activity during sustained grasp attempts (greater than ~2s) revealed a substantial dropoff in selectivity for grasp information over time, and participants performed better on sustained grasping tasks when using a toggle-based controller for the SRG compared to a controller that required continuous decoding of a grip state for grasp maintenance. The presence of SRG-induced proprioception was not detectable in our recordings from participants T11 and T5. However, passive movement of T17's hand with the SRG elicited significant modulation in neural activity. As anticipated, the presence of neural activity related to proprioceptive feedback in PCG during iBCI-enabled voluntary movement of the hand changes the activity available to the decoder, providing both a challenge and an opportunity for accurate decoding while driving limb motion in people with intact proprioception.

Disclosures: J. Gusman: None. **D. de Lucena:** None. **T. Hosman:** None. **K. Rao:** None. **C. Nicolas:** None. **A. Kapitonava:** None. **A. Acosta:** None. **D. Wagner:** None. **N. Hahn:** None. **D.B. Rubin:** None. **J.M. Henderson:** Other; JMH is a consultant for Neuralink and Paradromics, is a shareholder in Maplight Therapeutics and Enspire DBS, and is a co-founder and shareholder in Re-EmergeDBS., He is also an inventor on intellectual property licensed by Stanford University to Blackrock Neurotech and Neuralink.. **J.D. Simeral:** None. **C.E. Vargas-Irwin:** None. **C.J. Walsh:** None. **L.R. Hochberg:** Other; The MGH Translational Research Center has a clinical research support agreement (CRSA) with Axoft, Neuralink, Neurobionics, Precision Neuro, Synchron, and Reach Neuro,, for which LRH provides consultative input. LRH is a co-investigator on an NIH SBIR grant with Paradromics, and is a non-compensated member

of the Board of Directors of a nonprofit assistive, communication device technology foundation (Speak Your Mind Foundation). Mass General Brigham (MGB) is convening the Implantable Brain-Computer Interface Collaborative Community (iBCI-CC);, charitable gift agreements to MGB, including those received to date from Paradromics, Synchron, Precision Neuro, Neuralink, and Blackrock Neurotech, support the iBCI-CC, for which LRH provides effort..

Presentation Number: NANO48.02

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A4820R, A2295R)
NIH NIDCD (U01DC017844)
NIH NINDS (U01NS123101)
AHA (19CSLOI34780000)
Harvard School of Engineering and Applied Sciences
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government.
CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Neural control of a wearable soft robotic shoulder using an intracortical brain-computer interface

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Abstract: Intuitive neural control of external devices could potentially restore agency and independence for people living with neuromotor disorders. Intracortical brain-computer interfaces (iBCIs) have allowed people with paralysis to directly control robotic limbs using the activity of neuronal ensembles which reflects their intention to move. External robotic limbs, however, are often limited in portability outside of a clinical setting, and don’t achieve the ultimate goal of restoring control of one’s own hand or arm. Here we present neural control of shoulder abduction in both a virtual environment and a soft wearable robot (SWR). Neural activity was recorded using chronically implanted microelectrode arrays in the precentral gyrus of a participant in the BrainGate2 clinical trial. The SWR is a wearable, textile-based device equipped with inflatable actuators corresponding to the shoulder, elbow, and hand, enabling restoration of movement to the paralyzed limb. The SWR is compact, portable, and able to directly restore movement of the upper limb without additional surgical interventions. This system is also viable for a patient population including people with amyotrophic lateral sclerosis where degeneration of spinal motor neurons limits approaches based on functional electrical

stimulation. We developed a virtual platform to mimic the degrees of freedom afforded by the SWR and demonstrate closed-loop neural control. We then used joint-based decoding in concert with a cascade control architecture on the SWR, comprising an outer and inner control loop, to enable control of shoulder abduction from intracortical neural activity. The outer, kinematic control system provides an interface to control the arm's angle directly from the decoded joint angular velocity commands. This control signal is passed to an inner pressure control loop that reads data from device pressure sensors and actuates valves to achieve the desired joint angles. With both control systems working together, this cascade architecture balances and provides for effective translation of the decoded user intention to the SWR's kinematic output.

Disclosures: K. Rao: None. **H. Jenkins:** None. **B. Schornstein:** None. **J.T. Gusman:** None. **C. Nicolas:** None. **A. Acosta:** None. **E.K. Mann:** None. **J. Arnold:** None. **D. Pont-Esteban:** None. **H. Young:** None. **U. Civici:** None. **C. Walsh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.W. is an inventor on patent and patent applications filed by Harvard University that describes the inflatable soft robotic components in this research. **C.E. Vargas-Irwin:** None. **L. Hochberg:** F. Consulting Fees (e.g., advisory boards); The MGH Translational Research Center has a clinical research support agreement (CRSA) with Axoft, Neuralink, Neurobionics, Precision Neuro, Synchron, and Reach Neuro, for which LRH provides consultative input., LRH is a co-investigator on an NIH SBIR grant with Paradromics, and is a non-compensated member of the Board of Directors of a nonprofit assistive communication device technology foundation (Speak Your Mind Foundation)., Mass General Brigham (MGB) is convening the Implantable Brain-Computer Interface Collaborative Community (iBCI-CC), charitable gift agreements to MGB, including those received to date from Paradromics, Synchron, Precision Neuro, Neuralink, and Blackrock Neurotech, support the iBCI-CC, for which LRH provides effort.

Presentation Number: NANO48.03

Topic: E.05. Brain-Machine Interface

Support: NIH Grant UG3NS123135
Reach Neuro, Inc.

Title: Epidural cervical spinal cord stimulation for the restoration of upper limb motor function in post-stroke hemiparesis

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Abstract: Chronic stroke is an untreatable condition that severely impairs arm and hand use in 400,000 people each year in the US. Stroke causes loss of motor function by damaging the corticospinal tract, resulting in reduced cortical drive to motoneurons responsible for evoking

and coordinating muscle activity. We have shown that epidural electrical stimulation of the dorsal roots in the cervical spinal cord can be used to directly restore voluntary motor control in post-stroke patients. Stimulation of sensory afferent neurons can transynaptically recruit motor unit activity via intraspinal pathways such as reflex circuits. We hypothesized that by controlling the amount of afferent recruitment, motoneuron excitability can be tuned to enable residual cortical pathways to regain control of motoneuron activity. We have validated this concept through a 30-day, prospective pilot clinical study with 6 participants to-date. Participants were implanted with 2 linear epidural electrode arrays spanning spinal segments C4-T1. Stimulation was delivered for 3-4 hours per day, 5 days per week for 4 weeks. Patients saw marked improvements in single joint isometric torque production (up to 108% increase), 2 and 3 dimensional reaching performance (up to 40% increase in speed), and scored up to 15 points higher on the Fugl-Meyer upper extremity motor assessment compared to pre-study baseline. They were also able to perform functional tasks such as feeding themselves and opening a lock with a key. At the end of 30 days participants were explanted. During a 1-month post-study follow-up we also observed a durable retention of motor benefit indicating that the stimulation had a lasting "therapeutic" effect in addition to an immediate "assistive" effect. To achieve optimal results, the pattern of electrical stimulation needed to be customized for each patient's specific motor deficits. Reach Neuro is and early-stage startup company developing the Avantis platform, a neuromodulation tool designed to deliver targeted epidural stimulation for restoration of post-stroke motor control, that can be quickly and scalably programmed to the specific needs of each patient.

Disclosures: M.P. Powell: A. Employment/Salary (full or part-time):; Reach Neuro, Inc., Pittsburgh, PA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro, Inc., Pittsburgh, PA, USA. **E. Sorensen:** None. **N. Verma:** None. **E. Carranza:** None. **A. Boos:** None. **D.P. Fields:** None. **L.E. Fisher:** None. **P.C. Gerszten:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro, Inc., Pittsburgh, PA, USA. **E. Pirondini:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro, Inc., Pittsburgh, PA, USA. **M. Capogrosso:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reach Neuro, Inc., Pittsburgh, PA, USA.

Presentation Number: NANO48.04

Topic: E.05. Brain-Machine Interface

Support: NIH UG3NS12313501A1

Title: Spinal cord stimulation increases motoneuron excitability and improves upper limb motor function after stroke

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Abstract: Stroke is the leading cause of paralysis in the US and existing interventions yield underwhelming results in reducing post-stroke impairments. Many research groups, including ours, have used spinal cord stimulation (SCS) to improve motor function after spinal cord injury and now stroke. Despite showing striking improvements, the underlying mechanism of enabling voluntary motor control with SCS remains unclear. Our work offers initial insights into this question.SCS activates sensory afferents, which have mono- and poly-synaptic projections to spinal motoneurons. We hypothesized that by targeting these sensory afferents, sub motor-threshold SCS provides excitatory drive to the motoneuron pool, amplifying their response to supraspinal inputs weakened by stroke, thus restoring volitional control over these motor-units (MU). In this study, we directly measured the effects of SCS on MU excitability across a range of stimulation parameters. Secondly, we explored different methods of tuning SCS to enhance arm motor function. We hypothesized that closed-loop SCS, which uses phase-specific stimulation parameters, would produce even greater improvements in arm function than tonic SCS, as shown in our previous work, where the stimulation parameters were fixed across all phases of movement. Six participants with chronic post-stroke hemiparesis were implanted with 2 SCS leads in the epidural space of the cervical spinal cord. High-density EMG was used to obtain MU activity while participants performed isometric contractions of the arm and hand to follow target force traces with and without SCS. The participants also performed planar and 3D reaching and grasping movements over multiple sessions with and without SCS, where tonic and closed-loop SCS was applied. The peri-stimulus time histogram of MU firing corresponding to each SCS pulse, showed an increased probability of MU firing at approximately 7-15 ms latency following the SCS pulse, demonstrating that SCS provides transient excitatory drive to the motoneurons. Additionally, we observed a decrease in MU recruitment threshold with SCS, showing that this excitatory drive to motoneurons makes them more responsive to the residual supraspinal inputs. During functional movement tasks, we tested different methods of tuning SCS to improve motor control. The participants were able to reach faster and with smoother trajectories with tonic SCS than without. Closed-loop SCS further increased the speed and smoothness of hand trajectories. These results show that although tonic SCS was effective in promoting significant gains in voluntary arm motor function, closed-loop SCS further improved motor performance.

Disclosures: N. Verma: None. **E. Carranza:** None. **L. Borda:** None. **E. Sorensen:** None. **P. Yadav:** None. **R.M. de Freitas:** None. **M.P. Powell:** None. **J.A. Beauchamp:** None. **A. Boos:** None. **P. Gerszten:** None. **G.F. Wittenberg:** None. **L.E. Fisher:** None. **E. Pirondini:** None. **M. Capogrosso:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); REACH NEURO, INC., NeuroOne, NeuronOff, Iota Biosciences, Bionic Power.

Presentation Number: NANO48.05

Topic: E.05. Brain-Machine Interface

Support: NIH 1R01HD077220
NIH N01HD53403
NIH R01DC009899
VA B6453R
VAMR 5I01RX002654
DoD CDMRP SCIRP SC180308

Title: Enhancing BMI+FES Neuroprothesis via Exploration of the Neural Dynamics of Multi-joint Movements in People with Tetraplegia

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Abstract: The Reconnecting the Hand and Arm to the Brain (ReHAB) clinical trial integrates a Brain Machine Interface (BMI) with a Functional Electrical Stimulation (FES) system to restore volitional control of the upper limb in individuals with tetraplegia. The BMI translates signals from motor related areas of the brain into movement commands that are used to control muscle stimulation via the FES system. The BMI+FES system has been used by our study participants to successfully complete many activities of daily living including self-feeding, handshaking (greeting), object pick-and-place, and precise grasp force modulation. Our current focus is expanding the utility of the ReHAB system by increasing the complexity of movements that can be reliably performed. While we know motor cortex plays a primary role in orchestrating dexterous movements, the strategies it employs to do so are not well understood. Individual neurons have different tuning profiles across a range of single-joint movements, and large, overlapping populations of neurons are active no matter which joints are being moved. How, then, does motor cortex generate command signals for several joints simultaneously? Using intracortical microelectrode arrays, we recorded population spiking activity in the hand knob region of motor cortex while a human study participant with tetraplegia attempted various cued movements of their arm and hand. By comparing the neural state-space trajectories recorded during the course of single- and multi-joint movements, a simple cortical code is revealed. All trajectories, regardless of the joints being moved, follow a similar rotational path, though they traverse different regions of state-space (Location-Dependent Rotations decomposition, 87.1 ± 2.8% variance explained across conditions). Additionally, multi-joint trajectories closely follow the interpolated path between their constituent single-joint trajectories (1.1 ± 0.4 a.u. mean distance compared to 2.4 ± 1.2 a.u. mean distance to the single-joint trajectories). While simple, the strength of the relationship cannot be explained solely by the primary features of the activity of individual neurons ($p < 0.05$, compared to surrogate datasets that maintain covariant structure across time, neurons, and conditions but are otherwise random). The code is thus an emergent feature of the neural population that can only occur via coordinated activity between the neurons involved in controlling the upper limb. These findings shed light on the neural mechanisms underlying motor control and have significant implications for the development of advanced BMI-controlled neuroprosthetics for individuals with paralysis.

Disclosures: J. Krall: None. **B. Hutchison:** None. **W. Memberg:** None. **R.F. Kirsch:** None. **B. Ajiboye:** None.

Presentation Number: NANO48.06

Topic: E.05. Brain-Machine Interface

Support: Feinstein Institutes for Medical Research

Title: Towards long-term recovery of upper limb sensation using a bidirectional neural bypass in tetraplegia

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Abstract: The loss of motor and/or sensory function after spinal cord injury (SCI) can have profound impacts on quality of life. This case study involves a male 43-year-old participant with complete loss of movement and sensation below his injury level (C5 AIS A). The study examines the feasibility of restoring upper limb movement and sensation through a ‘double neural bypass,’ interfacing with and modulating both the spinal cord and sensorimotor networks with biomimetic stimulation patterns. Intracortical microelectrode (Utah) arrays were implanted in the motor and sensory cortices to record neural activity and induce intracortical micro-stimulation (ICMS) in S1, in conjunction with noninvasive transcutaneous spinal cord stimulation (tSCS) and peripheral vibrotactile stimulation. We hypothesized combining modalities may enhance recovery of sensation through neural plasticity mechanisms. On a given session day, excitability of both spinal and cortical networks were enhanced using targeted tSCS, followed by high-frequency S1 stimulation of task-specific electrodes. The electrodes were selected by analyzing cortical modulation during sensory tasks involving imagined and physical touch on the wrist, thumb, index and palm, picking the top 16 electrodes with most consistent modulation. Then, this biomimetic ‘cortical mirroring’ process involved priming these electrodes with 5-10 minutes of modulated high-frequency ICMS in each location. The sensory task was then repeated while undergoing active stimulation; on aforementioned areas, the participant received vibrotactile stimulation paired with location-specific ICMS. Encouragingly, this intervention led to the return of sensation in the wrist of the participant, feeling as low as 10g of applied force using Semmes-Weinstein monofilaments. The results suggest long-term plasticity effects may have occurred. To understand the mechanisms of action, we compared pre- and post-stimulation neural data in S1 in response to touch pressure on the participant’s hand and wrist. Despite an overall decrease in band-power, we observed enhanced neural modulation to applied pressure in the stimulated electrodes. This was characterized by an increase of electrodes showing task selectivity and higher neural feature amplitude post-stimulation. Results suggest

combining cortical and spinal cord stimulation can induce plasticity and facilitate recovery of function in patients with SCI. Individual contributions of each modality require further investigation, including temporal phasing between modalities, to refine rehabilitation strategies and enhance quality of life.

Disclosures: S. Wandelt: None. **S. Chandrasekaran:** None. **A. Jangam:** None. **Z. Elias:** None. **E. Ibroci:** None. **C. Maffei:** None. **D. Griffin:** None. **M.F. Glasser:** None. **J. Kim:** None. **J. Xu:** None. **P.D. Sharma:** None. **S. Bickel:** None. **S. Harkema:** None. **A.B. Stein:** None. **A.D. Mehta:** None. **C. Bouton:** None.

Presentation Number: NANO48.07

Topic: E.05. Brain-Machine Interface

Support: Fitzgerald Translational Neuroscience Fund

Title: Investigating novel neural features to use in an iBCI decoder linked to movement restoration in chronic hemiparesis due to stroke

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Abstract: Stroke is a major cause of disability worldwide and the disability is usually caused by impairments in voluntary movement. Our N-of-1 Cortimo trial (NCT03913286) showed that, using an intracortical brain-computer interface (iBCI), the primary motor cortex above a subcortical stroke can generate useful neural signals to drive an upper extremity (UE) effector. The participant showed improved reach, grasp, and release skills using iBCI control compared to electromyography (EMG)-based control. Achieving this proof-of-concept demonstration required navigating cognitive, affective, and perceptual deficits from the stroke. In this participant, we found that 1) the power spectral density (PSD) recorded by implanted arrays showed increased low frequency (LF) and decreased high frequency (HF) components when compared to PSD measured in subjects without cerebral stroke. The magnitude of this trend appeared distance-dependent with respect to the stroke site, with increased LF and decreased HF for the arrays closer to the stroke. 2) Intermittent bursts of LFP activity in the 9-14 Hz range were observed in the arrays closer to the stroke site. 3) We noticed frequent cross-channel bursts of action potentials with and without performing a task in all the arrays. 4) Intermittent 0.5 to 1 Hz kinematic tremors were seen during target-holding in a KinArm center-out task. The participant had variable cognitive processing speed: the timing of an instruction cue to the execution of an intended movement was delayed in a manner more variable than observed in intact people, complicating the use of any decoder that relied on accurate trial event markers. Offline combination of phase-amplitude coupling (PAC) between LF phases and HF (70-500 Hz) amplitudes performs better for reproducing the hand kinematics than only using PSD features alone. Also, by decomposing the full spectrum into periodic and aperiodic parts, peaks in LF ranges (5-14 and 17-30 Hz) were discovered for the trial participant. Incorporating the PAC of

those bands to gate the PSD features improved the performance of the decoder. Overall results suggest that developing an effective iBCI for the most common type of stroke (ischemic cerebral) must address a unique set of challenges compared to other causes of paralysis.

Disclosures: N. Shawki: None. **A. Napoli:** None. **C.E. Vargas-Irwin:** None. **B. Kopicko:** None. **C.K. Thompson:** None. **J.P. Donoghue:** None. **M.D. Serruya:** None.

Presentation Number: NANO48.08

Topic: E.05. Brain-Machine Interface

Support: EIC-Transition-Reverse Paralysis project
Reeve's foundation

Title: Interfacing brain-decoded motor intentions with the cervical spinal cord to restore voluntary arm and hand movements

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Abstract: Cervical spinal cord injury (SCI) alters the communication between the brain and the regions of the spinal cord that produce upper-limb movements resulting in permanent deficits in arm and hand functions. Epidural Electrical Stimulation (EES) has proven to promote recovery and/or to improve capabilities in humans both for lower and upper limb functions by targeting the large diameter afferent fibers where they enter the spinal cord. Moreover, a recent study has shown that enhanced lower limb neurological recovery could be observed when EES is synchronized with a patient's intentions through an artificial bridge between the brain and the spinal cord.Here, we implemented for the first time a similar technological framework, to restore voluntary control of the arm and hand motor activity. We implanted a participant suffering from an incomplete C4 spinal cord injury, with 2 spinal cord arrays (from C4 to T1) connected to two implanted pulse generators (ONWARD IPGs) and an electrocorticographic (WIMAGINE ECoG) device placed over the sensory-motor cortex. We optimized electromyographic activity

and induced kinematics with various stimulation parameters, building a heuristic myotome with frequential preferences for motor neurons activation. From the ECoG signals, we built algorithms to decode the participant's motor intention to extract up to 6 states. We then modulated the optimized EES parameters based on the state probabilities during 2 months of rehabilitation to promote recovery.We demonstrated the safety and feasibility of using the system in a first participant, and that the extended use of brain-controlled cervical EES rehabilitation supported neurological recovery in the arm and hand motor functions in a patient suffering from an incomplete SCI.

Disclosures: T. Collin: None. **V. Spagnolo:** None. **I. Sakr:** None. **N. Intering:** None. **J. Hervé:** None. **S. Hernandez Charpak:** None. **G. Carparelli:** None. **F. Martel:** None. **I. Yu:** None. **G.D. Dumont:** None. **P. Bessot:** None. **C. Jacquet:** None. **A. Watrin:** None. **L. Bole-Feysot:** None. **C. Sasportes:** None. **M. Simondin:** None. **S. Karakas:** None. **P. Carvalho:** None. **N. Hankov:** None. **A. Galvez:** None. **C. Haxaire:** None. **F. Sauter-starace:** None. **F. Becce:** None. **S. Carda:** None. **E. Ross:** None. **J. Squair:** None. **L. Asboth:** None. **R. Demesmaeker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EPFL. F. Consulting Fees (e.g., advisory boards); ONWARD Medical. **T. Aksenova:** None. **G. Charvet:** None. **J. Bloch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EPFL. F. Consulting Fees (e.g., advisory boards); ONWARD Medical. **H. Lorach:** None. **G. Courtine:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EPFL. F. Consulting Fees (e.g., advisory boards); ONWARD Medical.

Presentation Number: NANO48.09

Topic: E.05. Brain-Machine Interface

Support: Feinstein Institutes for Medical Research

Title: Towards integrating bidirectional brain-computer interface with transcutaneous spinal cord stimulation for long-term restoration of volitional movement in the human hand in tetraplegia

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Abstract: Regaining arm and hand function has been ranked as being of utmost importance by people with tetraplegia. However, functional recovery in upper-limb impairment after spinal cord injury (SCI) plateaus beyond 12-18 months post-injury. We have previously shown that transcutaneous spinal cord stimulation can bring about clinically-significant, persistent gains in

upper-limb muscle strength. Here, in a participant with C4/C5 AIS A SCI, we paired targeted tSCS with volitional activation of specific target muscles, namely biceps, triceps and finger flexors and extensors. The tSCS stimulation was targeted at the appropriate cervical level based on an extensive motoneuron pool recruitment mapping. Targeted tSCS paired with volitional activation of biceps resulted in 92% and 48% gains in volitionally generated force in the right and left biceps, respectively. Moreover, the participant demonstrated greater degree of range of movement, including being able to raise his arms up to his face potentially enabling activities such as self-feeding. However, improvement in tricep strength and synergistic arm/hand movements due to tSCS has proven elusive. Since active engagement is key in rehabilitation protocols and clinically-significant gains in movement, we hypothesized that measuring user intent, combined with real-time feedback, could help unlock further gains in additional muscles. Specifically, we explored the effect of using an intracortical brain-computer interface in combination with tSCS to facilitate long-term restoration of upper limb movement. First, we used methods that leverage machine learning and multimodal parcellation to guide the implantation of five microelectrode arrays into hand area in the primary motor and sensory cortices. After participant recovery, we conducted training sessions and subsequently built LSTM-based regression decoders to provide real-time feedback to increase engagement and refine descending/effect motor commands to the affected spinal cord networks and optimize tSCS patterns. The participant could match 3 levels of grasp force at an overall accuracy level of >70%. We explored the interaction of decoding of movement intent from the M1 neural activity and performing static and dynamic upper limb tasks. The approach developed in this study combines multiple neurotechnologies to promote neuroplasticity and long-term recovery. These methods and findings have broad implications and applications in not only spinal cord injury, but also in stroke recovery, traumatic brain injury with motor dysfunction, and peripheral nerve injury.

Disclosures: S. Chandrasekaran: None. **C. Maffei:** None. **Z. Elias:** None. **A. Jangam:** None. **S.K. Wandelt:** None. **E. Ibroci:** None. **P.D. Sharma:** None. **D. Griffin:** None. **J. Xu:** None. **S. Bickel:** None. **M.F. Glasser:** None. **A.B. Stein:** None. **S.J. Harkema:** None. **A.D. Mehta:** None. **C. Bouton:** None.

Nanosymposium

NANO49: Analytical Computational Tools

Location: MCP Room N426

Time: Tuesday, October 8, 2024, 1:00 PM - 4:15 PM

Presentation Number: NANO49.01

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

Title: Pattern recognition for exact synchronization in the brain applied over MEG recordings

Authors: *A. KATZ;
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Abstract: Identifying thoughts from different brain activities is a difficult challenge that many researchers in many labs around the world are engaged in. Successes are reported in identifying whether a person is thinking about one object (for example an elephant) or another object (like a car) .Previously, we presented for the first time a technology to identify if a person is thinking of the number 1,2,3,4 or 5 which is the most refined thought identification achieved so far.We used brain recording via MEG (Magnetoencephalography), while the MEG helmet surrounded the person's head (like a hat). We started by developing methodology and algorithms for characterizing properties of brain activity related to different numbers (e.g. number 2 and number 3) and distinguishing between brain activity during different visual stimuli of the same number (e.g. figure 3 or three circles). Guessing which one-digit number one is thinking of is done while the person is under MEG recording and a 100 percent accuracy can be reached after 45 seconds (on average) of trials, and for many cases after 15 seconds.
The newly developed methods included geometric characteristics-based encoding of MEG recordings and a multidimensional distance function that measures virtual distance among matrices with numerical entries.
By that, and by the new methods relying on previous developments, we show the exact synchronization between corresponding brain activities.

Disclosures: A. Katz: None.

Presentation Number: NANO49.02

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

Support: Simons Foundation Pilot Award 876513SPI
Israel Science Foundation 1432/19

Title: Contextual Feature Selection with Conditional Stochastic Gates

Authors: *R. SRISTI[1], O. LINDENBAUM[2], S. LIFSHITZ[3], M. LAVZIN[4], J. SCHILLER[5], G. MISHNE[1,6], H. BENISTY[7];
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Abstract: A pivotal challenge to computational neuroscience is identifying and tracking sub-populations of neurons that encode behavior, perception, and memory, across varying contexts such as task difficulty, sensing strategies, or temporal progression. Therefore, analysis tools that provide model interpretability, rather than prediction performance alone, are essential for the study of neuronal population activity. We propose a novel approach conditional-stochastic gates (c-STG) that combines predictive modeling with contextual feature selection. Our approach comprises two networks: a hypernetwork that learns the mapping between contextual variables and probabilistic feature selection parameters, and a prediction network, which can be linear or nonlinear, that maps the selected features to the response variable, e.g., trial outcome. c-STG can handle categorical (e.g., discrete trial condition), continuous (e.g., time within trial), and/or

multidimensional contextual variable. Our results show that c-STG outperforms population-wise, instance-wise, and context-specific feature selection techniques in terms of feature selection capabilities, prediction performance, and interpretability. We apply c-STG to calcium imaging data recorded from pyramidal neurons of layers 2-3 in the primary motor cortex in mice performing a hand-reach task for a food pellet, given an auditory cue. With c-STG, using a single model, we were able to consistently decode trial outcome from neural activity starting 2 seconds after the cue till the end of the trial and identify 12% of neurons as significant on either success or failure trials, thus reporting trial outcome. However, prior work required training of thousands of conventional models to arrive at these conclusions. In a similar experiment where mice received pellets flavored by - sucrose or quinine along with unflavored pellets, a single c-STG model identified which sub-population of neurons participated in outcome encoding as a function of flavor. We show that the encodings of sucrose and quinine flavors were most different (corr=0.4), while the encoding of unflavored pellets was more similar to sucrose (corr=0.7) than to quinine (corr=0.6). Overall, our findings demonstrate that c-STG is an interpretable tool for analyzing neuronal population activity and can provide novel insights into the way complex signals are encoded by brain activity.

Disclosures: R. Sristi: None. **O. Lindenbaum:** None. **S. Lifshitz:** None. **M. Lavzin:** None. **J. Schiller:** None. **G. Mishne:** None. **H. Benisty:** None.

Presentation Number: NANO49.03

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

Support: NIH Grant 1R01NS124224
NIH Grant R01NS115470
Travis Roy Foundation Boston, MA (Investigator Initiated)

Title: Hierarchical Bayesian estimation of motor evoked potential recruitment curves yields accurate and robust estimates

Authors: *V. TYAGI[1,2], L. M. MURRAY[4,6], A. S. ASAN[7], C. MANDIGO[3,8], M. S. VIRK[9], N. Y. HAREL[5,4,6], J. B. CARMEL[1,2,9], J. R. MCINTOSH[1,2,9];
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Abstract: Electrical and electromagnetic stimulation, including transcranial magnetic stimulation (TMS) and spinal cord stimulation (SCS), probe and modulate the neural systems that control movement. Key to understanding their effects is the muscle recruitment curve, which maps evoked potential size against stimulation intensity. Current methods to estimate curve parameters require large samples, however, collecting these is often impractical due to experimental constraints. Moreover, the conventional approach of modeling recruitment curves using sigmoidal functions forfeits estimation of the threshold, which is a key parameter for assessing changes in corticospinal excitability. Here, we present a hierarchical Bayesian framework that accounts for small samples, handles outliers, and returns posterior distributions

over curve parameters that quantify estimation uncertainty. It uses a rectified-logistic function that estimates threshold and outranks sigmoidal alternatives in predictive performance, as demonstrated through cross-validation on TMS and SCS data. The generative capability of our framework enables simulation of synthetic data that closely matches real data. In simulations, our method outperforms conventional non-hierarchical models, reducing threshold estimation error by up to 70% on sparse data. Additionally, compared to frequentist null hypothesis testing, it requires about 35% fewer participants to achieve 80% statistical power and produces up to 60% fewer false positives when detecting shifts in threshold. We present two common use cases involving electromagnetic stimulation data and provide a library for Python, called hbMEP, for diverse applications. By enhancing parametric estimation accuracy on sparse experimental data, our approach reduces the burden on participants by decreasing the necessary duration and the number of stimuli required to probe each individual's neuromuscular parameters, while simultaneously increasing the number of muscles across which these insights are obtained.

Disclosures: V. Tyagi: None. **L.M. Murray:** None. **A.S. Asan:** None. **M.S. Virk:** None. **N.Y. Harel:** F. Consulting Fees (e.g., advisory boards); RubiconMD. **J.B. Carmel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BackStop Neural, SharperSense. F. Consulting Fees (e.g., advisory boards); Pacira, Motric Bio, Restorative Therapeutics. **J.R. McIntosh:** None.

Presentation Number: NANO49.04

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

Support: NIH Grant 1UG3NS115637-01
NSF GRFP
DOD NDSEG

Title: Quantitative ethological profiling of the psychopharmacology and pain-alleviating effects of subanesthetic ketamine

Authors: *S. N. EWBANK[1], G. P. B. MUWANGA[1], D. N. GOPAL[1], B. J. YU[1], V. L. TAWFIK[2], R. D. AIRAN[1];
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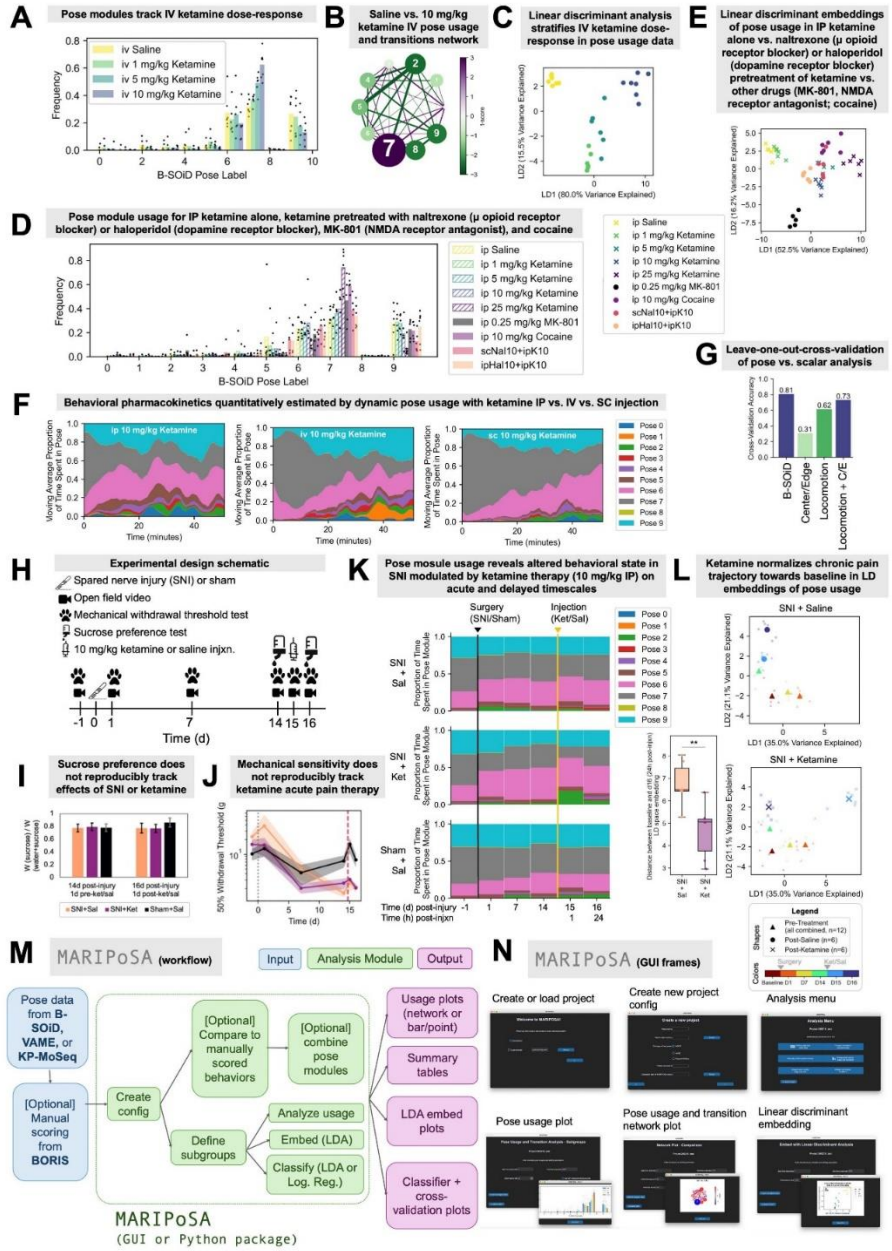
Abstract: Ketamine is an anesthetic, analgesic, and antidepressant drug. Given the poor face validity and reproducibility of rodent stress behavior assays, computational neuroethological approaches may afford deeper, richer descriptions of such a multifaceted drug. Here, we used four computational neuroethology tools - DeepLabCut, B-SOiD, VAME, and Keypoint-MoSeq - alongside standard behavioral assays to profile dose, receptor, and route-of-administration dependent acute effects of ketamine and multi-timescale systemic and rostral anterior cingulate cortex (rACC)-mediated effects of ketamine in the spared nerve injury (SNI) chronic pain model in male and female Sprague-Dawley rats (n=6-8/group). 1-10 mg/kg intravenous (IV) and intraperitoneal (IP) ketamine induced dose-dependent increases in locomotion pose modules and decreases in grooming pose modules which were modulated by naltrexone or haloperidol pretreatment (A-F). Linear discriminant analysis based on pose segmentation yielded higher classification accuracy in cross-validation than classifiers based on locomotion or center/edge

preference (G). We then measured longitudinal changes in open field pose module usage, mechanical sensitivity (von Frey, VF), and sucrose preference (SPT) in SNI versus sham rats before and after treating with IP saline, targeted ketamine delivery to rACC with our group's novel ultrasound (US) uncaging platform (1 mg/kg IV liposomal ketamine with rACC US [250 kHz, 1.1 MPa, 25% duty]), or systemic ketamine (1 mg/kg IV + rACC US or 10 mg/kg IP) (H). SNI induced dynamic pose module usage changes that were uncorrelated with VF and SPT and gave greater distinction between SNI and sham groups and between ketamine and saline on acute and delayed timescales (I-L). Finally, we introduce a toolkit for manageable and reproducible integrated pose segmentation analysis (MARIPoSA) to provide a harmonized pipeline for analyzing data from three popular pose segmentation tools (M-N). Together, these results provide a deep profile of ethologically relevant rat behavior in pain and its pharmacotherapy.



Disclosures: S.N. Ewbank: None. **G.P.B. Muwanga:** None. **D.N. Gopal:** None. **B.J. Yu:** None. **V.L. Tawfik:** None. **R.D. Airan:** None.

Presentation Number: NANO49.05

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

Support: Canadian Institute for Health Research (CIHR) (J.M.)
NSF/CIHR (NeuroNex Grant No. 2015276) (L.M., J.M.T.)
NSERC (L.M., J.M.T.)
Research in Autism from the Government of Ontario (J.M.T.)
ario (J.M.T.) The Canadian Foundation of Innovation (CFI) (J.M.T
BrainsCAN at Western University through the Canada First Research Excellence Fund (CFREF) (L.M.)
Compute Ontario (computeontario.ca) (L.M.)

Title: A mathematical language for spike patterns in large-scale recordings from Utah arrays and Neuropixels

Authors: *A. BUSCH[1], R. BUDZINSKI[2], J. A. MICHAELS[4], M. ROUSSY[2], R. A. GULLI[7], B. CORRIGAN[3], A. PRUSZYNSKI[5], J. C. MARTINEZ-TRUJILLO[8], L. E. MULLER[6];
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Abstract: Recent advances in multi-electrode recording technologies, such as Neuropixels, now allow recording simultaneously from hundreds or thousands of cells. This raises an important question: what is the correct level of description for patterns of spiking activity across these large neural populations? On the one hand, it is well known that features of sensory stimuli, such as position or orientation of a visual stimulus, can be decoded from the firing rates and receptive fields of individual neurons. On the other hand, recent work has found that onset latency of neural responses in visual cortex can robustly encode natural images [Yiling et al., Nat Comms, 2023] and that timing of individual spikes in motor neurons can dramatically change force production in muscle output [Srivastava et al., PNAS, 2017].
Several approaches have been developed to characterize spiking activity across simultaneously recorded neural populations, including firing rate decoding and neural state space analysis. It remains difficult, however, to characterize fine-scale patterns in spike times at the scale of hundreds or thousands of cells. The lack of a rigorous and complete mathematical language for spike-time patterns is the limiting factor.
Current methods for comparing spike trains, such as the Victor-Purpura distance [Victor and Purpura, JNeurophys, 1996], are useful for comparing spikes from a pair of cells, or from one cell over many trials, but do not scale well to consider patterns in spikes across hundreds or thousands of cells. Several computational approaches allow clustering neurons with similar responses for visualization, or detecting coincident spiking, but these methods often require specifying the temporal precision as a free parameter a priori, or require computationally expensive optimization algorithms.

We propose a novel mathematical framework for describing complex spiking patterns across many neurons, capturing both firing rate and precise spike timing. This method is applicable across many timescales, from short transient responses (~20 ms) to long stretches of activity across seconds. We define several mathematical operations, including: composing and decomposing spike patterns into simple basis elements, computing similarity between spike patterns, and robustly detecting structured sub-patterns, which we demonstrate in Neuropixel recordings from macaque motor cortex. We then apply this method to Utah array recordings from macaque prefrontal cortex, where this technique reveals how fine-scale structure in spike patterns across hundreds of cells transforms incoming sensory input into working memory activity and then to navigation.

Disclosures: A. Busch: None. **R. Budzinski:** None. **J.A. Michaels:** None. **M. Roussy:** None. **R.A. Gulli:** None. **B. Corrigan:** None. **A. Pruszynski:** None. **J.C. Martinez-Trujillo:** None. **L.E. Muller:** None.

Presentation Number: NANO49.06

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

Title: Generalizable Embeddings of Layer and Structure in Neuronal Activity

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Abstract: The mammalian brain exhibits complex, intricate organization, with distinct regions contributing to its overall architecture. Understanding the functions of these regions and their overlapping activity patterns is crucial for unraveling the mechanisms of brain function. This study explores the predictability of neural spike patterns to reveal regional characteristics, employing a supervised machine learning approach on publicly available electrophysiology datasets. Analyzing tens of thousands of neurons recorded with Neuropixels probes, we aim to uncover the extent to which regional influences are embedded in the spiking patterns of individual neurons.

Solely from their spike trains, we classify neurons into their regional, structural, and layer localizations using hierarchies of neural networks. The findings indicate that while brain regions exhibit distinct neural activity patterns, the differences are subtle and often require advanced computational approaches to uncover. We delve into how neurons' spiking patterns vary across cortical and subcortical regions. Localization is enhanced by leveraging knowledge of the anatomical hierarchy and the spatial proximity among neurons. Crucially, our models can generalize across animals, sensory stimuli, and even research groups, showcasing the robustness of the neural embeddings.

This research underscores the importance of computational methods in dissecting the subtle distinctions in neural activity that define brain regions. Our robustly generalizable results suggest the potential of this approach as a tool for electrode localization. More fundamentally, they indicate that a neuron's location, on multiple scales and dimensions, is reliably embedded in its activity.

Disclosures: A. Schneider: None. **G. Tolossa:** None. **K.B. Hengen:** None.

Presentation Number: NANO49.07

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

Support: Sandia LDRD
DOE ASC

Title: Language and software tool for large-scale modeling

Authors: *F. ROTHGANGER;
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Abstract: The brain is a complex system with many interacting components. The task of mathematically modeling this system can seem overwhelming, and arguably it is beyond human capability. However, we can work cooperatively and take advantage of computer tools to manage the complexity, remember relationships and analyze their higher-order interactions. N2A ("Neurons to Algorithms") is an effort toward this challenging goal. 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: A. Employment/Salary (full or part-time):; Sandia National Labs.

Presentation Number: NANO49.08

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

Support: Burroughs Wellcome Fund
NIH Grant T-32 NS076401

Title: Adaptive alignment of neural and behavioral latent dynamics

Authors: *J. GOULD[1], A. DRAELOS[2];
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Abstract: A fundamental goal of systems neuroscience is to obtain a mechanistic understanding of how neural circuit specificity and function regulate behavioral outcomes. Evidence suggests that this circuit-behavior relationship is mediated in part through low-dimensional dynamical patterns embedded in the neural activity of the brain [Cunningham 2014]. These patterns, or latent variables, have been strongly linked to behavior in multiple contexts, and their dynamics have proven to be useful in predicting the activity of the unembedded neural data [Pandarinath 2018]. However, these latent variables have only been partially characterized. Advances in neural measurement techniques provide an increasingly comprehensive picture of the joint

neural-behavioral system state [Manley 2024] and stimulation technology is similarly enabling the flexible perturbation of neural latent variables. Together, these technological advances make real-time causal interrogation of neural latent variables possible, assuming computational tools can be developed to capitalize on the new data.
We present an approach for real-time prediction of latent neural trajectories and simultaneously measured behavioral variables. In addition to modeling a shared latent space between the neural and behavioral data, we construct latent variables based on principled discovery heuristics. Our machine learning approach leverages a set of previously published methods, combining streaming dimensionality reduction, latent variable discovery, and tracking of resultant latent dynamics in real time (Bubblewrap, jPCA [Draelos 2021, Churchland 2012]). We consider the explicit inclusion of dynamical features that target high variance, rotational structure, and non-Gaussianity. We develop streaming formulations of all the above and benchmark their performance to ensure real-time performance speeds. We demonstrate our approach on simulated and experimental datasets including neural activity recorded via electrophysiology or calcium imaging during various behaviors ranging from continuous kinematics to discrete tasks. By adaptively aligning our models to ongoing features of interest, we show improved prediction of ongoing latent dynamics. We anticipate that these alignment strategies could be used in reverse to design optimal stimulations to test hypotheses about the dynamics of neural latents and their relationship to behavior.

Disclosures: J. Gould: None. **A. Draelos:** None.

Presentation Number: NANO49.09

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

Title: Whole Blood Transcriptomic Profiles to Predict LRRK2 Activity and Disease Severity in Parkinson's Disease Cohorts

Authors: *K. SREERAM[1], A. B. WEST[2];
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Abstract: Parkinson's Disease (PD) is a neurodegenerative disease that is characterized by the loss of dopaminergic neurons in the substantia nigra. More recently, studies have highlighted a role for peripheral immune responses in potentially modifying disease phenotypes and course. Genome-wide association studies have implicated genes linked to immune diseases and immune function, including the *leucine-rich repeat kinase 2 (LRRK2)* gene. LRRK2 can phosphorylate a subset of Rab GTPases, notably Rab10 highly expressed in immune cells, in the mediation of immunological signaling pathways and responses. It is not yet clear how LRRK2 activity manifested through Rab phosphorylation changes with disease states and immunological responses known to be altered in PD. To address this knowledge gap, we introduce a combined approach of whole blood RNA-seq analysis in deeply clinically phenotypes cohorts of PD patients and controls using machine learning models to identify genetic drivers of PD severity and LRRK2 activity. The goal is to identify robust panels of transcriptomic changes that predict LRRK2 activity, disease progression, and how they relate to one another. Weighted gene correlation network analysis (WGCNA) is being utilized to identify modules correlated with the level of phosphorylated Rab10 (pRab10)**,** a biomarker of LRRK2 activity. Starting with a cross

section of participants in the Parkinson's Disease Biomarker Program (PDBP), WGCNA analysis produced 27 gene module clusters of densely co-expressed genes, and module-trait relationships were quantified by measuring the Pearson correlation of each module eigengene with pRab10/total Rab10 ratios. WGCNA identified modules as significantly correlated with pRab/total Rab10 ratio. Gene ontology analysis revealed that the ratio of pRab10/total Rab10 associates with genes enriched for function in innate immune activation, neutrophil degranulation, and the release of several pro-inflammatory cytokines (i.e. IL-12). Associated modules also included the antigen presentation genes *HLA-B, HLA-C,* and *HLA-E,* which may be expressed in immune cells and dopaminergic neurons during inflammatory events. Additional inflammation profiles will be sought using machine-learning models that predict disease progression and decline within these patient cohorts. Through these studies, identification of pathways closely associated with disease severity and poor prognosis may be useful in therapeutic strategies that address these changes.

Disclosures: K. Sreeram: None. **A.B. West:** None.

Presentation Number: NANO49.10

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

Support: NIH RF1MH128695
NSF CAREER 2144475

Title: Neurotd: a deep learning approach to analyze multi-modal neuronal time series data

Authors: X. HUANG[1], Q. DONG[2], Q. CHANG[3], *D. WANG[4];
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Abstract: Studying temporal features neuronal activities is crucial for understanding functions of neurons as well as underlying neuronal circuits. To this end, the emerging techniques including calcium imaging in freely behaving animals, Neuropixel, and Patch-Seq generate multi-modal time-series data that depict the activities of single neuron, group of neurons, and behavior. However, many challenges exist, including the analysis of noisy, high-sampling-rate neuronal data, and the modeling of temporal dynamics across various modalities. To address these computational challenges, we developed NeuroTD, a novel deep learning approach to align multi-modal time-series datasets and infer cross-modality temporal relationships such as time delays/shifts. Particularly, NeuroTD integrates Siamese neural networks with frequency domain transformations and complex value optimization for the inference. We applied NeuroTD to three multimodal datasets for: (1) analyzing electrophysiological (ephys) time series measured by Neuropixel to identify time delays among neurons and construct neural circuits, (2) investigating neuronal activity and behavioral time series data derived from calcium imaging studies to establish causal relationships between neuronal activity and corresponding behavioral activity, and (3) exploring gene expression and ephys data of single neurons from Patch-Seq to identify gene expression signatures highly correlated with time shifts in ephys responses. Finally, NeuroTD is open source and available for general use.

Disclosures: X. Huang: None. **Q. Dong:** None. **Q. Chang:** None. **D. Wang:** None.

Presentation Number: NANO49.11

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

Support: Ford Foundation Postdoctoral Fellowship
NIH 1R01MH131534
Whitehall Foundation

Title: Cluster-aware machine learning of multiomics and neuroimaging for precision neuroscience and psychiatry

Authors: *A. M. BUCH, C. M. LISTON, L. GROSENICK;
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Abstract: Explainable machine learning of complex multimodal data is transforming the landscape of neuroscience research. In many cases, data heterogeneity across samples due to biological clusters is an important component of variation, and revealing these clusters and the biological factors that explain them is an important research approach. For example, in medical diagnoses, interpretable clustering of patients into distinct subtypes improves personalization of treatment. However, a combination of the well-known "curse of dimensionality" and the clustered structure of biomedical data together present a unique challenge in the high dimensional and limited observation regime common in datasets used in neuroscience. Embedding followed by clustering is popular, but this two-stage process often results in both suboptimal embeddings and degraded cluster separation, motivating a need for joint clustering and embedding approaches that are explainable, robust to technical variability, and generalizable. To overcome both challenges simultaneously we propose a simple and scalable approach to joint clustering and embedding that combines standard embedding methods with a convex clustering penalty in a modular way. Through both numerical experiments and real-world examples, we show that our approach outperforms traditional and contemporary clustering methods on highly underdetermined problems (e.g., with just tens of observations) as well as on large sample datasets. Thus our approach improves significantly on existing methods for identifying patient subgroups in multiomics and neuroimaging data.

Disclosures: A.M. Buch: None. **C.M. Liston:** None. **L. Grosenick:** None.

Presentation Number: NANO49.12

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

Support: Hungarian National Research, Development and Innovation Fund K113147
NKFIH K135837
NKFIH K137886
NKFIH PD143582
NKFIH RRF_2.3.1_21_2022_00015
Human Brain Project associative grant CANON NN118902
Hungarian National Brain Research Program 2017-1.2.1-NKP-2017-00002 and 3.0

Title: Unraveling the individual membrane current components and local microcircuit states of CA1 pyramidal cells using ground-truth recordings

Authors: *D. MESZENA[1,2], P. BOLDOG[3], K. FURUGLYAS[4,5], K. TÓTH[1], L. WITTNER[1,6], I. ULBERT[1,6], Z. SOMOGYVARI[3,5,7];
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Abstract: Measuring the spatiotemporal distribution of synaptic currents at the single-neuron level remains an ongoing challenge for electrophysiologists, awaiting a comprehensive solution. While it is relatively straightforward to calculate the density of the net membrane current, also known as the Current Source Density (CSD), from extracellular (EC) potentials measured by high-density electrodes, distinguishing between its two primary components poses significant challenges. The CSD consists of the transmembrane (ohmic or resistive) current and the capacitive current. The transmembrane current encompasses synaptic, active, and passive channel currents, while the capacitive current corresponds to charge accumulation on the membrane surface, typically manifesting as counter-currents accompanying a primary sink or source in the CSD distribution. Given the opposing signs of these co-occurring components, they tend to cancel each other. The superimposed current components result in a very low-amplitude extracellularly observable net membrane current (the CSD), which thus can only represent a small fraction of the total spatiotemporal distribution of membrane currents along the cell. Our recently developed membrane potential integration method offers a solution by enabling the inference of membrane potential and the distinction between the two CSD membrane current components - ohmic and capacitive - utilizing paired intra- and extracellular recordings. Applying this method to multiple experimental ground-truth recordings has unveiled the contribution ratio of transmembrane and capacitive currents, shedding light on the ratio between extracellularly observable and hidden currents. Furthermore, since the passive current is proportionate to the membrane potential, the membrane potential integration method allows for a finer subdivision of the transmembrane current, estimating the contributions of passive and active currents to the net membrane current. Our custom-designed ground-truth recordings, combined with full morphological reconstruction and the presented membrane potential integration method, may provide a panoramic view and detailed understanding of membrane potential dynamics and the input-output transformation CA1 pyramidal cells in their local microcircuitry.

Disclosures: D. Meszena: None. **P. Boldog:** None. **K. Furuglyas:** A. Employment/Salary (full or part-time):; Neunos ZRt, Szeged 6720, Hungary. **K. Tóth:** None. **L. Wittner:** None. **I. Ulbert:** None. **Z. Somogyvari:** A. Employment/Salary (full or part-time):; Neunos ZRt, Szeged 6720, Hungary, Axoncord LLC., Budapest, H-1048, Hungary.

Presentation Number: NANO49.13

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

Title: Exploring Neuronal Circuitry in Visual Perception and Cognition: The Role of FEAGI in Neuromorphic Modeling

Authors: *M. NADJI-TEHRANI[1], V. ZHANG[2];
[1]Neuraville Inc., Pittsburgh, PA; [2]Neuraville Inc., Irvine, CA

Abstract: Object differentiation, a fundamental capability of the human visual system, involves the accurate identification and prioritization of target objects within visually cluttered environments. This process, robust across variations in lighting, orientation, and distance, underscores the adaptability and sophistication of neuronal circuits in visual perception. The Framework for Evolutionary Artificial General Intelligence (FEAGI) embodies a neuromorphic approach designed to replicate and investigate these complex biological processes. This paper explores how FEAGI serves as both a model and a methodological tool in the study of the neural mechanisms underlying feature extraction, learning, memory, and object recognition. By simulating the architectural and functional aspects of human neural pathways, FEAGI provides valuable insights into the cognitive processes that govern perception and interaction with the environment. Although FEAGI effectively mimics several cognitive functions, ongoing developments are focused on enhancing its ability to recognize objects with high fidelity across various conditions and to reduce erroneous detections. By leveraging FEAGI, we aim to advance our understanding of the neuronal circuits that facilitate cognitive abilities, thereby contributing significantly to both the fields of artificial intelligence and neuroscience.

Disclosures: M. Nadji-Tehrani: A. Employment/Salary (full or part-time):; Neuraville Inc. **V. Zhang:** A. Employment/Salary (full or part-time):; Neuraville Inc..

Nanosymposium

NANO50: Evolutionary Perspectives on Neurodevelopmental Mechanisms and Circuitry

Location: MCP Room S404

Time: Wednesday, October 9, 2024, 8:00 AM - 9:45 AM

Presentation Number: NANO50.01

Topic: A.10. Development and Evolution

Support: SHARP Fellowship RG193211
ARC Linkage Project LP180100721

Title: The evolution of ultraconserved elements in vertebrates and their neurodevelopmental functions

Authors: *M. CUMMINS[1], R. EDWARDS[2], J. MATTICK[1];
[1]Sch. of Biotech. and Biomolecular Sci., Univ. of New South Wales, Kensington, Australia;
[2]Oceans Inst., The Univ. of Western Australia, Perth, Australia

Abstract: Ultraconserved elements (UCEs) were discovered two decades ago, arbitrarily defined as sequences that are identical over a length ≥200 bp in the human, mouse and rat genomes. The definition was subsequently extended to sequences ≥100 bp identical in at least three of five

mammalian genomes (including dog and cow), and shown to have undergone rapid expansion from ancestors in fish and strong negative selection in birds and mammals. Since then, many more genomes have become available, allowing better definition and more thorough examination of UCE distribution and evolutionary history. We developed a fast and flexible analytical pipeline for identifying UCEs in multiple genomes, dedUCE, which allows manipulation of minimum length, sequence identity, and number of species containing the UCE according to specified parameters. We suggest an updated definition of UCEs as sequences ≥100 bp and ≥97% sequence identity in ≥50% of placental mammal orders (12813 UCEs). By mapping UCEs to ~200 species we find that placental UCEs appeared early in vertebrate evolution, well before land colonisation, suggesting the evolutionary pressures driving UCE selection were present in aquatic environments in the Cambrian-Devonian periods. Most (>90%) UCEs likely appeared after the divergence of gnathostomes from jawless predecessors, were largely established in sequence identity by early Sarcopterygii evolution - before the divergence of lobe-finned fishes from tetrapods - and became near fixed in the amniotes. UCEs are mainly located in the introns of protein-coding and lncRNA genes involved in neurological and skeletomuscular development, enriched in cis-regulatory elements of human embryonic brain and spinal cord, and dynamically expressed throughout embryonic CNS development in human, mouse, and chicken.

Disclosures: M. Cummins: None. **R. Edwards:** None. **J. Mattick:** None.

Presentation Number: NANO50.02

Topic: A.10. Development and Evolution

Support: Allen Discovery Center for Human Brain Evolution, Paul G. Allen Frontiers Group
Howard Hughes Medical Institute Fellowship from the Helen Hay Whitney Foundation
Y. Eva Tan Fellowship from the Tan Yang Autism Center
NIH Grant K99MH136290-01

Title: Uncovering gene regulatory differences between human and chimpanzee neural progenitors

Authors: *J. H. T. SONG[1], A. CARTER[2], E. BUSHINSKY[1], S. BECK[1], J. E. PETROCELLI[2], M. E. GREENBERG[2], C. A. WALSH[1];
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Abstract: Although comparisons of human and non-human primate brains have identified thousands of molecular differences, it has been difficult to identify the human-specific sequence variants that underlie the dramatic modifications to brain size, connectivity, and function found in humans. One hurdle is that current comparative approaches cannot distinguish *cis*-regulated genes, which change in expression due to nearby sequence variants on the same DNA molecule, from *trans*-regulated genes, which change in expression due to changes in diffusible factors in the cellular environment (like the levels of *cis*-regulated transcription factors (TFs)). To distinguish *cis* from *trans* changes, we generated human-chimpanzee tetraploid stem cell lines as a genetic model where the human and chimpanzee genomes are in the same cellular environment

and only *cis*-regulated changes are observed. We have now used this system to profile *cis*- and *trans*-regulated genes and open chromatin regions in neural progenitor cells (NPCs), in order to identify genetic changes that underlie the expansion in size and neuron number in the human brain. Genes that are more highly expressed in humans are enriched for processes related to mitosis, consistent with increased neurogenesis in humans. We identify *cis*-regulated TFs, including *FOSL2* and *MAZ*, whose motifs are enriched at *trans*-regulated open chromatin peaks, suggesting that these TFs may be major drivers of epigenomic and transcriptomic rewiring between human and chimpanzee NPCs. To identify human-specific variants that underlie *cis*-regulated gene expression changes, we linked *cis*-regulated open chromatin peaks that contain derived sequence changes in humans to nearby *cis*-regulated genes. A CRISPR inhibition screen of 106 *cis*-regulated peaks identified species-specific enhancers, including one near *TNIK*. Further characterization of *cis*-regulated TFs and non-coding regions in NPCs, along with the application of this model to additional cell types and paradigms, will advance our understanding of how human-specific sequence changes contribute to increased brain size, as well as other phenotypes that have arisen in the human lineage.

Disclosures: J.H.T. Song: None. **A. Carter:** None. **E. Bushinsky:** None. **S. Beck:** None. **J.E. Petrocelli:** None. **M.E. Greenberg:** None. **C.A. Walsh:** None.

Presentation Number: NANO50.03

Topic: A.10. Development and Evolution

Support: NSF 2247939

Title: Evolution of NELL binding by dual-ligand responsive axon guidance receptor Robo.

Authors: *V. NAWROCKA[1], J. PAK[2], Y. PARK[3], K. NICKERSON[4], L. PRAKASH[6], A. JAWORSKI[5], E. OZKAN[1];
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Abstract: Robo receptors are common to all bilaterian groups as a major midline guidance receptor family, where their interactions with their secreted Slit ligands lead to the repulsion of growing axons. Recent work has identified NELLs as a new family of ligands for Robo3 in mammals, but not Robo1 and Robo2; NELL2 similarly effects a repulsive outcome via Robo3, but through an unrelated binding site on the Robo ectodomain. It is not known when NELL-Robo interactions evolved and if non-mammalian Robos are dual-ligand responsive receptors that can bind both NELLs and Slits. Here, we present an extensive analysis of extant Robo and NELL pairs across bilaterians and show that most deuterostome Robos, including Robo1, Robo2 and Robo3 orthologs, bind NELL, but protostome Robos do not. Unexpectedly, we observed that Robo ectodomains form condensates when mixed with cognate NELL ligands, a property conserved from cephalochordates to mammals. We also show that non-mammalian Robos are dual-ligand responsive, with implications that Robo activation mechanisms may be distinct or common to both ligands. These results also imply that Robo1/2 and Robo3 paralogs have specialized to take over distinct functionalities - Slit response and NELL response, respectively - of the ancestral chordate Robo receptor.

Disclosures: V. Nawrocka: None. **J. Pak:** None. **Y. Park:** None. **K. Nickerson:** None. **L. Prakash:** None. **A. Jaworski:** None. **E. Ozkan:** None.

Presentation Number: NANO50.04

Topic: A.10. Development and Evolution

Title: Cellular evolution of insect motor circuits: Motor neurons are constrained in number but flexible in their connectivity to muscles

Authors: *A. SHARMA, E. HECKSCHER;
The Univ. of Chicago, Chicago, IL

Abstract: Motor circuits comprise muscles, motor neurons, and premotor networks. Changes to these components' number, location, connectivity, and activity produce a vast diversity of motor behaviors across animals. Yet, our understanding of which features of motor circuits are fundamental (or, evolutionarily constrained) is limited by: (i) in-depth knowledge in only a few distantly related model organisms, (ii) muscles and neurons being soft tissues that do not fossilize, (iii) technical challenges in tracing connections between the periphery and central nervous system (CNS), (iv) redundancy in motor circuits making ablation/inactivation studies infeasible, (v) intractability of single-cell resolution, and (vi) ambiguity in identifying homologous cells. We leverage the simple motor systems and vast evolutionary diversity of larval insects to overcome these challenges and investigate how motor circuits evolve at the cellular level. The unparalleled knowledge of motor circuits in larval *Drosophila melanogaster* of the Order Diptera (two-winged flies) serves as our reference for comparing several species of the same Order (~250MY). Adapting techniques developed in *D. melanogaster*, we examine muscle anatomy and nerve branching using whole larval fillet dissections followed by immunostaining with *Phalloidin* (muscles) and *HRP* (nerves), and motor neurons via CNS dissections followed by immunofluorescence with *pMAD* (a pan-motor neuron antibody) and marker transcription factors for subsets of motor neurons. Additionally, we trace the connectivity of each muscle to its motor neuron partners by dye-fills of muscles in fillet preparations with *DiI*. We identify homologous cells based on anatomy: for muscles by relative position, orientation, and layering, and for motor neurons by relative position and the expression of marker transcription factors. We observe many changes in body wall musculature across Diptera, raising a fundamental question about how the nervous system adapts to these altered muscles. Here, we explore whether these changes are incorporated into motor circuits by producing new/different motor neurons or altered connectivity of existing motor neuron axons. In the species examined so far, the numbers and identities of motor neuron somata are conserved. However, axonal connectivity to muscles sometimes differs, even of motor neurons whose homologous muscle partners are present across species. Broadly, this shows that the constraint is in neuron number while flexibility is in connectivity. We aim to investigate this pattern's functional consequences and molecular mechanisms via future mutational studies in *D. melanogaster*.

Disclosures: A. Sharma: None. **E. Heckscher:** None.

Presentation Number: NANO50.05

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R00EY033402

Title: Monocyte invasion restricts neurogenesis from glia in the mammalian retina

Authors: *L. TODD[1], N. BLASDEL[2], S. BHATTACHARYA[1], T. A. REH[2];
[1]SUNY Upstate Med. Univ., Syracuse, NY; [2]Univ. of Washington, Seattle, WA

Abstract: Neurodegenerative diseases result in permanent disability because the human nervous system lacks the ability to regenerate lost neurons. However, some vertebrate species can regenerate their central nervous system and restore lost neuronal function. Regenerative species often replace neurons by de-differentiation of their glial cells back into a neurogenic state. In the retina, Muller Glia (MG) can regenerate functional neurons that can reverse blindness in fish and amphibians. However, in mammals, MG respond to injury with an inflammatory response rather than a regenerative one. Previous progress was made in engineering mouse MG to regenerate neurons by designing MG to overexpress Ascl1, a proneural transcription factor required for zebrafish regeneration. Ascl1 can stimulate adult mouse MG to regenerate neurons that functionally integrate into circuity. Using this mouse model of retinal regeneration, we have explored the role of the innate immune system in regulating adult neurogenesis. Although microglia are required for regeneration in zebrafish, we find that in mice, ablation of microglia significantly boosted neurogenesis. This differential response to neuroinflammation appears to be a key difference between fish and mammals.
In this study, we characterize the broader neuroimmune response to retinal degeneration and regeneration in the mouse retina. We perform scRNA-sequencing of immune cells that invade the injured retina. We show with transgenic reporter and knockout mice that monocytes are a major component of the neuroinflammatory response and restrict the capacity of glia to regenerate neurons. In CCR2 knockout mice, where monocytes are unable to colonize the retina, regeneration of neurons is significantly enhanced. We also identify Osteopontin (Spp1) as a molecule released by monocytes and received by MG that is sufficient to suppress neurogenesis. This work suggests that peripheral immune invasion into the injured nervous system restricts the regenerative capacity of mice, and modulation of this response may enhance regeneration in the mammalian retina.

Disclosures: L. Todd: None. **N. Blasdel:** None. **S. Bhattacharya:** None. **T.A. Reh:** None.

Presentation Number: NANO50.06

Topic: A.03. Stem Cells and Reprogramming

Support: NIH EY13010
NIH EY13012
NIH F32EY028012
NIH K99EY032269
NYU MacCracken Fellowship
NYU Dean’s Dissertation Ph.D. Fellowship
NYSTEM C322560GG

Title: Spatial patterning controls neuron numbers in the Drosophila visual system

Authors: *J. MALIN, C. DESPLAN;
New York Univ., New York, NY

Abstract: Neurons must be made in the correct proportions to carry out their functions within a circuit. In the *Drosophila* visual system, 20 classes of distal medulla (Dm) inhibitory interneurons are generated in reproducible numbers, from 5 to 800 cells per optic lobe. These neurons regulate the flow of visual information received from photoreceptors, and as such, their stoichiometry is important to their function. Dm neurons are born from a crescent-shaped neuroepithelium called the Outer Proliferation Center (OPC). This neuroepithelium is spatially subdivided into distinct domains along the anteroposterior axis; Vsx is expressed at the anterior edge, Optix/Six3 is expressed in the middle, and Rx is expressed at the posterior tips. The Rx subdomain can also be further divided based on the differential expression of the signaling molecules Dpp/BMP and Wg/Wnt. We used genetic fate mapping experiments to characterize the role of spatial patterning in the regulation of neuron number. We found that the size of the neuroepithelial domain from which a cell type is born scales directly with the number of Dm neurons it generates. Although spatial patterning is sufficient to explain most of the variation in cell number between Dm neuron types, it is unable to account for differences in cell quantity between less abundant neuron classes. Apoptosis mutants lead to small increases in Dm cell number, but not to a sufficient extent that it can account for cell number differences between types; this suggests that apoptosis fine tunes cell number, but that it does not set the initial numbers of cells generated. We found that an additional spatial signaling pathway divides the neuroepithelium into even smaller subdomains to produce greater cellular diversity. The transcriptional repressor and Dpp inhibitory target Brinker is expressed in a region whose boundaries cover the Vsx domain and the anterior 2/3 of the Optix domain. Overexpression or inhibition of Dpp or Brk expression is sufficient to change the ratios of Dm neurons produced; this suggests that morphogen signaling can generate smaller pools of stem cells to generate a greater number of cell types than previously appreciated. Finally, we show that the level of Dpp signaling that a stem cell is exposed to correlates to the fate adapted by its neural progeny, suggesting that differing Dpp levels are used to specify the least abundant cell types. While proliferation and death are often regarded as the main regulators of cellular abundance, our work suggests that multiple spatial patterning pathways can intersect to regulate cell number.

Disclosures: J. Malin: None. **C. Desplan:** None.

Presentation Number: NANO50.07

Topic: A.08. Development of Neural Systems

Support: Swedish Research Council Grant no:2021-00238

Title: Evo-devo diversification of cortical output channels

Authors: *S. M. SURYANARAYANA, X. AN, H. MOHAN, S. ZHAO, Z. HUANG;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: A hallmark of cortical circuitry is its unique access to downstream motor centers via extratelencephalic neurons (ET), enabling it to broadcast motor commands and orchestrate behavior. The developmental genetic mechanisms underlying the evolutionary amplification and diversification of ET types that assemble cortical output networks are not well understood.

Cortical glutamatergic projection neurons are generated from radial glial progenitors through either direct neurogenesis (dNG) or indirect neurogenesis (iNG) via intermediate progenitors (IPs). Across vertebrates, while direct neurogenesis is ubiquitous, indirect neurogenesis emerged since amniotes and has expanded tremendously in mammals, driving the evolutionary innovation of the isocortex. While the amplification of neuron production through IPs is inherent to iNG, how iNG expands the diversity of cortical output ET types is unexplored.We have recently revealed that ET cells are generated by both dNG/iNG. Using a novel genetic strategy, we are able to target direct and indirect derived ET cells, (ETd and ETi) in different cortical areas and examine their brain wide connectivity and function. We found that while ETd largely target conserved structures in the forebrain/midbrain, ETi extend their projections to the brainstem/spinal cord. Moreover, ETi diversify projections to distinct basal ganglia nuclei, neuromodulatory and lemniscal sensory processing centers. Therefore, ETi amplify and diversify cortical efferents to downstream action selection, diversification, and execution circuits. Preliminary evidence indicates that ETd/ETi form extensive projections to spinal cord in development and undergo pruning during early postnatal development to diversify projection targets. Input connectivity for ETd and ETi with dual-monosynaptic rabies tracing indicates they form distinct cortical microcircuit motifs. Optogenetic activation in the forelimb motor cortex shows that ETi generates forelimb and oro-facial movements as opposed to ETd. Furthermore, we are using dual-color 2-photon imaging in the forelimb motor cortex during a reach-grasp-to-drink task to simultaneously record ETd and ETi activity. Our results suggest that the evolutionarily more recent ETi, as “action diversifiers”, are key for the cortex to recruit downstream motor centers and orchestrate complex sequential motor behaviors.

Disclosures: S. M. Suryanarayana: None. **X. An:** None. **H. Mohan:** None. **S. Zhao:** None. **Z. Huang:** None.

Nanosymposium

NANO51: ALS Therapeutics

Location: MCP Room N427

Time: Wednesday, October 9, 2024, 8:00 AM - 9:45 AM

Presentation Number: NANO51.01

Topic: C.06. Neuromuscular Diseases

Title: Nx210c peptide: a promising drug candidate for neurodegenerative diseases and injuries

Authors: *S. LEMARCHANT[1], J. LE DOUCE[1], N. REBERGUE[1], S. MARIE[1], A. JANUS[1], Y. GODFRIN[2];
[1]Axoltis Pharma, Lyon, France; [2]AXOLTIS Pharma, Lyon, France

Abstract: NX210c is a 12-amino acid peptide designed from the type 1 thrombospondin repeats of the subcommissural organ-spondin, a CNS-specific glycoprotein involved in neuronal development during embryogenesis. Here, we used a combination of *in vitro* (cell cultures, electrophysiology) and *in vivo* experiments to examine the effects of NX210c on blood-brain barrier (BBB) leakage, neurodegeneration and synaptic dysfunction, key common pathogenic

traits of several brain and spinal cord disorders. NX210c reduced glutamate-induced excitotoxicity in cortical and hippocampal neurons of rat or human origins *in vitro* by promoting PI3K/mTOR survival pathway and reducing apoptosis, a neuroprotective mechanism mediated by β_1-integrins. In addition to saving neurons, NX210c also enhanced glutamatergic receptor post-synaptic currents which promoted synaptic transmission in acute mouse hippocampal slices in basal conditions or restored it in hypoxic conditions. Interestingly, NX210c effect on NMDA receptor post-synaptic currents was also mediated by β_1-integrins. Besides neurons, using several *in vitro* BBB models, we have shown that NX210c peptide increased the expression of tight junction proteins, claudin-5 and occludin, between endothelial cells and favorably modulated the integrity and permeability of the BBB, including in a primary human dynamic model exhibiting endothelial cells, astrocytes and pericytes. In a mouse model of aging used as an *in vivo* proof of concept of NX210c effect to repair a disrupted BBB, intraperitoneal injections of the peptide once a day for 5 days increased the protein expression of claudin-5 and occludin in the hippocampus and/or cortex. To conclude, NX210c strengthens the BBB, meanwhile protecting endangered neurons from neurodegeneration and synaptic dysfunctions. Accordingly, we have gathered preclinical proofs of concept of NX210c efficacy to reduce cognitive or motor deficits in various neurological disorders, including ALS. A good safety and tolerability profile of NX210c and pharmacological effects on BBB repair and neuroprotection were demonstrated in a phase Ib study in healthy elderly volunteers (2023). Altogether, this extensive package supports the planned phase II clinical trial evaluating NX210c efficacy in ALS patients (NCT06365216).

Disclosures: S. Lemarchant: None. **J. Le Douce:** None. **N. Rebergue:** None. **S. Marie:** None. **A. Janus:** None. **Y. Godfrin:** None.

Presentation Number: NANO51.02

Topic: C.06. Neuromuscular Diseases

Support: Revalesio Inc.

Title: Neuroprotective Effects of RNS60 in TDP-43 Pathology-Associated Amyotrophic Lateral Sclerosis

Authors: D. VESEVICK[1], B. HELMOLD[1], Z. FITZGERALD[1], A. AHRENS[1], J. LIU[2], S. GHOSH[3], A. KALMES[4], P. OZDINLER[1], ***M. GAUTAM**[1];
[1]Neurol., [2]Northwestern Univ., Chicago, IL; [3]Revalesio Corp., Tacoma, WA; [4]Revalesio Coproration, Tacoma, WA

Abstract: TDP-43 pathology is broadly observed in the cerebral cortex of familial and sporadic ALS patients. Mitochondrial dysfunction and increased astrogliosis/microgliosis are the most common problems in the motor cortex of ALS patients and in ALS mouse models with TDP-43 pathology, and they contribute to both upper and lower motor neurodegeneration. Upper motor neurons (UMNs) are critical for initiation and modulation of voluntary movement, and their degeneration begins much before symptom onset in TDP-43 associated-ALS. Studies have shown a direct link between health of mitochondria and astrogliosis. RNS60 is an experimental treatment for ALS that has been reported to induce mitochondrial biogenesis and ATP production in neurons *in vitro*. In this study, we investigated whether RNS60 improves mitochondrial stability and whether that leads to reduced astrogliosis and microgliosis in the

context of TDP-43 pathology. UCHL1-eGFP mice expressing eGFP in UMNs were bred with prpTDP-43^{A315T} mice to generate prpTDP-43^{A315T}-UeGFP mice, in which UMNs are genetically labelled with eGFP and display TDP-43 pathology. prpTDP-43^{A315T}-UeGFP and WT-UeGFP mice were treated with RNS60 or placebo intraperitoneally every other day from P30 until P90. Immunohistochemistry was performed on brain and spinal cord to quantitatively assess activated microglia (anti-Iba1) and activated astrocytes (anti-GFAP). Ultrastructural integrity of mitochondria was studied via electron microscopy. RNS60 treatment significantly a) improved mitochondrial ultrastructure, b) reduced the extent of astrogliosis and microgliosis in the motor cortex and spinal cord of prpTDP-43^{A315T}-UeGFP mice and c) protected health and stability of UMNs compared to placebo treated mice. These results suggest that RNS60 enhances mitochondrial stability, reduces gliosis, and thereby protects motor neurons.

Disclosures: D. Vesevick: None. **B. Helmold:** None. **Z. Fitzgerald:** None. **A. Ahrens:** None. **J. Liu:** None. **S. Ghosh:** None. **A. Kalmes:** None. **P. Ozdinler:** None. **M. Gautam:** None.

Presentation Number: NANO51.03

Topic: C.06. Neuromuscular Diseases

Support: NINDS R01 NS117968

Title: Discovery of N-terminal domain dependent small molecule modulators of TDP-43 puncta formation and cytoplasmic mislocalization

Authors: *N. NATHAN KOCHEN, M. MURRAY, E. E. LIAO, A. R. BRAUN, J. SACHS; Univ. of Minnesota, Minneapolis, MN

Abstract: TDP-43 pathological aggregates are found in ~97% of ALS patients spanning both familial and sporadic presentations of the disease. High-throughput screening (HTS) platforms targeting the TDP-43 conformational ensemble have focused on preventing its pathological aggregation associated with gain of toxic function. Recent developments in the field have shifted the focus towards loss of functional TDP-43 nuclear dimers and multimers capable of RNA binding and processing, mediated by loss of N-terminal domain (NTD) interactions, as a major contributor to amyotrophic lateral sclerosis (ALS) pathology. Here, we developed a set of fluorescence lifetime-based FRET (FLT-FRET) biosensors that probe for the NTD-dependent conformational ensemble of TDP-43. Screening full-length and NTD-null TDP-43 biosensors against the FDA-approved Selleck library, we identified three small molecules (ketoconazole, ginsenoside Rb1 and rifabutin) with known involvement in the cholesterol biosynthesis pathway that increase FRET in an NTD-dependent manner in HEK293T cells. Using fluorescence imaging we determined that these hits rescue relevant TDP-43 pathological phenotypes such as puncta formation and cytoplasmic mislocalization. Using transcriptomics and RT-qPCR under TDP-43 overexpression conditions, we show that our hits are able to rescue the downregulation of SREBP2, the master regulator of the cholesterol biosynthesis pathway and an mRNA binding target of TDP-43 with known implication in TDP-43-ALS. Overall, our results suggest that NTD-dependent modulation of the TDP-43 conformational ensemble is a promising therapeutic strategy to prevent TDP-43 pathology.

Disclosures: N. Nathan Kochen: None. **M. Murray:** None. **E.E. Liao:** None. **A.R. Braun:** None. **J. Sachs:** None.

Presentation Number: NANO51.04

Topic: C.06. Neuromuscular Diseases

Support: NIH/NINDS RO1-NS118145
NIH/NIGMS R35 GM118112

Title: Dysregulated PAD2 contributes to ALS pathogenesis through citrullination induced aggregation of MBP at arginine R-25

Authors: *I. O. YUSUF[1], W. CAMILLE[2], S. RAJORIA[3], P. THOMPSON[3], Z. XU[4];
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Abstract: Protein citrullination (PC) is a posttranslational modification that irreversibly converts protein-arginine to protein-citrulline and is catalyzed by a family of enzymes known as protein arginine deiminases (PADs). Mammals encode five PADs, and PAD2 is the most ubiquitous and dominant isoform in the central nervous system (CNS). We demonstrated previously that aberrant protein citrullination and PAD2 dysregulation are correlated temporally and spatially with disease progression in amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by motor neurons loss, paralysis, and death (PMID: 36076282, 38253209). While PC and PAD2 are increased in reactive astrocytes, they are decreased in neurons. Interestingly, PC is enriched in myelin protein aggregates. To determine the role of PAD2-induced citrullination in ALS pathogenesis, we crossed PAD2 knockout (KO) mice with $SOD1^{G93A}$ ALS mouse model and examined the consequent modulation on the clinical symptoms and pathology, using behavioral, molecular biology, electron microscopy and proteomics approaches for investigation. PAD2-deficiency led to a one-week extension of survival in male but an unchanged lifespan in female ALS mice. By contrast, body weight peaked about 3 weeks earlier in both male and female PAD2-deficient ALS mice, suggesting an earlier disease onset but a slowed progression. More motor neurons survived at the end stage in PAD2-deficient ALS mice. However, no significant change was observed in astrogliosis. Furthermore, myelin and axonal integrity were improved in PAD2-deficient ALS mice. Protein citrullination and myelin protein MBP aggregation were significantly reduced in PAD2-deficient ALS mice. Using antibodies against different MBP citrullinated sites, citrullination at arginine (R)-25 was enriched in aggregates but this enrichment was attenuated in PAD2-deficient ALS mice, suggesting PAD2 citrullination at R-25 induces MBP aggregation. Taken together, our results demonstrate that PAD2 induced citrullination contribute to ALS pathogenesis, in part, through citrullination at MBP R-25.

Disclosures: I.O. Yusuf: None. **W. camille:** None. **S. Rajoria:** None. **P. Thompson:** None. **Z. Xu:** None.

Presentation Number: NANO51.05

Topic: C.06. Neuromuscular Diseases

Support: FightMND 2022 Drug Development Grant

Title: Preclinical development of a CNS-permeable dual NLRP3 inhibitor and NRF2 activator for disease modification in Amyotrophic Lateral Sclerosis

Authors: R. GORDON[1], S. CAROEN[2], M. KUZNETSOVA[3], D. MONDHE[3], *B. ORONSKY[2];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating terminal adult-onset neurodegenerative disorder characterised by a progressive loss of motor neurons in the brain and spinal cord which erodes voluntary muscle control and movement, robbing patients of their autonomy and leading to death. Current treatments only provide minimal benefits in terms of ALS progression and there is an urgent need to develop more effective disease-modifying therapeutics.
The complex, multifactorial mechanisms of pathology which underpin ALS, and the failure of recent therapeutics, strongly suggest that targeting multiple pathological processes is critical to achieve neuroprotection and a meaningful change in disease progression. RRx-001 (nibrozetone) is a clinical-stage CNS permeable dual NLRP3 inflammasome inhibitor and NRF2 activator currently in development as a radio and chemoprotective agent.
In this study, we evaluated the therapeutic potential of RRx-001 for neuroprotection using a combination of pre-clinical animal models and mechanistic studies in isolated immune and neuronal cell cultures. We confirmed that nanomolar doses of RRx-001 were effective at reducing NLRP3-activation and inflammatory neuropathology triggered by neurotoxic C9ORF72 dipeptide aggregates (poly-GA) in immune cells.
Our studies in neuronal cells also confirmed that that RRx-001 improved mitochondrial function and NRF2 activation. In the SOD1 G93A mouse model of ALS, RRx-001 therapy reduced multiple markers of inflammasome activation, which drives disease progression in ALS, while increasing protective NRF2 pathways in the muscle and motor cortex. Once weekly dosing with RRx-001 at 2 to 5 mg/kg also improved exercise intolerance and markers of muscle injury such as malonaldehyde. Moreover, RRx-001 therapy improved motor deficits and increased median survival in SOD1 G93A ALS model.
Together, our initial results suggest that RRx-001 could be effective in slowing disease progression by blocking inflammasome activation which occurs in ALS, and, independently, by increasing protective NRF2 in muscle, blood, and the central nervous system.
Given that RRx-001 has been tested in almost 400 patients to date, with no dose limiting toxicities or related serious adverse events, our early results highlight the prospect of RRx-001 as a new disease-modifying therapeutic for ALS with the potential for rapid clinical translation.

Disclosures: R. Gordon: None. **S. Caroen:** A. Employment/Salary (full or part-time):; EpicentRx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpicentRx, Inc.. **M. Kuznetsova:** None. **D. Mondhe:** None. **B. Oronsky:** A. Employment/Salary (full or part-time):; EpicentRx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpicentRx, Inc..

Presentation Number: NANO51.06

Topic: C.06. Neuromuscular Diseases

Title: TDP-43 condensate modulation rescues TDP-43 loss of function in ALS patient-derived motor neurons and mouse models of TDP-43 proteinopathy

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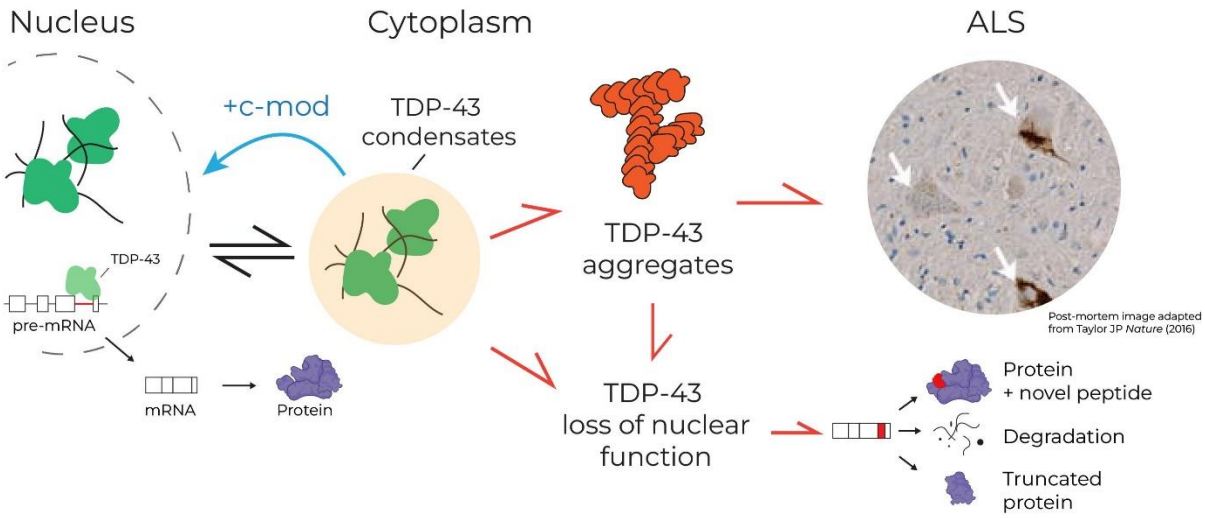
Abstract: Cytoplasmic inclusions of *TAR DNA Binding Protein 43* (TDP-43) occur in over 97% of Amyotrophic Lateral Sclerosis (ALS) patients. This mis-localization results in the aberrant splicing of multiple TDP-43 RNA targets including *STMN2* and *POLDIP3*, as well as neurite degeneration and cell death. We hypothesize that returning TDP-43 back to the nucleus via its de-partitioning from cytoplasmic condensates will rescue TDP-43 loss-of-function, thereby providing much-needed treatment to nearly all individuals with sporadic and familial ALS, irrespective of genetic background. To test this hypothesis, we developed a small molecule condensate-modulator (c-mod) to selectively de-partition TDP-43 from cytoplasmic condensates. C-mod treatment separates TDP-43 from G3BP1-containing stress granules under diverse stress conditions and rebalances splicing of *STMN2* and *POLDIP3* across 15 ALS patient-derived iPSC motor neurons (iPSC-MNs). In a 43-gene mRNA microarray and a 20-gene cryptic exon microarray, c-mod treatment broadly rebalances TDP-43-dependent gene expression and splicing respectively. Stress-induced neurite retraction and cell death are also mitigated in a concentration-dependent manner. In addition to ALS iPSC-MNs, we have tested c-mod efficacy in two mouse models of TDP-43 proteinopathy: traumatic brain injury (TBI) and mice with inducible expression of human TDP-43 lacking a nuclear localization signal (hTDP-43ΔNLS, rNLS8). C-mod treatment significantly reduces CSF neurofilament light chain, a promising biomarker for neurodegeneration, in both models. Furthermore, we observed decreased cytoplasmic TDP-43 in TBI mice receiving c-mod. We have also identified GFAP, neurofilament heavy chain, and MAG as plasma biomarkers that are suggestive of CNS injury and are rescued with c-mod. Together, our data indicate that targeting TDP-43 condensates can rescue TDP-43 loss of function and neurodegeneration under diverse environmental and genetic conditions to mitigate disease for most ALS patients.



Disclosures: J. Lai: A. Employment/Salary (full or part-time):; Dewpoint Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Dewpoint Therapeutics. **G. Welch:** A. Employment/Salary (full or part-time):; Dewpoint Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint Therapeutics. **C. Curran:** A. Employment/Salary (full or part-time):; Dewpoint Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint Therapeutics. **M. Wagner:** A. Employment/Salary (full or part-time):; Dewpoint Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint Therapeutics. **I. Klein:** A. Employment/Salary (full or part-time):; Dewpoint Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint Therapeutics. **V. Yu:** A. Employment/Salary (full or part-time):; Dewpoint Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint Therapeutics.

Presentation Number: NANO51.07

Topic: C.06. Neuromuscular Diseases

Support: ALS Association

Title: Novel chaperone-based gene therapy for ALS

Authors: *A. HISHIYA;
Sola Biosci., NATICK, MA

Abstract: Background: TDP-43 is a nuclear protein essential for the processing of DNA and RNA. In 97% of ALS cases, TDP-43 is mislocalized to the cytoplasm, where it accumulates and forms stress granules. These granules contain pathological forms of misfolded, phosphorylated, and C-terminal truncated TDP-43 that are resistant to degradation. Both the loss of function (due to the depletion of nuclear TDP-43) and the toxic gain of function (resulting from cytoplasmic TDP-43 aggregates) are believed to play crucial roles in the pathophysiology of neurodegeneration observed in ALS
Hypothesis We hypothesize that an engineered chaperone-based gene therapy targeting misfolded TDP-43 could serve as an effective treatment strategy for the broad ALS population. This approach focuses on rectifying the pathogenic forms of TDP-43, potentially addressing both the toxic gain of function and the loss of function effects central to ALS pathophysiology.
Methods/Results: SOL-257 is an AAV gene therapy that encodes a novel fusion protein composed of two functional domains: a unique targeting domain that specifically binds to misfolded TDP-43, and a protein folding activation domain that interacts with HSP70, a key cellular chaperone. This fusion protein functions as a co-chaperone, efficiently directing misfolded TDP-43 to HSP70 for either correct refolding or facilitated degradation. Following the establishment of in vitro proof of concept and successful in vivo expression of the SOL-257 fusion protein, we achieved in vivo proof of concept using a C9orf72-based mouse model. Administration of SOL-257 via lumbar puncture at three months of age—when mice begin to exhibit behavioral abnormalities and TDP-43 aggregates—resulted in a significant reduction in phosphorylated TDP-43 aggregates by nine months of age. Correspondingly, SOL-257 also

significantly ameliorated behavioral deficits in these mice. Conclusion: This in vivo proof of concept study demonstrated that SOL-257 gene therapy effectively prevents TDP-43-related toxicity in a mouse model of ALS. These results strongly support the continued preclinical development of SOL-257 for ALS treatment. Moreover, the adaptability of this gene therapy platform, which expresses engineered co-chaperones with tailored target specificities, holds promise for treating a range of protein misfolding diseases.

Disclosures: A. Hishiya: A. Employment/Salary (full or part-time):; SOLA Biosciences. 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.; ALS Association.

Nanosymposium

NANO52: Mechanisms of Cellular Stress and Degeneration

Location: MCP Room N228

Time: Wednesday, October 9, 2024, 8:00 AM - 10:15 AM

Presentation Number: NANO52.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R35127253

Title: Targeting ATXN2 to prevent cellular dysfunction in patient-derived models of spinocerebellar ataxia type 4

Authors: *M. GANDELMAN[1], S. PAUL[2], K. FIGUEROA[2], W. DANSITHONG[2], D. R. SCOLES[2], S. M. PULST[3];
[1]Dept. of Neurol., Univ. of Utah, Salt Lake city, UT; [2]Neurol., Univ. of Utah, Salt Lake City, UT; [3]Dept. of Neurol., Univ. of Utah, Salt lake city, UT

Abstract: Spinocerebellar ataxia type 4 (SCA4) is a rare inherited ataxia characterized by cerebellar and sensory ganglia neurodegeneration. It is caused by a dominant GGC-repeat expansion coding for poly-glycine in ZFHX3/ATBF1[1]. We analyzed a SCA4 patient brain and found neuronal intranuclear inclusions (NIIs) that were positive for ZFHX3, p62, ubiquitin and ATXN2, revealing a role for autophagy defects and proteostasis in this disease. Patient-derived induced pluripotent stem cells (iPSC) and fibroblasts mirrored these findings, with increased apoptosis, evidenced by cleaved PARP (cPARP), and increased phosphorylated mTOR, total mTOR, LC3, p62 and ATXN2, as detected by western blot analysis. Knockdown of ZFHX3 normalized these markers in both cell types, suggesting a causal role for ZFHX3. However, the multiple critical functions of ZFHX3 indicate therapeutic targeting is likely deleterious. ZFHX3 loss leads to neurodevelopmental disorders, epilepsy and cardiac arrhythmia, and ZFHX3 also acts as a tumor suppressor. We hypothesized that ATXN2 would constitute a better therapeutic target than ZFHX3. Expansions of the polyglutamine tract in ATXN2 cause SCA2 and increase the risk of ALS, and an antisense oligonucleotide targeting ATXN2 is currently in a phase 1/2 clinical trial for ALS. We found that in our SCA4 patient-derived iPSCs and fibroblasts

knocking down ATXN2 with an siRNA was sufficient to decrease cPARP, phosphorylated mTOR, total mTOR, LC3 and p62, consistent with reduced cell death and autophagy dysfunction. These results link the polyglycine expansion mutation in ZFHX3 to pathological pathways associated with ATXN2, a polyglutamine protein, establish autophagy dysfunction in SCA4 and reveal a role for ATXN2 as a potential therapeutic target treatment to prevent cerebellar neurodegeneration and improve patient outcomes in SCA4.
1-Figueroa, K.P., Gross, C., Buena-Atienza, E. et al. A GGC-repeat expansion in ZFHX3 encoding polyglycine causes spinocerebellar ataxia type 4 and impairs autophagy. Nat Genet (2024). https://doi.org/10.1038/s41588-024-01719-5

Disclosures: M. Gandelman: None. **S. Paul:** None. **K. Figueroa:** None. **W. Dansithong:** None. **D.R. Scoles:** None. **S.M. Pulst:** None.

Presentation Number: NANO52.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

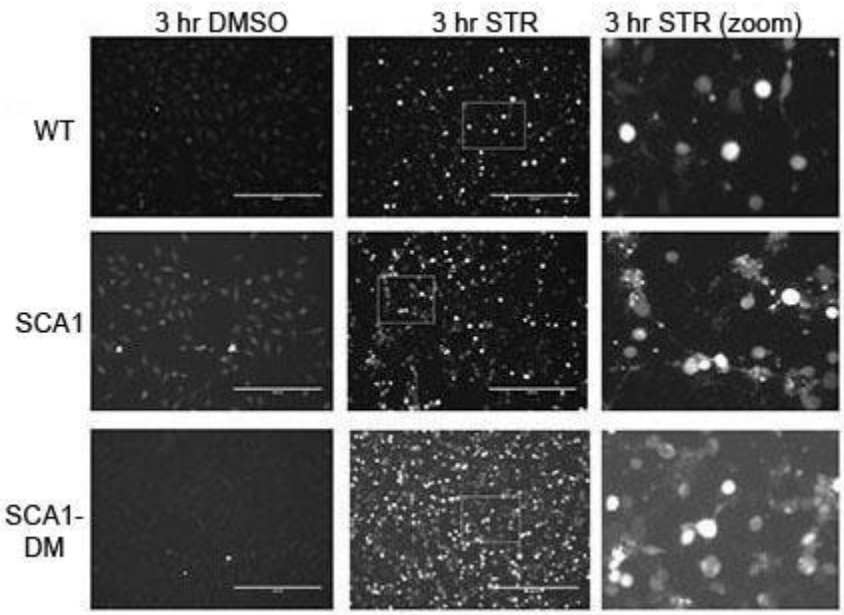
Support: NSF 2018952
NSF 1337280

Title: Beyond the nucleus: widespread mitochondrial and cytoplasmic dysfunction due to mutant ataxin-1 expression in Spinocerebellar ataxia type 1 cells

Authors: *S. LAGALWAR;
Neurosci., Skidmore Col., Saratoga Springs, NY

Abstract: Ataxin-1 (ATXN1) is a nuclear-cytoplasmic shuttling protein, which, when polyglutamine (polyQ) expanded, causes the progressive neurodegenerative disease Spinocerebellar Ataxia Type 1 (SCA1). While the role of nuclear ATXN1 as a repressor of transcription and regulator of splicing is well studied, its potential cytoplasmic role is more ambiguous. We previously demonstrated mitochondrial dysfunction- including altered respiration and enhanced oxidative stress- is associated with early SCA1 pathogenesis in mice. Intervention with the electron transport chain substrate succinic acid ameliorates Purkinje cell atrophy and cerebellar behavioral deficits. Additionally, recent published data using a cultured cell system supports direct interactions between mutant polyQ-expanded ATXN1 and mitochondrial proteins involved in apoptosis, oxidative phosphorylation, mitochondrial composition, and mitochondrial gene transcription. We therefore hypothesized that mitochondrial dysfunction in SCA1 may be at least partially due to cytoplasmic interactions between ATXN1 and mitochondria, rather than solely a result of mutant ATXN1's altered nuclear function or simply mitochondrial dysfunction being a toxic byproduct of disease. In order to characterize the extent of mitochondrial dysfunction due to mutant ATXN1 expression apart from a disease context, we stably-expressed mutant, nuclear-targeted ATXN1 in human cerebellar-derived cultured cells. Despite the short lifespan (~33 hours), mitochondria in mutant ATXN1-expressing cells reveal gross morphological, compositional, and physiological deficits. Additionally, stable expression of a readily degradable, cytoplasmic-targeted mutant ATXN1 selectively resulted in intermediate physiological phenotypes and altered mitochondrial protein composition therefore suggesting that irrespective of a disease context and ATXN1 nuclear

translocation, mitochondrial deficits still occur. We hope our current findings begin to provide insight into the multifaceted and multicompartmental role of ATXN1.



Disclosures: S. Lagalwar: None.

Presentation Number: NANO52.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NINDS 1R01NS129517-01

Title: Impairments in visual perception and cortical circuit function in a model of Parkinson's disease dementia

Authors: *A. THEINT[1], W. ZEIGER[2];
[1]Univ. of California Los Angeles, LOS ANGELES, CA; [2]Dept. of Neurol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Besides, motor deficits such as bradykinesia, tremor and rigidity, many patients experience non-motor symptoms such as cognitive impairment. One of the most affected cognitive domains in PD patients is visuospatial/perceptual function and it has been hypothesized that these deficits are attributable to the dysfunction of specific cortical circuits. However, mechanisms underlying cortical neuron dysfunction are not fully understood. PD is pathologically defined by the deposition of aggregated α-synuclein (α-syn) and neocortical α-syn pathology correlates with cognitive impairment and dementia. Thus, we hypothesize that α-syn pathology directly impairs neuronal activity in cortical circuits, leading to visuoperceptive impairments. To study PD-associated visuoperceptive impairments, we developed a model to monitor progression of α-syn pathology, visual cortical circuit function and visuoperceptive behavior in mice injected with α-syn pre-formed fibrils (PFF) into primary visual cortex (V1). PFF injections in V1 lead to time-

dependent formation of Lewy-like inclusions in V1 and spread to connected brain regions. Using longitudinal in vivo two photon calcium imaging in V1, we recorded visual-evoked activity of pyramidal cells in layer 2/3 while mice passively view drifting sinusoidal gratings. At 3 months post-injection (mpi) when cortical α-syn pathology peaks, we found fewer neurons responsive to visual stimuli, broader tuning, and lower orientation selectivity in PFF-injected mice compared to saline-injected controls. We also found that neurons are more strongly correlated and have lower spontaneous neuronal activity in PFF-injected mice compared to controls. We also measured visuoperceptive function using a novel head-fixed coherent motion discrimination "go/no-go" task. Both control and PFF injected mice can robustly discriminate motion direction of random dot kinematograms (RDKs) at 90% coherence. Introducing "test" trials with lower coherences, we are now quantifying coherent motion discrimination thresholds at different times after PFF injection as a measure of visuoperceptive function. Experiments are ongoing but we expect motion discrimination thresholds will be higher in PFF-injected mice at 3mpi compared to control mice. This would be consistent with our in vivo imaging results and would suggest that α-syn pathology in visual cortical circuits can directly impair visuoperceptive function. Together, our results will shed light on mechanisms by which α-syn pathology drives cortical circuit dysfunction and cognitive impairment in PD.

Disclosures: A. Theint: None. **W. Zeiger:** None.

Presentation Number: NANO52.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Assessing mitochondrial dynamics and function in Bak1 microexon knockout neurons

Authors: *Y. LAM[1], L. LIN[1], M. ZHANG[1], P. STOILOV[2], L. CHEN[3], S. ZHENG[1];
[1]Univ. of California, Riverside, Riverside, CA; [2]West Virginia Univ., Morgantown, WV; [3]USC, Los Angeles, CA

Abstract: Genetic regulation of neuron longevity is poorly understood. We previously found that intrinsic attenuation of neuronal apoptosis can be mediated through the neuron-specific, post-mitotic inclusion of *Bak1* microexon 5, resulting in the downregulation of outer mitochondrial membrane protein BAK1 as neurons mature. BAK1 is a pore-forming protein that releases cytochrome C from the mitochondrial intermembrane space during apoptosis. Germline knockout of microexon 5 results in post-mitotic BAK1 induction and increased apoptosis in the brain as well as perinatal lethality. We have generated a conditional allele for neural specific deletion of exon 5 which results in BAK1 protein expression in a subset of post-mitotic neurons. Forebrain knockout leads to progressive, region-specific neuronal degeneration. With the goal of understanding the mechanisms underlying neuronal loss, we examined how neuronal BAK1 expression resulting from microexon 5 deletion alters the behavior of mitochondria prior to obvious neuronal loss. Biochemical, microscopic, and metabolic studies show signs of mitochondria dysfunction in our Bak1 microexon 5 (Bak1-e5) conditional knockout mice, which express BAK1 protein post-mitotically. Studying the effects of Bak1 post mitotic expression on mitochondria dynamics and function could reveal greater insight into how neurons maintain their unique properties of energetic metabolism and lifespan.

Disclosures: Y. Lam: None. **L. Lin:** None. **M. Zhang:** None. **P. Stoilov:** None. **L. Chen:** None. **S. Zheng:** None.

Presentation Number: NANO52.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R35 NS122302
NIH Cellular and Molecular Biology Training Grant T32-GM007315
Rackham Regent's Training Grant

Title: Modeling early molecular features of Huntington's disease in human stem cell-derived, self-organizing, single-rosette cortical organoids

Authors: *R. G. POWERS, M. SIERRA, D. M. SEYFRIED, H. L. PAULSON;
Univ. of Michigan, Ann Arbor, MI

Abstract: Huntington's disease (HD) is one of nine fatal neurodegenerative diseases caused by expansion of a polyglutamine-encoding CAG repeat in the respective disease gene. No disease-modifying therapeutics exist for any polyglutamine disease, and our understanding of disease mechanisms remains a work in progress. Examination of postmortem human fetal brains has revealed that many age-related neurodegenerative diseases like HD can affect neurodevelopment. Furthermore, transcriptional dysregulation (TD) and somatic repeat instability (SRI), the process by which a disease gene's CAG repeat expansion increases over time in somatic cells, are thought to contribute to HD pathogenesis in the brain. The developmental timing and interrelationship of these two phenomena, however, are unknown. To examine the impact that TD and SRI have on early neurodevelopment in HD, we generated brain-like organoids derived from a panel of isogenic HD-human embryonic stem cell lines harboring the following CAG repeat lengths in the disease gene: 30 (disease absence), 45 (adult-onset), or 81 (juvenile-onset). Organoids were generated using a novel approach that results in self-organizing, single-rosette cortical organoids (SOSR-COs). The absence of multiple, primary rosettes reduces the heterogeneity and variability between organoids that is often seen in other approaches. This series of isogenic SOSR-COs were examined after one, three, and six months of differentiation, timepoints that correspond to distinct developmental stages. To examine SRI, DNA was extracted from organoids (n = 3 per genotype) and used to perform fluorochrome-labeled bulk-PCRs (n = 3 per organoid) that amplify the CAG repeat expansion in the HD disease gene, *HTT*. Brightfield images were taken of organoids at each timepoint (n = 5-25 organoids per genotype), from which equivalent diameters for individual organoids were calculated. At various timepoints, SOSR-COs with pathogenic CAG repeat lengths (45 or 81 CAGs) were significantly larger than those with a nonpathogenic length (30 CAGs; Tukey's multiple comparisons test, p-values < 0.001). In contrast, organoids harboring pathogenic CAG repeat lengths did not display SRI, as their repeat lengths remained constant over time. We are currently using a probe-based single-cell RNA sequencing platform and immunocytochemistry to characterize the transcriptomes and cytoarchitectures, respectively, of SOSR-COs. Collectively, this ongoing research will provide insight into how the HD mutation may alter various neurodevelopmental features, including cerebral cortical volumes and cell-type ratios.

Disclosures: R.G. Powers: None. **M. Sierra:** None. **D.M. Seyfried:** None. **H.L. Paulson:** None.

Presentation Number: NANO52.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R35NS116868
FLOYD Family Fund

Title: Progressive Mouse Models of SCA6: Insights from the humanized alpha1ACT Expanded polyQ Overexpression

Authors: *X. DU[1], **E. GAMA**[2], C. WEI[3], A. SIDKY[2], J. SUN[2], M. D. KOOB[4], K. A. BENZOW[4], C. M. GOMEZ[2];
[1]Univ. of Chicago, Chicago, IL; [2]Neurol., Univ. of Chicago, Chicago, IL; [3]Northwestern Univ., Chicago, IL; [4]Dept. of Lab. Med. and Pathology, Univ. of Minnesota, Minneapolis, MN

Abstract: Overexpression of alpha1ACT, the secondary protein product of *CACNA1A* gene, with an expanded polyglutamine (polyQ) tract (alpha1ACT$_{EXpolyQ}$) in cells causes cell death. Transgenic mice expressing humanized alpha1ACT $_{EXpolyQ}$ exhibit the wide based gait and thinning of the cerebellar molecular layer. To delineate the progressive disease caused by alpha1ACT$_{EXpolyQ}$, we engineered the PC-BAC alpha1ACT$_{EXpolyQ}$ mouse model utilizing a bacterial artificial chromosome (BAC) system. This model expresses the human C-terminus of the *CACNA1A* gene, bearing 33 CAG repeats under the control of the PCP2 promoter. PC-BAC alpha1ACT$_{Q33}$ mice develop normally, but from 3 to 9 months, they manifest robust progressive motor deficits, Purkinje cell loss, and progressive cerebellar atrophy. Notably, Purkinje cell loss predominantly occurs in anterior lobe, mirroring the pathological changes observed in SCA6 patients. Given that alpha1ACT expression is regulated by internal ribosome entry sites (IRES), we further refined the model to generate PC-IRES alpha1ACT$_{Q33}$ mice, in which *CACNA1A* IRES was used to control alpha1ACT expression which we have previously targeted using miRNA in an AAV hyperacute model. These mice exhibit very similar progressive phenotypes to those observed in PC-BAC alpha1ACT$_{Q33}$. Both PC-BAC alpha1ACT$_{Q33}$ and PC-IRES alpha1ACT$_{Q33}$ mouse models provide valuable insights into SCA6 pathogenesis, biomarker identification, and the evaluation of gene-targeting therapeutic strategies.

Disclosures: X. Du: None. **E. Gama:** None. **C. Wei:** None. **A. Sidky:** None. **J. Sun:** None. **M.D. Koob:** None. **K.A. Benzow:** None. **C.M. Gomez:** None.

Presentation Number: NANO52.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant IGNITE R61NS124965
R01NS097903
R35NS127253

Title: Staufen1 antisense oligonucleotides for treating tdp-43 proteinopathy

Authors: *D. R. SCOLES, S. PAUL, W. DANSITHONG, K. FIGUEROA, M. GANDELMAN, S. M. PULST;
Neurol., Univ. of Utah, Salt Lake City, UT

Abstract: The RNA binding protein STAU1 interacts with the ATXN2 protein and is overabundant in SCA2, TDP-43, and C9orf72 mutant patient fibroblasts (FBs) and mouse models as well as spinal cord from sporadic amyotrophic lateral sclerosis (ALS) patients. We found that STAU1 overabundance in these models is associated with impaired autophagic flux, which results from STAU1 directly interacting with the *MTOR* transcript enhancing its translation. We hypothesized that targeting STAU1 may be an effective approach for normalizing autophagy and other phenotypes common to many neurodegenerative diseases (NDDs). We screened 118 MOE gapmer ASOs targeting in the STAU1 coding region in HEK-293 cells. ASOs effective for lowering STAU1 by greater than 45% by quantitative PCR were further screened in SCA2 patient FBs, and for lowering human STAU1 in a BAC-STAU1 mouse model. Among the lead ASOs, ASO-45 also targets mouse *Stau1* and is useful for *in vivo* proof-of-concept testing in mouse models of NDD. The objective of this study was to determine the effect of targeting STAU1 as an approach for normalizing TDP-43 proteinopathy. ALS patient FBs with a A382T mutation in TDP-43 had 2-fold overabundance of STAU1, by western blotting (WB). Treatments of these cells with ASO-45 and two other lead *STAU1* ASOs normalized STAU1 and significantly improved expression of mTOR, p62, LC3-2, cleaved CASP3, and PERK & CHOP, two unfolded protein response proteins. Prp-TDP43-Q331K transgenic mice had 2-fold increased STAU1 in motor cortex (MC). Treatment of TDP43-Q331K mice at 8 wks of age by intracerebroventricular (ICV) injection of ASO-45 for 6 wks had normalized STAU1 abundance levels like in PBS treated wildtype littermates, and significantly improved GFAP and NEUN expression in MC by WB. TDP-43 abundance was also reduced in spinal cord of Prp-TDP43-Q331K mice treated 2 wks with ASO-45. Double-transgenic Thy1-TDP-43 mice have a severe phenotype with mean survival of 19 days and 2-fold STAU1 overabundance in brain. When treated by ICV injection with ASO-45 at age p3 the mean survival increased significantly to 22 days (15.8 % increased survival) with maximum survival of 26 days (log-rank test, $p<0.0001$). Treated mice also had a significantly better motor impairment score (ANOVA, $p<0.0001$). At the endpoint we observed significantly improved abundance of STAU1, NEUN, ATXN2, and cleaved CASP3 in brain by WB. We conclude that targeting STAU1 may be an effective strategy for treating disorders with STAU1 overabundance and TDP-43 proteinopathy characterized with impaired autophagic flux, including frontotemporal dementia (FTD), ALS and limbic-predominant age-related TDP-43 encephalopathy (LATE).

Disclosures: D.R. Scoles: None. **S. Paul:** None. **W. Dansithong:** None. **K. Figueroa:** None. **M. Gandelman:** None. **S.M. Pulst:** None.

Presentation Number: NANO52.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R35NS116868
FLOYD Family Fund

Title: Deciphering the Molecular Pathogenesis of SCA6: Insights from Multi-Omics Profiling of alpha1ACT with expanded polyQ

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Abstract: The presence of an expanded polyglutamine (polyQ) tract encoded by exon 47 of the *CACNA1A* gene is implicated in the development of spinocerebellar ataxia type 6 (SCA6). Alpha1ACT protein, a secondary protein product of *CACNA1A* gene, bears this expanded polyQ. This study explores the impact of alpha1ACT with expanded polyQ (n=33) (alpha1ACT$_{Q33}$) through a range of experiments and analyses spanning genomics, transcriptomics, proteomics, and epigenetics. Integrating genomic binding profiles and he transcriptional profiling of alpha1ACT$_{Q33}$ via ChIP-seq and RNA-seq, the study revealed that its involvement in neurodegeneration and DNA damage pathways, showing a time-dependent pattern. Proteomics analysis identified co-transcription factors of alpha1ACT$_{Q33}$ and the chaperons associated with its aggregation in nucleus. The miRNA/mRNA pinpointed the miRNAs that were affected by the expanded polyQ expansion. Further validation and localization of integrated target genes and proteins associated with alpha1ACT$_{Q33}$ were conducted in the alpha1ACT$_{Q33}$ mouse models. Integrated multi-omics profiles underscored the crucial role of alpha1ACT$_{Q33}$ in cerebellar degeneration, emphasizing both spatial and temporal aspects of its impact. These findings provide compelling evidence for the critical role of alpha1ACT$_{Q33}$ in the pathogenesis of SCA6.

Disclosures: E. Gama: None. **C. Wei:** None. **D. Pastor:** None. **J.N. Savas:** None. **A. Sidky:** None. **J. Sun:** None. **T.T. Thaxton:** None. **C. Flores:** None. **X. Du:** None. **C.M. Gomez:** None.

Presentation Number: NANO52.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 Grant 5R01NS089585-11
NIH T32 Grant 5T32AG071444-02

Title: Ribosomal profiling of motor neurons degenerating in ALS suggests a neuroprotective role for FGF21

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Abstract: The neuromuscular junction (NMJ) is a chemical synapse that is the site of skeletal muscle innervation by spinal motor neurons. This connection between the nervous system and muscle allows for coordinated motor function, and the maintenance of the NMJ is critical for maintaining musculoskeletal homeostasis. Under normal physiological conditions, spinal motor neurons have significant regenerative potential and can regrow axons in response to peripheral nerve injury. In diseases such as amyotrophic lateral sclerosis (ALS), the NMJ is dismantled and motor neurons selectively degenerate resulting in progressive muscle wasting and eventual fatal paralysis. We adapted the RiboTag methodology developed by Sanz *et al*. to perform ribosomal profiling of motor neurons in mice to assess how nerve injury and ALS affect motor neurons *in vivo*. One of the most highly upregulated transcripts was *Fgf21*, which was only induced in *Sod1*^{G93A} mice, making the transcript specific to ALS and not normal regeneration. *Fgf21* is a

stress-inducible hormone that is critically involved in glucose turnover, and its expression may cause metabolic alterations in ALS. Immunolabeling experiments in *Sod1*^{G93A} and *Tdp43*^{A315T} mice revealed that FGF21 protein is increased both in motor neuron cell bodies and in the periphery in motor axons and muscle. Transgenic mouse models where *Fgf21* is conditionally knocked out in *Sod1*^{G93A} motor neurons showed reduced motor neuron survival and NMJ innervation. Behavioral and survival trials with *Sod1*^{G93A} mice showed a dramatic reduction in locomomotion and lifespan when *Fgf21* was conditionally knocked out. Taken together, these data suggest *Fgf21* functions in a neuroprotective capacity in ALS pathology. We are evaluating the functions of *Fgf21* and the mechanisms in which it promotes motor neuron survival and skeletal muscle innervation and metabolism, with the ultimate goal of identifying new therapeutic strategies for ALS.

Disclosures: W. Stansberry: None. **E.A. Newell:** None. **B. Pierchala:** None.

Nanosymposium

NANO53: Spinal Cord Injury: Developing Therapeutic Strategies

Location: MCP Room N426

Time: Wednesday, October 9, 2024, 8:00 AM - 10:15 AM

Presentation Number: NANO53.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS124224
Travis Roy Foundation Boston, MA

Title: Comparison of epidural and transcutaneous spinal cord stimulation and their synergistic interaction with brain stimulation

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Abstract: Spinal cord stimulation (SCS) activates sensorimotor circuits and is primarily implemented with epidural or transcutaneous approaches. To date, direct comparisons of the methods have been lacking. We compared efficacy and selectivity of both methods in isolation, or combined with brain stimulation to integrate spinal sensory and descending motor activation. Efficacy was measured in terms of corticospinal facilitation or as recruitment curve threshold

and slope. Selectivity was measured as the relative activation of a target muscle compared with other muscles. We hypothesized that epidural SCS would facilitate brain stimulation both more effectively and selectively than transcutaneous SCS. We employed epidural SCS on 59 individuals undergoing elective cervical spine decompression surgery, recording responses from arm and leg muscles. We compared these to responses from transcranial magnetic stimulation paired with transcutaneous SCS in 15 individuals living with SCI and 14 individuals with no neurological deficit. Both SCS modalities were applied to generate suprathreshold motor evoked potentials (MEPs) and associated recruitment curves. SCS was applied independently as well as with brain stimulation at varied intervals in order to determine the optimal timing for synergistic effects. Pairing stimulation between the motor cortex and spinal cord produced MEPs that were larger than the sum of the brain-only and spinal-only responses regardless of injury severity [1]. However, the facilitation of paired stimulation was an order of magnitude larger for epidural than for transcutaneous SCS. We also observed that pairing with epidural stimulation is more selective than the brain- or spinal-only conditions, but this has not yet been observed with transcutaneous SCS. Epidural SCS, particularly when paired with brain stimulation, produces higher facilitation and selectivity, but the tradeoff between these approaches remains unexplored. It is currently unclear whether neuromodulation strategies will provide better recovery when they prioritize selectivity or efficacy. Selective activation might benefit patient recovery without causing excessive muscle activation or off-target effects, while more generalized activation of cervical circuits may be more efficacious and could be shaped by use. [1] McIntosh JR, Joiner EF, Goldberg JL, Greenwald P, Dionne, AC, Murray LM, Thuet E, Modik O, Shelkov E, Lombardi JM, Sardar ZM, Lehman RA, Chan AK, Riew KD, Harel NY, Virk MS, Mandigo C, Carmel JB. Timing dependent synergies between motor cortex and posterior spinal stimulation in humans. J. Physiol. In Press. 2024 April. doi:10.1113/JP286183

Disclosures: J.R. McIntosh: None. **L.M. Murray:** None. **E.F. Joiner:** None. **A. Dionne:** None. **R. Lehman, Jr.:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Medtronic, Stryker. F. Consulting Fees (e.g., advisory boards); Medtronic. **A.K. Chan:** None. **K. Riew:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biomet, Nuvasive, Amedica, Axiomed, Benvenue, Expanding Orthopedics, Happe Spine, Paradigm Spine, Spinal Kinetics, Spineology, Vertiflex. F. Consulting Fees (e.g., advisory boards); Happe Spine, Nuvasive. **M.S. Virk:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OnPoint Surgical. F. Consulting Fees (e.g., advisory boards); Depuy Synthes, BrainLab Inc. **N.Y. Harel:** F. Consulting Fees (e.g., advisory boards); RubiconMD. **J.B. Carmel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BackStop Neural, SharperSense. F. Consulting Fees (e.g., advisory boards); Pacira, Motric Bio, Restorative Therapeutics.

Presentation Number: NANO53.02

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Revealing mechanisms of non-invasive peroneal nerve stimulation in the human brain and spinal cord using functional ultrasound imaging

Authors: *K. A. AGYEMAN[1], D. J. LEE[2], A. ABEDI[2], Y. LO[2], J. RUSSIN[2], V. EDGERTON[3], C. LIU[2], V. N. CHRISTOPOULOS[4];
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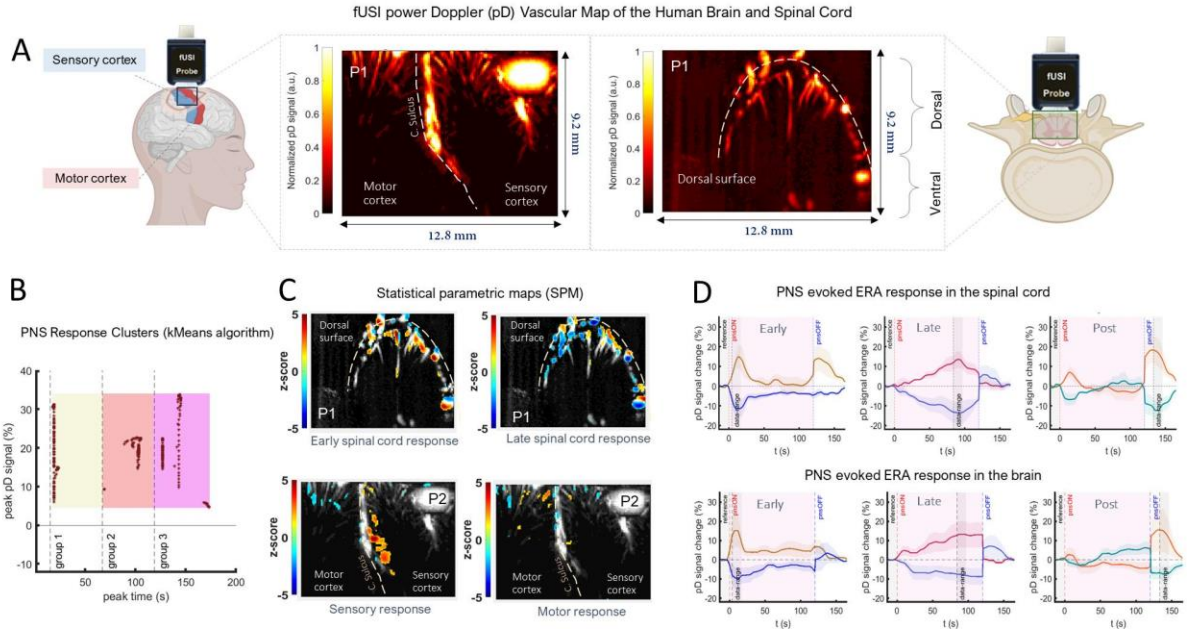
Abstract: Utilization of neuromodulation to treat neurological dysfunction associated with sensory-motor and autonomic functions has shown remarkable promise. Nonetheless, the mechanisms of action of responses in the human brain and spinal cord are not well understood, leading to suboptimal clinical outcomes. Additionally, research on the effects of neuromodulation on functional activity is limited due to susceptibility to artifacts arising from simultaneous stimulation with signal acquisition, physiological constraints, and technical challenges inherent to existing functional neuroimaging modalities.
Herein, we leveraged novel and enhanced sensitivity properties of functional ultrasound imaging (fUSI) to investigate the effects of non-invasive peroneal nerve stimulation (PNS) on brain and spinal cord functional organization (Fig. 1A). fUSI recordings were obtained simultaneously with stimulation (i.e., 4 cycles, 2 min ON - 1 min OFF, at 27 mA suprathreshold level) from 6 patients who underwent standard-of-care surgery for chronic back pain treatment.
Our findings demonstrated that PNS results in distinct spatiotemporal modulation and three stimulation-induced effect-states in the spinal cord and brain (i.e., early, and late - during stimulation, and post - after stimulation offset) across patients (Fig. 1B, D). From brain fUSI signals acquired simultaneously from sensory and motor cortices, we established that the effect groups correspond to functional activation of sensory and motor brain regions (Fig. 1C, bottom) - suggesting that PNS elicits sensory and motor activation within the brain and spinal cord.
This first in-human study is significant as it provides analytical capabilities to assess functional neural activity modulation coupled to non-invasive neuromodulation with a new level of precision in-vivo. Our characterization of distinct functional states opens new avenues to understand brain dysfunction, and effects of neuromodulation - ultimately vital for optimizing modulation parameters and developing closed-loop neurorehabilitation systems.



Disclosures: K.A. Agyeman: None. **D.J. Lee:** None. **A. Abedi:** None. **Y. Lo:** None. **J. Russin:** None. **V. Edgerton:** None. **C. Liu:** None. **V.N. Christopoulos:** None.

Presentation Number: NANO53.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research (CIHR)

Title: Effects of a Mediterranean-Ketogenic diet on CST plasticity and motor behavior in a pyramidotomy model of SCI

Authors: D. PARK[1], M. POOVATHUKARAN[1], S. NEMATI[1], B. KONDILES[1], M. LU[1], W. TETZLAFF[1], ***O. SEIRA**[2];
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Abstract: Spinal cord injury (SCI) causes motor, sensory and autonomic impairments, and global metabolic dysregulation. It is generally accepted that exercise and nutrition are the most effective health-modifying interventions for able-bodied people. However, exercise options are limited after SCI, and nutritional recommendations for the SCI population are largely opinions and not evidence-based. Work from our laboratory has shown that the induction of ketosis after SCI improves secondary neuropathology by decreasing oxidative stress, increasing antioxidants, reducing inflammation, and improving mitochondrial bioenergetics. Recent data from other laboratories showed that improved mitochondrial energy metabolism provides favorable conditions for axonal regeneration and CNS repair. In this study, we aimed to determine whether a presumed healthy diet combined with nutritional ketosis (Mediterranean-Ketogenic Diet, Med-KD) increases corticospinal tract (CST) sprouting and improves fine motor skills in mice when compared to an unhealthy Western Diet (WD). We performed a unilateral transection of the corticospinal tract in the pyramid of C57BL6 mice fed either of the two diets ad libitum for 7 weeks. Tape removal and grid walk tests were performed once a week. Two weeks before the endpoint, animals were traced with Dextran-488 (contralateral motor cortex) or BDA (ipsilateral) to evaluate compensatory sprouting of the CST axons at the level of the cervical cord (C5-C6) and compensatory sprouting and regeneration of the cortico-rubral pathway in the midbrain. As intended, our novel Med-KD induces ketosis as evidenced by increased blood β-hydroxybutyrate levels (BHB), and reduces body weight compared to the WD group. Preliminary sprouting data revealed no difference in sprouting between treatment groups at the level of the cervical cord or the midbrain. Finally, our behavioral data indicate some improvement in both the motor and sensory scores in the tape removal task and a trend towards a reduction in the percentage of mistakes in the grid walk test 1 week after injury in the Med-KD group. Thus our Med-KD regimen leads to increased ketosis and improved fine motor function in mice after SCI presumably by systemic metabolic effects independent of CST sprouting (i.e., enhanced mitochondria metabolism). Together, these findings suggest that the use of a healthy Med-KD might be a translatable and safe therapeutic strategy to promote improved motor outcomes after SCI.

Disclosures: D. Park: None. **M. Poovathukaran:** None. **S. Nemati:** None. **B. Kondiles:** None. **M. Lu:** None. **W. Tetzlaff:** None. **O. Seira:** None.

Presentation Number: NANO53.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation (599050)
NIH R01 NS034382-01
NIH R01 NS109552-01

Title: Motor response evoked by low frequency spinal cord stimulation are not affected by acute spinal cord injury

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Abstract: The neural circuitry of the spinal cord has a distinctive but repetitive structure that forms an especially promising target for controlling motor output via electrical stimulation. Furthermore, this structure allows the essential circuits for generation of movements to be preserved below the level of a spinal cord injury (SCI). Electrical stimulation approaches usually take advantage of another basic aspect of spinal anatomy, that all sensory axons enter the cord via a highly accessible location, its dorsal surface. Thus, dorsal electrical stimulation (DES) via surface electrodes provides effective activation of sensory axons without the need for penetrating electrodes.
The anatomical location of the motor pools (groups of motoneurons within the ventral horn) for muscles involved in locomotion has been identified. However, the map of surface electrical stimulation of the spinal cord and its functional output to different muscles is still relatively unknown. Importantly, it is still unknown whether the motor output generated by DES could change immediately following SCI. Before employing DES in individuals with acute SCI, these inquiries must be addressed.
In eight decerebrate cats with intact spinal cords, eight locations were stimulated starting from the caudal portion of lumbar segment L3 to the border of sacral segments S1 and S2. Stimulation was done 15 times at each location at 1 Hz (1ms square pulse) with stimulation amplitudes high enough to evoke muscle responses without causing tissue injury. EMG was measured in nine hindlimb muscles: soleus, tibialis anterior, lateral gastrocnemius, sartorius, medial gastrocnemius, vastus lateralis, biceps femoris posterior, gluteus medius, and pectineus. EMG peak to peak amplitude of the short-latency response (presumably monosynaptic) were used to assess muscle response. Then, the spinal cord was transected above L3 and the protocol was repeated to map the acute cord. Results showed that surface electrical stimulation at 1Hz did roughly follow the anatomical location; however, some muscles could be activated well beyond their anatomical pools. These results suggest that subdural electrical stimulation in the acute transected cord largely has the same effects as in the intact cord. Further research is needed to understand how the maps are affected by chronic spinal cord injury.

Disclosures: M. Chardon: None. **A. Mahrous:** None. **J.R. Patterson:** None. **M.D. Johnson:** None. **J.F. Miller:** None. **C. Heckman:** None.

Presentation Number: NANO53.05

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Exploring the neuromodulatory effects of invasive and non-invasive human spinal cord stimulation using functional ultrasound imaging

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Abstract: Spinal Cord Stimulation (SCS) is a widely employed neuromodulatory intervention for treating chronic back pain and improving sensorimotor and autonomic functions. Despite its extensive application, the precise mechanism through which SCS influences spinal cord activity remains inadequately understood, especially regarding the impact of diverse stimulation settings on underlying neurodynamics. This study aims to understand the effects of invasive and non-invasive SCS in the human spinal cord hemodynamics using functional ultrasound imaging (fUSI). To do so, we monitored the hemodynamic activity of the spinal cord in four (4) patients who underwent standard-of-care implantation of an epidural electrical SCS (ESCS) device (T10 partial laminectomy) under general anesthesia for chronic back pain treatment. The stimulation protocol involved five 30-second ON-OFF cycles of both unilateral and bilateral SCS at 3.0 mA current amplitude, 40 Hz burst frequency, and 250 μs pulse width. Two (2) patients received an enhanced bilateral stimulation at 4.5 mA and a lower intensity unilateral stimulation at 2 mA. Additionally, two (2) other patients underwent a targeted transcutaneous SCS (TSCS) at the L1 to L2 spinal level, structured into eight cycles: the initial four cycles at 50 mA, followed by four cycles at 100 mA, aiming to compare the hemodynamic effects of varying intensities. The results showed that fUSI is capable of detecting distinct neuromodulatory effects across stimulation types and intensities. In patients undergoing TSCS, elevating the intensity from 50 mA to 100 mA increases the hemodynamic response, emphasizing the significant impact of current intensity on neuromodulation. Moreover, while unilateral spinal cord stimulation affected the ipsilateral segment, it also triggered neural activation in the contralateral side. This suggests an intrinsic neural connection that facilitates cross-communication between the two sides of the spinal cord. This phenomenon was observed as both increased and decreased Power Doppler (PD) signal changes in the contralateral spinal segment, indicating a complex modulation of neural activity. Notably, right unilateral stimulation induced a greater PD signal change compared to the left, highlighting lateral differences in neuromodulation. Overall, preliminary findings showed that fUSI can effectively differentiate the hemodynamic responses induced by various SCS intensities and configurations, opening a new avenue to establish fUSI as a clinical monitoring technology for optimizing SCS protocols in chronic back pain and other neuropsychiatric disorder.

Disclosures: F. Zareian: None. **K. Agyeman:** None. **D.J. Lee:** None. **K. Wu:** None. **A. Abedi:** None. **Y. Lo:** None. **V. Edgerton:** None. **C. Liu:** None. **V.N. Christopoulos:** None.

Presentation Number: NANO53.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Neuroscience Program, the Office of Research and Graduate Studies, College of Medicine, the John G. Kulhavi Professorship in Neuroscience, and the E. Malcolm Field and Gary Leo Dunbar Chair in Neuroscience at Central Michigan University.

Title: Photobiomodulation as a Therapeutic Approach to Restore Motor Function following Spinal Cord Injury in Wistar Rats

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Abstract: Spinal cord injury (SCI) is the leading cause of disabilities worldwide, resulting in temporary orpermanent impairment of function. Despite various therapeutic strategies, including systemicmedication, surgical intervention, and rehabilitation therapy, enhancing neurological functionfollowing SCI remains limited and inconsistent. Photobiomodulation therapy (PBMT) hasemerged as a promising avenue due to its multifaceted benefits, including suppressinginflammation, repairing damage tissue, and providing analgesia. While initial preclinical studieshave shown potential of PBMT for axonal regeneration and inflammation reduction, there iscurrently no standardized protocol or timeline for its application in SCI treatment. We conductedthree studies aimed at optimizing PBMT as therapy for SCI. Firstly, we compared the effects ofone-week PBMT, two-weeks PBMT with treatments using methylprednisolone sodium succinate(MPSS) on motor function and inflammation in male rats with moderate compression SCI(Mojarad et al., Chem. Neuroanat., 2018). We found that two weeks of PBMT treatment was asefficacious as MPSS in motor recovery and inflammation reduction, without causing weight lossor mortality, suggesting long-term PBMT as a preferable option due to fewer side effects. Oursecond study investigated the efficacy of two PBMT protocols administered over two and fourweeks in male rats with moderate compression SCI (Janzadeh et al., Lasers in Med. Sci. 2023).The four-week PBMT showed greater effectiveness in alleviating SCI complications, such asneuropathic pain and motor dysfunction, compared to the shorter duration protocols, emphasizingthe importance of extended PBMT durations for optimal outcomes in motor function recovery. Ourthird study extended the PBMT timeline to seven weeks in both male and female rats with severecompression SCI (Mojarad et al., 2024). Daily PBMT administration throughout the experimentdemonstrated enhanced motor recovery compared to untreated groups, highlighting the potentialof extended PBMT durations in improving outcomes post-SCI. Our studies indicate that prolonged
PBMT therapy can significantly enhance motor recovery following SCI. Our ongoing studies areaimed at utilizing PBMT in combination with both neural stem cell transplantation and functionalelectrical stimulation to promote axonal regeneration, reduce inflammation, and restore functionalrecovery following SCI. Our results indicate that PBMT is an effective therapy, even followingsevere SCI, suggesting that it will enhance therapeutic outcomes when used in combination withother therapies.

Disclosures: N. Mojarad: None. **K. Reed:** None. **A. Uprety:** None. **S. Wright:** None. **B. Stewart:** None. **D. Doyle:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Presentation Number: NANO53.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSF Grant RES515698//DBI-2015317
HD084672-03
CHN Foundation Grant 649297

Title: Electrophysiological and Morphological Changes in Motoneurons due to Chronic Spinal Cord Injury: Implications for Spasticity Management

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Abstract: Chronic spinal cord injury (SCI) causes damage to descending motor tracts resulting in loss of volitional movement. In the months following the injury, patients experience symptoms of increased motor excitability, such as spasticity, hyperreflexia, and involuntary muscle spasms. Recent studies, including ours, have shown that motoneuron persistent inward currents (PICs) are increased and contribute to the development of muscle spasms in chronic SCI. To enable targeted interventions for management of spasms, we conducted a comprehensive investigation into changes in motoneuron electrical and morphological properties across various models of SCI. In adult mice (3 months old), we induced either a complete (transection) or incomplete (impact) SCI at the lower thoracic segments and allowed the animals to recover for six months establishing chronic SCI. Animals with incomplete injury were further categorized into low-function or high-function groups based on their motor recovery (BMS score). Whole-tissue sacrocaudal spinal cord was then extracted and used *ex-vivo* to study motoneuron properties via sharp microelectrodes. Tissue from age-matched animals with no prior spinal injuries served as the control. In addition, some animals were perfused with a fixative, and their lumbosacral cord tissue was processed for histological studies of motoneuron morphology. Our data revealed multiple changes in passive and active membrane properties of motoneurons in chronic SCI. These changes included reduced current threshold for action potential, decreased input conductance, and increased AHP amplitude and duration. In addition, motoneurons were divided into different types based on their repetitive firing behavior in response to slow ramp current injection. In models of chronic SCI, there was a significant shift to more excitable types with emergence of new firing patterns particularly in severe SCI models. This shift in excitability correlated with increased PIC amplitudes in voltage clamp as well as estimated PIC values in current clamp recordings. Moreover, these electrophysiological changes were paralleled by changes in motoneuron morphology further contributing to increased excitability. Our results highlight major changes in motoneuron properties and excitability in chronic SCI, which can predispose spasms. These findings indicate that modulation of motoneuron excitability can be a promising target for managing spasticity following SCI.

Disclosures: A. Mahrous: None. **D. Birch:** None. **V.M. Tysseling:** None. **C. Heckman:** None.

Presentation Number: NANO53.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS105725
NIH DP2 NS106663
Craig H. Neilsen Foundation 998694

Title: Rehabilitation enhances the structural dynamics of intracortical motor circuits and facilitates functional recovery after spinal cord injury

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Abstract: Spinal cord injury (SCI) triggers profound changes in the organization of motor cortex, resulting in expansion of body representations from above the injury level into de-efferented motor areas. Previously, through repeated mapping of evoked movements after cervical SCI in mice, we found a rehabilitation-dependent re-mapping of former hindlimb areas to accommodate forelimb movements disrupted by injury. The underlying circuit mechanisms facilitating this cortical reorganization, and the necessity for such broad reorganization for supporting functional recovery, remain unknown. In this study, we evaluated the effects of intensive skilled forelimb reach rehabilitation on recovery after C5 dorsal column SCI. Using *in vivo* two-photon imaging, we longitudinally tracked the structural plasticity of pre-synaptic axonal boutons of layer 2/3 intracortical neurons projecting from de-efferented hindlimb to forelimb motor areas over a period of 4 weeks. We observed increased survival of existing pre-injury boutons in response to rehabilitation. Additionally, we found a gradual increase in bouton number in response to 2 weeks of rehabilitation, followed by elimination and consolidation after 3 weeks. Rehabilitation also drove greater stability of newly formed boutons. These findings suggest that intracortical connections within the motor cortex undergo dynamic changes during rehabilitation from SCI. To assess the necessity of these intracortical connections in rehabilitation-mediated recovery after injury, we optogenetically silenced hindlimb motor cortex during single pellet reach. Silencing hindlimb motor cortex did not impact the success rate in intact mice prior to injury; however, hindlimb silencing disrupted the recovered function after 4 weeks of rehabilitation. These results indicate that intracortical reorganization is essential for rehabilitation-induced functional recovery after SCI. Together, our findings suggest that rehabilitation enhances the structural dynamics of intracortical neurons, thereby facilitating functional recovery post-SCI.

Disclosures: X. Guan: None. **E.R. Hollis:** None.

Presentation Number: NANO53.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Shaw Foundation

Title: Randomized control pediatric constraint induced movement therapy for children with brachial plexus injury: cognitive and upper extremity function changes

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Abstract: In the past we reported upper and lower extremity function changes from a constraint induced therapy (pCI) camp for children with brachial plexus injury (BPI). We reported on pre post and six months post performance of the experimental group. The objectives of this study were to a) contrast the performance of the experimental and the control group, and b) determine group related cognitive changes. This is a randomized control study including 17 children with BPI, 3-7 years of age, 9 randomly assigned in the experimental group (EG). No participant had history of other neuromusculoskeletal injury or previous CI exposure. All subjects could use the affected arm as gross assist during play and self care. Cognitively, they could follow two step commands. Treatment took place at a Children's Hospital. We delivered 30 hours of treatment (3 hours of treatment specific training over 10 days). Activities focused on gross, fine motor and self feeding skills. Control group (CG) participants had traditional occupational therapy (OT). Outcomes were measured using the Shrinner's Hospital Upper Extremity Evaluation (SHUEE), the GAITRite to assess gait and the Good Enough test to assess cognitive changes. EG participants were tested pre and post pCI, and after six months. CG participants were tested pre and after 30 hours of treatment (six months). Results were initially explored with discriminant analysis and then for simplicity and reporting with t-tests ($a<0.05$). This report focuses on the cognitive and SHUEE pre and post treatment results. Table 1 presents selected results based on the SHUEE and cognitive task that showed significant changes in either EG or CG groups.This is, to our knowledge, the first randomized control study investigating the immediate effects of pCI on the concurrent Cognitive and Upper Extremity function of children with BPI. The results demonstrate clear improvements in the cognitive and upper extremity performance. Furthermore, the results suggest superiority of pCI over the traditional OT approach in treating functional deficits for children with BPI.

Table 1. Means, standard deviations and p values for selected output parameters.

Parameter	Pre-Exp	Post-Exp	p	Pre-Cont	Post-Cont	p
	Mean(SD)	Mean(SD)		Mean(SD)	Mean(SD)	
Spontaneous Functional Analysis	71.6(9.9)	86.5(10.3)	.00	68.5(12.6)	73.4(16.7)	.36
Dynamic Positional Analysis	62.9(12)	79.7(9.1)	.00	69.6(9.3)	74.2(11.1)	.15
Cognitive age (normalized)	95.5(1)	99.3(2)	.02	90.9(12.3)	91.7(8.9)	0.44

Disclosures: T. Karakostas: None. **E.C. King:** None.

Nanosymposium

NANO54: Higher Visual Processing of Natural Stimuli

Location: MCP Room S106

Time: Wednesday, October 9, 2024, 8:00 AM - 11:30 AM

Presentation Number: NANO54.01

Topic: D.06. Vision

Support: imons Collaboration on the Global Brain award 542961SPI
NIH Grant R01EY022930
NIH Grant R01EY034723
NIH Grant RF1NS121913

Title: Visual cortical responses to image fragments are modulated by the exposure to the entire image

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Abstract: The visual system processes information through neurons that each respond to a small part of the visual scene. However, perception of a visual scene is an emergent property that is not easily explained by the sum of information within each composing fragment of the scene. Here, we tested the hypothesis that the responses of individual neurons to fragments of an image are influenced by the exposure to the entire image.We compared the responses of populations of macaque V1 and V4 neurons to image fragments before and after the monkey viewed the entire image. We measured neuronal responses to sequences of image fragments flashed within the joint receptive fields of the simultaneously recorded V1 and V4 neurons while the animals passively fixated. The animals then free viewed the entire image while we recorded gaze position and neuronal responses. After extensive experience free viewing the image, we repeated the measurements of neuronal responses to the fragments. Our data are consistent with the hypothesis that viewing the entire image modulates neuronal responses in ways that reflect knowledge of how the fragment fits into the whole rather than simply adaptation, familiarity, or repetition suppression. More specifically, after exposure to the whole image, V1 and V4 responses to image fragments are better predicted by 1) activations of convolutional neural networks trained on whole images, 2) higher level image features, and 3) the animals' gaze fixation patterns during free-viewing. These results are consistent with the idea that visual neuronal responses are modulated by knowledge of the entire image through, perhaps via feedback from areas higher in the processing hierarchy. Our findings therefore raise intriguing questions about the functional role of top-down feedback to visual cortex.

Disclosures: C. Xue: None. **M. Czarnik:** None. **M.R. Cohen:** None.

Presentation Number: NANO54.02

Topic: D.06. Vision

Support: Simons Collaboration on the Global Brain award 542961SPI
NIH R01EY022930
NIH R01EY034723
NIH RF1NS121913

Title: A novel, high-capacity memory task reveals behavioral and neuronal insights underlying image recognition memory

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Abstract: Primates have a remarkable ability to remember thousands of images after a single viewing. This form of high-capacity, recognition memory poses a considerable challenge for multiple reasons. One, it happens instantaneously, making it difficult to attribute the parts of the neural response related to sensory processing versus memory mechanisms. Second, feature dimensionality for natural images is extremely high and will greatly affect responses in visual areas thought to contribute. Third, primates excel at this task, making it hard to pinpoint aspects related to what is necessary for successful memory. Lastly, trial-to-trial variability, which has been linked to cognitive processes like decision-making, working memory, attention, and task switching, limits the coding capacity of a neural population and is thought to be a key reason why other cognitive processes have capacity limits magnitude lower than visual memory. To address these challenges, we designed a high-capacity memory task that allows us to analyze the neural and behavioral ingredients of memory on individual trials. Monkeys view images that are revealed slowly, one fragment at a time, and are rewarded for reporting whether or not they have seen the image before. This gradual reveal lengthens the decision process, giving us a glimpse of each decision as it is unfolding. The extended decision process makes it possible to associate distinct aspects of the visual stimulus or neural population responses with successful or unsuccessful memory encoding or retrieval. We use natural images with data-verified metrics, such as image labels, memorability scores, and similarity scores, to better understand visual modulations that might be occurring and/or guiding memory. We record from populations of neurons in visual cortex (mid-level area V4, which encodes many stimulus properties that impact visual memory) and parietal cortex (area 7a, which has been implicated in decision-making, learning, and memory). Our preliminary results suggest that the order and consistency with which image fragments are revealed, and the concomitant changes in the way image information is dynamically encoded neural population responses, impact visual recognition memory. We are using these data to identify neural coding schemes that account for behavior mediated by both low and high capacity cognitive processes. Understanding the neural basis of high capacity, one-shot learning has important applications for efforts to repair or enhance learning and memory in human patients and artificial systems.

Disclosures: G.F. DiRisio: None. **C. Xue:** None. **M.R. Cohen:** None.

Presentation Number: NANO54.03

Topic: D.06. Vision

Support: NIH Grant 1DP2EY035176-01
NSF CAREER 2143077

David and Lucile Packard Foundation
Kempner Research Fellowship
Victoria Quan Pre-doctoral Fellowship

Title: Factorized convolution model for neuronal tuning prediction and interpretation for natural images

Authors: *B. WANG[1,2], C. R. PONCE[2];
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Abstract: Convolutional neural networks have been extensively used to model neurons in the visual systems of primates and rodents. However, this is an ill-posed regression problem because the number of image-response pairs are often far fewer than the feature regressors. To solve this problem, previous neuron-modeling methods used unsupervised feature reduction (e.g., PCA) and penalized regression. Yet, these solutions discard the spatial structure of feature units for computational efficiency, usually leading to non-smooth or non-local weight structures, decreasing interpretability and generalization. Here, we describe a model with "supervised" feature reduction, which applies tensor factorization to a covariance tensor defining the relationship between image features and neuronal activations. This factorization identifies factors characterizing the spatial and feature selectivity of the neuron, providing a clear way to interpret the features selected by the neuron and a spatial attribution mask within the input image. This method is as efficient and accurate as previous penalized regression methods in predicting neurons and faster in training than previous factorized models. Moreover, it provides more accurate localization of receptive fields, which benefits interpretation of the preferred feature of neuron. To validate this method, we conducted closed-loop experiments on neurons recorded from V1, V4 and inferotemporal (IT) cortex of two primates. Neurons guided image synthesis using generative networks trained on natural images. The generated image-response pairs were used to train a factorized convolution model. We used these models to predict maximal activating image for the neuron. To identify necessary parts, we ablated components in these models (e.g. shuffled spatial masks) as controls and synthesized their maximal activating images. In the same session, we presented these predicted “optimal” images back to the animal and measured neuronal responses. We found that model-predicted images were more activating than controls. Consistent with many recent works, we found adversarially trained robust networks were effective in predicting the preferred images of the neurons. Using our factorization method, low-rank, localized read-out weights were able to predict the preferred images as well as dense readout weights, or the images obtained from online evolution optimization. In this manner, we are able to transform dense "black-box" model of a visual neuron into a low rank, part-based model, which was easier to describe and investigate, thus advancing both the ability to model neurons and to explain their tuning for natural images.

Disclosures: B. Wang: None. **C.R. Ponce:** None.

Presentation Number: NANO54.04

Topic: D.06. Vision

Support: PMRF

Title: Is It Boring to Look at A Face for More Than A Few Hundred Milliseconds?

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Abstract: Our visual system is extremely fast at perceiving faces. Within 200 milliseconds, even before the first eye fixation, we can identify faces in a scene. However, what we look at in faces beyond 200 ms is rarely explored, such as when we identify gender, age, and other details. In this study, we investigate the decodability of our visual system up to 1500 ms using EEG experiments.

We presented face stimuli (upright - female, male; inverted - female, and male) and house stimuli (upright and inverted) for 1500 ms with interstimulus intervals randomly set between 2500-3500 ms to six subjects (five male) in our preliminary study. EEG data were recorded using a 63-channel setup at a 2.5 KHz sampling frequency. We used two analytical tools to analyse the data - ERP and the invariant space of EEG graphs. As preprocessing, we first applied a bandpass filter with a cutoff frequency of 4-35 Hz, then performed mean rereferencing, and finally used an average filter. For the ERP analysis, we epoched the data from -200 ms to 1500 ms and performed baseline correction using the 200 ms signal before the onset of the stimulus, and finally averaged over subjects. For the analysis using the invariant space of the EEG graph, we first normalized the data using the logarithmic function to suppress spurious signals. Thereafter, we constructed the graph using correlations between consecutive windowed EEG data with a moving window length of 10 samples. Then, we performed eigenvector decomposition on the graph and plotted the distance between the invariant spaces of the EEG graph.

ERP analysis shows that beyond 600 ms, the differences between the different conditions are not significant and indicates that faces don't stimulate our visual system significantly differently than houses or inverted faces. However, this could be attributed to the very small number of subjects in the study. Therefore, we analyzed the invariant space of the EEG graph. This is an excellent way to deal with noisy EEG signals. The difference between the invariant eigenspaces of the EEG graph shows that the difference between male-house, male-inverted male, female-male, and female-inverted female is significantly greater than that of house-house, and house-inverted house invariant spaces, which are significant.

Our preliminary EEG study on face perception shows that faces indeed stimulate our visual system differently than houses, extending beyond mere recognition and classification by gender, age, and other features. However, further investigation is needed with more subjects. The future scope is to use eye-tracking tools to analyse where we look on faces beyond the first few fixations.

Disclosures: R. Dev: None. **S. Gupta:** None. **S. Kumar:** None. **T.K. Gandhi:** None.

Presentation Number: NANO54.05

Topic: D.06. Vision

Support: Simons Foundation grant (543061)
NSF CAREER grant (1844724)
NVIDIA hardware grant
Google TPU Research Cloud hardware grant

Title: Inter-animal transforms as a guide to model-brain alignment

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Abstract: The appropriate methods for aligning neural network models to the brain remain controversial. Ideally, a good alignment method should be powerful enough to enable accurate predictions of neural responses under a mapping from model units to neurons, while also being specific enough to distinguish the target system (e.g. a particular brain area) from other systems. It has generally been assumed that the goals of predictivity and specificity are in tension with each other, with methods that severely restrict the possible relationships between model and target being better for specificity, and more flexible methods yielding higher predictivity. We show that this apparent tension does not in fact exist. Fundamentally, this is because specificity requires not only distinguishing response patterns from different brain areas (i.e. separation), but also recognizing response patterns from the same brain area as being similar across subjects (i.e. identification). We evaluate a range of alignment methods for predictivity and specificity on a simulated population of mice and compare these results to real mouse data where possible. To evaluate predictivity, we compute the R^2 score when mapping responses between different subjects for the same brain area or model layer. To evaluate both aspects of specificity, we compute the silhouette score, which is 1 just in case responses of different types (e.g. different model layers) are scored as highly dissimilar compared to responses of the same type (e.g. the same model layer). We find that relatively flexible methods, like linear regression, exhibit greater specificity compared to stricter methods, while also enabling better predictions. Motivated by the idea that the correct balance between strict and loose is manifested by the empirical relationships between subjects in a population, we introduce an alignment method that incorporates known aspects of the biological circuit, specifically the nonlinear activation function. This further improves predictivity *and* specificity for the model population. We compare these results to a set of Neuropixels recordings for 31 mouse subjects in response to 118 naturalistic stimuli, where our proposed method again improves predictivity without reducing specificity compared to existing methods.

Disclosures: I. Thobani: None. **J. Sagastuy-Brena:** None. **A. Nayebi:** None. **R. Cao:** None. **D. Yamins:** None.

Presentation Number: NANO54.06

Topic: D.06. Vision

Support: Leon Levy Fellowship
NIH Grant EY022428

Title: Selectivity of neurons in macaque V4 to object and texture images

Authors: *J. D. LIEBER[1], L. PALMIERI[2], T. D. OLESKIW[2], E. P. SIMONCELLI[3], J. A. MOVSHON[2];
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Abstract: Humans and monkeys can effortlessly recognize and distinguish objects in everyday scenes. These abilities rely on neural computations in the ventral visual cortex. Specifically, previous studies implicate area V4 as an early site of selectivity for object shape. To explore this

selectivity, we “scrambled” 20 photographic images using techniques that preserve a set of statistics from the original image while discarding information about scene and shape. To create a continuum between scrambled textures and photographic images, we parametrically varied the size of the regions in which statistics were measured (the “scrambling regions”), which were blended to seamlessly cover the entire image. We presented these images to two awake, fixating macaque monkeys while recording responses from several hundred single units in area V4. On average, neurons responded equally strongly to photographic images and their scrambled counterparts, but the range of responses was larger for photographic images. To measure the ability of the neuronal population to discriminate between different scenes, we used linear classifiers to separate one image from the remaining set of 19. These classifiers more robustly discriminated photographic images than scrambled images. We conclude that the shape cues present in photographic images are encoded by V4 and contribute to the discrimination of scene identity. Next, to measure the sensitivity of the neuronal population to image structure, we used classifiers to discriminate photographic images from their scrambled counterparts. Discrimination was best between scrambled and photographic images, and much poorer between fully scrambled and partially scrambled images. We analyzed these images with an image-computable similarity metric that accurately predicts human judgments of image degradation (DISTS, Ding et. al. 2020). This measure of perceptual distance between image pairs reasonably predicted neural distances derived from our population responses. This demonstrates that V4 responses are highly sensitive to small, perceptually-salient deviations from photographic image structure. Finally, we measured the temporal evolution of scene discriminability and structural discriminability. Scene discriminability emerged at the onset of the visual response, while structure discriminability was delayed by ~20 ms. The delayed development of structure discriminability may be a consequence of recurrent processing within V4, or of feedback from downstream visual areas.

Disclosures: J.D. Lieber: None. **L. Palmieri:** None. **T.D. Oleskiw:** None. **E.P. Simoncelli:** None. **J.A. Movshon:** None.

Presentation Number: NANO54.07

Topic: D.06. Vision

Support: FKZ 01GQ2107
SFB 1233 Robust Vision
EXC 2064/1, project number 390727645
ERC 101041669
SFB 1456, project B05
R01 EY026927
IARPA D16PC00003

Title: Deep learning-driven tuning characterization unveils topological organization in V4

Authors: *K. F. WILLEKE[1,2], K. RESTIVO[3], K. FRANKE[1], N. KARANTZAS[1], A. S. ECKER[4], F. H. SINZ[4], A. S. TOLIAS[1];
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Abstract: Deciphering the brain's structure-function relationship is key to understanding the neuronal mechanisms underlying perception and cognition. The cortical column, a vertical organization of neurons with similar functions, is a classic example of primate neocortex structure-function organization. While columns have been identified in primary sensory areas using parametric stimuli, their prevalence across higher-level cortex is debated. A key hurdle in identifying columns is the difficulty of characterizing complex nonlinear neuronal tuning, especially with high-dimensional sensory inputs. Here, we asked whether area V4, a mid-level area of the macaque visual system, is organized into columns. We combined large-scale linear probe recordings with deep learning methods to systematically characterize the tuning of >1,600 V4 neurons using *in-silico* synthesis of most exciting images (MEIs), followed by *in-vivo* verification. We found that the MEIs of single V4 neurons contained complex features like textures, shapes, or even high-level attributes such as eye-like structures. Neurons recorded on the same silicon probe, inserted orthogonal to the cortical surface, were selective to similar spatial features, as expected from a columnar organization. We quantified the similarity of MEIs recorded in the same session using human psychophysics and by measuring MEI distance in a non-linear similarity space, learned with a contrastive loss. Moreover, the selectivity of the V4 neuronal population was clustered into distinct functional groups of shared feature selectivity, reminiscent of cell types. These functional groups closely mirrored the feature maps of units in artificial vision systems, hinting at shared encoding principles between biological and artificial vision. Our findings provide evidence that columns and functional cell types may constitute universal organizing principles of the primate neocortex, simplifying the cortex's complexity into simpler circuit motifs which perform canonical computations.

Disclosures: K.F. Willeke: None. **K. Restivo:** None. **K. Franke:** None. **N. Karantzas:** None. **A.S. Ecker:** None. **F.H. Sinz:** None. **A.S. Tolias:** None.

Presentation Number: NANO54.08

Topic: D.06. Vision

Support: NIH Grant EY031446
NIH Grant EY022428

Title: Neural code for natural planar shape in macaque V2 and V4

Authors: *T. D. OLESKIW[1,2], A. SUTTER[3], G. M. LEE[1], N. J. MAJAJ[1], J. H. ELDER[4], J. A. MOVSHON[1];
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Abstract: Planar shape - the silhouette contour of a solid body - carries rich information important for object recognition, including local (i.e., curvature) and global (i.e., statistical regularities spanning the contour’s length) shape cues. In the macaque, neurons in extrastriate area V4 encode the local curvature of figure-ground contrast, representing boundary conformation in an object-centered reference frame. However, it is not known whether V4 simply inherits its tuning from upstream visual areas or if this tuning is sensitive to global shape cues that may be computed within V4.
To answer this question, we recorded multi-unit activity from populations of V2 and V4 neurons

in the awake macaque, measuring responses evoked by simple synthetic shape stimuli used previously to characterize neural curvature tuning. Interestingly, we found strong tuning for local curvature not only in V4 as previously reported, but also in V2. This suggests that V4 might code global shape features by integrating over multiple curvature-tuned V2 afferents.
We also used a novel family of synthetic stimuli that match the local properties of natural shapes while varying their global regularities to dissociate local and global shape tuning. A mutual information analysis reveals model neurons trained on neural data extract information more efficiently from shapes with natural curvature distributions, suggesting an optimization to the ecological statistics of curvature.
To directly measure neuronal selectivity for natural shape, we recorded activity evoked in V4 neurons of one juvenile monkey by natural and synthetic shapes. Consistent with our model neuron analysis, synthetic shapes with natural curvature distributions evoked stronger responses than synthetic shapes with more random curvature distributions. Further, we found that natural shapes evoked stronger V4 responses than synthetic shapes with matching curvature statistics, indicating selectivity for global shape features. Together with preliminary behavioral data showing monkeys can readily discriminate synthetic contours, our findings demonstrate that V4 neurons preferentially encode the ecological statistics of both local and global object shape, challenging existing models of V4 shape selectivity.

Disclosures: T.D. Oleskiw: None. **A. Sutter:** None. **G.M. Lee:** None. **N.J. Majaj:** None. **J.H. Elder:** None. **J.A. Movshon:** None.

Presentation Number: NANO54.09

Topic: D.06. Vision

Support: NSERC Postdoctoral Fellowship to BRTR
National Eye Institute grant (R01-EY034432) to W.A.B.

Title: Visual features drive intrinsic memorability for abstract symbols

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Abstract: While prior work has investigated the stimulus properties that make pictures of objects, scenes, and faces memorable or forgettable, emerging work has turned to everyday visual symbols (e.g., !@#$%) as a new way to understand how memorability might operate for abstract concepts. It has been found that symbols are more memorable than their word counterparts (e.g., $ is better remembered than ‘dollar’), sharing many commonalities with how images are treated by memory. Here, we explored which low-level visual and high-level conceptual attributes drive the intrinsic memorability of 80 common symbols. To provide a metric of each symbol’s memorability, 248 online participants completed a continuous recognition memory test whereby responses were made whenever a visually presented symbol repeated. Split-half reliability tests confirmed that symbols do have intrinsic memorability: Certain symbols were reliably more memorable than others across individuals. Participants then completed a spatial arrangement task to indicate their representations of the visual similarity and semantic similarity of the symbol set. From this, we used the Euclidean distance between each pair of symbols as a proxy for how similar two symbols are, as judged by participants. First, we

tested for any organizational rules guiding participants' arrangements of the symbols. Principal components analysis revealed that pairwise distances between symbols in a spatial arrangement could be simplified into just a few major dimensions: thin to thick, straight to curvy, and good symmetry to poor symmetry. All three of these principal components were then validated objectively using computer vision analyses of low-level image features. Second, we tested how these features relate to the memorability of a symbol. Each of the three principal components significantly predicted the memorability of symbol images in a multiple regression. We conclude that the tested symbols were more likely to be memorable if they were thin, pointy, and have low vertical symmetry. Semantic features were also analyzed using a similar approach, indicating that particularly common symbols, and also those that represent salient concepts like religion or danger, are more memorable. Ratings of symbol iconicity—indexing how much a symbol looks like a physical object—did not predict memorability, contrary to expectations. Our results demonstrate that certain visual features of images contribute to their intrinsic memorability, and reveal that memory for abstract concepts can be altered dependent on their concrete visual representations.

Disclosures: B.R.T. Roberts: None. **W.A. Bainbridge:** None.

Presentation Number: NANO54.10

Topic: D.06. Vision

Support: NIH Grant R00EY031795
Incubator for Transdisciplinary Futures: Toward a Synergy Between Artificial Intelligence and Neuroscience

Title: Border ownership signals emerge in an artificial neural network trained to predict future visual input

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Abstract: To make sense of visual scenes, the brain must segment objects from background. This is thought to be facilitated by neurons in the primate visual system that encode border ownership (BO), i.e. whether a local border is part of an object on one or the other side of the border. It is unclear under which circumstances BO signals emerge in neural networks. In this study, we investigated whether BO signals exist in PredNet, a deep artificial neural network with an architecture inspired by predictive coding, that is trained to predict the next image frame of natural video sequences. We first mapped the classical receptive field (cRF) of units in PredNet and then used scenes with square objects to evaluate selectivity for BO, using a similar approach as in neurophysiology studies. We found that a significant number of PredNet units behave similarly to BO neurons in the brain: they respond differently to an identical contrast border in their cRF depending on which side the border belongs to, and this is independent from the polarity of luminance contrast across the border. We found that these BO units in PredNet share several other properties with BO neurons in the brain. First, BO signals in PredNet are remarkably tolerant to varying the border position in the cRF, object size and border orientation. Second, the activity of BO units is modulated by individual fragments of the square outside of

the cRF in a way that corresponds to the units' preferred side of BO. Third, BO signals persist even after the contextual information that defines the side of ownership is removed. Our discovery of BO units in PredNet suggests that these units aid in predicting future visual input. To test this, we performed ablation experiments on PredNet. We found that ablating BO units affected prediction accuracy more than ablating control units. Overall, we found that BO-selective units emerge in PredNet, an artificial neural network that is trained to predict future visual input, even though it was not explicitly trained to segment foreground from background, and that these units contribute to prediction. Our findings establish PredNet as a useful computational tool to study the computation of border ownership, and suggest that BO signals in the brain may be the result of evolutionary pressure to predict future input under dynamic visual conditions.

Disclosures: Z. Ye: None. **R. Wessel:** None. **T.P. Franken:** None.

Presentation Number: NANO54.11

Topic: D.06. Vision

Title: Modeling synaptic degeneration and neural plasticity in visual cortex

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Abstract: Neurodegenerative diseases of the visual system like Posterior Cortical Atrophy (PCA) and Visual Agnosia result in disproportionate volume loss in the occipital and posterior parietal lobes of the biological brain, with patients having difficulty in performing tasks like color discrimination, reading fragmented images, identifying faces, and perceiving boundary shapes (Milner et al., 1991). Task-optimized deep artificial neural networks (ANNs) of the brain have been developed to predict responses in the healthy ventral stream to much success, although computational models of the ventral stream under degeneration have been sparse. To this end, we use CORnet-S (Kubilius et al., 2019), an ANN, to simulate localized synaptic degeneration over time by progressively injuring 20% of the healthy convolution filter weights in areas V1, V2, V4, and inferotemporal cortex (IT) of the model across different experiments. Neural plasticity is incorporated by retraining healthy model weights on images from the ImageNet dataset (Deng et al., 2009). We analyze how performance on tasks such as face verification, noisy images, and pseudoisochroma get affected when different regions are lesioned, and what role neural plasticity plays in the process. Furthermore, we compute the optimal stimuli that activate various convolution filters in a layer, effective receptive field sizes, and neural predictivity scores (Freeman et al., 2013; Majaj et al., 2015) of different models with variously lesioned regions. We find that (1) different regions in the visual cortex have functionally different responses to tasks such as stimuli processing, shape discrimination, and face perception, with lesions in IT, for example, leading to a dramatic drop in performance on face perception using the Labeled Faces in the Wild (LFW) dataset (Huang et al., 2007) when compared to lesions in V1, V2, or V4; (2) even when a certain region in the visual cortex like V1 or IT is ~99.85% progressively lesioned, the cortex has a remarkable ability to recover task performance through perceptual learning; and (3) the visual cortex undergoes repurposing of regions adjacent to a lesioned region to achieve this: for example, when IT is lesioned, later convolution layers of V4 start becoming better at IT predictivity in the brain compared to those of a healthy V4, and their effective receptive field

sizes start becoming larger. These results not only provide an interpretability-based analysis of ANNs under lesioning, but also show that they can serve as computational models that can inform rehabilitation strategies for patients by considering the dynamic interplay between degeneration and plasticity in the visual cortex.

Disclosures: Y. Shah: None. **K. Tran:** None. **K. Kotar:** None. **D. Yamins:** None.

Presentation Number: NANO54.12

Topic: D.06. Vision

Support: NIH Grant MH128552

Title: Content-based modulation of medial temporal lobe theta oscillations during visual exploration

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Abstract: Memory involves translating perceptual experiences into memory traces that can guide future behavior. Not all information in the environment is treated equally, as affective and socially relevant stimuli are prioritized for perceptual and mnemonic processing. Such memory enhancement is thought to depend upon theta oscillations that coordinate activity across medial temporal and visual systems during encoding. However, the neural dynamics that support content-based prioritization of memories remain unknown. Using simultaneous eye tracking and intracranial recordings from human patients (n=6), we show content-based modulation of theta oscillations in the hippocampus and amygdala during a scene recognition task. Eye movements were phase-locked to theta oscillations in both structures, with greater phase-locking when viewing people within natural scenes. Robust phase-locking emerged as early the first fixation in both the amygdala ($\bar{r}$ = 0.32, 95% CI = [0.23 - 0.40], p = 0.01) and hippocampus ($\bar{r}$ = 0.34, 95% CI = [0.27 - 0.43], $p < 0.001$), suggesting theta oscillations as mechanism for content-based prioritization of information in memory.

Disclosures: J. Kragel: None. **J.L. Voss:** None.

Presentation Number: NANO54.13

Topic: D.06. Vision

Title: The contribution of semantic knowledge to visual change detection in the ventromedial temporal lobe

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Abstract: Ecologically significant attributes in a natural scene emerge not just from the perceptual information of isolated objects, but also from the semantic associations among objects

within the scene. Here, we examined how the human brain leverages these semantic features of scene elements to optimize visual change detection under different retrieval contexts. First, to quantify semantic features in diverse everyday scenes, Study 1 asked 1379 mTurk participants to label elements in 1026 scene images and provide subjective ratings of semantic relatedness among scene elements, scene complexity, valence, and arousal. Additionally, we calculated semantic associations among scene elements based on GloVe word embeddings, predictive of people's subjective reports of semantic relatedness. Next, Study 2 tested people's visual change detection task performance using these scene images in a typical recognition paradigm, where a test image could repeat a study scene ("repeated") or have an object added or removed from the scene ("changed"). Across 5280 mTurk participants, we found that people consistently detect changes in a certain scene depending on whether an object is added or removed. Participants had a higher likelihood of change detection when an object with high semantic relatedness was removed. However, they often failed to detect a change when an object with high semantic relatedness was added. Low-level features, scene complexity, valence, and arousal did not explain these observations. Finally, Study 3 investigated the neural underpinnings of this semantic contribution to visual change detection during memory retrieval in 16 participants who performed a similar task with intracranial recordings. We found that the deviation of ventromedial temporal lobe (vMTL) activity between encoding and retrieval of the scene image predicted accurate visual change detection. Furthermore, the magnitude of neural correlates to change detection in the vMTL mediated the effect of retrieval context (added vs. removed) and semantic relatedness on the overall change detection likelihood. Together, our findings indicate that visual change detection depends on the integration of ongoing sensory experiences and an observer's semantic knowledge, modulated by retrieval context in the vMTL.

Disclosures: M. Lichtenfeld: None. **W. Xie:** None. **S. Inati:** None. **C.I. Baker:** None. **K.A. Zaghloul:** None.

Presentation Number: NANO54.14

Topic: D.06. Vision

Support: NIH Grant R01EY026025
NIH Grant R01EY16187
NIH Grant R01EY25670
NIH Grant P30EY12196
Stuart H. Q. & Victoria Quan Fellowship

Title: Activity during natural vision in the inferior temporal cortex and potential non-visual codes

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Abstract: Animals use vision in complex, natural environments, and visual neurons may consequently encode information beyond pure visual (i.e., retinotopic) features. For example, mouse (but not macaque) V1 prominently encodes spontaneous movements. Meanwhile, the primate medial temporal lobe (MTL) harbors cells that jointly encode vision and space, such as

hippocampal spatial view cells and entorhinal gaze-based grid cells. We thus asked how neurons in the macaque inferior temporal cortex (IT) situated between V1 and MTL might encode information beyond pure visual features. We analyzed an extensive, open-access dataset where we let 13 macaques freely view natural images and recorded extracellular activities primarily in central and anterior IT. Beyond showing IT responses as predominantly retinotopic and feature-selective, we tested for specific non-purely-visual codes. First, we could decode the saccade directions from IT population activity above chance (mean peak performance of 55% for balanced binary decoding). However, decoding was even better using visual features encoded by a deep artificial neural network (DNN), suggesting that the decoding reflects behavioral biases as a function of the current view. Second, we developed a generalist computational model to detect potentially nonlinear, dynamic neural codes using a DNN sequence-to-sequence model (a Transformer). The unique contribution of each independent variable was assessed by cyclically permuting that variable to measure model performance changes (cross-validated across trials), thereby controlling for other modeling choices. To validate the model, we simulated specific neural codes, including ones nonlinear with regard to the input encoding (e.g., a gain field-like code is nonlinear when the gaze location and visual features are separate inputs). As expected, the DNN model outperformed a linear one (Ridge regression) in predicting simulated nonlinear codes. The DNN model further identified the specific variables underlying each simulated response type (retinotopic feature-selective, gain field-like, real-position, and gaze-based grid cells). In the IT data, the model identified visual features and time-since-image-onset as the top contributors, consistent with IT neurons as visual feature detectors evincing adaptation. Ongoing and future work can analyze the retinotopic feature selectivity in free viewing responses with movies. The generalist model can potentially help identify novel neural codes involving unobserved and even unobservable variables (e.g., internal states) by leveraging multi-area recordings to infer information reliably shared across the brain.

Disclosures: W. Xiao: None. **S. Sharma:** None. **G. Kreiman:** None. **M.S. Livingstone:** None.

Nanosymposium

NANO55: Neural Bases of Human Social Cognition and Connection

Location: MCP Room N227

Time: Wednesday, October 9, 2024, 8:00 AM - 11:00 AM

Presentation Number: NANO55.01

Topic: H.06. Social Cognition

Support: National Institute of Health, USA, Grant R01MH122611
National Institute of Health, USA, Grant R01MH123069

Title: Social interactions are tracked by hippocampal theta oscillations

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Abstract: Navigating social situations is an inseparable part of our lives that hinges on maintaining a coherent relationship narrative from temporally separated social interactions (Schafer and Schiller, 2018). These interactions can be represented as trajectories on an abstract map of two social dimensions - power and affiliation (Tavares et al., 2015). Neuroimaging evidence suggests that the hippocampus tracks these trajectories (Schafer et al., 2023), but its electrophysiological correlates, relevant for understanding social navigation in disorders affecting social abilities and to relate these insights to well-established insights from spatial navigation, are not known.
Using a social interaction game (Tavares et al., 2015), we studied social navigation in epilepsy patients via stereo-EEG (n=5; mean age 50; 1 M). The game followed a fictional storyline and required participants to make decisions affecting their relationship with five characters. Each decision changed either how affiliated the participants were with a character or how much power the characters held relative to the participants. Effectively, the decisions moved characters from a neutral point to a position of higher/lower affiliation/power relative to the self.
Via GLM, we examined task-relevant changes in local field potentials' spectral power right before the social decision was made by relating task behavior to hippocampal theta activity from 1 to 0.5 seconds before the decision, compared between and across characters and decision types within subjects.
We found that theta power modulation corresponded to decision type as well as to character identity. Further, the location of a character on the abstract map, relative to the self, was tracked by hippocampal theta power.
These findings identify electrophysiological correlates of social navigation in the human hippocampus that are similar to the correlates of spatial navigation and provide a candidate signal for studying disorders affecting social abilities.

Disclosures: P. Jain: None. **S.E. Qasim:** None. **I. Saez:** None. **D. Schiller:** None.

Presentation Number: NANO55.02

Topic: H.06. Social Cognition

Title: Time spent in conversation over meals predicts default network function: Evidence from a passive mobile-sensing and fMRI study

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Abstract: Sharing meals with others, often referred to as ‘commensality,’ fosters social connection and may have been essential to human brain evolution. Yet, how shared meals influence the brain remains unknown. Shared meals may impact social brain mechanisms through the conversations they afford. If so, then time spent in conversation over meals should affect the default network- a set of interconnected cortical regions reliably associated with the social exchange of ideas. To test this possibility, we capitalized on a dataset that combines passive-mobile sensing with neuroimaging. Undergraduate participants had an application on their phone for two months that unobtrusively captured their time spent in conversation as well as their location, on and around campus. In between these two months, participants completed a resting state scan. Time spent in conversations around meals (e.g., at cafeterias and restaurants) during the prior month predicted greater functional connectivity in the dorsomedial default

network subsystem, specifically between the left inferior frontal gyrus (LIFG) and the rest of the subsystem regions. This relationship was preferential to: (1) time spent in conversation over the past (vs. future) month, (2) connectivity with the LIFG (vs. other dorsomedial subsystem nodes), (3) the dorsomedial subsystem (vs. other default network subsystems), (4) eateries (vs. other locations), and (5) time spent *around conversation* at eateries, rather than time at eateries more generally. Exploratory analyses further revealed results were driven by a ventral portion of the LIFG, with peaks in voxel-connectivity associated with language, social, and affective processes, as determined by the meta-analytic platform, NeuroSynth. Time spent in conversation over meals may exercise social cognitive processes supported by the dorsomedial subsystem, a network key to social communication, understanding, and connection.

Disclosures: D.M. Bhatt: None. **M.L. Meyer:** None.

Presentation Number: NANO55.03

Topic: H.06. Social Cognition

Support: NIMH Grant R01MH129648

Title: Seeing is not being: Actively participating in an interaction changes social perception relative to passive viewing

Authors: *Q. LIANG, R. VARRIER, T. BENSON, Z. SU, E. JOLLY, E. S. FINN;
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Abstract: Humans are social beings, excelling at understanding social information and engaging in social interactions. Recent work has proposed that there is a third visual pathway in the human brain specialized for social perception, suggesting that the human brain is sensitive to social information as early as primary sensory processing. However, most evidence supporting this pathway is based on passive-viewing paradigms, overlooking possible differences in this perception process between people as external observers versus active participants in the interaction. In this study, we created simple visual scenes where one agent (represented as a dot) might be chasing another dot and set up two conditions - passive-viewing and interactive conditions - to address this possible difference. In both conditions, to quantify participants' sensitivity to social information, we parametrically manipulated the directness of the chase, i.e. the degree to which the chasing dot heads directly towards the chased dot (more direct chase) versus deviates from this perfect path (more subtle chase). In the passive-viewing trials, participants watched a 6s animation of two moving dots and rated how much they thought one dot was chasing the other versus moving independently. In the interactive trials, participants controlled one of the dots with their mouse, explored the scene for 6s, and then rated how much they thought the other dot was chasing the dot they controlled. Behavioral results showed that, in both conditions, participants were more likely to rate the scenes as more social when the chase was more direct. However, compared to the passive-viewing condition, in the interactive condition, participants perceived high-directness scenes as even more social and, conversely, low-directness scenes as more non-social, suggesting that actively engaging in social interactions might boost the sensitivity to social information and confidence in social decisions. Moreover, we found that participants stayed closer to the chasing dot when the chase was less direct in the interactive condition, which might reflect an information-gathering strategy that enhanced their

perception. We further investigated this process using fMRI, and found that a region in the lateral occipitotemporal cortex (LOTC) hypothesized to be part of the third visual pathway tracked the subjective socialness ratings, underscoring its role in representing the perception of social interactions. In summary, our findings highlight that participating in an interaction might enhance the sensitivity of social perception compared to passively watching it and that LOTC might be the key region supporting this process.

Disclosures: Q. Liang: None. **R. Varrier:** None. **T. Benson:** None. **Z. Su:** None. **E. Jolly:** None. **E.S. Finn:** None.

Presentation Number: NANO55.04

Topic: H.06. Social Cognition

Support: National Institute of Mental Health, U.S.A. (R01-MH112357)
National Institute of Mental Health, U.S.A. (DP1-HD091948)
Ministry of Education (MOE) in Taiwan (Higher Education Sprout Project)
National Science and Technology Council, Taiwan (113-2410-H-038-002-)

Title: How a speaker herds the audience: multi-brain neural convergence over time during naturalistic storytelling

Authors: *C. CHANG[1], S. A. NASTASE[2], A. ZADBOOD[3], U. HASSON[4];
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Abstract: Storytelling—an ancient way for humans to share individual experiences with others—has been found to induce neural synchronization among listeners. In our exploration of the dynamic fluctuations in listener-listener (LL) coupling throughout stories, we uncover a significant correlation between LL coupling and lagged speaker-listener (lag-SL) coupling over time. Using the analogy of neural pattern (dis)similarity as distances between participants, we term this phenomenon the "herding effect": like a shepherd guiding a group of sheep, the more closely listeners mirror the speaker's preceding brain activity patterns (higher lag-SL similarity), the more tightly they cluster together (higher LL similarity). This herding effect is particularly pronounced in brain regions where neural synchronization among listeners tracks with moment-by-moment behavioral ratings of narrative content engagement. By integrating LL and SL neural coupling, this study reveals a dynamic, multi-brain functional network between the speaker and the audience, with the unfolding narrative content playing a mediating role in network configuration.

Disclosures: C. Chang: None. **S.A. Nastase:** None. **A. Zadbood:** None. **U. Hasson:** None.

Presentation Number: NANO55.05

Topic: H.06. Social Cognition

Support: NSF GRFP
K99MH120257
R00MH120257

Title: Real-world social inputs trigger shifts in neural activity patterns and reinterpretations of ambiguous stimuli

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Abstract: Multivariate neural representations track subjective percepts of information. While work has explored these processes in basic perceptual tasks, the dynamics of social contexts—marked by the influence of external opinions (e.g., a friend's viewpoint)—remain less understood. Here we tested how exposure to others' interpretations can induce meaningful neural shifts that predict reinterpretation (change in subjective percept). To this goal, subjects (n=40) were scanned (fMRI) as they generated interpretations for ambiguous stimuli and then processed interpretations from others. These were sourced in real-time based on the subject's own inputs, effectively mimicking real-world social interactions; natural language processing ensured a balanced trial distribution, exposing subjects to others' interpretations that spanned a semantic range from very similar to very dissimilar from their own. Specifically, in our paradigm, subjects first interpreted a stimulus. Then, on experimental trials only, they read another possible interpretation and rated the likelihood of either just their own (for control trials) or both their own and the other's interpretation (for experimental trials; Fig. 1a). Ratings were contrasted defining reinterpretation. Neural shifts were quantified via cosine distances between multivoxel patterns in cortical nodes, before and after exposure to the other's interpretation (or not, in control trials; Fig. 1b). Results show that, compared to control trials, exposure to another's interpretation, regardless of similarity to one's own or whether it prompted reinterpretation, evoked widespread neural shifts across the brain. On experimental trials, greater neural shifts, especially in the frontoparietal and default mode networks, predicted a behavioral shift toward the other person's interpretation, particularly when presented with more diverging interpretations. This highlights the impact of social information on neural responses, showing that integrating others' interpretations can drive measurable changes in how stimuli are processed.

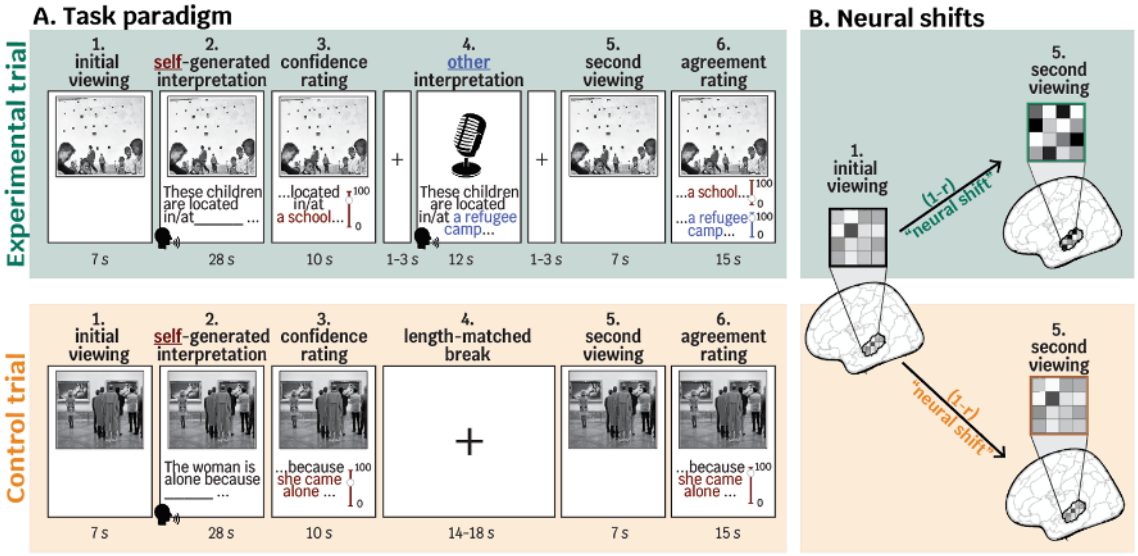

Figure 1.
A. *Task Paradigm.* Subjects are presented with a different image on each trial. Differences between experimental and control trials are displayed. All interpretations are generated by filling in the blanks of a "MadLibs"-style passage with three blanks. Alternative interpretations follow the same format and are sourced from other subjects. Data collection was conducted in an iterative design.
B. *Computing neural shifts.* Neural shifts were computed as the cosine distance between multivariate patterns when looking at the same stimulus across phases (within trial; phase 1 to phase 5). In control trials, this is a repetitive viewing. In experimental trials, this is after being exposed to an alternative interpretation.

Disclosures: C. Sava-Segal: None. **T. Benson:** None. **E.S. Finn:** None.

Presentation Number: NANO55.06

Topic: H.06. Social Cognition

Support: ISF 103/22 (V.K.D.)
NYU Grossman School of Medicine (P.G.)
Stern School of Business (A.B.)
NYU Shanghai (A.B.)
J.P. Valles (A.B.)

Title: A Game Theoretic Foundation for the Psychophysical and Neurometric Study of Social Interactions

Authors: *V. KURTZ DAVID[1], A. BRANDENBURGER[2], P. W. GLIMCHER[1];
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Abstract: Finite cognitive capacity limits human reasoning when engaging in social interactions. However, how do particular cognitive resources, across multiple cognitive domains, limit social interactions? Here, we investigate how adult humans reason in social strategic interactions when forced to integrate across cognitive domains associated with distinct cortical networks. Combining cognitive neuroscience with paradigms from game theory we decomposed each

interaction into separable social and non-social cognitive demands. We use this separation to develop a new psychophysical model that rests on economic definitions of production functions. Briefly, we posit that when one increases the depth of the social analysis, that individual must incur a cognitive cost and our approach enables us to measure how that cost is distributed amongst multiple cognitive or neural domains. To accomplish this, we allow individuals to have distinct innate capacities in each of these domains, and for these capacities to trade-off against each other within an individual. We utilized the well-studied economic Ring Game (Kneeland, 2015) to directly measure how individuals manage varying demands across the social and non-social cognitive dimensions. In the Ring Game, each subject's payoff depends on their own choices and the choices of the next player in the ring, thus creating a social dependency between players in the game. The number of players in each ring determines the social burden of the task, while the structure of choice alternatives imposes arithmetic non-social demands. In two studies employing this approach we show that it provides new traction on human behavior in social settings. In the first, we present subjects with an array of social interactions, which vary stepwise in their social and non-social cognitive demands. In the second, we manipulate the cognitive resources available to subjects by varying processing times. Both social and non-social demands are revealed as fundamental contextual factors for the depth of the social analysis, and behave lawfully with psychophysical regularity. Subjects trade-off these capacities in a constrained manner - with a great heterogeneity across individuals - as task demands vary. Our results extend classical psychophysics and theories of individual decision-making into the domain of social reasoning. We are currently applying this framework to a recently collected neuroimaging dataset with the prospects of a network-level mapping of subjects' utilization of social and non-social cortical resources. Our framework could be of use to a suite of computational psychiatric inquiries of disorders of social cognition such as Autism

Disclosures: V. Kurtz David: None. **A. Brandenburger:** None. **P.W. Glimcher:** None.

Presentation Number: NANO55.07

Topic: H.06. Social Cognition

Support: NIH Grant F31MH123123
NIH Grant R01MH122611
NIH Grant R01MH123069

Title: The Social Navigation Task as a window into real-world social function

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Abstract: We navigate social relationships daily, making decisions that change our affiliation and power relations with others. Over the course of a relationship, these changes trace out a trajectory in abstract social space. Here, we use a narrative-based choose-your-own-adventure game, the Social Navigation Task, to study social trajectories in simulated relationships and how this relates to real-world social dynamics. In two independent fMRI samples, individual relationships were tracked with sequences of hippocampal states, such that each relationship's history was captured by its own neural trajectory. The number of unique hippocampal states was related to the structure of real-world social networks, with fewer unique states corresponding to

simpler social networks. We then studied the same behavioral geometry in two large online samples. We found that abstract social distance related both to self-reported social avoidance and the same measures of social network structure that tracked hippocampal activity, suggesting task-based social navigation relates to real-world social function. Moreover, participants' impressions of the characters also tracked the task dynamics: language analysis with a large language model found that semantic structure reflected the affiliation and power relationship dynamics, with more negative sentiment about the relationships related to greater social avoidance. These results demonstrate that the Social Navigation Task offers a unique window into social behavior, connecting task-based measures of social decision-making and brain activity to real-world social function.

Disclosures: M. Schafer: None. **D. Schiller:** None.

Presentation Number: NANO55.08

Topic: H.06. Social Cognition

Support: NIMH R01MH114904
NIMH R21MH127284

Title: Neural alignment during real-time conversation among friends and strangers

Authors: *L. MWILAMBWE-TSHILOBO[1], L. TSOI[2], S. SPEER[1], S. BURNS[3], E. FALK[4], D. TAMIR[1];
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Abstract: Introduction: Conversations are central to human social connection. Although conversations with a close friend may differ from those with a stranger, both can foster closeness. When people feel close to one another or share experiences, the neural activity of their brains becomes more aligned. What occurs during a conversation that leads to a successful social interaction, and does neural alignment differ based on the social relationship context of the conversation partners? Here, we examine neural dynamics between dyads of friends and strangers during real-time conversation and explore how conversation relates to later feelings of social connection. **Methods:** We analyzed fMRI hyperscanning data from dyads (N=30 self-identified friends; N=27 strangers) engaged in a real-time conversation. The conversation task consisted of two conditions: a natural, free-flowing, and a pre-scripted condition. During the free-flowing conversation, dyads were given specific prompts as conversation starters and took turns speaking to each other. For the scripted conversation, the partners in a dyad were given the script from a conversation of another dyad to read out loud (control condition). To examine how conversation aligns the brain across all dyads, we measured whole-brain intersubject correlation (ISC) between dyads in each condition. **Results:** Across all dyads, naturalistic conversation led to greater alignment in brain regions associated with social cognition (e.g., temporoparietal junction, inferior parietal lobule, bilateral dorsal medial prefrontal cortex) and language (inferior and middle frontal gyrus), compared to scripted conversation. Neural alignment in these regions was similar for friend and stranger dyads. Next, we examined how socially connected partners in each dyad felt towards each other after the conversation using self-reported responses rating the enjoyment of the conversation and similarity and closeness to their partner. Friends enjoyed their

conversation more and felt similar and closer to their partner than strangers. **Conclusion:** Here, we explore interpersonal neural alignment in friends and strangers during real-time conversation. Generally, conversation resulted in greater interpersonal brain alignment. Our results suggest that while friends and strangers shared a common alignment pattern during conversation, they differed in how socially connected they felt afterward. Future work will assess differences in the relationship between neural alignment and social connection outcomes between friends and strangers.

Disclosures: L. Mwilambwe-Tshilobo: None. **L. Tsoi:** None. **S. Speer:** None. **S. Burns:** None. **E. Falk:** None. **D. Tamir:** None.

Presentation Number: NANO55.09

Topic: H.06. Social Cognition

Support: R21MH127284

Title: Finding Agreement: fMRI-hyperscanning reveals that dyads diverge in mental state space to align opinions

Authors: *S. P. H. SPEER[1], H. SENED[2], L. MWILAMBWE-TSHILOBO[2], L. TSOI[3], S. BURNS[4], E. FALK[5], D. P. TAMIR[2];
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Abstract: Many prize synchrony as the ingredient that turns a conversation from a debate into a delightful duet. Yet, learning from other people's diverging opinions can also foster understanding and facilitate collaboration and progress, suggesting that the process matters. Agreement depends on the ability to anticipate others' intentions and emotions. By scanning two people simultaneously engaging in joint decision-making, we captured the emergent and bidirectional nature of these interactions. We tested how two debaters explore each other's minds, and how that exploration aligns opinions.In two 10-minute conversations, dyads (N = 60) discussed how to best allocate money to five solutions to two controversial public health problems while being scanned. Both partners were instructed to either *persuade* their partner or *compromise* with each other. Partners individually allocated money before and after the conversation, enabling us to track how the conversation helped them find agreement. Results showed that conversation improved agreement: participants agreed more after the conversations. Instructions to compromise amplified this effect: Participants in the compromise condition agreed significantly more.Next, we tested how conversation aligns opinions. We hypothesized that flexibility and novelty are key to agreement. That is, dyads may diverge in order to align. We tested this hypothesis by tracking exploration of mental state space throughout the conversation. We used a model that decodes mental states from whole-brain patterns, allowing us to decode each person's 'location' in mental state space at each moment. Exploration was computed as the distance between the two speakers at each moment of time across the whole conversation, where increasing distance represents a higher dyadic exploration.Exploration of mental states reliably predicted changes in opinion and agreement. Individuals who explored a larger mental state space agreed more relative to less exploratory participants. Instructions to compromise amplified exploration and ultimate agreement relative to instructions to persuade.

Together, these results suggest that trying to find agreement may require more exploration, something that happens naturally when people are motivated to compromise but not persuade.

Disclosures: S.P.H. Speer: None. **H. Sened:** None. **L. Mwilambwe-Tshilobo:** None. **L. Tsoi:** None. **S. Burns:** None. **E. Falk:** None. **D.P. Tamir:** None.

Presentation Number: NANO55.10

Topic: H.06. Social Cognition

Support: 1R01AT010627

Title: Insights into drug addiction dynamics: a Natural Language Processing analysis of movie recall in people with Heroin Use Disorder

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Abstract: Previous studies have shown that drug addiction is associated with enhanced salience attribution to an individual's substance of choice, including substance-related cues, along with a decline in attention to other (non-drug-related) stimuli, in the natural environment. Naturally generated speech can provide unique insights into a person's current psychological state, such as following exposure to salient drug stimuli. In this research we utilized advanced Natural Language Processing (NLP) tools to extract psychological features from natural speech and to use them to distinguish between affected and healthy individuals. 59 participants with heroin use disorder (HUD) in inpatient medication-assisted treatment and 32 healthy control participants (HC) viewed the first 17 minutes from the movie *Trainspotting*, heavily featuring heroin use and addiction, then verbally recalled the events and their subjective experience of the movie. In all subjects procedures were repeated at a second session, 15 weeks later (while the HUD group was still in treatment). The verbal recordings were transcribed and analyzed via the BERTopic algorithm, which automatically extracts topics from the transcripts and determines the probability of each topic within a specific transcript. Out of the 32 extracted topics, 6 showed significant ($p<.05$) group differences in prevalence (HUD vs. HC, across both sessions). Specifically, compared to HC, HUD participants were more likely to talk about drug usage in the movie ($\chi^2=7.38$) and their personal experience with drugs ($\chi^2=8.21$). HC participants, conversely, were more likely to speak about other, non-drug-related scenes from the movie ("the dog", "the robbery", the character "Spud", all $\chi^2 \geq 5.21$), as well as their philosophical view of the movie ($\chi^2=7.15$). Next, for each individual/session, we calculated the expected probability of using each topic (32 in total), analyzed via a 2 (group) X 2 (session) ANOVA. Two topics showed both a significant (all $p<.05$) group main effect and an interaction between group and session. One topic related to drug usage (Main effect: $F(1,57)=3.99$; Interaction: $F(1,57)=4.30$) and the other to one of the characters in the movie (Main effect: $F(1,57)=6.35$; Interaction: $F(1,57)=4.14$). In both topics, the interaction was explained by session changes in the HUD group who became more similar to the HC group in the second session. Taken together, the observed convergence of speech patterns between the HUD and HC groups over time implies potential changes in

cognitive processing and salience attribution with treatment. Overall, these NLP results provide a uniquely rich perspective on core symptoms of drug addiction.

Disclosures: S. Pais: None. **R. Tikochinski:** None. **S.G. King:** None. **G. Kronberg:** None. **N. McClain:** None. **Z. Zhang:** None. **N. Alia-Klein:** None. **R. Reichart:** None. **R.Z. Goldstein:** None.

Presentation Number: NANO55.11

Topic: H.06. Social Cognition

Support: NIH Grant MH109329

Title: Multivariate Pattern Analysis of Amygdala Predicts Psychopathy in Incarcerated Females During an Empathy Task

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Abstract: The amygdala plays a crucial role in most clinical neuroscience models of psychopathy, as it is associated with emotion recognition and regulation. However, many neuroimaging studies, especially the ones with high statistical power, found a null relationship between the amygdala and psychopathy. This challenges the theoretical framework. This lack of consensus may be due to the limitation of focusing on the overall activation of the amygdala rather than investigating the pattern of activity within amygdala. Therefore, voxel-based analysis is needed to investigate the activity pattern within the amygdala and identify potential clusters of neighboring voxels that can predict psychopathy. The current study collected fMRI data from 36 incarcerated women from a North American correction institution with an empathy for pain task. A three-level whole-brain univariate analysis was first conducted to examine the brain activity across groups (high-psychopathy group and control group) and different conditions of empathy for pain task (self-pain, self-no-pain, other-pain, other-no-pain). No significant differences were found in the amygdala, as hypothesized. However, significant outcomes were found in the voxel-based multivariate pattern analysis (MVPA). Specifically, results demonstrate classification accuracy of around 60% for the binary classification between the high-psychopathy and the control groups across all four empathy conditions by leveraging both traditional models (decision tree, random forest, logistic regression) and deep-learning models. The study demonstrates the added value of conducting multivariate pattern analysis within amygdala compared to common univariate analysis.

Disclosures: H. Ma: None. **M.S. Cohen:** None. **J. Li:** None. **K.A. Kiehl:** None. **J. Decety:** None.

Presentation Number: NANO55.12

Topic: H.06. Social Cognition

Support: STI2030-Major Projects (2022ZD0205104 to L.Z.)
NSFC (32071095 to L.Z.)

Title: Interpersonal neural alignment reveals a computational shortcut to large-scale coordination

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Abstract: Human coordination is unparalleled in its scale and flexibility. We are able to act complementarily for common goals, sometimes with millions of unrelated individuals, across diverse social contexts. Such ability is crucial for complex societies, yet its neural and cognitive bases remain largely unknown. Building on a longstanding hypothesis that coordination is facilitated by a 'tacit agreement' about the external world—an implicit, broadly shared consensus on beliefs, norms, and practices—we provide both neural and behavioral evidence for a computational shortcut to large-scale coordination. Using a well-established measure of interpersonal neural alignment previously employed to study correlated brain activity in socially interacting animals, we approximate public consensus by intersubject similarity in stimulus representation. We expect that if public consensus supports large-scale coordination, intersubject neural alignment in one group should predict coordination in another group, and if it serves as a shortcut for bypassing complex reasoning, scenarios with strong consensus should yield faster coordination than those lacking consensus and relying on reasoning. A total of 542 subjects played a classic Pure Coordination Game online, where they needed to select one out of 4 landscape images to match the choices of other players. Concurrently, a separate group of 34 underwent fMRI scans, passively viewing these images without making decisions. The strength of neural alignment when passively viewing each image, measured by intersubject pattern correlation (ISC), was used to predict online coordination choices, eliminating possible inherent correlations arising from directly assessing ISC during social interactions. The analysis identified a set of brain areas, especially the posterior cingulate cortex (PCC), where stronger ISC during passive viewing predicted higher coordination choice probability. This prediction was specific to coordination, absent when subjects made personal choices. Moreover, by manipulating decision scenarios based on PCC alignment in the fMRI sample, we could control coordination choices in an entirely new group, indicating a direct link between interbrain alignment and large-scale coordination. Computational modeling further revealed faster coordination in scenarios with stronger PCC alignment than those relying on reasoning, supporting the idea of public consensus providing a decision shortcut. The data suggest that large-scale coordination may be supported by a shared understanding of the world commonly coded in the PCC, which can be flexibly assembled and compared in service of social behavior.

Disclosures: C. Wang: None. **H. Wang:** None. **Y. Bi:** None. **L. Zhu:** None.

Nanosymposium

NANO56: Human LTM: Encoding and Retrieval III

Location: MCP Room S103

Time: Wednesday, October 9, 2024, 8:00 AM - 11:15 AM

Presentation Number: NANO56.01

Topic: H.07. Long-Term Memory

Title: On a roll: Successful retrieval primes the brain to retrieve other memories via dopaminergic responses

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Abstract: What ongoing cognitive and neural states prepare us to recall? Research in human memory has illuminated factors that set the stage for successful episodic memory formation, however, little work has investigated why our ability to retrieve varies from one moment to the next. Animal models suggest that slowly changing concentrations of modulatory neurotransmitters, including acetylcholine, dopamine, and norepinephrine, may shape retrieval. In line with this possibility, our group previously found that novelty-a form of stimulus salience associated with neuromodulator release-decreased associative retrieval ability for multiple seconds (Patil & Duncan 2018). While dopamine and norepinephrine have been shown to enhance retrieval in response to novelty (Giovannini et al. 2001), acetylcholine is the only novelty-evoked neuromodulator theorized to suppress retrieval by inhibiting pattern completion (Hasselmo et al. 1995). In this study, we sought to uncover the neural mechanisms driving novelty's negative impact on retrieval, hypothesizing a critical role of novelty-evoked cholinergic nuclei activity in decreasing subsequent retrieval ability. We used functional magnetic resonance imaging (fMRI, n=29) to test whether activation of key modulatory nuclei mediates novelty's suppression of pattern completion, indexed with encoding-retrieval similarity (ERS). In line with previous research, participants recalled fewer places and faces associated with words when novel objects preceded these word cues ($t=-2.31$, $p<0.05$). Preceding novel objects also reduced ERS across the ventral stream, consistent with suppressed pattern completion (all $t>2.16$, $p<0.05$). However, contrary to our hypothesis, neuromodulatory centers did not signal novelty in this task. Surprisingly, we found that both dopaminergic and cholinergic nuclei responded more to familiar objects, and the response in dopaminergic regions uniquely mediated the effect of novelty on subsequent ERS in the medial temporal lobe (MTL) cortex (indirect effect (ab)=0.0004, $p<0.05$). These findings reflect the motivational significance familiar stimuli may carry in memory tests. Identifying how familiarity-induced dopaminergic nuclei activity prepares the brain to reinstate memories opens new avenues for memory modulation.

Disclosures: M. Dougherty: None. **A. Patil:** None. **K.D. Duncan:** None.

Presentation Number: NANO56.02

Topic: H.07. Long-Term Memory

Support: NSERC
CIHR
Brain Canada Foundation Grant
Vanier Canada Graduate Scholarship

Title: Category learning builds behaviourally relevant attentional templates

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Abstract: Attention determines the success of concept learning while learned concepts modulates attention. The focus of the most prominent theories of concept learning has been the involvement of attention during learning. Attention filters the relevant information and alters the feature dimensions in psychological space to create or update concepts. Yet, it remains unclear how concepts stored in memory guide attention. We capture this phenomenon in attentional templates that emerge from learning, storing goal-relevant representations and aiding attention allocation. Healthy young adults learned to categorize complex visual items - cartoon bears - in two separate learning tasks. Participants then completed a test block wherein each trial began with a cue, indicating which learning task should be used to categorize the upcoming visual item. Half of the time, a probe trial followed the cue, where instead of the features of the visual items, a small arrow appeared at a feature location that was relevant (i.e., valid probe location) or irrelevant (i.e., invalid) for the cued category task. The speed of participant responses to valid versus invalid probe locations was leveraged to assess attentional templates guided by learned concepts. Successful learners were faster at responding to valid probe locations than invalid, demonstrating the deployment of attentional templates that emerged from the concept of the cued learning task. This response time benefit was associated with the complexity of learning; the benefit was reduced for cued tasks with more diagnostic features consistent with a template that divides attention resources among multiple potential locations. Importantly, the deployment of attentional templates was linked to learning performance such that higher learning accuracy yielded larger response time benefit. These results provide empirical evidence for the emergence of attentional templates from concept learning. Concepts stored in memory guide attention and this phenomenon is influenced by the dynamic learning process, top-down signals, and task-demand. This paves the way for understanding the neural signatures of the dynamic link between attention and learning.

Disclosures: M. Gumus: None. **Z.Y. Lee:** None. **M.L. Mack:** None.

Presentation Number: NANO56.03

Topic: H.07. Long-Term Memory

Support: NIH Grant R01EY034436-01

Title: Investigating timescale changes in the brain during repeated film viewing

Authors: *N. AL-ZAHLI[1], M. ALY[2], C. BALDASSANO[1];
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Abstract: The brain processes our continuous experiences by segmenting them into discrete events. Lower-order brain regions tend to segment experiences at finer timescales, whereas higher-order regions do so at coarser timescales. Many of our experiences, however, are not entirely unique and may be repeated: we can watch the same movie more than once or listen to the same song on repeat. How does the brain modify its event hierarchy when experiences are repeated? Here, we explore whether the brain's event representations become more granular, less granular, or remain stable with event repetition. The "fine-tuning" hypothesis suggests that brain regions become more sensitive to finer details and lower-level features with repeated exposure to a stimulus, leading to smaller, more frequent events. Conversely, the "chunking" hypothesis

suggests that brain regions may integrate events into broader, more generalized representations with repeated exposure. To test these accounts, we analyzed data from 30 human participants who underwent functional magnetic resonance imaging (fMRI) while watching three 90-second clips from “The Grand Budapest Hotel” six times each. Using hidden Markov models within searchlights across the entire brain, we identified event timescales for each viewing. We then tested for significant changes in the timescales of event processing across multiple viewings. Most brain regions exhibited stability in their preferred timescale across repeated viewings, indicating fixed event representations for both novel and familiar events. However, a smaller set of regions showed flexible event representations that became more or less granular with clip repetitions, supporting both the “fine-tuning” and “chunking” hypotheses in different brain regions. Notably, a region in the right temporal lobe consistently detected more events with repeated viewings across different movie clips, indicating a tendency to fine-tune its representations with experience regardless of stimulus content. These results enhance our understanding of the stability vs. flexibility of event segmentation in the brain, showing that stability, fine-tuning, and chunking all occur across different regions with repeated stimulus exposure. Rather than treating timescales in the brain as inherent or fixed, our findings highlight the importance of treating them as flexible with experience.

Disclosures: N. Al-Zahli: None. **M. Aly:** None. **C. Baldassano:** None.

Presentation Number: NANO56.04

Topic: H.07. Long-Term Memory

Support: NIH R01MH133732

Title: The relationship between event boundary strength and pattern shifts across the cortical hierarchy during naturalistic movie-viewing

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Abstract: The human brain effortlessly chunks continuous, information-rich experience into meaningful units (events), which have been revealed to crucial to successful episodic memory encoding (Baldassano et al., 2017; Ezzyat & Davachi, 2010). Phenomenologically, however, the moments where one event is perceived to end and a new one begins are not always sharply identifiable. In other words, it is not obvious how to reconcile this phenomenological uncertainty with the notion that events are discrete. Are cortical responses to event boundaries modulated by perceived strength? Alternatively, do cortical responses discriminate between only two states, i.e., a boundary state and a no-boundary state? To answer this, we used fMRI to investigate changes in multi-voxel patterns at event boundaries with three strengths (weak, moderate, and strong) during naturalistic movie-viewing (N = 17; “Filmfest” dataset; Lee & Chen, 2022). Event boundary strength was determined by agreement across an independent group of human observers, following the methods in a prior study (Ben-Yakov & Henson, 2018). We investigated brain responses in regions along the cortical hierarchy of information processing hierarchy that was defined by temporal receptive windows (Hasson et al., 2008). We found that the magnitude of multi-voxel pattern shifts was clearly scaled with boundary strength in auditory processing areas with short timescales ($F(2, 32) = 62.35$, $p < .001$). We observed the significant differences

between every pair of event boundary strength categories in auditory processing areas ($p = .006$ for weak $p < .001$ for weak vs. strong; $p < .001$ for moderate vs. strong; Bonferroni corrected). However, cortical response to event boundaries were weakly modulated by boundary strength in high-level cortical areas, such as in the default mode network with long timescales ($F(2, 32) = 5.10$, $p < .05$). The effect was primarily driven by the difference between moderate and strong event boundaries ($p = .010$; Bonferroni corrected). Taken together, the results suggest that there is a gradient in brain responses to event boundary strength along the cortical hierarchy such that graded response profile is manifest in low-level cortical areas with short timescales.

Disclosures: Y. Lee: None. **J. Chen:** None.

Presentation Number: NANO56.05

Topic: H.07. Long-Term Memory

Support: Office of Naval Research N00014-17-1
NIH R01AG062438
McDonell Center for Systems Neuroscience

Title: Meaningful moments during film viewing are represented and remembered similarly across participants

Authors: *A. UPADHYAYULA[1], J. M. ZACKS[1], J. M. HENDERSON[2,3], Z. M. REAGH[1]; [1]Psychological & Brain Sci., Washington Univ. in St. Louis, St. Louis, MO; [2]Dept. of Psychology, [3]Ctr. for Mind and Brain, Univ. of California, Davis, CA

Abstract: How do people represent and remember complex experiences? Recent work on event cognition suggests that comprehenders parse activity into meaningful units and knit those units into coherent structures. An intriguing possibility is that event units are represented as a scaffold of key moments that distill the essentials of an activity stream. Not much is known regarding how events are represented. Here, we introduce a novel storyboard paradigm to investigate these representations. Participants watched 50 video clips (approximately 1-2 minutes each) from an episode of a television show (BBC's *Sherlock*, S1E1: *A Study in Pink*). They were then asked to create a storyboard that conveyed the underlying story by selecting individual frames within each clip. A separate group of participants was asked to segment the episode into meaningful events. Participants showed strong agreement about which frames to include in the storyboard. Importantly, these frames were not the same as the event boundaries. To test the hypothesis that storyboard frames can form a scaffold of key moments to represent a sequence, we analyzed an open fMRI dataset containing both the encoding and recall of the same video clips. Within the Posterior Medial Cortex (PMC), the pattern of activity across observers was more strongly aligned at moments that were more likely to be chosen as storyboard frames. In contrast, event boundaries were not significant predictors of cross-observer pattern alignment. Further, during recall of the movie, patterns in the PMC were more strongly related to storyboard moments than to moments not chosen for storyboards. These results suggest that information that is consistent across observers during comprehension and reinstated during recall disproportionately reflects a small number of moments that are chosen as storyboard frames to convey the essentials of the activity.

Disclosures: A. Upadhyayula: None. **J.M. Zacks:** None. **J.M. Henderson:** None. **Z.M. Reagh:** None.

Presentation Number: NANO56.06

Topic: H.07. Long-Term Memory

Support: Spanish Government, MCIN/ AEI /10.13039/501100011033
SEAS Interdisciplinary Research Seed (SIRS) Funding
HFSP Fellowship, LT0037/2024-L

Title: The strength of theta power at event boundaries predicts the long-term retention of event memories

Authors: *M. SILVA[1], X. WU[4], M. SABIO-ALBERT[5], E. CONDE BLANCO[6,7], P. ROLDAN RAMOS[6], M. CARRENO[7,6], N. AXMACHER[8], L. FUENTEMILLA[9], J. JACOBS[2], C. BALDASSANO[3];
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Abstract: Ongoing experience is transformed into discrete memory episodes through the process of event segmentation, in which temporally-extended information is bound together into a coherent event representation. The underlying neural mechanisms of this process remain unknown, but recent work has identified the theta rhythm as a potential mechanism for organizing and maintaining sequential information. Theta power in the human hippocampus has been shown to reliably increase for successful episodic memory encoding, and individual elements within an event may be sequentially activated at different phases of the theta oscillation. Here, we leverage the anatomical precision of intracranial electroencephalographic recordings from epileptic patients (N = 10, 4 female, mean age = 29.1) to assess the presence of hippocampal theta modulations at event boundaries during the encoding of a 45-min movie film (the first episode of BBC's Sherlock) and how this modulation might impact long-term memory for an event. We found increases in hippocampal theta (4-8 Hz) power at event boundaries and that the strength of theta power predicted whether an event would be later remembered (cluster-based permutation test, $p < 0.001$). The presence of these theta modulations around movie boundaries gives support to rodent models ascribing a key role for hippocampal theta power shifts in memory function and enhances our comprehension of the neurophysiological underpinnings through which continuous experience may be organized into discrete long-term memory events.

Disclosures: M. Silva: None. **X. Wu:** None. **M. Sabio-Albert:** None. **E. Conde Blanco:** None. **P. Roldan Ramos:** None. **M. Carreno:** None. **N. Axmacher:** None. **L. Fuentemilla:** None. **J. Jacobs:** None. **C. Baldassano:** None.

Presentation Number: NANO56.07

Topic: H.07. Long-Term Memory

Support: DFG project 437219953 supported S.M.
R01 MH112357 awarded to U.H. and K.A.N.

Title: Tracing the neural underpinnings of memory search across slowly unfurling states

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Abstract: Human episodic memory can span long periods of time, for instance, when we search through memory for our keys. Such long timescale memories entail structure that segments a continuous stream of information into discrete events (Zacks et al. 2007). Previous work suggests that event-structure in the perception and memory of long narratives is characterized by slowly unfolding neural states (Baldassano, Neuron 2017). However, neural underpinnings of episodic memory are often fast-lived and unfold at millisecond timescales (e.g., Roux et al., elife 2022). How are fast memory processes orchestrated at naturalistic timescales? Here we bridge the gap between fast neural processes and long timescales with human electrocorticography: Ten patients watched a movie and answered naturalistic interview questions that prompted memory-search across segments in the movie (asking: “after X, when does Y happen next?”; compare Michelmann et al., 2023, Psychological Science). Based on slow components that captured spatiotemporal patterns, we performed a state-segmentation (Geerligs et al., Neuroimage, 2021) of the neural data during movie-viewing. The boundaries between neural states predicted the shared perception of the movie’s event-structure in a separate norming sample (N=195) and preceded behavioral responses by approximately 1.3 seconds. During the interview, states from corresponding moments in the movie were reinstated on several cortical channels that were localized in areas that overlapped substantially with the default mode network. As patients scanned their memories for an answer, neural states then unfurled in a forward direction making it possible to investigate the neural correlates of state-transitions during memory-scanning: State transitions on cortical channels were marked by a decrease in power-spectral density in low frequencies (3-30 Hz) and were preceded by power decreases in hippocampus in the same frequencies (preceding state-transitions in cortex by 760-510 ms). Connectivity analysis revealed a statistically significant relationship between power decreases in hippocampus and cortex suggesting information-flow from hippocampus to cortex underlying state-transitions.

Disclosures: S. Michelmann: None. **P. Dugan:** None. **W.K. Doyle:** None. **D. Friedman:** None. **L. Melloni:** None. **C.K. Strauss:** None. **S. Devore:** None. **A. Flinker:** None. **O. Devinsky:** None. **U. Hasson:** None. **K. Norman:** None.

Presentation Number: NANO56.08

Topic: H.07. Long-Term Memory

Support: ONR Grant N00014-15-1-0033

Title: Natural language processing captures shared recall interpretations associated with shared neural patterns at encoding and recall

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Abstract: During encoding and recall of naturalistic events people instate shared representations throughout higher order cortical regions of the brain. These shared representations are thought to reflect shared mental models of the encoded and recalled events. We explored whether neural representations are more similar in participants who recall events similarly to each other. Participants were scanned using fMRI as they encoded two 15-minute movies and verbally recalled those movies either immediately or after a 2-day delay. Intersubject pattern similarity (ISPS) was calculated from fMRI data for four default mode subnetworks previously shown to be connected to the hippocampus. Verbal recall was transcribed, segmented into events, and fitted to topic models. This produces a vector of "topic activations" that represents the abstract concepts present in recall for each event. We calculated how similar each participant's recall was compared to the group by correlating the vector of topic activations for each subject, for each event to the group. Further, we manually scored each recall transcript for details using published methods. We found that recall topic similarity is strongly associated with ISPS at encoding in the DMN, suggesting that shared neural representation during encoding lead to more similar recall of events at retrieval. Topic similarity was also associated with ISPS at recall in the posterior medial cortex. This suggests that when participants recall events more similarly after a delay, their pattern of brain activation is also more similar. However, ISPS was not related to the number of details recalled suggesting that shared representational patterns between people are not simply reflective of how well something is remembered, but how similarly it is remembered to others.

Disclosures: J. Kim: None. **C. Ranganath:** None. **A. Barnett:** None.

Presentation Number: NANO56.09

Topic: H.07. Long-Term Memory

Support: NIH Grant F99NS118740-01

Title: Evidence for music-evoked reactivation of naturalistic events

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Abstract: In our daily lives, music serves as a powerful cue capable of evoking memories from our past. However, replicating this phenomenon in a lab setting has proven to be a challenge, as implied by inconsistent findings from previous studies (Echaide et al., 2019). Furthermore, research on the neural correlates of music-evoked memories is rather limited, although existing work points to a critical role of mPFC (Janata, 2009, Belfi et al., 2018), a core region of the default mode network (DMN). Furthermore, recent work on narrative comprehension revealed that activity patterns in a broader set of DMN regions were related to successful reinstatement of

high-level narrative events (Chen et al., 2017, Baldassano et al., 2017). Interestingly, however, the research on music-evoked episodic memory and narrative event perception has yet to produce a unified theory of the neural mechanisms underlying music-evoked retrieval of real-world information. In our study, we sought to investigate how music shapes naturalistic event understanding and memory, by collecting fMRI data while participants (N=50, 29 female) viewed a feature-length film (Eternal Sunshine of the Spotless Mind) either with the film score intact or entirely removed. Importantly, this film was chosen because the film score contains songs that repeat throughout the film. Our hypothesis was that areas of the DMN would show evidence of reactivation in response to repeated musical themes, which would be associated with an improvement in the recall for the film events. In keeping with this hypothesis, our results show that subregions of the DMN including angular gyrus exhibit evidence of music-evoked reactivation ($t=3.96$, $q<0.05$); preliminary analyses also indicate that reactivation is linked to improved memory for the reactivated events on a subsequent free recall test.

Disclosures: J.A. Williams: None. **E.H. Margulis:** None. **C. Baldassano:** None. **U. Hasson:** None. **J. Chen:** None. **K. Norman:** None.

Presentation Number: NANO56.10

Topic: H.07. Long-Term Memory

Support: NINDS T32- NS115672

Title: Event boundaries and interactive inference shape the content and organization of memory

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Abstract: Though our daily experiences are continuous, we often remember the past as snapshots. Event segmentation theory describes these snapshots as being formed in response to prediction errors. In this framework, one has an internal model predicting ongoing observations. When the predictions and observations differ by a large margin, an event boundary occurs, and the model is altered or replaced. Previous studies show that these event boundaries affect episodic memory in various ways, interfering with temporal order memory, and enhancing item recognition. Commonly, these boundaries are examined during passive viewing tasks. However, very little is known about how these processes operate in interactive events where inference is necessary. To investigate this, we designed a novel rule inference task that enabled us to assess memory around shifts in rules that elicited event boundaries. Critically, the stimuli in this task were unique common words, enabling us to test episodic memory for specific items as a result of the experienced event structure. In the first experiment, participants (N=73) performed a free recall task after the rule inference task. Contrary to theoretical predictions, we found that people were less likely to recall items that were encoded immediately after an event boundary. Moreover, recall was organized by event structure, with boundary and pre-boundary items forming anchoring points as individual words were retrieved. In another set of experiments (N=91), we replicated this decrease in memory for the post-boundary item using a recognition task. Here, we examined whether this post-boundary memory deficit is related to increased

inference demands caused by a rule change. Indeed, explicitly informing participants of the sequence of rule shifts eliminated this deficit. Our results imply that event segmentation during interactive events is different than during passive viewing, and that under some circumstances, event boundaries might *inhibit* rather than *enhance* memory for specific items.

Disclosures: A.B. Karagoz: None. **W. Kool:** None. **Z.M. Reagh:** None.

Presentation Number: NANO56.11

Topic: H.07. Long-Term Memory

Title: Functional network integration mediates arousal-dependent memory enhancement during narrative perception

Authors: *J. PARK[1], J. KE[1], K. GOLLAPUDI[1], I. PAPPAS[2], Y. LEONG[1];
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Abstract: People tend to have vivid, detailed memories of emotional events. Prior studies examining this phenomenon have focused on the role of individual brain regions, particularly the amygdala and the hippocampus. Recent work, however, have begun to consider the relationship between whole-brain interactions and memory encoding. In this study, we test the hypothesis that heightened functional integration across large-scale brain networks mediates arousal-dependent effects on episodic memory. We used two publicly available fMRI datasets (n = 17 and 15) where participants watched audiovisual movie clips and verbally recalled what they remembered. The continuous movie clips were divided into events defined by major shifts in the narrative. For each event, we constructed an unweighted, undirected graph from the pairwise functional connectivity matrices separately for each participant. We then computed the average participation coefficient (PC) across all brain regions as a measure of overall network integration. High average PC indicates a brain state with high levels of intermodular connectivity across the brain. As a continuous measure of memory performance, we converted movie annotations and participants' recall transcripts into text embeddings, and calculated memory fidelity of each event as the cosine similarity between the movie and recall embeddings. Using Bayesian multilevel modeling, we found that average PC while viewing an event was positively associated with subsequent memory fidelity (posterior prob. > 0.95). Follow-up analyses indicated that these effects were driven by increased inter-network integration between multiple canonical functional brain networks. We then collected behavioral ratings of emotional arousal (n = 30), which were positively associated with both average PC and memory fidelity (posterior prob. > 0.95). Furthermore, the effects of emotional arousal on memory fidelity were mediated by whole-brain functional integration at encoding (posterior prob. > 0.95). Results survived controlling for activity in the amygdala and the hippocampus, suggesting a contribution of whole-brain functional integration above and beyond that of localized brain regions. Altogether, our results suggest that emotional arousal enhances memory encoding through facilitating increased functional integration across brain networks. Our study highlights the importance of considering whole-brain network interactions in understanding emotional influences on memory in naturalistic contexts.

Disclosures: J. Park: None. **J. Ke:** None. **K. Gollapudi:** None. **I. Pappas:** None. **Y. Leong:** None.

Presentation Number: NANO56.12

Topic: H.07. Long-Term Memory

Support: NIH R01MH133732

Title: Neural correlates of prediction potential during movie-viewing

Authors: *Y. HUANG[1,1], A. HU[1], B. BELLANA[2], J. CHEN[1];
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Abstract: The ability to make predictions about upcoming events is critical for evolutionary survival and everyday functioning. However, our environment varies in the extent to which it encourages or requires strong predictions at any given moment. In this study, we examine the neural states that signal when our environment affords a strong prediction—when there is high prediction potential—in the context of complex, naturalistic events.
To examine this, we leveraged an open fMRI data set (N = 17) watching six films (Lee & Chen, 2022). We collected a separate behavioral dataset in which subjects watched the same films but were periodically stopped and asked to predict what would happen next. For each film, subjects watched 60 seconds, then were asked to predict the next 30 seconds. Counterbalancing stop points produced 30 predictions for every 10-second interval. As the potential for making predictions might depend on the interpretation of the period leading up to the prediction, we collected a parallel description dataset in which subjects described what happened in the 30 seconds prior to the same prediction stop points.
To assess prediction potential, we computed agreement among subjects' predictions ("consensus"). We chose this metric rather than prediction accuracy because films are designed to be surprising at times; a consensus among viewers indicates that the current moment elicits a strong prediction, regardless of accuracy. We obtained prediction consensus by transforming the prediction texts into embedding vectors with Universal Sentence Encoder (USE) and calculating the pairwise cosine similarity of vectors, which showed stable split-half reliability of r=0.56. Description consensus was calculated in the same way (r = 0.32). Prediction consensus and description consensus were somewhat correlated (average r=0.25).
Description consensus—the degree of shared interpretation of the current moment—was correlated with activity throughout the default mode network (DMN). Meanwhile, prediction consensus—the extent of shared predictions about upcoming events—correlated with activity in hippocampus, parahippocampal cortex (PHC), retrosplenial cortex (RSC), and medial prefrontal cortex (mPFC). These findings indicate that DMN activity is high when environmental input enables coherent interpretation of the current event; conversely, a set of brain areas related to episodic and schematic knowledge signal when there is high potential for making strong predictions, even if predictions are not actually demanded at that moment.

Disclosures: Y. Huang: None. **A. Hu:** None. **B. Bellana:** None. **J. Chen:** None.

Presentation Number: NANO56.13

Topic: H.07. Long-Term Memory

Support: National Science Foundation BCS-2043740
Social Sciences Research Center Faculty Seed Grant Program at the University of Chicago

Title: Comprehension of causal structure in narratives

Authors: *H. SONG[1], J. KE[2], Y. LEONG[2], M. D. ROSENBERG[2];
[2]Dept. of Psychology, [1]Univ. of Chicago, Chicago, IL

Abstract: Comprehending a narrative involves continuously integrating ongoing events into a situational model based on their causal relationships. Theories posit that comprehension involves inferring causal links between events (Graesser et al., 1994), and behavioral evidence suggests that causally related past events are retrieved from memory when comprehending an event (Suh & Trabasso, 1993). However, neural evidence is limited on how these causal relationships are represented and integrated in memory during narrative comprehension. How does the brain piece together the information we accumulate from the world to construct a causally coherent situational representation? During functional magnetic resonance imaging, 36 participants indicated moments of insight by pressing an "aha" button (Song et al., 2021) while watching a temporally scrambled television episode. After watching several scenes, participants verbally explained their reasons for each button press. Findings based on hidden Markov model-based event segmentation (Baldassano et al., 2017) revealed significant changes in cortical representation patterns approximately 2 s prior to aha button presses, with 16 out of 100 cortical parcels showing a higher likelihood of pattern shifts (FDR-$p < 0.05$). This suggests that insight accompanies updates to the situational model. Participants with a higher likelihood of cortical pattern shifts ~2 s prior to button presses better recounted the scrambled story in its original sequence after the scan (controlling for the number of button presses; $rho = 0.344$, $p = 0.046$). When explaining the reasons for each insight moment, participants frequently mentioned past events that were causally related to the current event (53.54 % ± 23.37% of aha moments). These causally related events were represented with similar multivariate voxel activity patterns, even when controlling for semantic and perceptual similarities (15 out of 100 cortical parcels; FDR-$p < 0.05$). Interestingly, the representation patterns of the retrieved events were not transiently reinstated at insight moments but were evident throughout the encoding of the event in which the insight occurred (no significant difference in pattern similarities between the past and current events at aha moments and the rest of the encoding period). This supports a slow build-up of memory integration, rather than a transient reinstatement, in updating situational representations to better reflect the overall causal structure in narratives. Together, this study provides a neural account of comprehension as a dynamic updating of situational representations to reflect the causal structure in narratives.

Disclosures: H. Song: None. **J. Ke:** None. **Y. Leong:** None. **M.D. Rosenberg:** None.

Nanosymposium

NANO57: Network Dynamics and Molecular Mechanisms in Neurodevelopmental Resilience and Function

Location: MCP Room N426

Time: Wednesday, October 9, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO57.01

Topic: A.08. Development of Neural Systems

Support: NIH R01 MH116950-04

Title: Behavioral resilience in children with sensory over-responsivity is associated with elevated endogenous and reduced exogenous functional MRI network connectivity

Authors: *H. CHOI[1], R. POWERS[4], M. C. LAZERWITZ[4], J. WREN-JARVIS[5], L. CAI[2], A. SADIKOV[1,3], A. BRANDES-AITKEN[4], R. CHU[6,1], K. J. TRIMARCHI[4], R. D. GARCIA[4], E. J. MARCO[4,7], P. MUKHERJEE[1,3];
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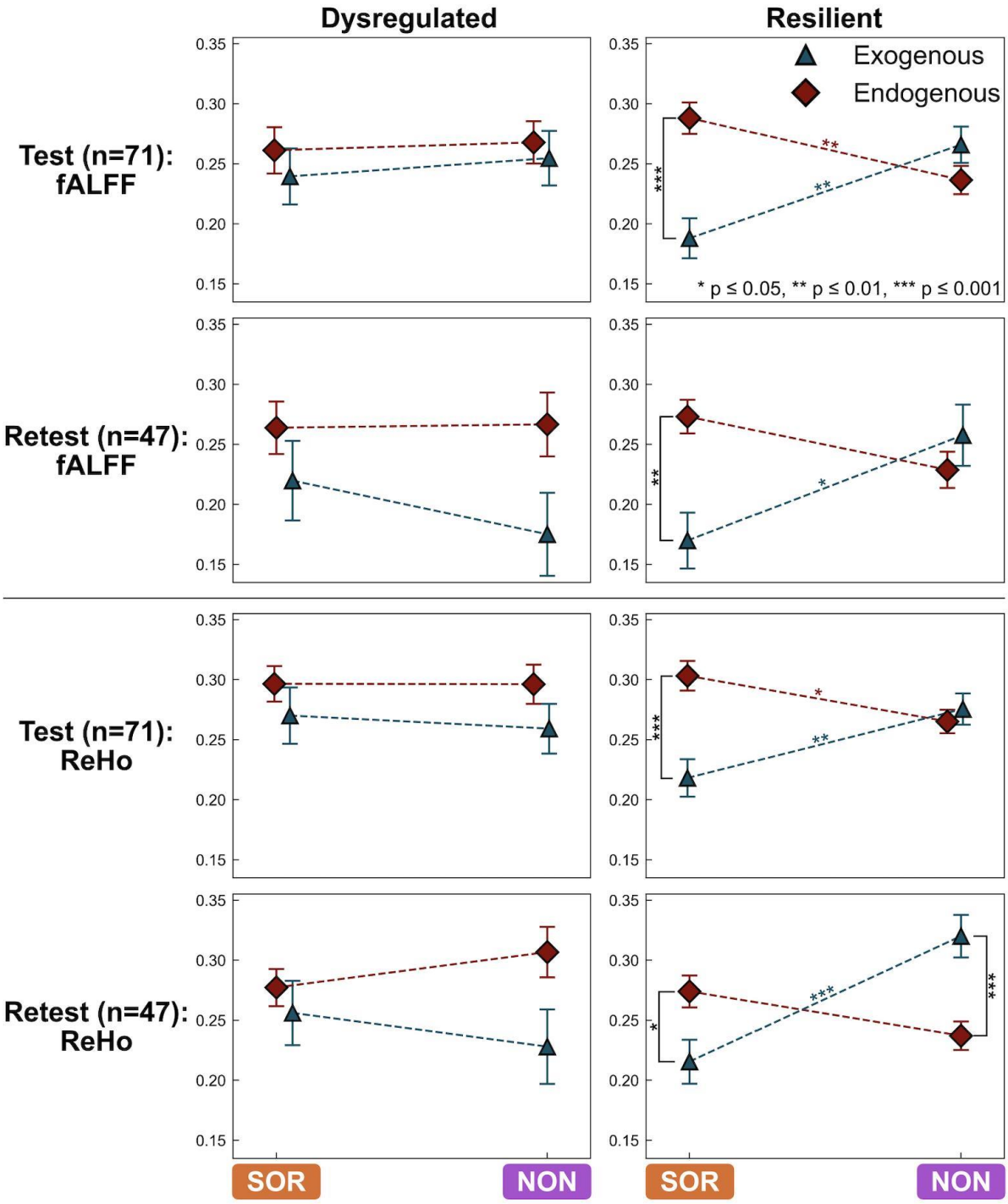
Abstract: Sensory over-responsivity (SOR) has been increasingly gaining attention within the neuroscience community due to the growing recognition of its convergence with autism spectrum disorder, attention-deficit/hyperactivity disorder, anxiety, and depression. To our knowledge, this is the first resting-state fMRI study of SOR children (ages 8-12) without autism. We test the hypothesis that functional connectivity (FC) is weakened in exogenous (externally-influenced) brain networks and strengthened in endogenous (internally-influenced) networks of SOR youth to compensate for sensory processing challenges. Participants were assigned to SOR and non-SOR groups using the Sensory Processing 3-Dimensions Assessment and scanned on a 3T Siemens Prisma scanner for 6 minutes using multiband echo-planar imaging. Rigorous image quality assurance, fMRIPrep preprocessing, image quality control, and denoising were performed, resulting in a sample of 83 participants (mean age: 10.47 ± 1.59; 30 females; 39 SOR). Local FC metrics of fractional amplitude of low-frequency fluctuations (fALFF) and regional homogeneity (ReHo) were computed for the 7 exogenous (cerebellar; 3 somatomotor; 3 visual) and 7 endogenous (central executive, default mode, dorsal attention, left and right frontoparietal, salience, and ventral attention) FC networks generated using independent component analysis. Dysregulated and resilient clusters were delineated from k-means clustering of the Behavior Assessment System for Children-3 scores (n=71; 25 females; 31 SOR; 48 resilient). Our results indicate that resilient SOR youth exhibit reduced local FC of exogenous networks and elevated local FC of endogenous networks for both fALFF and ReHo, including on a retest fMRI acquisition (n=47) [**Figure**]. SOR individuals in the behaviorally dysregulated cluster display intermediate levels of network FC. Hence, decreased exogenous local FC and

increased endogenous local FC may represent a neural signature of resilience to behavioral dysregulation in SOR.



Disclosures: H. Choi: None. **R. Powers:** None. **M.C. Lazerwitz:** None. **J. Wren-Jarvis:** None. **L. Cai:** None. **A. Sadikov:** None. **A. Brandes-Aitken:** None. **R. Chu:** None. **K.J. Trimarchi:** None. **R.D. Garcia:** None. **E.J. Marco:** None. **P. Mukherjee:** None.

Presentation Number: NANO57.02

Topic: A.08. Development of Neural Systems

Support: Sheryl and Dan Tishman Postdoctoral Fellowship
Simons foundation
Philippe Foundation
R01

Title: Arousal state influence on spontaneous and visually-evoked network activity across postnatal development

Authors: *M. SALMI, E. SABRI, R. BATISTA-BRITO;
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Abstract: Sensory perception allows us to receive and integrate information from the environment, a process that starts right from birth. Indeed, newborns can interact with their environment and perform sensorimotor tasks soon after birth, without any previous experience of patterned sensory input. The first postnatal weeks in mice are a critical time window for cortical circuit assembly and as consequences to prepare sensory encoding. In sensory cortex such as the primary visual cortex (V1), critical transitions in network dynamics occurred during early postnatal development. Indeed, through the time window surrounding eye-opening onset (P9 to P15) the V1 network activity transitions from highly synchronized burst events triggered by retinal wave (recurrent spontaneous activity coming from the retina) to an asynchronized network activity ready to encode external visual inputs. In adult rodents, it was previously reported that in V1 area, both spontaneous and sensory-driven network activity dynamics are highly arousal state-dependent. However, relatively little is known about how the arousal state influence on cortical network dynamic emerges through early postnatal development. Understanding how neurons assemble into circuits that developmentally prepare to encode visual information accordingly to arousal states is still poorly understood and is likely to provide critical insight not only into how these circuits function, but also how they malfunction in various neurodevelopmental disorders such as autism, schizophrenia, and epilepsy. To study this critical question, we tracked V1 activity evolution in pup mice through early postnatal development. Both spontaneous and sensory-evoked neuronal population activity were recorded from before (P9) to after (P18) eye opening by combining both *in vivo* head-fixed, dense electrophysiological extracellular (LFP/SUA) and 2P calcium imaging recordings. V1 activity was recorded along with the monitoring of facial motion to assess the transition between low and high arousal states. Before eye opening, both spontaneous and visually-evoked activity dynamics are not influenced by facial motion. While at eye-opening onset both spontaneous and visually evoked activity get enhanced by facial motion, in line with what was previously described in the adult V1. Our findings suggest that eye-opening is a critical developmental turning point for arousal state influence onto cortical network dynamics at the time when the network activity is also transitioning from synchronized to asynchronized and from not visually to visually driven. In other words when external sensory inputs encoding emerges in V1.

Disclosures: M. Salmi: None. **E. Sabri:** None. **R. Batista-Brito:** None.

Presentation Number: NANO57.03

Topic: A.08. Development of Neural Systems

Support: ROGER DE SPOELBERCH Prize 2021

Title: Tracking activity of the same neurons throughout development reveals the emergence of stable neocortical representations

Authors: *J. MAJNIK[1], S. ZANGILA[2], R. A. COSSART[3], J.-C. PLATEL[4];
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Abstract: Brain development is a dynamic process that unfolds with great variability across individuals. In mice, sensory systems develop rapidly after birth, with active whisking and eye openning occuring during the second postnatal week. Acute recordings of neural activity have revealed important global principles of this developmental period, most striking being the sparsification of neocortical dynamics. Due to the limitations of acute recordings however, an understanding of this developmental process unfolding within a cortical circuit of a single individual is still lacking.To overcome this gap we developed a protocol allowing us to record the activity of the same neurons throughout early neocortical development using longitudinal 2-photon calcium imaging. We recorded in barrel cortex, starting from P7 and repeated the recording daily up to P17, each session consisting of a period of spontaneous and evoked activity, delivering tactile stimuli to the whiskers. We also simultaneously tracked behaviour using videography. To match the recorded cells across days we developed a novel open-source algorithm (Track2p), specifically tailored to development, overcoming challenges such as brain growth and changing cell morphologies. The algorithm yielded on the order of hundreds of neurons per mouse identified across all days. The statistical properties of the matched cells' activity displayed the canonical phenomena of this period at the level of a single individual (decorrelation, loss of spatial structure etc.).We next turned to decoding analysis to investigate the representational properties of the circuit. Studying the relationship between spontaneous acitvity and behaviour showed a striking rise in state-dependence at the onset of active sensation. Once developed, this representation is stable, with the same neurons exhibiting state-coupling, allowing for cross-day decoding. Our preliminary analysis of evoked activity also shows similar characteristics in terms of reliability, sparsity and stability across days at later ages, which is essential for guiding sensory behaviours controlled by downstream cirucits.Using longitudinal imaging of a matched neuronal population, we managed to track the development of a sensory system at the level of single individuals. We showed a progressive maturation of response across days, culminating in stable and reliable respresentations at later ages. Based on the described results, we suggest that during this period the role of neocortical activity patterns switches from supporting activity dependent developmental mechanisms to enabling adult-like sensory processing.

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

Presentation Number: NANO57.04

Topic: A.08. Development of Neural Systems

Support: National Science Foundation Grant No. IOS-1921065
National Institutes of Health NINDS R01NS118562
HHMI-Helen Hay Whitney Foundation Fellowship

Title: Neuronal identity control at the resolution of a single transcription factor isoform

Authors: *N. SMOLIN[1], M. DOMBROVSKI[2], B. W. HINA[1], A. MORENO-SANCHEZ[3], R. GOSSART[4], C. R. CARMONA[4], A. REHAN[2], R. H. HUSSEIN[2], P. MIRSHAHIDI[2], J. AUSBORN[3], Y. KURMANGALIYEV[4], C. R. VON REYN[1,3];
[1]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA; [2]Dept. of Biol. Chem., UCLA, Los Angeles, CA; [3]Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [4]Dept. of Biol., Brandeis Univ., Waltham, MA

Abstract: Although mechanisms that contribute to cellular identity by patterning neural progenitors have been well established, how identity is finalized and maintained is not well understood. In post-mitotic cells, “terminal selectors” are transcription factors hypothesized to determine final identity characteristics, but which transcription factors function as terminal selectors and the level of control they exert over different terminal characteristics is not well established. To investigate how terminal selectors affect morphology, gene expression, and synaptic specificity, we use the model organism *Drosophila melanogaster*. Through differential expression analysis from scRNA-seq data, we uncovered highly differential expression of one transcription factor, *broad*, between two otherwise very similar visual system cell-types, Lobula Plate Lobula Columnar neuron type 1 and 2 (LPLC1 and LPLC2). Using the UAS/GAL4 system, we were able to overexpress *broad* in LPLC2 and knock down *broad* in LPLC1, allowing us to study how broad modulates identity between these two cell-types. In both cases, perturbations of *broad* caused morphologies from one cell type to resemble the other. Using scRNA-seq, we determined how *broad* overexpression impacts transcription and found that *broad*-LPLC2 cells were now more transcriptionally similar to native LPLC1 cells than LPLC2 cells. Using whole-cell electrophysiology with optogenetics, we found that LPLC2 cells made novel, functional connections with an LPLC1-specific presynaptic partner. We then focused on whether *broad*’s effects are isoform specific, and narrowed down *broad-z4* as the isoform expressed in LPLC1 that is sufficient to induce a comprehensive change in identity—changing gene expression, morphology, and synaptic specificity—when altered. We therefore establish a novel role for the transcription factor *broad* as a terminal selector in *Drosophila melanogaster*. Our work identifies a single isoform as the smallest unit underlying an identity switch, which may emerge as a conserved strategy replicated across developmental programs. These findings may have major implications for understanding neurodevelopmental and neuropsychiatric disorders that may arise from complications in establishing the correct cell type identity.

Disclosures: N. Smolin: None. **M. Dombrovski:** None. **B.W. Hina:** None. **A. Moreno-Sanchez:** None. **R. Gossart:** None. **C.R. Carmona:** None. **A. Rehan:** None. **R.H. Hussein:** None. **P. Mirshahidi:** None. **J. Ausborn:** None. **Y. Kurmangaliyev:** None. **C.R. von Reyn:** None.

Presentation Number: NANO57.05

Topic: A.08. Development of Neural Systems

Support: Doctoral scholarship - Centre-Val de Loire Region - France

Title: Mapping the sensory spectrum: unraveling variability in young children's brain responses

Authors: *L. MICHEL, M. SASSIER-ROUBLIN, C. WARDAK, M. LATINUS;
Univ. de Tours, INSERM, Imaging Brain & Neuropsychiatry iBraiN U1253, TOURS, France

Abstract: Early childhood is a sensitive period of development, marked by a high degree of cerebral plasticity, and by high between and within individual variability in the acquisition of cognitive and social abilities. Electroencephalography (EEG) can be used to study this cerebral variability and has revealed not only a high degree of heterogeneity between children, but also some intra-individual variability in certain children. Our aim was to characterize EEG individual profiles of young children between 2,5 and 6 years old. We recorded event-related potentials evoked by sensory stimulations in different sensory modalities (visual, auditive and tactile), and with different levels of complexity (stimuli of low perceptual level, complex and social stimuli). 23 children (13 girls) without neurodevelopmental disorder diagnosis, aged 47.8 months (± 10.5) [30 64] partook in the study. For each participant, we analyzed ERPs peak amplitudes and latencies for each condition (modality by complexity level). In addition, we calculated the median absolute deviation and the inter-trial consistency as measures of intra-individual variability. A hierarchical cluster analysis on the Euclidian distance calculated using all EEG parameters between each participant revealed 4 clusters. Two multinomial logistic regression were tested to evaluate the effects of cognitive parameters (index of visuo-spatial reasoning, sound discrimination, age) and sensory parameters (Dunn's sensory score - SP, mean ITC level) respectively, on cluster membership. In the 1st model, we found that age ($F(3,30) = 7.8$, $p = 0.056$) and sound discrimination ($F(3,30) = 15.4$, $p=0.015$) partly explains cluster membership. One cluster comprised only children under 50 months, with good homogeneous sound discrimination scores while two of the other clusters mainly comprised older children with heterogeneous sound discrimination scores. The 2nd model revealed that cluster membership was driven by interactions of SP tactile scores with audio scores ($F(3, 30) = 14.6$, $p < 0.01$), and with visual scores ($F(3,30) = 27.9$, $p < 0.001$). Neurophysiological sensory profiles of young children appeared to differ mainly according to age and therefore may reflect cerebral maturation. Yet, they also seem to be linked to children's cognitive abilities and sensory profiles. Multisensorial EEG profiles appear a good measure of a child development. EEG profiles could help better understand the developmental trajectory of children, in particular those with neurodevelopmental disorders. The main limitation of this work is the limited number of participants, considering the wide heterogeneity of cerebral development.

Disclosures: L. Michel: None. **M. Sassier-Roublin:** None. **C. Wardak:** None. **M. Latinus:** None.

Presentation Number: NANO57.06

Topic: A.08. Development of Neural Systems

Support: NIH RO1 EYE030458-3
NIH F32 EY034358-01

Title: A kinase at a crossroads: The serine/threonine kinase LKB1 drives neurotransmitter switching in the visual system.

Authors: *R. MACKIN[1], M. A. SAMUEL[2];
[1]Baylor Col. of Med., Houston, TX; [2]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 diseases of the central nervous system (CNS), 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, Starburst Amacrines (SACs) specifically, is sufficient to induce this conversion, which increases the dopaminergic population. Thus, cholinergic and dopaminergic interneurons require LKB1 to regulate post-mitotic neural identity. Interestingly, removal of Tyrosine Hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, from SACs results in a complete absence of TH+ cells in the retina except in the periphery. These results indicate a novel mechanism by which crosstalk between cholinergic and dopaminergic retinal interneurons influence the identity of each neuronal type post mitotically. These mechanisms may possibly be manipulated to modulate these two neuromodulators to expand therapeutic options in dopamine-related diseases of the CNS.

Disclosures: R. Mackin: None. **M.A. Samuel:** None.

Presentation Number: NANO57.07

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01 NS028182

Title: Establishing the roles of molecular specificity and cell surface affinity in motor axon guidance in the developing fruit fly neuromuscular system

Authors: *A. B. LEBOVIC[1], J. PRIEST[1], R. ZHANG[2], A. M. OLECHWIER[1,3], R. A. CARRILLO[4], E. OZKAN[1];
[1]Dept. of Biochem. and Mol. Biol., [2]Dept. of Mol. Genet. and Cell Biol., [3]Dept of Biochem. and Mol. Biol., [4]Mol. Genet. and Cell Biol., Univ. of Chicago, Chicago, IL

Abstract: The development of the neuromuscular system is dependent on a microscopic landscape controlled by cell-surface and secreted proteins (CSSPs). Neurons rely on CSSPs for directional growth and migration, cell adhesion, the formation of synaptic connections, and muscle innervation. Eight Beaten Path (Beat) and 14 Sidestep (Side) proteins make up two families of CSSPs, controlling axon branching and muscle innervation in Drosophila melanogaster by interacting with each other between neighboring cells. Misregulation of these proteins leads to the failure of axons to navigate to their targets, incorrect development of the optic lobe, and selective loss of synaptic connections in the central nervous system. It is unknown how these proteins achieve binding specificity with select partners and if their specific interactions lead to proper connectivity. To answer these questions, we established expression and purification protocols for Beat and Side ectodomains, and demonstrated a direct physical

interaction for several pairs using size-exclusion chromatography and surface plasmon resonance. We then determined the first structure of a Beat-Side complex, that of Beat-Vc with Side-VI. Using a structure-based rational approach, we engineered point mutations to break the binding between Beat-Ia and Side-I and validated these variants in vitro and in vivo for proper folding and trafficking. Using both an overexpression strategy and endogenous engineering of the mutations, we demonstrated that the Beat-Ia-Side-I interaction is required for the connectivity of motor neurons with muscles in Drosophila larvae. We also observed that Side-I ectodomain dimerizes, which results in the dimerization of neuronal Beat-Ia receptors. Therefore, we propose receptor dimerization as a putative mechanism of activation of Beat receptors. Elucidating the mechanisms for Beat-Side-mediated trans-cellular interactions can reveal not only their purpose in motor axon guidance, but further uncover mechanisms underlying cell adhesion, the formation of synaptic connections, and muscle innervation.

Disclosures: A.B. Lebovic: None. **J. Priest:** None. **R. Zhang:** None. **A.M. Olechwier:** None. **R.A. Carrillo:** None. **E. Ozkan:** None.

Nanosymposium

NANO58: Aging: Molecular and Cellular Changes

Location: MCP Room S106

Time: Wednesday, October 9, 2024, 1:00 PM - 4:00 PM

Presentation Number: NANO58.01

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

Support: R01NS064131

Title: A transgenic C. elegans model of neurodegenerative TMEM106B proteinopathy.

Authors: *B. KRAEMER[1], R. RIORDAN[2];
[1]Univ. of Washington Sch. of Med., Kent, WA; [2]Univ. of Washington, kent, WA

Abstract: Genetic variation of lysosomal protein, transmembrane protein 106B (TMEM106B) has long been known as a risk factor for a diverse range of neurodegenerative disorders, especially FTLD with progranulin (GRN) haplo-insufficiency, though the mechanisms involved are not yet understood. Recently, through advances in cryo-electron microscopy (cryo-EM), aggregates of the C-Terminal domain of TMEM106B (TMEM CT) were shown to make up previously unidentifiable protein aggregates in the brains of human FTLD, AD, progressive supranuclear palsy (PSP), and dementia with Lewy Bodies (DLB) patients. To investigate TMEM CT aggregation propensity, neurodegenerative potential, and interactions with other proteinopathies we generated a new transgenic C. *elegans* proteinopathy model pan-neuronally expressing the TMEM CT fragment constituting the fibrillar core found in FTLD-TDP cases. We observe that C. *elegans* pan-neuronal expression of TMEM CT causes neuronal dysfunction as evidenced by behavioral analysis. TMEM c-terminal fragments accumulate as detergent insoluble material. Behavioral dysfunction was accompanied by neurodegeneration as illustrated by loss of GABAergic neurons. To investigate the mechanisms driving TMEM106B

proteinopathy, we aimed to explore the impact of GRN loss on the neurodegenerative effect of TMEM CT expression. To this end, we generated TMEM CT expressing C. *elegans* with PRGN loss of function. We found that loss of PGRN did not alter TMEM phenotypes confirming PGRN loss of function is upstream of TMEM aggregation. We also observed TMEM neurodegenerative phenotypes exhibit a distinct set of genetic modifiers as strong suppressors of tauopathy such as spop-1, sut-2 sut-6 show only modest modification of TMEM proteinopathy. Taken together, our data suggest expression of TMEM CT in C. *elegans* neurons provides a useful model for the functional characterization of TMEM106B proteinopathy in neurodegenerative disease. Comparison with other proteinopathy models will inform potential disease mechanisms.

Disclosures: B. Kraemer: None. **R. Riordan:** None.

Presentation Number: NANO58.02

Topic: C.01. Brain Wellness and Aging

Support: Howard Hughes Medical Institute

Title: Neuron-specific CRISPR genetics to study the role of mitochondrial dynamics in aging Drosophila neurons

Authors: *S. RICHHARIYA[1], D. SHIN[1], M. SCHLICHTING[1,2], M. ROSBASH[1];
[1]Dept. of Biol., Brandeis University/HHMI, Waltham, MA; [2]Northwestern Univ., Boston, MA

Abstract: Mitochondria are dynamic organelles that undergo fission and fusion for many reasons, including energy demands and stress. Altered mitochondrial dynamics are associated with several neurodegenerative diseases, yet the relationship of mitochondrial dynamics to neurodegeneration and aging is poorly understood. We are addressing these issues *in vivo* using CRISPR-Cas9 based methods to efficiently disrupt mitochondrial dynamics by targeting genes for mitochondrial fission and fusion in specific brain neurons in *Drosophila melanogaster*. Because perturbing mitochondrial dynamics in broad sets of neurons caused lethality, we explored the consequences of altering mitochondrial dynamics in the clock neurons of *Drosophila*, which are largely inessential for viability. Circadian behavioral assays identify an age-dependent impairment of neuronal function with loss of fusion. To further understand the cellular effects of disrupted mitochondrial dynamics, we performed transcriptomic analysis of clock neurons deficient in mitochondrial fusion and fission, in young and old clock neurons. Consistent with the behavioral effects, loss of fission had only a minor influence on the transcriptome, whereas loss of fusion led to much more dramatic effects on several genes, with bigger changes observed in older neurons. The transcriptional changes and the affected genes suggest that gene expression mechanisms compensate for the lack of fusion. Indeed, a CRISPR-Cas9 double gene knockout strategy indicates that the key up-regulated gene *Ldh* compensates for the decrease in mitochondrial function due to chronic loss of fusion. This upregulation also prevents neurodegeneration. These findings are being validated in other types of cells using adult and tissue specific CRISPR. Future work will utilize this system to further explore the functions and interactors of the mitochondrial fission-fusion pathway in neurons.

Disclosures: S. Richhariya: None. **D. Shin:** None. **M. Schlichting:** None. **M. Rosbash:** None.

Presentation Number: NANO58.03

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG056597
UAB Integrated Center for Aging Research

Title: Increased levels of extracellular matrix proteins associated with extracellular vesicles from brains of aged mice

Authors: A. K. KAPLELACH, C. F. MURCHISON, K. KOJIMA, J. MOBLEY, ***A. ARRANT**;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Extracellular vesicles (EVs) regulate multiple aspects of brain function, serving as a mode of intercellular communication and as a pathway for disposal of cellular debris. While these functions serve to maintain healthy brain function, they may also contribute to diseases affecting the brain. For example, EVs may promote cancer metastasis to the brain or spread pathologic proteins in neurodegenerative disease. Aging is the greatest risk factor for neurodegenerative diseases and many other conditions affecting the brain, but the effects of aging on brain EVs are not well understood. Aging-associated changes such as inflammation, cellular senescence, and impairment of autophagy alter the production and contents of EVs in other contexts. Levels of EVs in plasma may change with age and have deleterious functional effects. There may also be changes in levels of subtypes of brain EVs in aged animals. In a prior study, we observed trends for age-dependent changes in the protein contents of brain EVs between young (3 month-old) and middle-aged (12 month-old) mice. In this study, we investigated whether brain EV protein contents further change with aging by analyzing brain EVs from 4-, 12-, and 22-month-old mice. We detected no changes in EV levels, but observed age-dependent changes in EV proteins. EV preparations from brains of aged (22-month-old) mice contained higher levels of extracellular matrix proteins than EVs from young (4-month-old) mice, with similar trends occurring in 12-month-old mice. Specifically, levels of hyaluronan and proteoglycan link proteins (Hapln) 1 and 2 were elevated in EVs from aged mice, as were levels of several chondroitin sulfate proteoglycans (CSPGs), which interact with Hapln1 and 2. Analysis of extracellular matrix in several brain regions of aged mice revealed increased immunolabeling for aggrecan, but a general reduction in labeling with *Wisteria floribunda* agglutinin, which binds to chondroitin sulfate. These data are consistent with prior studies showing changes to the composition of extracellular matrix in aged brains, which might contribute to age-related cognitive decline. Our findings reveal a novel association of EVs with these changes in the extracellular matrix of the aging brain.

Disclosures: A.K. Kaplelach: None. **C.F. Murchison:** None. **K. Kojima:** None. **J. Mobley:** None. **A. Arrant:** None.

Presentation Number: NANO58.04

Topic: C.01. Brain Wellness and Aging

Support: Medical College of Wisconsin (MCW) Imagine More Award
MCW Research Affairs Committee
5520482 Advancing a Healthier Wisconsin Endowment project titled

Developing Innovative Translational Research Programs in Clinically Relevant Neurological Disorders

Title: Skin Collagen Reduction Drives Selective Aspects of Aging in Cutaneous Sensory Neurons

Authors: *M. M. KRISHNA[1,2], A. FRANITZA[2], B. STUART[1], L. E[1,2];
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Abstract: Despite significant progress in revealing the molecular processes during aging-associated neuronal degeneration, our understanding of the upstream mechanisms that drive neuronal aging remains limited. Our study aims to address this by examining interactions between neurons and non-neural tissues during aging, using the *Caenorhabditis elegans* PVD neuron, a cutaneous mechanosensory neuron, as a model. We observed progressive excessive dendritic branching in the PVD as wild-type animals aged. This excessive branching was functionally correlated with age-related deficits in proprioception. To examine the skin's potential role in the aging of nearby neurons, we focused on a reduction in skin collagens, a commonly observed age-related change across species. We found that loss-of-function mutations in two skin collagen genes, *dpy-5* and *col-120*, induced early-onset PVD excessive branching. A similar early-onset branching was seen via adulthood RNAi knockdown, suggesting that skin collagen's role in PVD dendritic integrity is aging-associated and not developmental. Skin-specific rescue experiments also confirmed the tissue-specific role of skin collagens in regulating aging-associated branching. Further, overexpressing these genes mitigated the severity of excessive branching at an advanced age, pointing to a neuroprotective role for these skin collagens during aging. Interestingly, in collagen mutants, we found no significant differences in aging-associated neuritic beading, another degenerative phenotype in aging PVD neurons, implying that skin collagens' role is phenotype-specific. We also examined two other cutaneous sensory neurons, the ALM and PLM, and found that only the ALM displayed increased branching in collagen mutants, suggesting neuron-subtype specific vulnerability to skin collagens' influence. A candidate RNAi screen to identify downstream factors revealed that reduced expression of a neuronal cell adhesion molecule, *rig-3*, also induced early-onset branching in PVD neurons. When *rig*-3 was knocked down in collagen mutants, it did not further enhance the excessive branching, suggesting that it functions in the same genetic pathway as the skin collagen genes to regulate PVD neuron aging. Our study demonstrates the causative role that age-related reduction in skin collagens play in neuronal aging. We also highlight the selectivity of skin collagen's impact, implying that selective neuron vulnerability in aging may be mediated by distinct non-neural signals. This emphasizes the importance of exploring multi-tissue strategies to comprehensively address the complexities of neuronal aging.

Disclosures: M.M. Krishna: None. **A. Franitza:** None. **B. Stuart:** None. **L. E:** None.

Presentation Number: NANO58.05

Topic: C.01. Brain Wellness and Aging

Support: AG072977
AG045571

AG067781
AG073153

Title: Enhanced Integrity of Basal Forebrain Cholinergic System in Cognitive SuperAgers

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Abstract: Cognitive decline is well documented as a normal phenomenon of "typical" human aging. However, some elderly appear to avoid this age-related cognitive decline. We have coined the term 'SuperAger' to refer to individuals over the age of 80, whose performance on tests of episodic memory is at least equivalent to healthy 50-65 year-olds, and on tests of other cognitive domains at least equivalent to their cognitively average aged peers. In a previous study, we described the presence of age-related alterations in the basal forebrain cholinergic system in the form of abnormalities in cortical cholinergic axons, such as ballooned terminals and thickened axons, and accumulation of phosphorylated tau in basal forebrain cholinergic neurons (BFCN). This study investigated the integrity of the basal forebrain cholinergic system in SuperAgers (N=6 per group). The total density of cholinergic axonal abnormalities, visualized using acetylcholinesterase histochemistry and quantified using unbiased stereology in the middle frontal gyrus and the entorhinal cortex, was significantly less in SuperAgers when compared with cognitively average elderly ($p=0.0036$). This result was primarily driven by differences in counts of ballooned terminals ($p=0.0179$). No significant differences were observed in thickened axons ($p>0.05$). Accumulation of phosphorylated tau in the BFCN was investigated using the PHF1 antibody, which recognizes tau phosphorylated at Ser396/404 / Thr181. The number of magnocellular basal forebrain neurons containing PHF1 immunoreactivity was significantly lower per section in SuperAgers compared with cognitively average elderly ($p = 0.03$). Systematic qualitative observations revealed no consistent differences in the density of BFCN or cortical cholinergic axons between SuperAgers and cognitively average elderly. These findings indicate maintained integrity of the basal forebrain cholinergic system in SuperAgers when compared with cognitively average controls. They are consistent with our earlier reports indicating an overarching resistance of SuperAger brains to involutional processes that characterize normal human brain aging. Given the known involvement of the basal forebrain cholinergic system in cognitive processing of memory, the preserved integrity of this system is a likely contributor to the greater memory capacity of SuperAgers.

Disclosures: I. Ayala: None. **T. Gefen:** None. **R.J. Castellani:** None. **P. Jamshidi:** None. **E.J. Rogalski:** None. **M. Mesulam:** None. **C. Geula:** None.

Presentation Number: NANO58.06

Topic: C.01. Brain Wellness and Aging

Support: Searle Innovator Grant to Dr. Romanova
Shapiro Foundation grant to Dr. Romanova

Title: Mapping meningeal vasculature in non-human primates

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Abstract: Background. Dura is the outermost layer of the meninges that envelope the brain. It is highly vascularized with a high content of extracellular matrix (ECM). Vessels of the outer dural layer comprise an extensive, parallel intracranial vascular bed outside the brain and subarachnoid space. Most of our knowledge of dural vasculature comes from rodent models. Rodent meninges that contain dura are readily available, small, thin and optically transparent. These characteristics permit imaging in whole-mount flat preparations. Technical barriers, however, remain high for imaging studies of the meninges of larger mammals. This is especially true for the primate dura. Dura in non-human primates (NHP) and humans is large, thick and opaque. These characteristics limit options for routine high-resolution imaging and leave unanswered questions about the architecture of vascular beds, extracellular matrix (ECM) and distribution of the resident cells in both health and aging. In our work, we provide solutions for these technical barriers using new clearing and imaging protocols to successfully visualize structural components of NPH dura in their entirety. Methods. Here we used novel approaches to tissue clearing and resonance scanning confocal imaging of large areas with the sickness over 1000 μM. Results. This is the first study to analyze vascular structures, ECM and resident cells in the whole-mount specimens of NPH dura in young and aged animals. Our approach revealed extensive and dense vascular networks in NHP dura with the resolution sufficient for visualization of the smallest vessels. We also characterized localization and phenotype of the lymphatic vessels, ECM and the cells that reside in the dural environment. Our imaging approach revealed changes in dural vasculature and ECM in aging in primates. Conclusions. We developed clearing, mounting and imaging protocols that permitted panoramic fluorescence-based microscopy of NPH dura. These new techniques are directly applicable to primate models of neurodegenerative diseases with a focus on the complex interplay between meningeal arteries, veins, lymphatics and other structural components of the dura.

Disclosures: L. Romanova: None. **C. Janson:** None. **S. Muller:** None. **J. Kordower:** None.

Presentation Number: NANO58.07

Topic: C.01. Brain Wellness and Aging

Support: NSF Grant 2233539

Title: The Impact of Intestinal Occluding Junction Modulation on Aging and the Brain

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Abstract: The world population is aging, with the number of people over 65 more than doubling to 1.57 billion by 2050, making a concomitant elevation in numerous age-related pathologies,

including neurodegenerative diseases such as Alzheimer's Disease, highly likely. Because of this, a clearer understanding of the pathophysiological changes accompanying aging, and the discovery of novel therapeutics to assist in aging phenotypes, are absolutely essential. Aging is a process marked by a continuous decline in multiple physiological functions, including the 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. These investigations add more information about the impact of the gut on tissue outside the gut and begin 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. Current work utilizes cellular and molecular biological methodologies to build upon current knowledge to address crucial questions at the intersection between microbial dysbiosis, epithelial integrity, inflammation, protein aggregation, neurodegeneration, and disease, with the ultimate goal of discovering novel therapies that may enhance barrier function, healthspan, and lifespan.

Disclosures: D. Fomby: None. **S. Le:** None. **C. Auby:** None. **H. Moore:** None. **C. Patton:** None. **A. Salazar:** None.

Presentation Number: NANO58.08

Topic: C.01. Brain Wellness and Aging

Support: NIH-NINDS-R01NS108810
NIH-T32GM142520
NIH/NINDS-T32NS041218

Title: Analyses of the dorsal striatum and hippocampus during normal aging reveal a differential expression of inflammatory markers and perineuronal nets implicating a difference in regional plasticity.

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Abstract: Objective: This study aims to investigate the effects of aging on inflammatory changes and perineuronal net (PNN) dynamics in two brain regions implicated in neurodegenerative disorders (NDDs): the dorsal striatum (dStr) and hippocampus (hpc). **Methods:** Male wild-type mice aged to 4 and 22 months underwent behavioral tests, RNA

quantification, and immunohistochemistry (IHC) targeting PNNs, aggrecan (ACAN), parvalbumin (PV), and neuroinflammation markers. **Results:** Both regions showed elevated pro-inflammatory markers, gliosis, senescence, and complement-related markers including IL-6, TNF-α, and P16. The dStr exhibited elevated markers of phagocytosis (CD68*)* and CCL5, while the hpc showed IL-1β, MMP-3, and TIMP1 upregulation. Increased PNN-PV-ACAN co-localization occurred in the hpc, indicating decreased plasticity, while the dStr displayed persistent plasticity of PNNs and PV interneurons. Furthermore, regional analysis of the hpc revealed a significant increase in the intensity and number of PV cells with PNNs in the CA2 region of the hpc of aged mice, while no regional or age differences were observed in the dStr. **Conclusions:** Aging leads to region-specific increases in inflammation, with the hpc showing a marked increase in PNNs potentially limiting plasticity, suggesting a therapeutic avenue for hippocampal-dependent memory deficits. Furthermore, the CA2, a region important in the local excitatory circuitry of the hpc, may be a specific area of interest in understanding the E/I balance within the hpc. Conversely, the dStr maintains plasticity of PNNs and PV interneurons. These findings lay groundwork for further exploration in an Aging & Inflammation model to identify novel targets in NDD pathology progression. **Scientific Rigor:** Sample sizes were 5 male mice per group. IHC regions of interests ranged from 3 to 10 sections/mouse. Biological variables such as sex of experimental subjects were considered, and sex differences are currently being assessed in another cohort of animals. Data was tested for normality and Student t-tests or ANOVAs with Tukey tests were performed when applicable.

Disclosures: Z.A. Colon: None. **K.A. Maguire-Zeiss:** None.

Presentation Number: NANO58.09

Topic: C.01. Brain Wellness and Aging

Title: Investigating Hallmarks of senescence in the aging brain using a novel rna-protein multiomic co-detection assay

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Abstract: During early brain development, progenitor stem cells in specific brain regions like the subventricular zone and the Dentate Gyrus of the hippocampus differentiate into various neuronal and glial cell types. Through precise regulation and signaling, these progenitor stem cells contribute to rapid brain development and play a crucial role in learning, memory, and cognition. In contrast, aging in the brain is marked by long, gradual changes in structure, function, and biochemistry, affecting cognitive capacities. As the brain ages, it undergoes synaptic pruning, alterations in neuronal connectivity, a decline in neurogenesis, and increased oxidative stress and inflammation. These changes contribute to a significant decrease in cognition, memory, and learning. Due to the lack of robust methods for detecting biomarkers of aging, the role of stem cell and senescence markers is not well studied. Assessing the aging brain requires a multiomic strategy to identify these unique aging-associated markers and their interactions with other cells within the hippocampus..Using the flagship single-cell spatial

Multiomic LS RNAscopeTM technology, target gene and protein expression can be visualized simultaneously *in situ* on the same tissue section using an automated workflow on a Leica BOND Rx instrument. With this novel Tyramide Signal Amplification-based co-detection assay, we visualized up to 6 RNA and protein marker panels on mouse hippocampal sections of young and aged mice.The neurogenesis path was tracked by utilizing RNA probes targeting different stages of progenitor stem cells, such as radial glial cells (*Ascl1*), neuroblasts *(Calb2*), proliferation *(Top2a*), and neuronal maturation (*Prox1*). RNA probes targeting markers for senescence *(P16, Cdkn2d*) and immune activation (*Tnfa*) along with cell profiling antibodies (NeuN, IBA1, and GFAP), were also included in the panel. Aging hallmarks in the brain tissue were characterized by studying the co-expression of RNA and protein markers. Co-expression of senescence and cell type-specific markers enabled us to assess the effect of aging on the hippocampal microenvironment. We observe distinct differences in aging-related signatures between young and old mouse brains.The assay offers a powerful technique for visualizing target RNA biomarkers in specific cell types identified by cell-marker protein expression. This tool is valuable for multiomic analysis and accurate interrogation of complex tissues such as the brain to obtain insights into novel prognostic and therapeutic biomarkers in neurodegenerative disorders.

Disclosures: D. Wakhloo: None. **J. Yu:** None. **A. Dikshit:** None. **G. Kim:** None. **S. Deshpande:** None. **J.V. Pulliam:** None. **L. wang:** None. **M. Srinivasan:** None.

Presentation Number: NANO58.10

Topic: C.01. Brain Wellness and Aging

Title: Preliminary effects of improving specific cognitive domains with American elderberry juice in mild cognitive impairment patients

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Abstract: In prior nutritional diet interventions with high antioxidant/anti-inflammatory properties (anthocyanin-rich foods, e.g., blueberries and elderberries) findings showed a decreased risk in memory loss and cognitive decline. However, the effects of anthocyanin-rich foods on cognition in a mild cognitive impairment (MCI) population is limited. Therefore, we examined preliminary effects of American elderberry juice on various cognitive domains in MCI patients in a double-blind placebo-controlled trial. For 6-months in a randomized double-blind clinical trial, MCI patients (*n*=24, Mage=76.33±6.95) received elderberry (*n*=11) or placebo (*n*=13) juice (5mL) 3-times-a-day. Patients completed various cognitive tasks at baseline, and 3- and 6-month follow-ups, including: Hopkins Verbal Learning Test (HVLT), Boston Naming Test (BNT), Rey Complex Figure Test (REY-CFT), Anagrams, and Visuospatial Problem Solving task (VPS). Scores from each cognitive measure were calculated as a *z*-score. Cognitive total correct *z*-scores for each cognitive domain were averaged to calculate a global cognitive score. Global scores for cognitive flexibility [average of Anagram and VPS latency (ms) correct *z*-scores], verbal recall (average *z*-score for HVLT recall subscores), verbal recognition (average

z-score for HVLT recognition subscores), and delayed recall (average of HVLT and REY-CFT delay recall *z*-scores) were also computed. Multilevel models examined the interaction between condition (elderberry/placebo) and time (baseline/3-months/6-months) on various cognitive domains. Interactions were further evaluated by Kenward-Roger pairwise tests. There was a significant interaction between condition and time on overall cognitive flexibility (p=.049). Specifically, in the elderberry condition (not placebo) there was a trend (p=.08) towards a significant decrease (better) in global cognitive flexibility latency abilities from baseline (M=30.10, SE=5.96) to 6-months (M=16.7, SE=6.85). The interaction between condition and time were not significantly associated with global cognition, recall, recognition, or memory scores (ps>.05). In MCI patients, elderberry (not placebo) juice may provide cognitive flexibility benefits. Inflammation has previously been associated with poor cognitive functioning, and the anti-inflammatory properties in elderberry juice may attenuate the negative impact of inflammation on cognitive flexibility. The promising preliminary cognitive findings provide support for larger prospective studies to further investigate potential mechanisms of action in elderberries to mitigate cognitive decline in MCI populations.

Disclosures: M. Musich: None. **A. Curtis:** None. **B. Ferguson:** None. **A. Thomas:** None. **C.M. Greenlief:** A. Employment/Salary (full or part-time):; Employment: University of Missouri and the National Science Foundation. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Grant: National Institute of Food and Agriculture – USDA (current funding), National Science Foundation (current funding). **J.I. Shenker:** None. **D.Q. Beversdorf:** F. Consulting Fees (e.g., advisory boards); Quadrant Biosci, YAMO Pharma, Impel Pharma, Scioto Biosci, Stalicla Biosci.

Presentation Number: NANO58.11

Topic: C.01. Brain Wellness and Aging

Support: BrightFocus Foundation Grant
NIH/NIA RF1AG046205

Title: Apoe genotype differentially impacts the benefits of calorie restriction in aging

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Abstract: Background: The efficacy of Calorie Restriction (CR) in enhancing cognition, promoting healthy aging, and extending lifespan is well-established. Yet, it remains unclear whether the *APOE* (apolipoprotein E) genotype, a known modifier for aging and age-related disorders, influences the beneficial effects of CR in countering aging. **Methods:** We examined humanized APOE mouse models (referred to as E2, E3, and E4 mice) subjected to ad libitum (AL) feeding or 30% CR feeding starting from 12 months of age (N= 11-20/ genotype /group, mixed sex) for 6-8 months. We used the Comprehensive Laboratory Animal Monitoring System (CLAMS) to monitor energy expenditure, assessed anxiety and memory through various behavioral tests, and profiled gene expression in the brain through bulk RNA sequencing. The findings from the transcriptome analysis were validated using immunostaining and western

blotting. **Results:** CLAMS measurements showed that CR significantly decreased the metabolic rate in E3 and E4 mice, but not E2 mice, during the non-feeding period of the CR group compared to the AL group. Behavior tests revealed that CR reduced anxiety and enhanced associative memory in E3 and E4 mice. Furthermore, brain transcriptomics revealed a pronounced upregulation of cholesterol synthesis in CR groups of E3 and E4 mice, potentially linked to enhanced myelination and differentiation of oligodendrocyte precursor cells based on the downstream functional prediction of the differentially expressed genes. Consistently, in an independent cohort, we observed increased myelin intensity (MBP+), number of oligodendrocytes (ASPA+) and oligodendrocyte precursor cells (Pdgfra+) in CR groups of E3 and E4 mice, but not in E2 mice. **Conclusions**: Our study highlights the role of *APOE* genotypes in modulating the diverse effects of CR on energy metabolism, cognition, and myelination, providing a crucial foundation for considering *APOE* genotypes in developing prevention strategies for aging and age-related disorders.

Disclosures: W. Qiao: None. **N. Zhao:** 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/NIA R01AG066395 (PI), NIH/NIA RF1AG046205 (PI), NIH/NIA RF1AG056130 (MPI), Cure Alzheimer's Fund (PI), BrightFocus Foundation Grant (PI).

Presentation Number: NANO58.12

Topic: C.01. Brain Wellness and Aging

Support: DA056288
DA13137
DA059310
NS100624
GM109091

Title: Selective inhibition of CDK8/19 Mediator kinase improves hippocampal senescence

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Abstract: Alleviating the onset of aging-associated disease, such as: Alzheimer's disease, Parkinson disease, etc., has become the major goal in neuroscience studies. Previously, we demonstrated that selective inhibition of CDK8/19 Mediator kinase using a medicated diet containing brain permeable CDK8/19 inhibitor (Compound 25) improves age-related cognitive decline in mid-aged mice (10 months old). In the current study, we investigated the effect of Compound 25 on senescence (telomere length, senescence-associated β-galactosidase activity (SA-β-gal), autophagy dysfunction, and Tau phosphorylation to enhance our understanding of CDK8/19 Mediator kinase activity relative to cognitive decline. First, a postmortem evaluation

of SA-β-gal in the CA1 region of the hippocampus revealed significantly decreased SA-β-gal in mice treated with Compound 25. Next, telomere shortening, and mitochondrial copy number displayed an improvement with CDK8/19 Mediator kinase inhibition, compared to control group, supporting treatment-induced improvements in age-related hippocampal senescence. Interestingly, P62, a negative feedback marker of autophagy was significantly decreased in hippocampus in the Compound 25-treated group (less P62 accumulation and inclusion) compared to control group. Furthermore, alleviation of hippocampal P_2X_7R level (expressed in microglia) indicated a lower level of microglia activation and inflammation in mice treated with Compound 25. Additionally, the distribution of total Tau and Tau phosphorylation markers (Ser396, Ser404, and Ser202) in the hippocampus were assessed, and the results showed that Tau phosphorylation at Ser404 site was decreased after CDK8/19 Mediator kinase inhibition relative to control group, with no changes at Ser396 and Ser202 sites of Tau phosphorylation. Overall, our study provided comprehensive phenotypes of CDK8/19 Mediator kinase inhibition in determining hippocampal-associated spatial learning and memory function, and pharmacological treatments able to effectively slow the onset and development of age-related cognitive and neuronal decline.

Disclosures: R. Booze: None. **H. Li:** None. **K.A. McLaurin:** None. **C.F. Mactutus:** None. **A.C. Sharko:** None. **M. Chen:** None. **E. Broude:** None. **I. Roninson:** None.

Nanosymposium

NANO59: ALS and Motor Neuron Diseases

Location: MCP Room N427

Time: Wednesday, October 9, 2024, 1:00 PM - 3:30 PM

Presentation Number: NANO59.01

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R35NS132179

Title: Whole genome screen in human iPSC derived neurons for regulators of nuclear cytoplasmic transport identify genes that control TDP-43 transport and links to novel sporadic ALS genes

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Abstract: Nucleocytoplasmic transport (NCT) dysfunction is an early event in Amyotrophic lateral sclerosis (ALS) pathogenesis. Current understanding of the NCT primarily stems from studies conducted in mitotic cells such as yeast and immortalized cell lines. This leaves a substantial gap in our understanding of NCT in postmitotic neurons and the neuron-specific

vulnerabilities linked to various neurodegenerative diseases. To address this, we utilized fluorescence-activated cell sorting (FACS)-based whole genome screening on neurons derived from induced pluripotent stem cells (iNeurons). Overcoming the challenge of NCT reporter leakage during FACS, we developed a novel system called INFINITE (Induced Freezing in Nucleus of Import/Export Reporter), which accurately maintains the integrity of NCT reporter levels in isolated nuclei over extended sorting durations. Employing this system alongside CRISPR interference (CRISPRi) and a newly developed compact dual-guide sgRNA whole genome library, we conducted screens at multiple stages of neuronal differentiation. Through next-generation sequencing of sgRNAs from variously sorted FACS fractions, we pinpointed a set of neuron-specific NCT regulators, validating over 90% of these using individual gRNAs. Further, to explore the potential influence of these regulators on TDP-43 transport, crucial in ALS, we knocked down these genes in iNeurons and assessed TDP-43 localization and its role in preventing cryptic exon accumulation and identified neuronal TDP-43 NCT regulators. Furthermore, we validated that the protein levels of the newly identified hits were indeed reduced in the motor cortex of ALS patients compared to age-matched non-ALS controls. This investigation not only advances our understanding of NCT in neurons but also opens new avenues for developing therapeutic strategies to correct NCT defects and mitigate neuronal loss in ALS.

Disclosures: L. Ruan: None. **J.F. Reyes:** None. **M. Elrick:** None. **M. Ward:** None. **J.D. Rothstein:** None.

Presentation Number: NANO59.02

Topic: C.06. Neuromuscular Diseases

Title: A druggable genome-scale optical pooled screen for STMN2 cryptic exon expression in a TDP-43 depletion ALS cell model

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Abstract: One of the hallmarks of amyotrophic lateral sclerosis (ALS) pathology is the cytoplasmic aggregation and loss of nuclear function of the RNA-binding protein TDP-43 in patient motor neurons. Nuclear depletion and cytoplasmic accumulation of TDP-43 are known to affect splicing of downstream genes, leading to truncation or inclusion of cryptic exons (CEs) in critical neural genes such as STMN2. We modeled TDP-43 depletion in iPSC-derived motor neuron cells with a 48-hour, 1uM treatment of siRNA targeting the TARDBP gene. We also developed a fluorescence in situ hybridization (FISH) assay and computational pipeline to measure STMN2 full length (FL) and cryptic exon (CE) transcript abundance at the single-cell level. Using this assay setup, we performed an optical pooled CRISPR knockout screen of 1,924 genes in the druggable genome for targets that could reduce CE expression and potentially recover correct splicing and downstream gene function. We quantified STMN2 CE and FL levels and identified CRISPR guides of 3.6 million TARDBP siRNA-treated and 619 thousand nontargeting siRNA-treated cells in this screen, using high-throughput computer vision pipelines to read out and match phenotypes and CRISPR perturbations. By calculating gene- and guide-

level residuals for STMN2 CE and FL FISH dots relative to nontargeting KO controls, we were able to identify positive control genes (STMN2, TARDBP, and AGO2) as well as novel protein hits that regulate STMN2 CE and FL transcript expression in both NT and TARDBP siRNA-treated conditions. These hits are currently undergoing validation in various formats (imaging, multi-electrode array, and qPCR) to confirm their effects on biomarker phenotypes as well as functional firing readouts. In summary, we developed a human iPSC-based model of TDP-43 depletion, conducted a FISH-based high-throughput optical pooled screen to detect downstream STMN2 splicing defects, and used these approaches to detect potential genetic revertants of ALS pathology.

Disclosures: C.V. Hao: A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **F. Yi:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **S. Sivanandan:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **J. Liu:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **Y. Kwan:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **B. Leitmann:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **A. Ewer:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **M. Vuppalapaty:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **E. Krauel:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **I. discovery labs:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **S. Sances:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **S. Tu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **M.R. Salick:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified

mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **N. Ranu:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb. **A. Kaykas:** A. Employment/Salary (full or part-time):; insitro. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); insitro. Other; Discovery Collaboration with Bristol Myers Squibb.

Presentation Number: NANO59.03

Topic: C.06. Neuromuscular Diseases

Support: Active Against ALS
ALS Finding a Cure

Title: Characterizing the role and mechanisms of gene fusions in amyotrophic lateral sclerosis

Authors: *T. PETROZZIELLO[1], S. HUNTRESS[1], J. LEMANSKI[1], A. CASTILLO-TORRES[1], C. DE ESCH[1], D. GAO[1], M. CUDKOWICZ[1], J. BERRY[1], R. MOURO PINTO[1], G. SADRI-VAKILI[2];
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Abstract: Although our knowledge of the mechanisms underlying amyotrophic lateral sclerosis (ALS) is growing, the genetic cause of over 80% of the disease is still unknown. A potential genetic cause may be due to genomic structural variants, such as gene fusions, which occur when two independent genes are juxtaposed to form a new one. Recently, we have demonstrated an enrichment of gene fusion events in ALS post-mortem brain and spinal cord by assessing publicly available RNA-Seq datasets using STAR-Fusion. Building on these findings, we leveraged the entire cohort of samples from Target ALS and the New York Genome Center ALS Consortium and performed paired RNA-Seq and whole-genome sequencing (WGC). Our results identified 426 ALS-specific gene fusions, 84 control-specific fusion, and 305 shared fusions between control and ALS and we demonstrated that the odds ratio of identifying a fusion in ALS sample is significantly greater than in controls. Currently, we are leveraging our paired RNA-Seq and WGS to determine whether an ALS-specific fusion is caused by genomic rearrangements and whether these alterations could contribute to gene fusions and ALS etiology. For example, we have begun to assess the molecular consequences of one of the most recurrent gene fusions in ALS: the inter-chromosomal fusion between YY1 Associated Factor 2 (YAF2) and RING1 and YY1 Binding Protein (RYBP), genes involved in chromatin remodeling and transcriptional regulation. YAF2 and RYBP interact with the polycomb group (PcG) proteins, and they both catalyze the ubiquitination of histone H2 ($H2AK119ub_1$). To determine the consequences of their fusion, human neuroblastoma SH-SY5Y cells were transfected with YAF2, RYBP or the YAF2-RYBP fusion plasmids and binding to PcG proteins were measured by co-immunoprecipitation and immunocytochemistry followed by immunofluorescence. Our results revealed that the interaction between YAF2-RYBP fusion and RING1A was increased, and, in turn, there was a decrease in $H2AK119ub_1$ levels, suggesting that YAF2-RYBP may alter the chromatin landscape. Ongoing RNA-Seq and transposase-accessible chromatin with sequencing (ATAC-Seq) will determine whether YAF2-RYBP fusion alters chromatin accessibility and thereby gene expression in ALS.

Disclosures: T. Petrozziello: None. **S. Huntress:** None. **J. Lemanski:** None. **A. Castillo-Torres:** None. **C. de Esch:** None. **D. Gao:** None. **M. Cudkowicz:** None. **J. Berry:** None. **R. Mouro Pinto:** None. **G. Sadri-Vakili:** None.

Presentation Number: NANO59.04

Topic: C.06. Neuromuscular Diseases

Support: DoD-W81XWH-22-1-0271

Title: A Semi-High Throughput Drug Discovery Platform Using Diseased Corticospinal Motor Neuron Health as a Readout, Facilitates Drug Discovery Efforts for Upper Motor Neuron Diseases

Authors: C. QUINTANILLA[1], B. HELMOLD[2], G. EKMEN[1], Z. Y. EROL[1], S. F. DUNNE[3], ***P. OZDINLER**[4];
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Abstract: Corticospinal motor neurons (CSMN), also known as the upper motor neurons (UMNs), are clinically relevant neuron populations. They play important roles in the initiation and modulation of voluntary movement, and their degeneration is a hallmark of numerous UMN diseases, such as amyotrophic lateral sclerosis, hereditary spastic paraplegia, and primary lateral sclerosis. Despite their clinical importance, none of the preclinal assays have considered their improved health as a prelude to any clinical trial, and none of the compounds that are tested for UMN diseases in clinic had information on their potential of improving diseased UMN health. To overcome this challenge and to build better and translational preclinical assays, we fist generated fluorescent CSMN that are diseased due to different underlying causes, by crossbreeding UCHL1-eGFP mice with well-characterized UMN disease models, that have proven CSMN vulnerability and progressive degeneration. We then established cultures on glass-bottom 96-well plates, in which diseased CSMN can be distinguished among other cells and neurons in culture. Upon treatment with riluzole, edaravone and NU-9, we investigated cellular responses of diseased CSMN to drug and/or compound treatment, using increased axon outgrowth and enhanced arborization/branching as an established readout for improved neuronal health. High throughput imaging and automated image analyses enable unbiased cellular assessment of CSMN and their neuronal health in a more robust and semi high-throughput drug discovery platform. Our results reveal that diseased CSMN respond to drug treatment. The responses of CSMN diseased due to different underlying causes, such as misfolded SOD1 toxicity, TDP-43 pathology, lack of Alsin function, mutations in the profilin gene and spastin gene differ greatly with response to treatment, suggesting that one treatment strategy may not be able to address the needs of the motor neurons that are diseased due to different underlying causes. Our studies not only set the stage for developing preclinical assessments for UMN diseases, but also help determine compounds that are best appropriate to a given cellular dysfunction or pathology.

Disclosures: C. Quintanilla: None. **B. Helmold:** None. **G. Ekmen:** None. **Z.Y. Erol:** None. **S.F. Dunne:** None. **P. Ozdinler:** None.

Presentation Number: NANO59.05

Topic: C.06. Neuromuscular Diseases

Support: School of Psychology and Neuroscience, University of St Andrews (UK)
MRC UKRI (UK)
Louis-Hansen Foundation (DK)

Title: Spatial transcriptomics reveals early changes in inhibitorysynaptic transmission in Amyotrophic Lateral Sclerosis

Authors: S. MORA[1], R. MONTAÑANA-ROSELL[3], S. NEWELL[5], M. FERNANDES[2], T. ALS[5], K. THORSEN[5], C. IMIG[3], I. ALLODI[4,1];
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Abstract: Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by the degeneration of somatic motor neurons (MNs). As the final output of the brain, MNs directly connect to the muscles, but they are activated by a complex network of excitatory and inhibitory spinal interneurons (INs). Previous research in our lab (1) showed that spinal V1 spinal inhibitory INs, positive for Engrailed 1 (En1) transcription factor (2) are affected in the disease prior to muscle denervation and MN degeneration (1): specifically, they lose their connections to MNs. Inhibitory IN dysfunctions can trigger maladaptive MN hyperexcitability, resulting in increased intracellular Ca^{2+} levels, oxidative stress, and endoplasmic reticulum stress, extensively reported in ALS (3). However, either MN- or V1 IN-related mechanisms might underlie the loss of synaptic inputs. To understand which cell type initiates degeneration, spatial transcriptomics where performed on V1 INs and MNs at three different timepoints: postnatal days 45, 63 and 83, in the $SOD1^{G93A}$ ALS mouse model (4), with Wild-type littermates used as controls. The novel GeoMX Digital Spatial Profiling platform (Nanostring) (5) , coupled with RNAscope, enabled us to isolate V1 INs and MNs, and the whole transcriptome was obtained from En1+ (V1) and Chat+ (MN) neurons. Bioinformatic analysis was performed while maintaining spatial resolution of neurons at the different timepoints. Differential gene expression (DGE) and enrichment analysis performed in control V1 INs and MNs showed a differential signature in the overall synaptic machinery of the two populations: specifically, transcripts involved in neurosecretion, synaptic vesicle priming, pre and post synaptic element regulation were preferentially expressed in V1 INs. Moreover, DGE performed in ALS mice over time revealed changes in transcripts expression, such as *Unc13a*, *Unc13c*, *Snap25* and *Stxbp1*. Together, these results report differences in the synaptic machinery of V1 INs and MNs, and highlight the contribution of early changes at synaptic levels regarding V1 INs in ALS.
References: 1) Allodi I et al 2021 *Nature Communications* 12, 3251 2) Gosgnach S et al 2006 *Nature* 440(7081):215-9 3) Jensen DB et al 2020 *Journal of Physiology* 598 (19), 4385-4403 4) Gurney ME et al 1994 *N Engl J Med* 331:1721-2 5) Beechem JM (2020) *Methods in Molecular Biology* vol 2055
Funding: School of Psychology and Neuroscience, University of St Andrews (I.A., UK), MRC UKRI (I.A., UK), Louis-Hansen Foundation (I.A., Denmark).

Disclosures: S. Mora: None. **R. Montañana-Rosell:** None. **S. Newell:** None. **M. Fernandes:** None. **T. Als:** None. **K. Thorsen:** None. **C. Imig:** None. **I. Allodi:** None.

Presentation Number: NANO59.06

Topic: C.06. Neuromuscular Diseases

Support: MOE-T2EP30220-0014
NRF2019-NRF-ISF003-3221

Title: Splice-at: an integrated pipeline for detection and quantification of aberrant transcripts with novel splicing events

Authors: *S.-C. LING[1,2], Y. LIM[1], I. AGRAWAL[1], J. HO[3];
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Abstract: Splicing misregulation, such as the inclusion of previously unknown cryptic exons, is implicated in numerous diseases. Recent advances in tools have enabled accurate and efficient detection of such splicing alterations in disease phenotypes. However, the quantification and differential analyses of splicing alterations remain at a splice event level, thus preventing a complete view of the effects on the downstream transcriptomic landscape. Here, we present a novel and integrated pipeline, SpliCeAT, that (1) detects and quantifies differential splicing events from short-read bulk RNA-seq data, (2) augments the canonical transcriptome with novel transcripts containing these differential splicing events, and (3) performs transcript-level differential analysis to identify and quantify aberrant cryptic exon-containing transcripts based on this “augmented transcriptome”. Using TDP-43, an ALS/FTD-associated RNA-binding protein, as an example, we identified and catalogued aberrant splicing events in embryonic mouse brains. The accuracy of this integrated pipeline was further confirmed and validated with long-read isoform sequencing. Furthermore, by comparing neuronal TDP-43 knockouts in mice to an available human dataset with TDP-43 pathology, we identified 4 common genes, namely, Kalrn/KALRN, Poldip3/POLDIP3, Rnf144a/RNF144A, and Unc13a/UNC13A, with cryptic exons. In summary, our method provides a clearer view of how novel splice events are incorporated on the transcript level, thereby enabling more complete profiling of transcript-level modifications.

Disclosures: S. Ling: None. **Y. Lim:** None. **I. Agrawal:** None. **J. Ho:** None.

Presentation Number: NANO59.07

Topic: C.06. Neuromuscular Diseases

Title: Extracellular vesicle release contributes to shaping the immunophenotype of C9orf72knock-out mice.

Authors: *M. CICARDI[1], D. TROTTI[2];
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Abstract: The hexanucleotide repeat expansion in the intron 1a of the C9orf72 gene is the most common cause of familial and sporadic amyotrophic lateral sclerosis (ALS). As a result of this mutation, the encoded protein levels are reduced, producing C9orf72 haploinsufficiency.

C9orf72 reduction leads to lysosomal damage, dysregulation in autophagy levels, and impaired lipid metabolism. The autophagy pathway is tightly related to the release of extracellular vesicles (EVs) from cells. The release of small EVs (exosomes) results from the fusion of the multi-vesicular bodies with the plasma membrane, and thus, changes in autophagy are paralleled by alterations in EV release. We hypothesized that C9orf72 haploinsufficiency alters the release of EVs and their content, contributing to the state of neuroinflammation present in C9orf72-linked ALS. We used C9orf72 knock-out (KO) mice from which we cultured primary neurons, astrocytes, and microglia. After bringing these primary cells to maturation, we isolated EVs from cell culture media by ultracentrifugation. Surprisingly, only C9KO microglia showed increased EV release, while neurons and astrocytes showed no differences. We also analyzed lipidomic and proteomic profiles, finding no difference between groups in any cell types. We then hypothesized that different EV production in C9KO microglia could affect the presence and the fate of the immune cells in the central nervous system (CNS). By flow cytometry, we thus immunophenotyped the immune cell populations in the cortices and the spinal cord of C9orf72 WT and KO mice. By multi-parameter reduction analysis, we identified multiple populations in the different genotypes. Strikingly, we found many differences between control and C9KO mice at 8 months of age. To understand if EVs contribute to these differences, we treated C9KO mice with two different EV release inhibitors: DPTIP and GW4869. Notably, we observed that some of the immune cells identified in C9KO mice revert their phenotype to one more resembling the one that characterizes WT animals. We did not find changes in the behavior of mice treated with EV release inhibitors. Based on our work, we have concluded that C9orf72 plays a role in regulating the release of EVs and immune cell features in the central nervous system. Our findings suggest that the absence of C9orf72 can lead to dysregulation of EVs, which in turn can affect the immune cell phenotypes observed in C9KO mice. These results highlight the importance of understanding EV biology in order to better comprehend the neuro-immune interactions involved in ALS.

Disclosures: M. Cicardi: None. **D. Trotti:** None.

Presentation Number: NANO59.08

Topic: C.06. Neuromuscular Diseases

Support: AFM-Telethon Research Grant #25060
AFM-Telethon Trampoline Grant #23648
NeuroImpulse IDIBELL 2022

Title: Deciphering the role of extracellular matrix in amyotrophic lateral sclerosis pathophysiology

Authors: *J. A. ORTEGA;
Pathology and Exptl. Therapeut., Univ. of Barcelona, Barcelona, Spain

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating adult-onset neurodegenerative disease characterizedby the degeneration of upper and lower motor neurons (MNs) that leads to muscle weakness, spasticity,and atrophy. Although extensive number of studies during the last decades have uncovered multiplemechanisms associated to this disease, the frustrating reality is that no effective treatments are yet available. Luckily, the rapid development of OMIC

technologies have allowed to identify new promising molecular targets for therapeutic intervention associated to protein homeostasis, RNA processing or DNA damage, among others. Although these studies have also identified alterations in extracellular matrix (ECM) components in the ALS context, little is known about their contribution to the disease. The ECM is an intricately arranged scaffold of secreted proteins and complex sugars that in the central nervous system regulates the maintenance of the neural functions and plays a central role in key events after injury or in disease. The structural and chemical changes in the composition of the ECM can affect axonal regrowth as well as communication, migration, and survival of multiple cellular components that are involved in motor function control in the spinal cord. Nonetheless, studying the ECM is particularly challenging because of its biochemical and biophysical complexity, leading us to hypothesize that its role in ALS might have been considerably underestimated. In this study, we have combined distinct profiling techniques to decipher changes in the ECM components of the spinal cord that specifically occur in ALS. We have also investigated the contribution of ALS-associated ECM proteins to MN degeneration using distinct ECM signal-mimetic strategies on human induced pluripotent stem cells derived MNs. Results obtained so far encourage us to further pursue rationally driven designs of ECM mimetic matrices to better understand ALS pathophysiology and to design novel therapeutic approaches.

Disclosures: J.A. Ortega: None.

Presentation Number: NANO59.09

Topic: C.06. Neuromuscular Diseases

Support: ALS Association
Live Like Lou Foundation

Title: Role of mutant SPTLC1 in juvenile amyotrophic lateral sclerosis

Authors: *D. PANT[1], J. PARAMESWARAN[2], M. LONE[4], T. HORNEMANN[5], J. JIANG[3]; [2]Cell Biol., [3]Dept. of Cell Biol., [1]Emory Univ., Atlanta, GA; [4]Inst. of Clin. Chemistry, Univ. Hosp. Zürich, Schlieren, ; [5]Univ. Hosp. Zurich, Zurich, Switzerland

Abstract: Juvenile amyotrophic lateral sclerosis (JALS) is a rare condition affecting motor neurons, marked by their gradual deterioration. Recent findings have linked mutations in the human SPTLC1 gene to JALS. This gene encodes Serine Palmitoyltransferase, a vital enzyme in sphingolipid production. Studies have revealed that missense and deletion mutations in SPTLC1 are linked to ALS onset in childhood. Notably, these mutations occur within the gene's transmembrane domain, leading to an overproduction of sphingolipids. However, the precise mechanism by which changes in sphingolipid levels contribute to the disease remains unclear. We analyzed SPTLC1-JALS mutation using both in vitro and in vivo models. Through targeted lipidomics studies, we identified alterations in sphingolipid profiles in the SPTLC1-JALS mutant. These findings suggest that mutations in SPTLC1 disrupt sphingolipid metabolism, causing cellular abnormalities that ultimately result in neuronal degeneration in JALS. Our goal is to delve deeper into the role and regulation of sphingolipid signaling and biology in ALS. By understanding how sphingolipids impact ALS development, we can pinpoint targets for novel therapies aimed at preventing or mitigating the severity of ALS and related neuropathies.

Disclosures: D. Pant: None. **J. Parameswaran:** None. **M. Lone:** None. **T. Hornemann:** None. **J. Jiang:** None.

Presentation Number: NANO59.10

Topic: C.06. Neuromuscular Diseases

Support: Associazione Girotondo ODV
SMArathon Onlus

Title: Time-dependent alteration of projection neurons due to lack of SMN protein significantly impacts the cortical cytoarchitecture in a murine model of Spinal Muscular Atrophy

Authors: *R. SCHELLINO[1,2], A. CARETTO[1,2], G. IEZZI[1,2], M. BOIDO[1,2], A. VERCELLI[1,2]; [1]Neurosci., Univ. degli Studi di Torino, Torino, Italy; [2]Neuroscience Institute Cavalieri Ottolenghi, Orbassano, Italy

Abstract: Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disease of the early childhood, caused by the mutation/deletion of the survival motor neuron (SMN1) gene. The lack of functional SMN protein was believed to specifically determine the selective degeneration of lower motor neurons (MNs), resulting in progressive skeletal muscle denervation. However, recent research in animal models and patients revealed that the brain is also impacted by SMN deficiency, thus the involvement of cortical alterations in SMA needs to be clarified. Therefore, in this study we focused on the sensorimotor cortex of SMAΔ7 mice, as a severe model of SMA, to examine how SMN protein deficiency affects survival and organization of cortical projection neurons in time. We analyzed early (postnatal day 5) and late (P11) symptomatic animals, comparing SMA mice with their wild-type (WT) littermates (N>4 each group). We used immunofluorescence to identify neuronal subtypes and retrograde tracers for morphological analysis of projection neurons. To investigate whether SMN depletion can affect projection neurons since cortical development, we performed thymidine-analogues (EdU) labeling in the dams at different embryonic time points (E12, E14, E15, E17) and mapped EdU+ cell distribution in the cortical layers of pups. We confirmed a 47% (± 3.8) reduction in projection neuron density in layer V of SMA cortex at P11. Indeed, looking at different projection neuron subtypes, we found a reduction of 50% (± 6.2) and 36% (± 2.8) of corticospinal (Ctip2-positive) and callosal (Satb2-positive) neurons respectively, together with a reduction of vGlut1 signal of about 38% (± 4.6), suggesting that SMN loss affects upper MNs as well. Moreover, SMA cortical cells also show alteration in some morphological traits. Although the same analyses in early symptomatic brains (P5) suggest that these changes occur concurrently with spinal MN death and are not manifested earlier (with a reduction of cortical projection neurons by only 14% in SMA mice), the evaluation of cell birthdating and distribution by thymidine analogues are helping us in revealing possible alterations in SMA cortex already at developmental stages. Indeed, preliminary data show a different distribution of EdU+ cells born at E14 and E15 in the cortical layers of SMA brain compared to WT. Overall, we found an alteration in cortical cytoarchitecture in SMA which could contribute to the etiopathology of the disease. Understanding the involvement of the cerebral cortex in the pathogenesis of SMA will help to understand any developmentally-related cognitive or neuropsychological deficits associated with the disease, along with motor deficits.

Disclosures: R. Schellino: None. **A. Caretto:** None. **G. iezzi:** None. **M. Boido:** None. **A. Vercelli:** None.

Nanosymposium

NANO60: Spinal Cord Injury: From Transplantation to Recovery

Location: MCP Room S404

Time: Wednesday, October 9, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO60.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH 5R01NS119297-03
NIH 3R01NS119297-03S1
MN OHE SWIFT 232024

Title: Development and application of 3D printed spinal neural progenitor cell scaffolds for spinal cord injury.

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Abstract: Spinal cord injury (SCI) is associated with profound changes in sensory and motor performance that severely impact quality of life, often leaving patients paralyzed and with little chance of complete recovery. Cell transplantation is among the most promising treatments for SCI given its potential for targeted neural regeneration and repair in sensorimotor systems. However, a lack of host to graft integration can limit the capacity for survival, regeneration, and connectivity. Combinatorial approaches that utilize scaffolds to provide a support structure for axonal extension may provide the necessary balance of cellular and structural support needed to promote meaningful improvement. Our lab has previously utilized a multi-channel silicone scaffold in which human induced pluripotent stem cell (hiPSC) derived spinal neural progenitor cells (sNPCs) can be 3D-printed in precise configurations. We report here our results characterizing these 3D-printed sNPC scaffolds and their applications to target repair in sensorimotor circuits. Using growth factor mediated differentiation, distinct populations of region-specific spinal neurons localized to both dorsal sensory and ventral motor circuits are derived from hiPSCs and then printed directly into scaffold channels. We demonstrate the importance of cell type, cell density, bioink composition, and scaffold material considerations for bioprinting outcomes that optimize viability and integration. These cells form 3D assembloids within the scaffold and express markers consistent with neural (NeuN, Mapp2, Tubb3), astrocyte (S100B), and oligodendrocyte progenitor (APC) identity. Mature scaffolds (>20 days) produce a diverse array of functionally active spinal neuronal subtypes. Cell-laden scaffolds may subsequently be used for both *in vitro* and *in vivo* applications for the study and advancement of spinal cord therapeutics. This combinatorial approach with both neural cell therapy and bioengineering demonstrates a novel technology for the field of spinal regeneration and advancement of the translation of cell transplantation therapies.

Disclosures: A. Huntemer-Silveira: None. **H. Kim:** None. **W. Chai:** None. **A. Parr:** None.

Presentation Number: NANO60.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS, NIH; R01-NS104291
NIH; T32-NS121768

Title: Temporal dynamics of donor-host connectivity in fetal spinal tissue transplants for SCI repair

Authors: *A. HALL[1], A. NICEFORO[2], V. C. OGBOLU[3], K. SCHARDIEN[4], L. V. ZHOLUDEVA[5], L. QIANG[3], M. A. LANE[6];
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Abstract: Cervical spinal cord injuries can severely disrupt motor, sensory, and autonomic functions, critically impairing breathing by compromising the phrenic motor network. Current therapeutic interventions such as activity-based therapy and neural stimulation offer limited improvements as they rely on spared neural tissue and do not address the fundamental anatomical damage. Cell transplant strategies, particularly using fetal spinal cord (FSC) tissue, present a promising alternative by potentially repairing the damaged anatomy and introducing new neuronal circuits to reinnervate areas below the injury. Our research hypothesizes that transplantation of neural progenitor cells, enriched in spinal interneurons (SpINs), can facilitate tissue repair, and establish donor-host synaptic connectivity. Although previous studies have shown that transplants derived from embryonic (E)13.5 spinal cords differentiate into diverse SpINs and glia, contributing to functional recovery within a month, the long-term sustainability of these newly formed donor-host connections and their effectiveness remain underexplored. Here, E13.5-GFP tissue was transplanted into adult rats one week after a lateralized cervical contusion. We employed transsynaptic pseudorabies virus (PRV) tracing to map the synaptic integration between transplanted cells and the host phrenic network at 1 (short term) and 12 (long term) months post-transplantation. Functional recovery of the phrenic circuit was assessed via diaphragm electromyography (dEMG). PRV tracing revealed donor to host synaptic integration one-month post-transplantation, but this integration was lost at 12-months post-transplantation. Diaphragm activity at 1- and 12-months corresponded with the level of synaptic integration between transplanted cells and the host phrenic network. The findings from this study address the critical knowledge gap concerning the long-term efficacy of FSC transplants. In addition, the promising outcomes observed with FSC transplants and ongoing work provide a compelling premise for future studies exploring human spinal interneuron-rich organoids in SCI repair, as they may offer enhanced translational merit in clinical settings.

Disclosures: A. Hall: None. **A. Niceforo:** None. **V.C. Ogbolu:** None. **K. Schardien:** None. **L.V. Zholudeva:** None. **L. Qiang:** None. **M.A. Lane:** None.

Presentation Number: NANO60.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: State of Minnesota Office of Higher Education's Spinal Cord Injury and Traumatic Brain Injury Research Program

Title: Neurod1-mediated functional and neuroprotective effects after subacute spinal cord injury

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Abstract: Spinal cord injury (SCI) is a globally prevalent concern with no available treatments that restore nervous system function. As such, SCI research has focused on approaches that target regeneration of the spinal cord to promote functional recovery after injury. One such experimental approach, viral delivery of proneural factors for in vivo glia-to-neuron reprogramming, has emerged as a potential strategy for central nervous system (CNS) restoration. Published studies have identified NeuroD1, a developmental proneural factor, as sufficient to convert astrocytes into neurons both in vitro and in several animal models of CNS injury and disease. Significantly, however, there is only one published study using NeuroD1 to reprogram astrocytes for SCI, but this study did not assess motor or sensory functional recovery. Separately, other studies have reported an inconsistency in reprogramming efficiency and questioned the cellular origin of "reprogrammed" cells. In response, viral dosage and intervention timing have been identified as factors capable of influencing reprogramming efficacy and reproducibility, potentially explaining the reported inconsistencies. We hypothesize that the AAV9-NeuroD1 platform is capable of restoring nervous system function following SCI through astrocyte-to-neuron reprogramming and neuroprotection. Here we employed a two-vector, AAV9 DIO/FLEx-based delivery platform for selective expression of NeuroD1-mRuby2 (reprogramming) or mRuby2 alone (control) in GFAP-expressing reactive astrocytes after moderate SCI in female rats. The viruses were administered 1 week post-contusion (WPC) in the subacute stage of injury at one of two viral titers (10^{13} or 10^{11} GC/mL). Histological analysis was used to examine the extent of successful viral transduction throughout the spinal cord lesion area. Basso, Beattie, and Bresnahan (BBB) scoring was employed to assess motor function whereas the Von Frey filament and cotton swab tests were used to assess sensory function. Motor and sensory functional analysis of subacute NeuroD1-treated rats did not reveal any functional recovery up to 6 WPC. Preliminary analysis of spinal cord anatomical restoration revealed a NeuroD1-mediated decrease in the spread of the lesion cavity. Future work will continue to explore the spatial resolution and extent of reprogramming throughout the spinal cord along with assessing the additional potential neuroprotective effects of NeuroD1 treatment.

Disclosures: A. Roman: None. **M. Sorensen:** None. **A. Parr:** None. **A.W. Grande:** None. **W.C. Low:** None.

Presentation Number: NANO60.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation, SCIRTS Fellowship #890819

Title: Manipulating Post-Traumatic Astrogliosis to Improve Spinal Cord Injury Outcomes in Aging

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Abstract: Spinal Cord Injury (SCI) is the second most common cause of paralysis and places a significant life-long burden on patients. The average age of incidence has increased placing great importance on understanding SCI in an aging population. Experimentally, SCI is regularly modeled in young adult animals in contrast to the aging human population, hampering translation of research to the clinic. One hallmark of SCI is the astroglial scar. Age-dependent alteration in astroglial dynamics is likely to impact the efficiency of astrogliosis, and diminish the ability to quickly sequester the SCI lesion. This may decrease recovery potential and lead to worse outcomes. Understanding these age-dependent changes in the acute phase of SCI is highly significant to provide strategies to target this early response in aging animals. Mitochondria play important roles in aging and the progression of SCI. Dysfunctional astrocytic mitochondria may have a significant impact on the functional state of aging astrocytes. STAT3 is involved in reactive astrogliosis and mitochondrial activity, and is decreased with age. We hypothesize that the onset of astrogliosis is delayed and less effectual at sequestering the lesion with age, which impairs functional recovery. Using isolation and culture of primary astrocytes, we have analyzed mitochondrial changes in young, aging and old mice. We observed that astrocyte mitochondrial activity is altered with age, with an increased mitochondrial membrane potential ($\Delta\Psi m$), reduced expression of OXPHOS proteins, increase in respiration, and ATP retention. We previously observed an age-dependent reduction in STAT3 expression and increase in its inhibition (PIAS3). Current data demonstrates manipulating STAT3 expression alters mitochondrial activity in isolated primary astrocytes. The manipulation of STAT3 and mitochondria effects astrocytes' ability to close artificial wounds in vitro, and the increase in STAT3 in primary astrocytes via deletion of SOCS3 (a negative regulator of STAT3) improves neurite growth in both young and old neurons. Increasing STAT3 also rescues mitochondrial activity in the presence of Rotenone, and STAT3 deletion exacerbates reactive oxygen species (ROS) production in astrocytes in vitro. In vivo the deletion of SOCS3 in astrocytes at the site of a T8 SCI expedited astrocyte border formation and improved hind-limb recovery. We postulate that by targeted manipulation of STAT3 in astrocytes after SCI, we can stimulate transient astrogliosis in acute injury in both young and aging mice to improve border formation and functional recovery.

Disclosures: T.C. Sutherland: None. **D. Horvat:** None. **A. Sefiani:** None. **C.G. Geoffroy:** None.

Presentation Number: NANO60.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Board of Governors Regenerative Medicine Institute

Title: An induced pluripotent stem cell-based therapy improves aging- and Alzheimer's disease-related outcomes in mice

Authors: *V. MOSER[1], L. DIMAS-HARMS[2], J. INZALACO[2], S. BELL[2], S. PARKER[3], C. N. SVENDSEN[2];
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Abstract: We and others have previously shown that young blood products like plasma or bone marrow improve cognition and neural health in mouse models of aging and Alzheimer's disease (AD). However, the practical use of these products is reduced by their inherent limited availability. Thus, we have focused on developing a similar young blood product from induced pluripotent stem cells (iPSCs), as this allows for unlimited production of the cells. Specifically, we have generated iPSC-derived mononuclear phagocytes (iMPs), which includes both monocytes and macrophages, as these cells are known to have important functional changes in aging and AD. Short-term intravenous infusions of iMPs significantly improved performance in learning and memory tasks in aging mice, as well as reduced neuroinflammation and restored the health of a key population of hippocampal neurons. We have now tested this cell therapy in the 5xFAD mouse model of AD, both as a prevention and intervention strategy. Specifically, 5xFAD mice were treated with iMPs beginning either at 3 months when pathology is first starting, or at 9 months when these animals already have significant AD-like pathology. iMP treatments rescued cognition at both timepoints and improved AD-associated changes in neural health and neuroinflammation in 9-month-old mice. Proteomic analysis of plasma and single nucleus RNA sequencing of hippocampus revealed potential targets underlying iMPs' mechanism of action. Thus, this iPSC-based therapy holds promise as both an early intervention strategy and as a potential treatment.

Disclosures: V. Moser: None. **L. Dimas-Harms:** None. **J. Inzalaco:** None. **S. Bell:** None. **S. Parker:** None. **C.N. Svendsen:** None.

Presentation Number: NANO60.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Veterans Affairs Grant I21RX004085

Title: The effect of 600 Hz sacral root stimulation on lower urinary tract function in an individual with spinal cord injury

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Abstract: Bladder dysfunction is an unmet challenge that is highly prioritized by individuals with spinal cord injury (SCI). Sacral anterior root stimulation (SARS) is effective in achieving bladder emptying for many individuals with SCI and difficulty emptying their bladder. However, individuals with reflex urethral sphincter contractions during bladder emptying require a rhizotomy, which is cutting the sensory nerves to stop the reflex urethral sphincter contractions. Cutting the sensory nerves yields downsides of inhibiting certain desirable reflexes, such as sexual, bowel, and loss of any remaining pelvic sensations. Stimulation at 600 Hz is believed to block synaptic transmission, which could be used to reduce urethral sphincter pressure and promote bladder emptying. With a study participant who was previously implanted with a SARS device and has had a rhizotomy, SARS was tested at 600 Hz frequency to determine the effect on urethral sphincter and bladder function during a single session. We used clinically standard

urodynamics and anorectal manometry methods to measure urethral pressure, anal sphincter pressure, bladder pressure, rectal pressure, and pelvic floor electromyogram (EMG) simultaneous with electrical stimulation of the sacral roots at 20 or 600 Hz. The mean urethral sphincter pressure in response to 20 Hz and 600 Hz SARS were 100±47 cmH2O and 77±32 cmH2O, respectively ($p = 0.67$). Anal sphincter pressures and pelvic floor EMG were similarly reduced from 38±8 to 4±8 cmH2O ($p = 0.026$) and from 406±27 to 132±31 mV ($p= 0.00097$), respectively. Stimulation frequency did not affect bladder or rectal pressures. This experiment is a first-in-human proof-of-concept study. While these data are promising, increasing our sample size is needed to draw accurate conclusions on the effects of 600 Hz stimulation on lower urinary tract function.

Disclosures: C. Kim: None. **R.F. Hoey:** None. **D. Bourbeau:** None.

Presentation Number: NANO60.07

Topic: C.11. Spinal Cord Injury and Plasticity

Title: A hybrid hPSC differentiation strategy for region-specific spinal neurons

Authors: *N. IYER;
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Abstract: Diverse spinal cord neurons along the body axis coordinate sensory processing, proprioception, and motor control, but human pluripotent stem cell (hPSC) differentiation methods often overlook the critical regional specificity of these cells. We previously developed a direct differentiation method to efficiently generate any spinal neuron along the rostrocaudal (R/C) axis; however, protocol duration, user expertise, and cell line-specific optimizations remain impediments to rapid experimental iteration. In addition, the preferential differentiation into glia versus neurons following *in vivo* transplantation remains a significant hurdle in efforts to restore depleted neuronal populations and form functional relays. Here we apply a dual-strategy combining direct differentiation and direct reprogramming with a TET-on NGN2 system to efficiently generate region-specific spinal neurons. Transduced hiPSCs are first patterned to neuromesodermal progenitor cells corresponding to discrete R/C regions. Region-specific spinal progenitors can then be patterned to dorsal or ventral progenitors by concentration dependent application of BMP4 of Shh agonists. Concurrent application of doxycycline enables swift transition to post-mitotic neurons, including motor neurons and locomotor interneurons from the ventral spinal cord, and proprioceptive or sensory interneurons from the dorsal regions. This method enhances efficiency while reducing media costs and can be rapidly applied to patient derived hiPSCs for personalized screens. We expect that this hybrid differentiation strategy will provide an efficient source of cells for both *in vitro* models and future transplantation applications.

Disclosures: N. Iyer: None.

Presentation Number: NANO60.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NS104442
Sanford Consortium CIRM Fellowship

Title: Transcriptome of Non-Human Primate Corticospinal Neurons Informs Therapeutic Window for Stem Cell Grafts

Authors: *A. GARBUZOV[1], E. SINOPOULOU[1], E. S. ROSENZWEIG[1], R. KAWAGUCHI[2], M. J. CASTLE[1], D. H. GESCHWIND[3], M. H. TUSZYNSKI[1];
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Abstract: How adult cortical neurons respond to injury and which pathways mediate survival, regeneration, and re-establishment of connectivity after axonal lesion remains a fundamental question in the field of spinal cord injury. We began to address this question by using the Glt25d2-eGFP-L10a mouse line to isolate RNA specifically from Layer 5 cortical neurons. Following spinal cord injury, we showed that corticospinal tract (CST) neurons revert to an embryonic-like state upon injury and that contact with a neuroprogenitor graft extends this pro-regenerative state to 21 days (Poplawski et al., 2020). The extent to which this result is generalizable to other organisms, including humans, is of fundamental importance. How accurately murine transcriptional studies reflect what happens in humans is still unknown. Do CST neurons of human patients show a similar window for intervention? To answer these questions, we developed a viral-based approach that allows for RNA isolation from any neurons that can be targeted by AAV injection. When applied to corticospinal neurons, this sequential immunoprecipitation-based approach allows for purification of RNA from whole motor cortex that is enriched in neuronal genes, depleted in glial genes, and enriched in Layer 5 marker genes. After validating this approach in rats, we applied it non-human primates (NHPs) to isolate RNA specifically from the corticospinal neurons of the hand/arm region in the primary motor cortex. We collected RNA from cynomolgus monkeys 3, 10, and 20 days after C7 hemisection spinal cord-injury as well as from uninjured controls. Together, this data shows that the CST response to injury in NHPs is very similar to mouse. The NHP response also involves the upregulation of embryonic genes and suggests that the window for optimal therapeutic intervention in primates is closing by day 20 post-injury. .

Disclosures: A. Garbuzov: None. **E. Sinopoulou:** None. **E.S. Rosenzweig:** None. **R. Kawaguchi:** None. **M.J. Castle:** None. **D.H. Geschwind:** None. **M.H. Tuszynski:** None.

Presentation Number: NANO60.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R21 NS111393-01A1
VA Merit Review Award I01RX02089-A2
VA BRAVE funds
NYS SCIRP DOH01-PART6-2023-00005
NIH R01 AI119090
NIH R44 DA051278

Title: Targeted-delivery of nanomedicine-enabled methylprednisolone to injured spinal cord promotes neuroprotection and functional recovery and mitigates adverse effects after acute spinal cord injury in rats

Authors: *W. QIN[1], W. ZHAO[2];
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Abstract: Introduction: Spinal cord injury (SCI) is a catastrophic medical problem that leads to loss of sensory, motor, and autonomic functions. To date, no therapy has been proven to be efficacious in fully restoring neurological functions post SCI. Systemic high-dose methylprednisolone (MP) treatment improves neurological recovery after acute SCI in both animal and human. The utility of high-dose systemic MP in acute SCI remains controversial due to its modest effect on functional recovery and the accompanying adverse effects. To overcome the limitation of MP therapy, we have developed a *N*-(2-hydroxypropyl) methacrylamide copolymer-based MP prodrug nanomedicine (Nano-MP) that can selectively deliver MP to the inflamed SCI site when administered systemically in a rat model of acute SCI, aiming to improve the therapeutic efficacy of MP while minimizing unwanted distribution to and actions on other tissues, thereby, reducing untoward side effects. **Methods:** Nano-MP was synthesized (Fig 1). Immediately after SCI, a single dose of Nano-MP was administered to the SCI rats via intravenous (i.v.) injection. Unconjugated MP and vehicle were subjected to a bolus i.v. injection and followed by 24-h infusion with the Bracken protocol. **Results:** After a single i.v injection, Nano-MP preferentially accumulated to the SCI injury site, sequestered (Fig 2) and retained mainly by $CD11^+$ infiltrating inflammatory cells. The Nano-MP significantly inhibited lipid peroxidation and inflammation of the injured spinal cord, resulting in reduced neuronal damage and enhanced neuroprotection after acute SCI. Compared to conventional i.v delivery of free MP, Nano-MP administration provided better functional improvement after acute SCI in rats and had far fewer systemic adverse side effects—that is, reduced muscle atrophy, bone loss, and less carbohydrate intolerance. **Conclusion:** Nano-MP is a promising drug candidate that is more effective and safer than standard MP therapy and it may be translated as a new treatment for acute SCI patients with improved functional recovery.

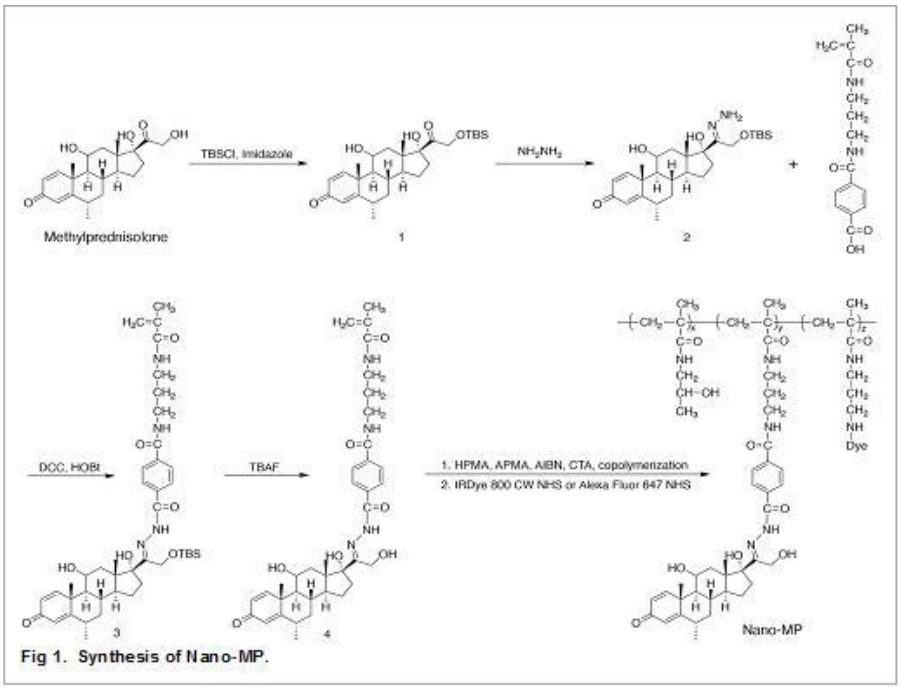

Fig 1. Synthesis of Nano-MP.

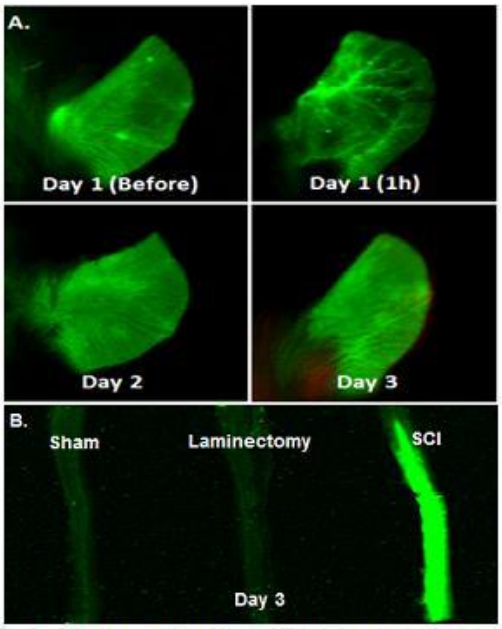

Fig 2. Selective uptake of Nano-MP in injured spinal cord. (A) Detection of Nano-MP in vivo at different time points post-injection. Male rats underwent complete T4 spinal cord transection or laminectomy only. Nano-MP-IRDye was injected via tail veins immediately after SCI. Blood vessels in rat ear were visible soon after injection and were not detectable at days 2 and 3. (B) Nano-MP was detected in the injured spinal cord at day 3, but not that in sham or laminectomy-only rats. Images were taken using a LI-COR imaging system (X4). N=10

Disclosures: W. Qin: None. **W. Zhao:** None.

Nanosymposium

NANO61: Stress and the Brain: From Pregnancy to Adulthood

Location: MCP Room S401

Time: Wednesday, October 9, 2024, 1:00 PM - 2:15 PM

Presentation Number: NANO61.01

Topic: G.05. Mood Disorders

Title: Effect of maternal SSRI exposure during pregnancy on the gut microbiome of offspring in rats

Authors: *D. KROPP[1], M. E. GLOVER[2], K. A. UNROE[3], G. E. HODES[4], S. M. CLINTON[2]; [1]Virginia Technol. SoN, Blacksburg, VA; [2]Virginia Technol., Blacksburg, VA; [3]Translational Biol., Med., and Hlth., Virginia Technol., Blacksburg, VA; [4]Neurosci., Virginia Technol., Blacksburg, VA

Abstract: Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed to people suffering from mood disorders, and are commonly prescribed during pregnancy (Zbigniew Marchocki et al., 2013). Despite this, little is known about the effect that serotonin reuptake inhibition has on the developing offspring. Furthermore, SSRIs have been shown to cause alterations in the gut microbiome of people taking them (Shen et al., 2021). This poses an important question about how SSRIs are affecting the gut microbiome of mothers during pregnancy. Gestation and birth are critical times for the transfer of microbiota from mother to offspring (Mueller et al., 2015). Because of this, we sought to understand what specific bacteria (down to the genus level) change due to SSRI exposure in utero, and if these changes differ by sex of the offspring. We investigated this by giving pregnant Sprague Dawley mothers citalopram, then using next generation shotgun sequencing methods on feces to quantify gut microbiome alterations in male and female offspring, as well as the mothers. Adult female Sprague Dawley rats randomly received either citalopram dissolved in drinking water (10 mg/kg/day); or vehicle (tap water) for a week prior to and through mating, as well as pregnancy and the postpartum period. On P40, fecal samples were collected (n=14-20/condition/sex) from male and female offspring. DNA from the samples was extracted and sequenced using an Illumina Novaseq 6000. Sequencing analysis was performed by filtering the reads by quality score, removing host genomic data, and quantifying relative abundances using Metaphlan 4.0 (Aitor Blanco-Míguez et al., 2023). We expect that small changes in the gut microbiome of SSRI treated mothers will be amplified in the offspring. We also expect that male and female rats will show sex specific alterations to the gut with males showing a greater level of change compared to controls.

Disclosures: D. Kropp: None. **M.E. Glover:** None. **K.A. Unroe:** None. **G.E. Hodes:** None. **S.M. Clinton:** None.

Presentation Number: NANO61.02

Topic: G.05. Mood Disorders

Support: NINDS: F31NS132558 (AMC)
Hope For Depression Research Foundation (EJN
Howard Hughes Medical Institute (HHMI) IM
R01 MH129306 (EJN)
R01 MH129306-01 (EJN)
R01 MH116900-04 (IM)

Title: Cell Type-Specific Roles of H3 Serotonylation in Postnatal Neurodevelopment

Authors: *A. CUNNINGHAM[1], J. CHAN[2], E. BRINDLEY[2], L. HOLT[2], L. SHEN[2], E. J. NESTLER[2], I. S. MAZE[2];
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[2]Neurosci., Mount Sinai Sch. of Med., New York, NY

Abstract: The serotonergic (5HTergic) system and its homeostasis in early life are critical to establishing the proper architecture of the developing brain and early life stress (ELS) can alter these precise developmental trajectories and increase lifetime risk for affective disorders. We now know 5HT not only works through receptors but also forms covalent bonds with histone H3 tail forming the epigenetic modification H3 serotonylation (H3 Ser.). While we know H3 Ser. regulates normal patterns of neurodifferentiation *in cellulo* and brain transcriptional profiles *in vitro,* its functional roles during postnatal neurodevelopment, a critical period of neuroplasticity, have largely been unexplored, and the impact of environmental stimuli (aberrant or otherwise) on this modification during early life remains unknown. Here, we aim to elucidate how H3 Ser. contributes to postnatal neurodevelopmental gene expression, and whether perturbations of the mark by ELS may result in altered gene expression and increased vulnerability to stress-related behavioral phenotypes. Leveraging FANS-coupled genome-wide analyses (CUT&RUN, RNA-sequencing) in mPFC of male and female mice we characterized H3 Ser. developmental and ELS-induced differences in H3 Ser. genomic enrichment and its role in regulating transcription. Interestingly, normal developmental differences of H3 Ser. enrichment and those perturbed by ELS were observed to be more pronounced in males *vs.* females in both neurons and glia and were accompanied by changes in gene expression. Strikingly, in glia, ELS increased the number of differential loci by 150-fold in adolescence, representing the most pronounced patterns of differential enrichment observed across all conditions. Upstream transcription factor analyses of ELS-induced differential peaks in glia during adolescence predicted OLIG2, (oligodendrocyte transcription factor 2) - a master regulator for oligodendrocyte lineage specification - as a top associated transcription factor. In males, H3 Ser. displays increased Olig2 promoter binding in response to ELS at adolescence compared to age-matched controls. We also find that *Olig2*+ cells exhibit changes in H3 Ser. intensity accompanied by increased oligodendrocyte precursor cells, suggesting that increased H3 Ser. enrichment at the *Olig2* loci may disrupt oligodendrogenesis. These findings provide novel insights into how precisely H3 serotonylation regulates neurodevelopment, as well as the mechanisms through which disruptions in this PTM cause aberrant pathophysiological states.

Disclosures: A. Cunningham: None. **J. Chan:** None. **E. Brindley:** None. **L. Holt:** None. **L. Shen:** None. **E.J. Nestler:** None. **I.S. Maze:** None.

Presentation Number: NANO61.03

Topic: G.05. Mood Disorders

Support: KAKENHI JP22H03532
KAKENHI JP21H00198
KAKENHI JP21K19707
KAKENHI JP23K05978
AMED JP23ak0101197
Shionogi & CO., Ltd.
NIH R01MH118297

Title: Sustained antidepressant effects of ketamine metabolite entail GABAergic inhibition-mediated molecular dynamics in aPVT

Authors: A. KAWATAKE-KUNO[1,2], H. LI[2], H. INABA[2], M. HIKOSAKA[2], T. UEKI[3], H. MORISHITA[1], G. OHTSUKI[2], S. UCHIDA[3,2];
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Abstract: Ketamine is emerging as a novel treatment for treatment-resistant depression. Despite the rapid and sustained antidepressant effects of ketamine and its metabolites, their underlying cellular and molecular mechanisms remain unclear. Here, we aimed to decipher the mechanisms of the sustained antidepressant-like effects of (*2S,6S*)-hydroxynorketamine (S-HNK) in repeatedly stressed animal models. First, we observed that S-HNK ameliorated anhedonia and social deficits in mice that were susceptible to repeated stress, with the antidepressant effects sustained over 28 days. Next, we constructed a brain-wide mapping of cFos, an index of neural activities, in mice treated with S-HNK and identified the anterior paraventricular nucleus of the thalamus (aPVT) as a novel brain region responsible for the long-lasting actions by S-HNK. To identify the molecular mechanisms within the aPVT responsible for inducing the sustained antidepressant actions of S-HNK, we conducted comprehensive gene expression analysis, including RNA-seq, and performed gain/loss-of-function experiments using adeno-associated viruses (AAVs). This allowed us to examine the causal relationships between the long-lasting antidepressant actions of S-HNK and the molecular mechanisms identified through RNA-seq as potentially vital for eliciting the antidepressant effects. Ultimately, we discovered that S-HNK induces mRNA expression of extrasynaptic $GABA_A$ receptors and subsequently enhances $GABA_A$-receptor-mediated tonic currents, leading to the nuclear export of histone demethylase KDM6 and its replacement by histone methyltransferase EZH2. This process increases H3K27me3 levels, which in turn suppresses the transcription of genes associated with G-protein-coupled receptor signaling. Thus, our findings uncovered the comprehensive cellular and molecular mechanisms in aPVT underlying the sustained antidepressant behavioral effects of ketamine metabolites. This study may support the development of potentially effective next-generation pharmacotherapies to promote sustained remission of stress-related psychiatric disorders.

Disclosures: A. Kawatake-Kuno: None. **H. Li:** None. **H. inaba:** None. **M. Hikosaka:** None. **T. Ueki:** None. **H. Morishita:** None. **G. Ohtsuki:** None. **S. Uchida:** None.

Presentation Number: NANO61.04

Topic: G.05. Mood Disorders

Support: Alexander von Humboldt, Equipment grants
CNPq
Capes

Title: Sexually dimorphic reponses of stressed flies to antidepressants

Authors: F. BOZ ECKERT[1], J. E. COSTA[1], F. TRICHES[1], P. R. SUMAN[2], K. R. KAUN[3], ***C. LINO DE OLIVEIRA**[4,5];
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Abstract: Model organisms are non-human species, which allow for mechanistic understanding of human diseases and treatments. Flies, like Drosophila melanogaster, have contributed to the understanding of genetics for over a century and, more recently have been useful for investigating the mechanistic basis of neuroscience and pharmacology. Flies possess neurotransmitters and neurobiological structures similar to vertebrates. Previous studies indicate that flies also react to stressors by reducing locomotion, sucrose preference and food intake. Similar to rodents, the reversal of stress-induced sequelae in flies can be achieved by antidepressant treatments. The aim of this study was to evaluate reversal by fluoxetine treatment of chronic variable stress -induced sequelae in flies. For this purpose, fluoxetine (2.5, 5, 10 mM) was fed to male or female flies exposed to stressors (combination of food deprivation, cycle reversal, cold, heat) for 3 days before locomotion and sucrose preference was measured in a lane-maze assay for 40 min. Untreated flies were control group for drug treatment. Unstressed flies were controls for stressed groups. According to expected, locomotion was smaller in stressed flies as compared to unstressed and untreated ones. However, fluoxetine treatment failed to reverse it. Contrasting to expected, sucrose preference was larger in stressed males as compared to unstressed ones. In females, sucrose preference was similar in stressed and unstressed groups. However, stressed female flies treated with 2.5 mM fluoxetine displayed larger preference for sucrose as compared to untreated ones. Intracellular contents of 5-HT increased in the nervous system of female flies while decreased in males when treated with 10 mM fluoxetine. In summary, conflicting to the initial hypothesis, preference for sucrose was larger in stressed males or females as compared to unstressed files. Moreover, fluoxetine treatment was sinergic with stressors in female flies preference for sucrose. Therefore, female flies seem less sensitive to the putative anti-stress effects of fluoxetine compared to males.

Disclosures: F. Boz Eckert: None. **J.E. Costa:** None. **F. Triches:** None. **P.R. Suman:** None. **K.R. Kaun:** None. **C. Lino De Oliveira:** None.

Presentation Number: NANO61.05

Topic: G.05. Mood Disorders

Title: Sex-specific and duration-dependent effects of chronic variable stress on brain anatomy and behavior.

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Abstract: The incidence and symptomatology of depression differs between sexes. In the rodent model for depression known as chronic variable stress (CVS), anxiety- and depressive-like phenotypes are observed after 6 days of stress in females and 21-28 days in males. However, stress-induced adaptations at the whole-brain level, and how they differ by sex and duration remain unexplored. Here, we investigated behavioral and neuroanatomical adaptations (using in vivo magnetic resonance imaging; MRI) induced by 15 or 28 days of CVS in male and female 9 week old C57BL/6 mice. Using a 7T Bruker preclinical scanner, we acquired T1w anatomical (100um^3 voxels) and resting state functional scans (EPI: Echo Planar Imaging, TR 1000ms, 250x250x500um resolution), and behavioral assays (open field test for anxiety-like behavior, and social preference test for social withdrawal) at baseline and 24-hrs after CVS (1hr daily stressors: 100 foot shocks, tail suspension; n=15 sex/group). Linear mixed effects models examined longitudinal behavioral and neuroanatomical (Jacobian determinants from deformation-based morphometry) changes, followed by False Discovery Rate (FDR) correction. After 15 days of CVS, stressed mice spent less time in the center (Fig. 1A). After 28 days, stressed females traveled less distance (Fig. 1D), and spent more time immobile compared to stressed males (Fig. 1G). Both durations led to less weight gain (Fig. 1F and H). In both sexes and durations, we observed significant changes in the hippocampus, lateral septum and amygdala. Sex-specific effects were found in the prefrontal cortex and insula for females, and nucleus accumbens and thalamus for males (Fig. 1I-L). Despite exposing both sexes to the same stress protocol, our whole-brain characterization elucidated minimally overlapping sex- and duration specific stress-induced adaptations in neuroanatomy and behavior at 15 or 28 days.

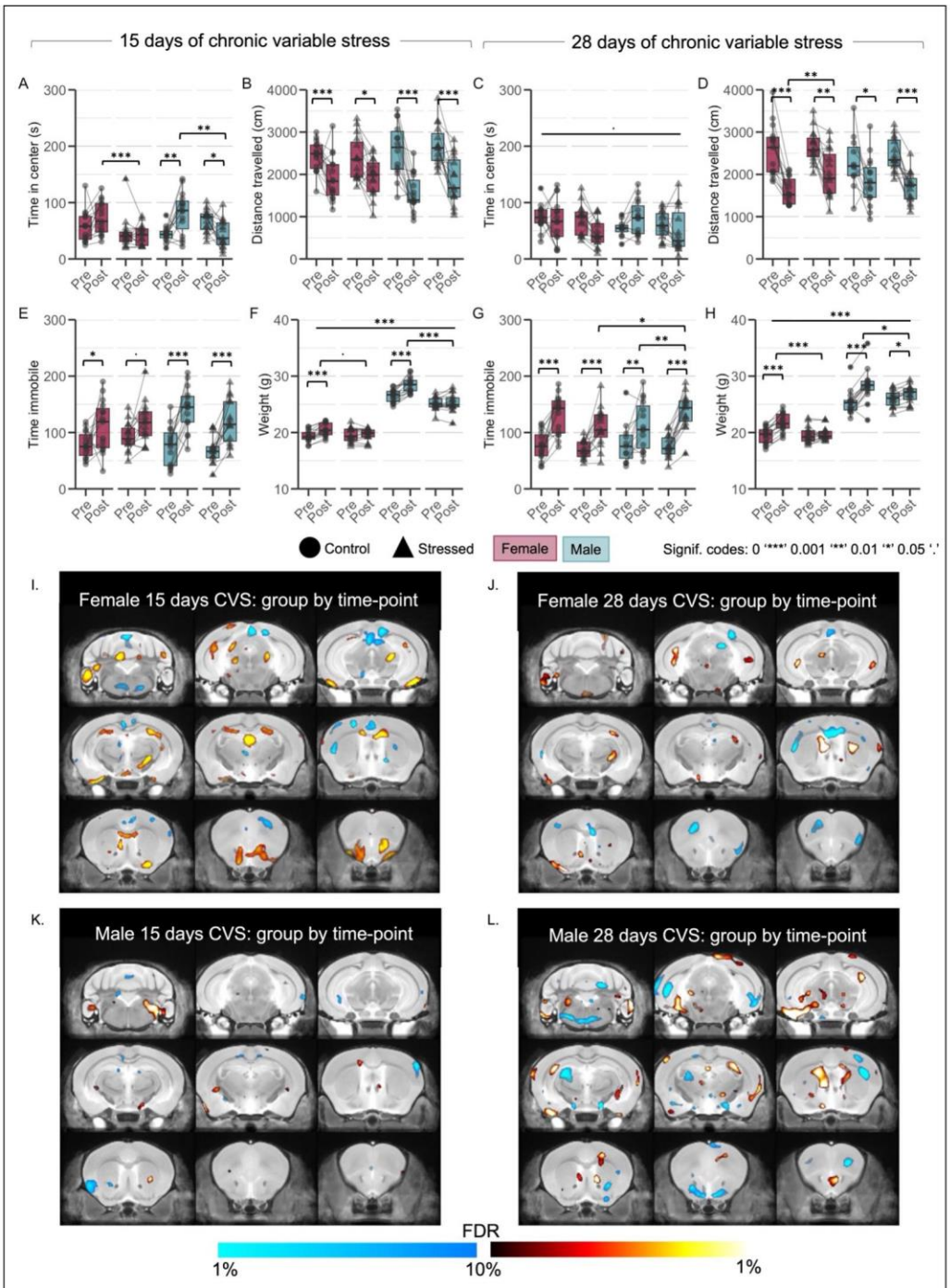

Figure 1. A and C) Time spent in the center of the open field arena (Total time of test: 300 seconds). B and D) Distance traveled in the open field. E and G) Time spent immobile during the test. F and H) Weight in grams before and after CVS. I-L) Significant neuroanatomical changes induced by 15 or 28 days of CVS in females (I and J) and males (K and L). Each inset shows positive and negative t-statistics for volumetric brain changes: group by timepoint interaction thresholded between at 10-1% FDR, overlaid on DSURQE template. Areas in warm colors (red-yellow) represent areas in which stress mice have larger volumetric increases relative to controls, whereas cool colors (blue) represent the opposite relationship.

Disclosures: L. Herrera Portillo: None. **D.R. Gallino:** None. **K. Bradshaw:** None. **R.C. Bagot:** None. **M. Chakravarty:** None.

Nanosymposium

NANO62: Opioid Addiction: From Neural Mechanisms to Novel Therapies

Location: MCP Room S103

Time: Wednesday, October 9, 2024, 1:00 PM - 3:00 PM

Presentation Number: NANO62.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-DA050908
NIH-DA056804

Title: Dysfunction in prelimbic cortex to ventral tegmental area projection mediates early social isolation stress-induced susceptibility to heroin relapse

Authors: *Y. WANG[1], S. YUE[2], Z. WANG[3];
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Abstract: Early life adversities induce persistent changes in the brain and increase vulnerability for many psychiatric disorders including substance use disorders. The hallmark characteristic of substance use disorder is the persistent uncontrollable drive to seek the drug that leads to eventual relapse. Our previous studies have reported that chronic early social isolation (ESI) during adolescence potentiates heroin-seeking behavior in mice. However, the underlying circuit and molecular mechanisms are still unknown. Here, we found that ESI stress aggravates heroin abstinence-induced neuronal dysfunction in prelimbic cortex (PrL) to ventral tegmental area (VTA) projecting neurons. Additionally, activation of PrL-VTA projections attenuated ESI-potentiated heroin seeking, which is accompanied by recovered neuronal firing and excitatory synaptic transmission in these neurons. Furthermore, ESI stress and heroin abstinence convergently affected the expression of genes regulating morphogenesis and metabolic processes, with Tmsb4x as one of the hub genes mediating ESI-potentiated heroin seeking. Interestingly, ESI stress and heroin abstinence interactively altered the expression of genes regulating cell cycle and DNA damage response, with Mcm3 and Mcm7 as hub genes. Together, our study showed that ESI stress-induced susceptibility to heroin relapse is associated with the ESI-potentiated neuronal dysfunction in PrL-VTA projections; and that this neuronal dysfunction is accompanied by gene transcriptional changes within PrL-VTA circuits.

Disclosures: Y. Wang: None. **S. Yue:** None. **Z. Wang:** None.

Presentation Number: NANO62.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Circuit-specific mechanisms underlying pain-facilitated fentanyl use

Authors: *R. H. TEICH[1], J. A. HIGGINBOTHAM[2], J. MORON-CONCEPCION[3];
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Abstract: The opioid crisis, currently fueled by synthetic opioids like fentanyl, has disproportionately impacted men. We recently found that pain contributed to excessive fentanyl use in male rats and this was associated with enhanced ventral tegmental area (VTA) dopamine neuron activity elicited by fentanyl self-administration. VTA dopamine neuron activity can be regulated by excitatory and inhibitory inputs, but it remains unclear how these inputs modulate VTA dopamine activity in this phenomenon. Inputs to the VTA from the prefrontal cortex (PFC) and nucleus accumbens (NAc) represent major sources of excitation and inhibition, respectively. Thus, we tested the hypothesis that excitatory PFC-to-VTA input activity is enhanced in a time-dependent manner during fentanyl self-administration, while inhibitory NAc-to-VTA input activity is reduced over time, thereby leading to time-dependent increases in fentanyl-evoked VTA dopamine activity in males with pain.To target these pathways, a retrogradely transported GCaMP virus was injected into the VTA of male and female Long Evans rats, and an optic fiber was implanted into either the shell of the NAc or the medial PFC (mPFC). IV catheters were then implanted in the right jugular vein to permit intravenous fentanyl delivery and Complete Freud's Adjuvant (CFA) was injected in the hind right paw of each rat to produce persistent inflammatory pain. Rats underwent three weeks (M-F) of daily 2-hour fentanyl self-administration, wherein each response on a designated lever resulted in a fentanyl infusion (5μg/kg/infusion or 2μg/kg/infusion). Wireless in vivo fiber photometry was used during self-administration to detect calcium transient activity during the self-administration of fentanyl in the PFC-to-VTA or NAc-to-VTA pathways. Photometry recordings occurred throughout the training sessions at least once per week and DF/F signals were aligned with fentanyl-reinforced lever responses.Interestingly, fentanyl-evoked NAc-to-VTA responses were time-dependently enhanced, suggesting alternative sources of inhibitory regulation of VTA DA activity play a critical role in this process. Similarly, our preliminary data indicates that PFC-to-VTA inputs may also increase over time, potentially implicating a role for this circuit in driving increases in VTA dopamine responses to fentanyl. These findings add to our understanding of the balance between excitatory and inhibitory mechanisms regulating VTA dopamine activity in the context of opioid reward in pain states and may shed light on potential therapeutic targets to lessen the impact of the opioid crisis.

Disclosures: R.H. Teich: None. **J.A. Higginbotham:** None. **J. Moron-Concepcion:** None.

Presentation Number: NANO62.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R01DA052953
NIDA Grant 5R01DA037216
Department of Veterans Affairs Merit Award IO1BX004440
Roy J. Carver Charitable Trust

Title: Asic1a opposes synaptic and behavioral responses to opioids

Authors: *M. J. FULLER[1], N. R. ANDRYS[1], S. C. GUPTA[2], A. GHOBBEH[1], C. J. KREPLE[3], R. FAN[1], R. J. TAUGHER-HEBL[4], J. J. RADLEY[5], R. T. LALUMIERE[6], J. A. WEMMIE[1]; [1]Psychiatry, Univ. of Iowa, Iowa City, IA; [2]Psychiatry, Univ. of Iowa, Iowa, IA; [3]Neurol., Univ. of Wisconsin, Plymouth, WI; [4]Psychiatry, The Univ. of Iowa, Iowa City, IA; [5]Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA; [6]Dept. of Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: Drugs of abuse produce rearrangements at glutamatergic synapses in brain regions important for drug-seeking behavior, including the nucleus accumbens core (NAcc). Our previous work suggested that Acid-Sensing Ion Channel 1A (ASIC1A) in the NAcc opposes cocaine-evoked synaptic effects as well as cocaine-seeking behavior, but its role in opioid responses is largely unknown. We therefore tested whether ASIC1A contributes to opioid-evoked behavioral and synaptic effects. We tested acute locomotor responses to several opioids and conditioned place preference (CPP) to oxycodone. Compared to *Asic1a*$^{+/+}$ mice, *Asic1a*$^{-/-}$ mice had increased locomotor activation to acute administration of oxycodone, morphine, and heroin and increased oxycodone CPP. These data suggest that ASIC1A opposes behavioral responses to multiple different opioids. Because our previous studies indicated that loss of ASIC1A altered synaptic responses to cocaine, including effects on the AMPAR/NMDAR ratio and dendritic spines in NAcc medium spiny neurons (MSNs), we wondered if it would alter synaptic effects of opioid exposure. To answer this question, we administered oxycodone to *Asic1a*$^{+/+}$ and *Asic1a*$^{-/-}$ mice for 5 days, and after 10 days of withdrawal tested AMPAR/NMDAR ratio and analyzed dendritic spine morphology. We found that oxycodone withdrawal increased AMPAR/NMDAR ratio in *Asic1a*$^{+/+}$ mice, but had the opposite effect in *Asic1a*$^{-/-}$ mice, reducing AMPAR/NMDAR ratio. We found no effect of oxycodone withdrawal on dendritic spine density or morphology. However, it reduced spine volumes in both *Asic1a*$^{+/+}$ and *Asic1a*$^{-/-}$ mice, and to a similar degree. We conclude that ASIC1A plays an important role in opioid-induced behaviors and that its effects are not specific to a singular opioid. Additionally, ASIC1A modulates opioid-induced effects on glutamatergic receptors in NAcc MSNs, which may underlie its role in behavior. ASIC1A opposes both behavioral and synaptic effects of opioids, and this work suggests ASIC1A could be a potential therapeutic target for opioid use disorder in humans.

Disclosures: M.J. Fuller: None. **N.R. Andrys:** None. **S.C. Gupta:** None. **A. Ghobbeh:** None. **C.J. Kreple:** None. **R. Fan:** None. **R.J. Taugher-Hebl:** None. **J.J. Radley:** None. **R.T. LaLumiere:** None. **J.A. Wemmie:** None.

Presentation Number: NANO62.04

Topic: G.09. Drugs of Abuse and Addiction

Support: F31DA053774
R21DA057695

Title: A Novel Gene Therapy-Based Approach for Treating Opioid Use Disorder

Authors: *K. CLEMENZA[1], M. FARRELL[2], A. FRYC[3], S. M. NICOLA[4], L. L. SJULSON[5]; [1]Neurosci., [2]Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [3]Neurosci., Albert

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[5]Neuroscience, Psychiatry, Albert Einstein Col. of Med., Bronx, NY

Abstract: Opioid use disorder is a debilitating condition that poses a substantial, and growing, public health burden. Although effective medications exist, they typically require strict lifelong adherence, which is unrealistic for many people suffering with this disorder. There is thus an urgent need to develop new interventions that confer long-term protection from a short-term administration. To this end, we have developed a novel gene therapy-based strategy using a mutant "Low-Affinity Mu Opioid Receptor (LAMuOR)" that can be conceptualized as an inhibitory DREADD that is activated by high-affinity exogenous opioids of abuse. We use adeno-associated viral vectors (AAVs) to express LAMuOR in dopaminergic neurons of the ventral tegmental area in mice to test the hypothesis that LAMuOR suppresses opioid-induced dopamine release and opioid use disorder-related behaviors. We find that LAMuOR-expressing mice exhibit reductions in fentanyl-induced dopamine release in the nucleus accumbens, with the highest AAV dose group exhibiting suppression below baseline levels. Alternatively, LAMuOR was not found to impact cocaine-evoked dopamine elevations in most AAV doses, indicating that baseline dopamine signaling is intact in those groups. LAMuOR-expressing mice also exhibited reductions in fentanyl-induced hyperlocomotion, while opioid drug-independent activities like cocaine- and drug-naive open field locomotion and sucrose preference were unaffected. Further, preliminary results indicate that LAMuOR-expressing mice exhibit reductions in oral oxycodone self-administration in the home cage. Together, these results begin to demonstrate the promise of LAMuOR as a potential new treatment strategy for opioid use disorder. Our next steps will focus on testing LAMuOR's ability to suppress previously acquired operant opioid self-administration in rats, which is considered a gold-standard model of addiction-like behavior, to further substantiate LAMuOR's clinical viability.

Disclosures: K. Clemenza: None. **M. Farrell:** None. **A. Fryc:** None. **L.L. Sjulson:** None.

Presentation Number: NANO62.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Intramural Program

Title: Oxycodone self-administration is associated with differential mRNA expression of potassium channels in the prefrontal cortices of rats that do not escalate their drug intake.

Authors: *J. CADET[1], M. T. MCCOY[2], A. DAIWILE[3], B. N. LADENHEIM[4], A. WABREHA[5];
[1]Natl. Inst. On Drug Abuse/ NIH, Baltimore, MD; [2]Mol. Neuropsychiatry Res. Br., DHHS/NIH/NIDA/IRP, Baltimore, MD; [3]MNRB, NIH, NIDA IRP, Baltimore, MD; [4]NIH/NIDA Addiction Res. Ctr., Baltimore, MD; [5]NIH/NIDA IRP, Baltimore, MD

Abstract: The prevalence of opioid use disorder (OUD) has skyrocketed because the increased prescribing of opioid agents for chronic pain due to neurological and cancer diagnosis and treatment. OUD is characterized by loss of control of drug taking and continued drug use in the presence of adverse consequences. OUD is also a chronic relapsing disorder due to molecular and biochemical alterations in neurocircuits that regulate emotional states and trigger craving. The cost of this chronic brain disorder has cause major health and financial crises throughout the

USA and other countries. The Cadet laboratory is seeking to understand the molecular neurobiology of OUD by using oxycodone self-administration (SA) by rats. In the present study, we trained Sprague-Dawley rats self-administered oxycodone for 20 days according to short- (ShA, 3 h) and long-access (LgA, 9 h) paradigms. Animals were euthanized 2 h after the last session of oxycodone SA. Brain regions were dissected and then used in various biochemical and molecular assays. Herein, we present quantitative PCR data on the expression of several mRNAs in the prefrontal cortex (FC). Rats given short access to oxycodone did not escalate their drug intake. Rats given long access to the drug escalated their oxycodone intake but separated into lower (LgA-L) or higher (LgA-H) oxycodone takers, with LgA-L showing little or no escalation of oxycodone intake. Measurements of potassium channel mRNAs revealed significant increases in *Kcnd3, Kcnma1, and Kcnk1*expression in the PFC of LgA-L rats. Other genes of interest included *Ago3, CacNA2D1, Grip2, and Slc16A7* that showed increases only in the ShA group. The increased expression of the mRNAs of some potassium channels in the PFC suggests that these potassium channels might serve to suppress neurotransmission in a prefrontal-subcortical circuit that regulates oxycodone SA and might account, in part, for the lack of oxycodone escalation in these rats despite being given 9h access to the drug.

Disclosures: J. Cadet: None. **M.T. McCoy:** None. **A. Daiwile:** None. **B.N. Ladenheim:** None. **A. Wabreha:** None.

Presentation Number: NANO62.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Analyzing activity-dependent gene plasticity in opioid addiction using MRI

Authors: *V. PHI VAN, A. JASANOFF;
MIT, Cambridge, MA

Abstract: Our ability to develop, learn, and adapt depends on the brain's capacity to change over time. To analyze plasticity processes over time in individual animals, we introduced a novel technique that enables immediate-early gene (IEG) activity to be mapped noninvasively using magnetic resonance imaging (MRI). Using this method, called IEG BLUsH, we interrogated the spatiotemporal relationships between neural activity and plasticity gene induction in an opioid administration paradigm. IEG activity and functional MRI (fMRI) signals were recorded following fentanyl treatments lasting several days (Figure 1). Results revealed overall correspondence of plasticity and activity signatures, but with marked dissociations in brain regions including prefrontal and insular cortex, where IEG induction was particularly strong. Over a week of fentanyl exposure, expression of the IEG reporter increased further in prefrontal cortex, reflecting ongoing engagement of plasticity mechanisms during this period. Interestingly, latency of IEG turn-on decreased over days in some brain regions, suggesting that repeated drug exposure primes the brain for activation of plasticity-related genetic programs. These results show how drug stimuli reshape the brain over time scales from seconds to days, and indicate how IEG BLUsH can help characterize complex patterns of genetic regulation underlying neuroplasticity and other processes in the brain.

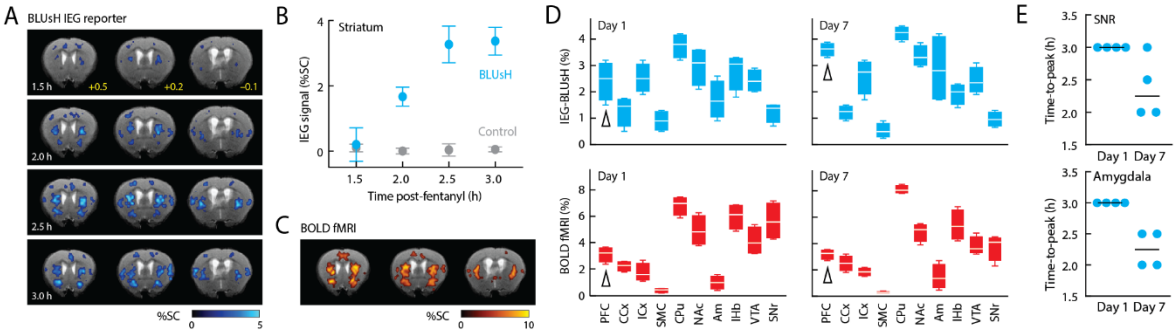


Figure 1: In response to fentanyl a buildup of BLUsH signal over 3 h can be observed in frontal cortical and subcortical regions with exemplary quantification (cyan) in striatum versus control (B). Conventional fMRI response to stimulation in the experiment of (A), showing similar regional pattern of hemodynamic responses (C). (D) Comparison of IEG-BLUsH (top panels) and drug-stimulated fMRI activity (bottom panels) from a pilot set of four animals imaged on day 1 (left) and day 7 (right) of fentanyl treatment. Open arrowheads highlight PFC signals, for which the ratio of BLUsH to fMRI activity increases over time. (E) Individual time-to-peak measurement of 4 animal reveals

Disclosures: V. Phi van: None. **A. Jasanoff:** None.

Presentation Number: NANO62.07

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA037897
T32 DA028874
F31 DA058451

Title: A novel role for GLP-1R-expressing interpeduncular circuits in relapse, avoidance, and anxiety-like behaviors during fentanyl abstinence

Authors: *R. HERMAN[1], V. CHINAKA[1], A. POTHIKAMJORN[1], H. D. SCHMIDT[2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept Psychiatry, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Given that the interpeduncular nucleus (IPN) expresses glucagon-like peptide 1 receptors (GLP-1Rs) and GLP-1R agonists reduce opioid seeking, we hypothesized that activation of GLP-1Rs in the IPN would attenuate fentanyl reinstatement. We trained male and female rats to self-administer intravenous fentanyl (1.25 µg/kg/infusion) for 21 days. Once fentanyl taking was extinguished, rats were pretreated with intra-IPN infusions of the GLP-1R agonist Ex-4 (0.01 or 0.1 µg) before fentanyl reinstatement tests. We showed that intra-IPN infusions of Ex-4 dose-dependently decreased fentanyl seeking at doses that did not affect body weight, chow intake, or pica. Additionally, we found that GLP-1Rs and MORs are both expressed on GABAergic IPN neurons that project to the laterodorsal tegmental nucleus (LDTg). Given that GLP-1Rs are excitatory, these results support a circuit whereby IPN GLP-1R activation attenuates fentanyl reinstatement by activating GABAergic IPN projections to the LDTg. Therefore, we hypothesized that chemogenetic activation of the IPN-LDTg pathway would attenuate fentanyl reinstatement. Chemogenetic activation of IPN neurons that project to the LDTg attenuated fentanyl seeking, confirming a role of the IPN-LDTg projection in fentanyl-

seeking behavior. Interestingly, we found that activation of IPN GLP-1Rs during fentanyl withdrawal produced anxiolytic effects (as measured in the elevated plus maze), but had no effect on conditioned taste avoidance or conditioned place preference. However, chemogenetic activation of the IPN-LDTg projection induced conditioned place preference for the CNO-paired side. We are currently using an AAV expressing the Cre-dependent inhibitory DREADD hM4D(Gi) in GAD:Cre rats to examine the effects of cell-type specific inhibition of the GABAergic IPN-LDTg projections on fentanyl reinstatement and Ex-4 efficacy. We are also using fiber photometry to measure cell-type specific calcium dynamics in IPN GABAergic neurons during fentanyl reinstatement and Ex-4 treatment. Overall, this work identifies a novel pathway underlying fentanyl-seeking behavior, provides new insight into the complex role of the IPN in regulating reward, anxiety, and avoidance, and supports the use of GLP-1R agonists as a potential treatment for fentanyl use disorder.

Disclosures: R. Herman: None. **V. Chinaka:** None. **A. Pothikamjorn:** None. **H.D. Schmidt:** None.

Presentation Number: NANO62.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA006214
NIH Grant 7T32DA055569

Title: Suvorexant co-treatment prevents development of negative affect with chronic oxycodone while preserving analgesia.

Authors: *K. A. NEWMAN[1], G. S. ASTON-JONES[2];
[1]Rutgers Univ. Grad. Program In Neurosci., Piscataway, NJ; [2]Brain Hlth. Inst., Brain Hlth. Inst. Rutgers Univ., Piscataway, NJ

Abstract: Prescription opioids are commonly used to treat chronic pain but can lead to withdrawal and negative affect following dependence, and to development of opioid use disorder (OUD). We developed a rodent model of prescription opioid-associated negative affect, showing that 3wk of oxycodone (oxy) treatment is associated with hyperalgesia and anhedonia-, anxiety-, and depressive-like behavior in acute abstinence. Compared to saline-treated controls, oxy rats had increased numbers of orexin-expressing neurons. Here, we tested whether attenuation of orexin signaling during chronic oxy treatment would prevent negative affect while preserving the analgesic properties of prescription opioids. Adult male Long Evans rats (n=5/group) were injected with Freund's Complete Adjuvant (FCA) in the hindpaw (s.c) and given 2wk of twice-daily saline or oxy (3mg/kg, i.p.), in conjunction with daily oral vehicle (veh) or the dual orexin antagonist suvorexant (suvo). During acute abstinence, rats were evaluated on the von Frey test (hyperalgesia), saccharin preference test (anhedonia), elevated plus maze (anxiety-like behavior), and forced swim test (depressive-like behavior). Rats were also tested for mechanical analgesia in the FCA pain model with von Frey 15min following acute oxy. We found that increased negative affect seen in oxy+veh compared to saline+veh rats ($p=0.058$) tended to reverse in oxy+suvo rats (oxy+suvo vs saline+veh, $p=0.98$), indicating that co-treatment with suvo reversed the increased negative affect produced by the chronic oxy. In pain model rats, acute oxy induced mechanical analgesia as expected (oxy+veh compared to sal+veh rats, $p<0.05$), and suvo co-

treatment did not significantly affect this analgesic action of oxy (von Frey thresholds between oxy+suvo and oxy+veh rats, p=0.75). Together, these results indicate that daily co-treatment with suvo prevents oxy-associated negative affect development but does not affect analgesia induced by oxy. Current experiments are evaluating whether daily suvo co-treatment prevents other indices of OUD after chronic oxy (including increased demand for opioids), and also if suvo co-treatment attenuates the increase in orexin cell numbers seen with chronic oxy. These experiments support the adoption of suvo as an adjunct to oxy treatment for chronic pain to prevent OUD development.

Disclosures: K.A. Newman: None. **G.S. Aston-Jones:** None.

Nanosymposium

NANO63: Computational Models

Location: MCP Room N227

Time: Wednesday, October 9, 2024, 1:00 PM - 3:45 PM

Presentation Number: NANO63.01

Topic: H.03. Decision Making

Support: ZIA MH002928

Title: Reconfiguration of population dynamics in macaque prefrontal cortex during reinforcement learning

Authors: *S. WANG[1], Y. ZHAO[1], R. BARTOLO[2], F. PEREIRA[1], B. B. AVERBECK[1]; [1]NIMH/NIH, Bethesda, MD; [2]Lab. of Sensorymotor Res., NIH, Natl. Eye Inst. (NEI), Bethesda, MD

Abstract: In reinforcement learning (RL), animals can flexibly change their choice preferences as they learn through trial and error. However, it remains elusive how populations of neurons reconfigure their collective activity patterns to generate adaptive choice behavior as animals learn. Theoretical work suggests that attractor dynamics in networks can account for the choice process. Attractor models operationalize the idea that decision-related neural activity evolves in a way that can be modeled as an object frictionally sliding on a landscape with basins separated by a hill. The object starts on top of the hill and is pushed into one of the two basins by evidence in favor of one of the choices. The basin that the object ends up in reflects the chosen option. Artificial network simulations predict that, as animals learn, the attractor basins get deeper and the energy barriers between the two choices become higher, which reflect a learned preference for one option over another. Despite recent empirical studies in support of the attractor model, it is still an outstanding question how attractor network dynamics in the brain change as animals learn.

In this project, we investigated how choice-related attractor dynamics develop during learning. We trained two rhesus monkeys on a variant of the two-armed bandit reversal learning task. In this task, monkeys were asked to choose between two options that were associated with different probabilities of reward. Monkeys had to determine which option was better via trial and error.

We simultaneously recorded hundreds of neurons using high-channel-count multielectrode arrays in the dorsal lateral prefrontal cortex (dlPFC) of rhesus monkeys while they performed the task. RL models were fit to the behavioral data to extract the trial-by-trial value estimate of the two options. As animals learned, the value difference between the two options became larger. We then analyzed population dynamics of prefrontal neurons in the state space spanned by the firing rates of individual neurons. To address the challenge of interpreting high-dimensional neural activity, we used both a supervised method in which we estimated local linear dynamics around the mean population trajectory in small time bins, and an unsupervised method using neural network models in which we fit non-linear dynamics over a longer time period. Results from both methods supported the hypothesis that attractor landscapes become deeper as animals learn, while each method yielded additional complementary information. With converging results from both methods, we provide neural evidence of a reconfiguration of attractor dynamics in macaque prefrontal cortex during learning.

Disclosures: S. Wang: None. **Y. Zhao:** None. **R. Bartolo:** None. **F. Pereira:** None. **B.B. Averbeck:** None.

Presentation Number: NANO63.02

Topic: H.03. Decision Making

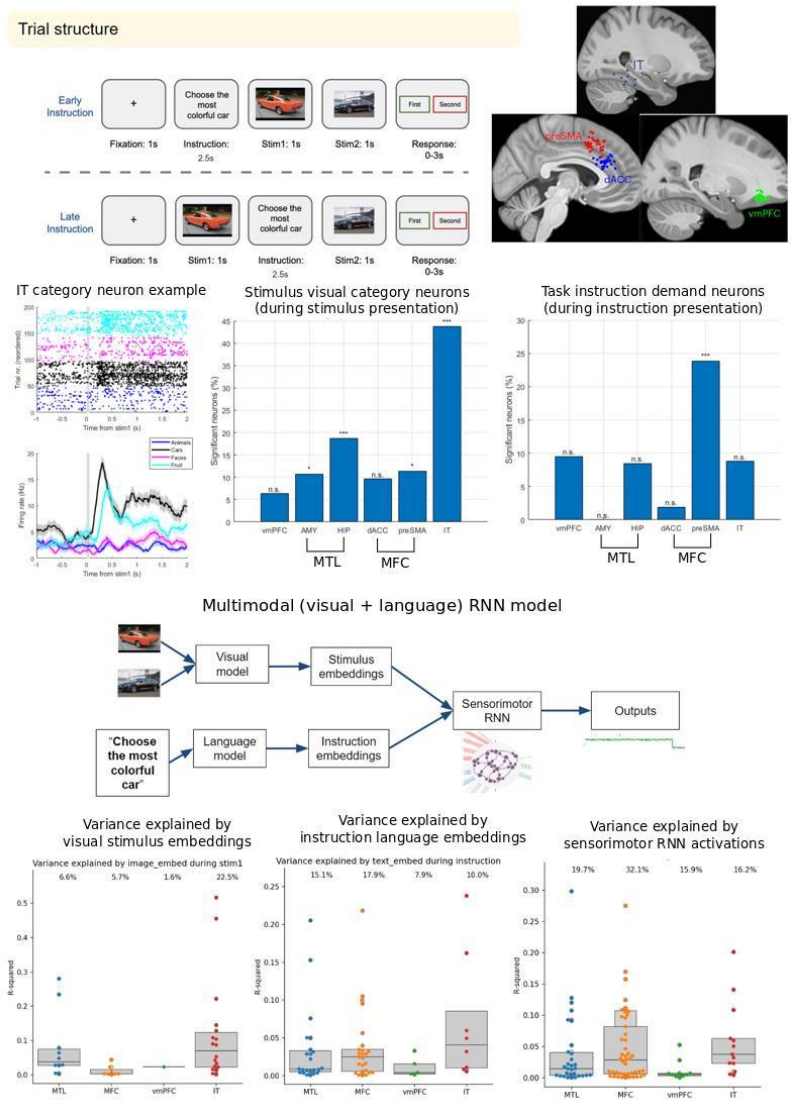
Support: ARL Human Guided Intelligent Systems grant (W911NF-23-2-0067)
Strengthening Teamwork for Robust Operations in Novel Groups (STRONG) grant (W911NF 22-2-0148)

Title: Top-down task instructions dynamically mediate flexible behavior in human single neurons

Authors: *T. AQUINO[1], R. RIVELAND[2], R. KIM[3], A. N. MAMELAK[4], A. POUGET[5], N. RUNGRATSAMEETAWEEMANA[6], U. RUTISHAUSER[7];
[1]Columbia Univ., New York, NY; [2]Univ. of Geneva, Geneva, Switzerland; [3]Neurol., Cedars-Sinai Med. Ctr., Los Angeles, CA; [4]Cedars-Sinai Med. Ctr., Los Angeles, CA; [5]Univ. Hosp. of Geneva, Geneva, Switzerland; [6]Biomed. Engin., Columbia Univ., New York, NY; [7]Dept. of Neurosurg., AHSP #6432, Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Decision making in complex settings requires flexible integration of various bottom-up and top-down signals about stimulus identities, task demands and environmental contexts. Humans are able to quickly switch between opposing task demands and ignore irrelevant information by utilizing information contained in language instructions, but the mechanism of integration between stimuli, instructions, decision making, and motor planning is not fully understood in human neurons. To study these processes, we recorded single neuron activity from intracranially implanted epilepsy patients, from inferotemporal, medial frontal (MFC), prefrontal, and medial temporal (MTL) areas, while they performed a flexible decision making task. In this task, visual stimuli were presented while instructions were manipulated for the relevant information of interest to be attended by the patient, as well as for the order in which instructions were presented, to gauge the impact of the presence and contents of top-down information. Our results indicate a hierarchical distinction between activity patterns in inferotemporal versus medial frontal cortex in coding bottom-up, and, subsequently, top-down

task-relevant information. While hippocampal and inferotemporal neurons coded bottom-up stimulus features, medial frontal neurons flexibly responded to task demands while reading instructions and performing decisions. Lastly, we trained multimodal (visual and linguistic) RNNs to perform the same exact task, and found that sensorimotor unit activations successfully explain variance in medial frontal cortex neurons during instruction reading and decision-making. Additionally, while visual embeddings of stimuli and text predicted activity in IT, language embeddings of task instructions predicted activity of medial frontal cortex neurons. Put together, these results provide a mechanistic account for top-down and bottom-up sensorimotor integration, utilizing a novel combination of human intracranial data aligned with multimodal RNN representations.



Disclosures: T. Aquino: None. **R. Riveland:** None. **R. Kim:** None. **A.N. Mamelak:** None. **A. Pouget:** None. **N. Rungratsameetaweemana:** None. **U. Rutishauser:** None.

Presentation Number: NANO63.03

Topic: H.03. Decision Making

Title: Overcoming sensory-memory interference in artificial and biological neural networks

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Abstract: Memories of recent stimuli are crucial for guiding behavior. However, the same sensory pathways that receive information to be remembered are constantly bombarded by new sensory experiences, and it remains largely unknown how the brain overcomes interference between sensory and memory representations. Here we report which mechanisms might be at play in artificial and biological networks that are robust to sensory-memory interference. We examined recurrent neural networks (RNNs) that were either hand-designed or trained using gradient descent methods, and compared our results with neural data from two macaque experiments. We found an infinite RNN solution space, that included gating of the sensory inputs, modulating synapse strengths to achieve a strong attractor solution, and dynamic coding of feature preference, such that, at the extreme, cells invert their tuning over time. Neural data from macaque brain area medial superior temporal (MST) was most aligned with the Gating + Inversion of Tuning solution. This solution was also consistent with experimental results from monkey behavior. Taken together, our results help elucidate how recurrent neural networks are able to solve the problem of sensory-memory interference using a combination of both static and dynamic codes, and suggest MST may play a role in this computation.

Disclosures: A. Zahorodnii: None. **D. Mendoza-Halliday:** None. **N. Qian:** None. **R. Desimone:** None. **C. Cueva:** None.

Presentation Number: NANO63.04

Topic: H.03. Decision Making

Support: K. Lisa Yang ICoN Center
NIMH-MH122025

Title: Neural Foundations of Mental Simulation

Authors: ***A. NAYEBI**[1], R. RAJALINGHAM[2], M. JAZAYERI[3], G. YANG[1];
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Abstract: Humans and animals have a rich and flexible understanding of the physical world, which enables them to infer the underlying dynamical trajectories of objects and events, plausible future states, and use that to plan and anticipate the consequences of actions. However, the neural mechanisms underlying these computations are unclear. We combine a goal-driven modeling approach with dense neurophysiological data and high-throughput human behavioral readouts that contain thousands of comparisons to directly impinge on this question. Specifically, we construct and evaluate several classes of sensory-cognitive networks to predict the future

state of rich, ethologically-relevant environments, ranging from self-supervised end-to-end models with pixel-wise or object-slot objectives, to models that future predict in the latent space of purely static image-pretrained or dynamic video-pretrained foundation models. We find that "scale is not all you need," and that many state-of-the-art machine learning models fail to perform well on our neural and behavioral benchmarks for future prediction. In fact, only one class of models matches these data well overall. We find that neural responses are currently best predicted by models trained to predict the future state of their environment in the latent space of pretrained foundation models optimized for dynamic scenes in a self-supervised manner. These models also approach the neurons' ability to predict the environmental state variables that are visually hidden from view, despite not being explicitly trained to do so. Finally, we find that not all foundation model latents are equal. Notably, models that future predict in the latent space of video foundation models that are optimized to support a diverse range of egocentric sensorimotor tasks, reasonably match both human behavioral error patterns and neural dynamics across all environmental scenarios that we were able to test. Overall, these findings suggest that the neural mechanisms and behaviors of primate mental simulation have strong inductive biases associated with them, and are thus far most consistent with being optimized to future predict on reusable visual representations that are useful for Embodied AI more generally.

Disclosures: A. Nayebi: None. **R. Rajalingham:** None. **M. Jazayeri:** None. **G. Yang:** None.

Presentation Number: NANO63.05

Topic: H.03. Decision Making

Support: NSERC Discovery Grant: RGPIN-2020-05105
NSERC Discovery Accelerator Supplement: RGPAS-2020-00031
Arthur B. McDonald Fellowship: 566355-2022
CIFAR Canada AI Chair; Learning in Machine and Brains Fellowship
This research was enabled in part by support provided by Calcul Québec the Digital Research Alliance of Canada
The authors acknowledge the material support of NVIDIA in the form of computational resources

Title: Learning with non-Euclidean synaptic plasticity

Authors: *J. CORNFORD[1], R. POGODIN[3], A. GHOSH[1], O. CODOL[4], K. SHENG[5], B. A. BICKNELL[6], G. LAJOIE[7], B. A. RICHARDS[2];
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Abstract: Synaptic plasticity imposes a cost on neuronal circuits. This cost exists at a biophysical metabolic level, but also in terms of the circuit’s ability to perform future and past tasks. As such, there is a pressure for synaptic weight configurations to not change “too much” during learning, but how should we formulate this plasticity cost in algorithmic models of neural circuits? In deep learning with gradient descent the cost is Euclidean. Here we show when training recurrent neural networks (RNN) to perform cognitive tasks that a Euclidean weight-

change cost is not consistent with experimentally observed neural circuit properties. In contrast, learning with an entropic non-Euclidean cost of synaptic plasticity results in Dale's Law, multiplicative synaptic weight changes, and log-normal-like connection strengths. Further in line with biology, these networks are also more robust to weight sparsification. Finally, we show in both multicompartmental neuron models and RNNs that learning with non-Euclidean synaptic plasticity is better when there are many irrelevant features - as is common in the brain. As such, our work suggests biological features of neural circuits can be accounted for by the implicit cost of synaptic changes. Moreover, we find there are situations where biologically inspired learning outperforms current deep learning approaches.

Disclosures: J. Cornford: None. **R. Pogodin:** None. **A. Ghosh:** None. **O. Codol:** None. **K. Sheng:** None. **B.A. Bicknell:** None. **G. lajoie:** None. **B.A. Richards:** None.

Presentation Number: NANO63.06

Topic: H.03. Decision Making

Support: NSF Grant 2152260

Title: Graph Neural Networks for Decoding Reward Prediction Error

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Abstract: Decision making is a critical cognitive task that we all perform on a daily basis, and is disrupted in neuropsychiatric disorders like addiction and depression. Because decision making involves the coordinated activity of multiple brain regions, investigating the functional connectivity between these regions can lay the foundation for pharmacological and surgical interventions to treat disorders involving decision making. The most common methods to assess the functional connectivity between brain regions are coherence, phase synchrony, correlation and Granger causality. These methods require simplifying assumptions about the neural data that are known to be incorrect, including linearity of the functional connectivity, lack of directionality of the connection or independence of the interactions. Graph neural networks have had great success modeling complex networks in other fields and naturally lend themselves to modeling the functional interactions of different brain regions. We collected intracranial electroencephalogram data from subjects during a decision-making task that asks the subjects to consider gambling away a fixed reward for a potentially more lucrative reward. After the participant makes their choice and gets their reward, we calculate reward prediction error, a metric that measures the difference between the expected reward given win probability and choice selection and the actual received reward. We show that graph neural networks, which model systems as nodes that pass information between them, can predict reward prediction error significantly above chance, calculated by bootstrapping the data. We find evidence that interregional information flow carries sufficient information to predict reward prediction error. These results suggest that Graph Neural Networks can be used to asses neural encoding of events with high emotional valence.

Disclosures: L. Peters: None. **J.A. Overton:** None. **I. Saez:** None. **K.A. Moxon:** None.

Presentation Number: NANO63.07

Topic: H.03. Decision Making

Support: NIH Grant R21MH120805

Title: Categorizing Decision-Making Behavior Via Recurrent Neural Network Prediction Accuracy

Authors: *J. LI[1], J. P. O'DOHERTY[2], J. COCKBURN[2];
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Abstract: Computational modeling of behavior holds promise for phenotyping decision-making processes that may be subserved by distinct neural underpinnings. Clarifying individual differences in decision-making behavior could thus allow researchers to better relate neural mechanisms to distinct aspects of decision-making. A common approach to modeling behavior relies on manual model specification with relatively few points of variation provided by estimated parameters (e.g., the learning rate in a reinforcement learning model), which reflects a priori assumptions about the data generation process that could result in misattribution of the data generation process to the inaccurate model, or of variance to the incorrect parameter. The current study proposes a data-driven method to inform model specification using recurrent neural networks (RNNs) and support vector machine (SVM) classifiers. We posited that tuning RNNs using simulated data to detect specific response patterns associated with computational mechanisms of interest can help distinguish among candidate strategies in behavioral data. To test this hypothesis, we trained a set of RNNs on data generated by distinct learning and decision-making algorithms (e.g. reinforcement learning, win-stay/lose-shift) in a probabilistic reversal learning task. We then tested them on holdout data simulated from various candidate strategies to generate prediction accuracies, which served as features for an SVM classifier to infer strategy membership. Results showed that the SVM correctly classified a heterogeneous sample of unseen data along multiple distinct prototypical strategy dimensions embedded in the RNNs. Furthermore, SVM classification accuracy and confidence recapitulated the degrees of uncertainty in the generative models, such that variances explained by the generative models tracked SVM-predicted probabilities of the most likely class memberships. In summary, the RNN-SVM pipeline can uncover latent strategy topography from observations, thereby constraining the model and parameter spaces of computational models that could in turn reveal finer-grained individual differences in decision-making behavior. Thus, the RNN-SVM pipeline could complement theory-driven modeling approaches to reveal dissociable behavioral signatures of learning and decision-making that may map onto distinct neural computations.

Disclosures: J. Li: None. **J.P. O'Doherty:** None. **J. Cockburn:** None.

Presentation Number: NANO63.08

Topic: H.03. Decision Making

Support: Simons Collaboration on the Global Brain (SCGB) 543045
Simons Collaboration on the Global Brain (SCGB) 543049
Simons Collaboration on the Global Brain (SCGB) Transition to Independence Award 1155867

Title: Flexible multitask computation in recurrent networks utilizes shared dynamical motifs.

Authors: *L. DRISCOLL[1,2], K. V. SHENOY[3], D. SUSSILLO[2];
[1]Allen Inst. for Neural Dynamics, Seattle, WA; [2]Stanford Univ., Stanford, CA; [3]Electrical Engineering, Bioengineering, Neurobio. & Neurosurg., Stanford Univ. & HHMI, Stanford, CA

Abstract: Flexible computation is a hallmark of intelligent behavior. Yet, little is known about how neural networks contextually reconfigure for different computations. Here we identified an algorithmic neural substrate for modular computation through the study of multitasking artificial recurrent neural networks. Dynamical systems analyses revealed learned computational strategies mirroring the modular subtask structure of the training task-set. Dynamical motifs, which are recurring patterns of neural activity that implement specific computations through dynamics, such as attractors, decision boundaries and rotations, were reused across tasks. For example, tasks requiring memory of a continuous circular variable repurposed the same ring attractor. We showed that dynamical motifs were implemented by clusters of units when the unit activation function was restricted to be positive. Cluster lesions caused modular performance deficits. Motifs were reconfigured for fast transfer learning after an initial phase of learning. This work establishes dynamical motifs as a fundamental unit of compositional computation, intermediate between neuron and network. As whole brain imaging studies simultaneously record activity from multiple specialized systems, the dynamical motif framework will guide questions about specialization and generalization.

Disclosures: L. Driscoll: None. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Scientific Advisory Board, Heal Inc. (2015 - 2022), Consultant / Advisor & Co-founder, Neuralink Inc. (2016 - 2022), Consultant / Advisor (2020-), Scientific Advisory Board (2016-2020), CTRL-Labs Inc (acquired by Facebook Reality Labs, Facebook), NYC, NY & Menlo Park, CA (2016 - 2022), Scientific Advisory Board, The University of Washington's Center for Sensorimotor Neural Engineering (a National Science Foundation Engineering Research Center) (2016 - 2022), Scientific Advisory Board, Inscopix Inc. (2018 - 2022), Scientific Advisory Board, MIND-X Inc. (2018 - 2022). **D. Sussillo:** A. Employment/Salary (full or part-time):; Adjunct Prof. Sussillo is a research scientist at Meta Reality Labs (for CTRL-Labs, within MRL).

Presentation Number: NANO63.09

Topic: H.03. Decision Making

Support: R00NS092972
R01NS121409
R01NS122969
Whitehall foundation
Brain and Behavior Research Foundation
R21NS135361

Title: Recurrent neural network models of decision-related dynamics in macaque premotor and prefrontal cortex

Authors: *C. CHANDRASEKARAN[1], J. C. KAO[2], M. KLEINMAN[3], K. LEE[4], N. CARR[5], **T. WANG**[6];

[1]Boston Univ., Boston, MA; [2]Electrical and Computer Engin., UCLA, Los Angeles, CA; [3]UCLA, Los Angeles, CA; [4]Psychological and Brain Sci., Boston Univ., Boston, MA; [5]Biomed. Engin., Boston Univ., Boston, MA; [6]Anat. & Neurobio., Boston Univ., Boston, MA

Abstract: We will demonstrate the utility of recurrent neural networks (RNNs) to understand neural dynamics underlying perceptual decision-making, our ability to choose and perform actions based on sensory cues and context to achieve behavioral goals. We trained macaque monkeys to discriminate the dominant color of a central static checkerboard composed of red and green squares and report their decision by reaching to and touching the target that matches the color of the checkerboard. By randomizing target configurations, we decoupled color choice (red vs. green) from action choice (left vs. right). We used two task variants: TF and CFD. In TF, the targets appear first before the checkerboard and in the CFD task, the order is reversed and the checkerboard appears before the targets. While monkeys performed these tasks, we recorded in the dorsal premotor cortex (PMd; monkeys T and O; TF task) and dorsolateral prefrontal cortex (DLPFC; monkeys T, V, Z; TF, and CFD tasks), and used RNNs to derive a mechanistic understanding of these neural dynamics.

DLPFC neural activity in the TF task covaries with target configuration, color choice, and action choice. In contrast, PMd neural activity is only correlated with action choice. We trained a multi-area RNN model of decision-making with targets and checkerboard inputs to the first area and action choice signals as output from the third area. RNN dynamics in the first area resembled DLPFC, whereas the third area resembled PMd and only had action choice signals. This filtering of color but not action choice emerged because of an information bottleneck. Action choice is propagated from area 1 to 2 (and thus from 2 to 3) because it is aligned to the potent space of connectivity matrix between areas 1 and 2, whereas the color choice signals are more aligned with a random axis (eLife, 2023).

Finally, we trained a low-rank RNN (lr-RNN) to perform both the TF and CFD tasks. The lr-RNN predicted that color evidence from the checkerboard and target configuration should be encoded in orthogonal axes and end in distinct initial conditions depending on color and target configuration. After the onset of the second stimulus, neural activity should evolve in an action choice subspace. Consistent with this prediction, color evidence and action choice were encoded in orthogonal axes in DLPFC during the first epoch. Before the onset of the second epoch, neural activity was separated as a function of target configuration and color evidence. In the second epoch, activity again separated as a function of action choice for the two different tasks. Collectively, these results highlight the important mechanistic insights that RNNs provide into decision-related neural dynamics.

Disclosures: C. Chandrasekaran: None. **J.C. Kao:** None. **M. Kleinman:** None. **K. Lee:** None. **N. Carr:** None. **T. Wang:** None.

Presentation Number: NANO63.10

Topic: H.03. Decision Making

Support: James Simons Foundation Grant 543057SPI
National Institutes of Health grant R01MH062349
ONR grant N00014-23-1-2040.

Title: Flexible gating between subspace by a disinhibitory motif: a neural network model of internally guided task switching

Authors: *Y. LIU, X.-J. WANG;
New York Univ., New York, NY

Abstract: Behavioral flexibility relies on the brain's ability to switch rapidly between multiple tasks, even when the task rule is not explicitly cued but must be inferred through trial and error. The underlying neural circuit mechanism remains poorly understood. We investigated recurrent neural networks(RNNs) trained to perform an analog of the classic Wisconsin Card SortingTest. The networks consist of two modules responsible for rule representation and sensorimotor mapping, respectively, where each module is comprised ofa circuit with excitatory neurons and three major types of inhibitory neurons.We found that rule representation by self-sustained persistent activity across trials, error monitoring and gated sensorimotor mapping emerged from training. Systematic dissection of trained RNNs revealed a detailed circuit mechanism that is consistent across networks trained with different hyperparameters. The networks' dynamical trajectories for different rules reside in separate subspaces of population activity; they become virtually identical and performance was reduced to chance level when dendrite-targeting somatostatin-expressing interneurons were silenced, demonstrating that rule-based gating critically depends on the disinhibitory motif.

Disclosures: Y. Liu: None. **X. Wang:** None.

Presentation Number: NANO63.11

Topic: H.03. Decision Making

Support: Swartz Fellowship for Theory in Neuroscience

Title: Neural Circuit Underlying Economic Decisions: Insights from a Computational Model

Authors: *A. BATTISTA[1], C. PADOA-SCHIOPPA[2], X.-J. WANG[1];
[1]New York Univ. Ctr. For Neural Sci., New York, NY; [2]Neurosci., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Understanding the neural underpinnings of economic choices is fundamental in neuroscience. We present a computational model that sheds light on the neural circuitry instantiating the decision process. Our study utilizes excitatory-inhibitory recurrent neural networks trained with state-of-the-art reinforcement learning algorithms to simulate decision processes in which individuals assign values to available goods and make choices based on their subjective preferences. Analysis of trained networks reveals three types of neurons reminiscent of those observed in the primate orbitofrontal cortex (OFC). These neurons encode the value of individual goods, the value of the chosen good, and the choice outcome. Furthermore, the dynamics of the networks are low-dimensional, with the relevant dimensions - which explain most of the variance - associated with decision quantities. We extend the model to more complex choice paradigms, including tasks involving multiple features (e.g., quantity and probability), ternary, sequential, and bundled offers. Our network can generalize to new situations akin to real-world decision scenarios. This model challenges previous theoretical assumptions by revealing a heterogeneous activity among the three types of neurons. Unlike prior models, our

network does not impose constraints on the functional role of excitatory and inhibitory cells. In particular, it allows excitatory or inhibitory neurons to encode the chosen value. This divergence aligns with observations of decision-related neurons in OFC and highlights the importance of considering more diverse neural responses in economic choice contexts. Importantly, we uncover a categorical representation of decision variables, contrasting with a category-free representation found in other prefrontal areas. A recurrent connectivity matrix analysis reveals a highly structured, low-rank pattern indicating low-dimensional population dynamics within the network. We further develop a reduced circuit model based on our trained networks, illustrating winner-take-all dynamics as a mechanism for decision-solving. Our study offers novel insights into the neural circuits and dynamics underlying economic decisions. The findings provide a foundation for future investigations, offering testable predictions that could be examined in future experimental studies.

Disclosures: A. Battista: None. **C. Padoa-Schioppa:** None. **X. Wang:** None.

Nanosymposium

NANO64: Mechanisms of Working Memory and Cognitive Control in Prefrontal Circuits

Location: MCP Room N228

Time: Wednesday, October 9, 2024, 1:00 PM - 3:15 PM

Presentation Number: NANO64.01

Topic: H.05. Working Memory

Support: NIH Grant EY026924
NIH Grant NS113073
NIH Grant EY014800
NIH T32 Grant EY024234
Research to Prevent Blindness

Title: Manipulation of dopamine-mediated activity within prefrontal cortex alters its oscillatory synchrony with visual areas during working memory

Authors: *I. VANEGAS, K. L. CLARK, B. NOUDOOST;
Ophthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Manipulating dopamine-mediated activity within the frontal eye fields (FEF) of the prefrontal cortex has been shown to alter the processing of visual information in extrastriate cortex, V4. Considering the known role of dopamine D1 receptors (D1Rs) in modulating persistent activity in prefrontal areas, the signature of working memory (WM), we explored how manipulating D1R-mediated activity in the FEF influences the capacity of visual signals to guide WM-dependent behavior.
Two macaque monkeys performed a version of a memory-guided saccade task with distractors. In this task, the monkeys fixated on a central spot while a peripheral visual target appeared at the memory location. During a delay period, a distracting visual stimulus appeared at a varying location. The monkeys had to remember the target location, disregard the distractor, and make a

saccade towards the target location at the end of the delay period. Targets and distractors were placed either within or outside the overlapping response field (RF) of simultaneously recorded FEF and V4 areas. We recorded spiking activity and local field potentials in V4 and FEF before and after manipulating D1R-mediated activity using a local injection of the D1R antagonist SCH23390. Oscillatory synchrony between and within cortical areas was assessed through phase-amplitude coupling, phase-phase locking, and spike-phase locking.
Our results indicate that altering D1R-mediated activity in the FEF is sufficient to change WM-dependent behavior. Prior to D1R manipulation, the monkeys successfully ignored distractors and accurately selected the target location. Following D1R manipulation, a bias emerged towards selecting distractors presented within the FEF RF, highlighting the role of prefrontal D1R-mediated activity in determining the entry of visual targets into WM. Our findings suggest that it is not the level of neuronal activity in V4 that governs behavior, but rather the synchrony between FEF and V4 influenced by D1R-mediated activity within the FEF. Specifically, oscillatory synchrony within V4, and the locking of FEF and V4 phases in the beta band appeared as the main predictor for determining whether a visual stimulus entered WM as a target. These results indicate that pharmacological manipulation of FEF with drugs altering its persistent activity is sufficient to enhance its oscillatory synchrony with visual areas and drive a change in WM-dependent behavior.

Disclosures: I. Vanegas: None. **K.L. Clark:** None. **B. Noudoost:** None.

Presentation Number: NANO64.02

Topic: H.05. Working Memory

Support: Brain and Behavior Research Foundation YI award to I.A.
University of Minnesota MnDrive Initiative

Title: Causality of brain oscillations in human working memory learned from brain stimulation studies

Authors: M. WISCHNEWSKI[1], T. A. BERGER[2], A. OPITZ[2], ***I. ALEKSEICHUK**[2,3];
[1]Exptl. Psychology, Univ. of Groningen, Groningen, Netherlands; [2]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN; [3]Psychiatry & Behavioral Sci., Northwestern Univ., Chicago, IL

Abstract: Working memory (WM) is a complex cognitive system associated with multiple neuroanatomical areas, including frontal, parietal, temporal, and occipital cortices, and related brain oscillations, encompassing theta, alpha, and gamma rhythms across the above-mentioned areas. These lists run long due to the distributed nature of WM but also - the associative nature of most evidence. Classic cognitive research involves correlating task actions with neuroimaging. Being fundamentally important, such studies are limited in isolating the causal bottlenecks of WM processing. Transcranial alternating current stimulation (tACS) is a non-invasive technique that influences brain oscillations via ephaptic coupling by applying electric fields at specific frequencies. This technique is particularly effective for influencing large-scale brain networks, making it suitable for studying the causal role of brain oscillations in functions like working memory. While tACS is non-invasive, computational modeling can precisely estimate the induced electric field at any point in the brain, revealing the applied “dose” per each anatomical area. So, we can assess the causal relationship between regional functional changes and

behavioral consequences. After an extensive literature search in PubMed and Google Scholar, we identified 28 placebo-controlled tACS studies in adults during a working memory task. We extracted 64 stimulation experiments with specific electrode montages, intensities, and frequencies. These parameters guided our modeling of each experiment in a virtual population of 100 head models representing a range of normal anatomies and tissue conductivities using a novel meta-modeling framework based on SimNIBS. The resulting electric field maps were parcellated and used as predictors against known effects on working memory from corresponding studies. A non-parametric test probed the significance of the electric field to working memory performance regression. Our findings show that high theta oscillations over prefrontal areas (orbitofrontal, medial frontal, and inferior frontal sub-areas) and low theta oscillations in temporal areas (medial temporal cortex and insula) significantly improved working memory. The same was observed for gamma rhythms in occipitoparietal areas. Interestingly, gamma stimulation over prefrontal areas decreased working memory performance. Our results highlight the significant causal role of prefrontal and temporal theta rhythms as well as posterior gamma rhythms for WM. This approach provides a solid foundation for future research into the mechanisms of WM in humans.

Disclosures: M. Wischnewski: None. **T.A. Berger:** None. **A. Opitz:** None. **I. Alekseichuk:** None.

Presentation Number: NANO64.03

Topic: H.05. Working Memory

Support: NIH Grant MH118929
NIH Grant EB026953
NSF NCS
Simons Foundation
JASSO (Japan Student Services Organization)

Title: Robustness of PFC networks under patterned microstimulation perturbations

Authors: *J. SOLDADO MAGRANER, Y. MINAI, M. A. SMITH, B. M. YU;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: In the prefrontal cortex (PFC) delay period activity has been linked to the maintenance and control of stimulus information in working memory. A mixture of stable and dynamic mnemonic representations are found in such delay period activity. However, the function of these rich internal representations is poorly understood. Here, we probed the dynamics of PFC's mnemonic representations with a diverse set of activity perturbations, and measured their consequence on behavior. For this, we developed a patterned microstimulation protocol in monkeys implanted with multi-electrode arrays, and electrically stimulated different electrodes in the array while the monkeys performed a memory guided saccade task. We found that each stimulation pattern pushed the PFC activity in different directions and triggered transient responses lasting hundreds of milliseconds with diverse relaxation dynamics. The relaxation brought back the responses to specific locations in neural state space which encoded the different memory conditions. However, the activity did not always recover in several dimensions, including dimensions that reflected memory encoding. Despite the widespread impact of

perturbations on PFC population activity, the performance of the monkey remained unaffected. The highly distributed mnemonic code in PFC, with complex but widespread tuning across the neural population, made it possible to find a subspace of the activity that preserved the mnemonic information. Thus, PFC displays robustness to a wide range of activity perturbations, and its characteristic heterogenous code might underlie such robustness.

Disclosures: J. Soldado Magraner: None. **Y. Minai:** None. **M.A. Smith:** None. **B.M. Yu:** None.

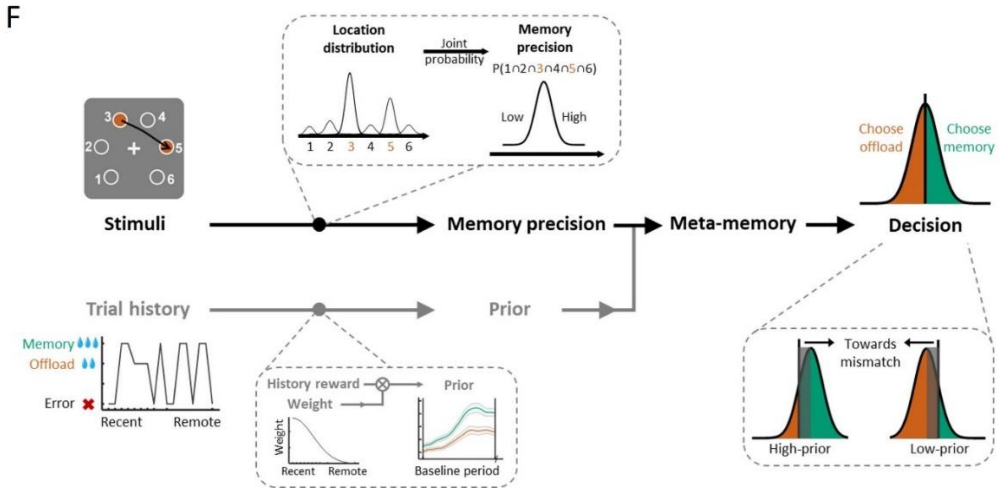
Presentation Number: NANO64.04

Topic: H.05. Working Memory

Title: Metacognitive Control of Sequence Working Memory in Macaque Prefrontal Cortex

Authors: *C. NING, G. FU, Y. ZHANG, L. WANG;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: The ability to reflect on one's own memory is known as metamemory. Whether metamemory is inherent to memory quality or requires additional machinery or computation remains largely open. We investigated the metacognitive control mechanism of sequence working memory (WM) using two-photon calcium imaging in the prefrontal cortex of macaque monkeys, who were trained to memorize spatial sequences spanning a range of (lengths) difficulties. In some trials, after viewing the sequence, monkeys could opt out of the memory for a smaller reward to reflect their confidence in WM quality (meta-WM). We found that PFC neurons represent the sequence WM precision through probabilistic population coding, predicting the reported accuracy of each sequence. Crucially, the prefrontal responses were also highly correlated with the probability of opt-out decision, representing metacognitive judgments of WM quality. Furthermore, the prefrontal responses before viewing the sequence encode the prior expectations resulting from the reward received from history trials. Thus, WM quality, prior, and meta-WM are implemented within the same prefrontal circuits, and their integration leads to metacognitive decisions, providing a novel, general framework for understanding metamemory computations.



Disclosures: C. Ning: None. **G. Fu:** None. **Y. Zhang:** None. **L. Wang:** None.

Presentation Number: NANO64.05

Topic: H.05. Working Memory

Support: R01 MH121480
Mount Sinai Seed Funds

Title: Representational gradients of mnemonic chunking in primate lateral prefrontal cortex

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Abstract: Self-organized behaviors often rely on working memory to maintain and manipulate information in mind step-by-step. Although the cumulative information held in working memory is limited by capacity constraints, cognitive strategies such as mnemonic chunking can structure the information and mitigate this constraint. Here, we investigated how neural ensembles in primate lateral prefrontal cortex (LPFC) structure information in sequential behaviors guided by chunking strategies. To do this, we trained two monkeys to perform a spatial self-ordered target selection task. Monkeys were trained to saccade to eight identical targets on a screen, one at a time in any order, to collect a one-time reward from each target. This required them to use working memory to update reward-target contingencies and prepare for the next target selection. From target selection patterns, we quantified chunking tendencies with modularity index (MI), which is the strengths of subgroup segregations when targets are assigned to two chunks. Behaviorally, we found that reaction times were longer when transitioning between subgroups than within subgroups, suggesting a chunking boundary can be identified. In addition, error rates were lower within the same chunk compared to those transitioning between chunks, and negatively correlated with MI, suggesting that stronger chunking improved task performance. Neurons were recorded from four 64-channel Utah arrays implanted along with LPFC, including dorsal principal sulcus and prearcuate gyrus. We assessed how ensemble codes and dimensionality of neural encoding changed across the rostro-caudal extent of LPFC. We found that ensemble activity represented target locations with more distinct structure in posterior compared to anterior LPFC, and this corresponded to the individual target locations. In contrast, there were lower-dimensional representations in anterior LFPC. Cross-category decoding revealed better decoding of behaviorally-identified chunks in anterior LPFC, suggesting that chunking information is more strongly represented in the anterior regions. Overall, our results are consistent with a rostro-caudal abstract-to-concrete gradient of ensemble representations in LPFC that may be critical for flexible, intelligent behaviors.

Disclosures: F. Chiang: None. **E.L. Rich:** None.

Presentation Number: NANO64.06

Topic: H.04. Executive Functions

Support: NSERC Discovery Grant

Title: A review and meta-analysis of fMRI studies of proactive and reactive cognitive control

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Abstract: The dual mechanisms of control (DMC) theory postulates that cognitive control is not a unitary mechanism, but has two modes, proactive control and reactive control. Proactive control refers to the process of selecting and maintaining goal-relevant information in anticipation of conflict or a cognitively demanding event. In contrast, reactive control refers to the process whereby control processes are transiently activated after the onset of conflict or interference. The DMC theory posits that different brain regions are involved in proactive and reactive control. That is, proactive control is thought to be subserved by sustained and anticipatory activation of the lateral prefrontal cortex (lPFC), while reactive control is thought to be associated with transient activation of the lPFC as well as activations in other brain regions, including the anterior cingulate cortex and the posterior parietal cortex. Here, we conducted a review and activation likelihood estimation (ALE) meta-analysis of studies that have investigated the brain regions involved in proactive and reactive control in healthy adults. The ALE meta-analysis showed that the lPFC was consistently recruited in studies of both proactive and reactive control. In line with the DMC theory, reactive control was also found to be associated with activations in a broader set of brain regions, including the insula and the superior and inferior parietal lobules. In contrast to the predictions of the DMC theory, proactive control was also associated with activations in a wider set of brain regions, including the medial frontal gyrus and the inferior parietal lobule. This finding suggests that the conceptualization of the brain regions that are important for proactive control might need to be modified to include other brain regions in addition to the lPFC.

Disclosures: M. Kusi: None. **V. Goghari:** None.

Presentation Number: NANO64.07

Topic: H.04. Executive Functions

Support: NIH Grant R01 NS102201

Title: Lexical inhibition after semantic violations recruits a domain-general inhibitory control mechanism

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Abstract: Language processing is incremental. As language signals - e.g., words in a sentence - unfold, humans predict and activate likely upcoming input to facilitate comprehension. Prediction not only accelerates understanding, but also prompts reassessment in the case of prediction error, fostering learning and refining comprehension skills. Therefore, it is paramount to understand what happens when predictions are violated - e.g., a sentence ends in an unpredicted word. One theory, which we test here, is that the originally-predicted word is actively inhibited after semantic violations. Furthermore, we tested whether this purported lexical inhibition process is achieved by a domain-general mechanism - i.e., one that also inhibits other processes (e.g., movement). We combined a semantic violation task, in which highly-constrained sentences primed specific words, but sometimes continued otherwise, with a motoric

stop-signal task. Across two experiments, semantic violations significantly impaired simultaneous action-stopping. This implies that lexical and motor inhibition share the same process. In support of this view, multi-variate EEG decoding showed early overlap in neural processing between action-stopping (motor inhibition) and semantic violations (lexical inhibition). Moreover, a known signature of motor inhibition (the stop-signal P3) was reduced after this initial overlap period, further suggesting the presence of a processing bottleneck. These findings show that semantic violations trigger inhibitory processing, and suggest that this lexical inhibition recruits a domain-general inhibitory control mechanism. This provides a new perspective on long-standing debates in psycholinguistics, extends the range of a well-characterized cognitive control mechanism into the linguistic domain, and offers support for recent neurobiological models of domain-general inhibitory control.

Disclosures: H. Sanchez Melendez: None. **K. Hendrickson:** None. **Y. Choo:** None. **J. Wessel:** None.

Presentation Number: NANO64.08

Topic: H.04. Executive Functions

Support: NINDS (1 R01 NS114405-01; NS095741)
NIMH (1 P50 MH094258; 1 R21 MH120441-01; 2T32MH019113-29A1)
NIGMS (T32GM108540)
NIH (1S10RR028821-01)

Title: Working memory and fluid reasoning rely on largely overlapping brain networks: A lesion network mapping study.

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Abstract: Working memory and fluid reasoning are critical aspects of higher-order human cognition that support adaptive behavior. Knowledge of their neural mechanisms is critical for advancing basic and translational cognitive neuroscience. Psychometric studies have demonstrated strong correlations between working memory and fluid reasoning, and functional MRI research suggests that these abilities share largely overlapping neural correlates, including in the left middle frontal gyrus and left inferior parietal lobe. Lesion studies have not explicitly compared the critical neural correlates of these abilities, and extant evidence has used either small samples or has not considered the network-level effects of brain lesions. Here, we used data from the Iowa Neurological Patient Registry to create lesion network maps, which are statistical maps linking cognitive deficits to lesion-associated brain networks. Cognitive data comprised the total scores from the Digit Span (n=648) and Similarities (n=596) subtests from the Wechsler Adult Intelligence Scales, which measure auditory-verbal working memory and verbal reasoning, respectively. We derived lesion-associated networks for each patient by using their lesion locations as seed regions-of-interest in resting-state functional connectivity fMRI, and diffusion weighted imaging-based tractography based on Human Connectome Project data. We used FSL's Permutation Analysis of Linear Models tool to create voxel-wise general linear

models whose statistical significance was determined through 1000 permutations and False Discovery Rate corrections. Maps for Digit Span and Similarities were highly spatially correlated for results based on both resting-state fMRI (r = 0.92) and diffusion tractography (r = 0.82), with the most robust overlap in the left pars opercularis and the left posterior middle frontal gyrus. In either imaging modality, only Digit Span was associated with damage to networks involving the left auditory cortex and supramarginal gyrus. There were no findings uniquely associated with the Similarities test. Our results support the notion that working memory and fluid reasoning depend on largely overlapping brain networks. However, the discordance involving the early auditory cortex suggests that verbal fluid reasoning does not specifically depend upon auditory cortex mechanisms for maintaining sound in working memory. Future studies could examine whether these conclusions generalize to other sensory modalities.

Disclosures: M. Bowren: None. **K. Langbehn:** None. **J. Bruss:** None. **K. Manzel:** None. **D. Tranel:** None. **A.D. Boes:** None.

Presentation Number: NANO64.09

Topic: H.05. Working Memory

Support: 1R01NS116589
T32-NS058280

Title: Practice crystallizes unstable working memory representations

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Abstract: Working memory (WM) is crucial for temporarily holding and manipulating information, yet the long-term neural mechanisms of WM are poorly understood. In our study, mice were trained on a task that required them to remember and differentiate sequences of odors during a five-second delay. Disrupting secondary motor neurons during late delay period drastically degraded their performance. Calcium imaging of several brain areas, including the secondary motor cortex (M2), revealed the emergence of selective and mixed-selective neurons vital for maintaining memory throughout the delay. Using volumetric light bead microscopy, we recorded the activity of over 70,000 neurons simultaneously and tracked their dynamics over 10 days as the expert mice continued practicing the task. We show that although the WM representations initially fluctuated, they stabilized through continuous practice.

Disclosures: A. Bellafard: None. **G. Namvar:** None. **J.C. Kao:** None. **A. Vaziri:** None. **P. Golshani:** None.

Nanosymposium

NANO65: *In Vivo* Voltage Imaging and Network Dynamics

Location: MCP Room S102

Time: Wednesday, October 9, 2024, 1:00 PM - 2:45 PM

Presentation Number: NANO65.01

Topic: I.04. Physiological Methods

Support: NIH BRAIN grant U01NS120822
NIH BRAIN grant UF1NS107610
NIH BRAIN grant U19NS104590
NSF NeuroNex grant DBI-1707261

Title: Imaging of population-level, high-frequency voltage dynamics in multiple neuron classes of behaving mammals.

Authors: *S. HAZIZA[1], R. T. CHRAPKIEWICZ[2], Y. ZHANG[1], V. KRUZHILIN[2], M. J. SCHNITZER[3];
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Abstract: Recent methods for optical voltage imaging allow studies of neural spiking at a resolution of single action potentials. However, optical instruments for monitoring the aggregate, population-level voltage dynamics of identified neuron-types have lacked the sensitivity to track high-frequency (≳10 Hz) voltage waves and oscillations. This technology gap has impeded studies of how specific neuron-types shape the spatiotemporal dynamics of the brain's rich set of electrical oscillations. Here, we present two TEMPO (Transmembrane Electrical Measurements Performed Optically) technologies with unprecedented sensitivity for monitoring high-frequency transmembrane voltage activity up to ~100 Hz, from one or two cell-types in behaving animals. TEMPO has 3 key ingredients: (1) Co-expression of one (or two) fluorescent genetically encoded voltage indicators to track neural activity and a reference fluor to track optical artifacts (*e.g.*, from hemodynamics and brain motion); (2) A dual-color fluorescence sensing or imaging apparatus; (3) Computational unmixing of optical artifacts from the fluorescence voltage traces. Notably, TEMPO differs from extracellular electric field potential recordings, which capture contributions of multiple unidentified neuron-types, are influenced by electrode shape, orientation, and composition, and include volume-conducted signals originating up to ~1 cm away from the recording electrode. We built fiber-optic and imaging systems for TEMPO studies of neural dynamics in freely moving and head-fixed behaving mammals, respectively. The fiber-optic apparatus, termed 'uSMAART' (ultra-Sensitive Measurement of Aggregate Activity in Restricted cell-Types), attains ~10-fold greater sensitivity than prior fiber photometry systems. The imaging system captures population-level neural voltage activity across a ~7-mm-wide field-of-view with a spatial resolution >10 times finer than the densest electrocorticography recordings. With these instruments, we resolved propagating voltage waves and oscillations up to high-gamma (~100 Hz) frequencies, as well as cross-frequency coupling between oscillations of distinct frequencies in the transmembrane potentials of individual neuron-types. With dual cell-

type TEMPO, we characterized the joint dynamics of excitatory and inhibitory neurons during hippocampal ripples and visual cortical processing. Overall, TEMPO technologies will allow neuroscientists to examine how specific neuron-types interact to shape the brain's electric field dynamics and to probe the contributions of voltage waves and oscillations to animal behavior in both health and disease.

Disclosures: S. Haziza: None. **R.T. Chrapkiewicz:** None. **Y. Zhang:** None. **V. Kruzhilin:** None. **M.J. Schnitzer:** None.

Presentation Number: NANO65.02

Topic: I.04. Physiological Methods

Support: NIH R34 NS128890
NIH U01 NS120822
BBRF NARSAD Young Investigator

Title: Voltage dynamics of distinct cortical subtypes and their real-time interplay in sensorimotor integration

Authors: *M. KANNAN[1], G. VASAN[2], P. DEVARAJU[2], K. MCCLUSKEY[2], A. KANG[2]; [1]Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Animals' perception of their environment relies not only on the hierarchical flow of sensory information from peripheral receptors to sensory cortical areas but also on contextual information flow from circuits that encode sensory surround, behavioral goals, and/or internal states. In actively viewing animals, visual input alone is a poor predictor of activity in visual cortex, where contextual factors such as locomotion and self-generated visual feedback from body movements elicit large neural responses. The lateral posterior thalamus (LP), a higher-order visual thalamic nucleus and the rodent homolog of the primate pulvinar, is implicated in visuomotor integration, and signals carried by LP axons are thought to modulate sensory responses even at the earliest stages of visuocortical processing (V1). Here, we identify the postsynaptic targets of LP among cortical excitatory and inhibitory neurons in V1. To examine the differential role of identified cortical subtypes in visuomotor integration, we use our newly described suite of mutually compatible genetically encoded voltage indicators (GEVIs) for *simultaneous*, high-speed (>0.6 kHz), multipopulation voltage recordings in head-restrained mice, running in a virtual environment. By extracting their concerted voltage dynamics at single-neuron, single-spike resolution, we make inferences on their distinctive roles in visuomotor integration, the temporal characteristics of their responses at visuomotor mismatch onsets, as well as the time-varying functional connectivities across cortical subtypes. We further test whether the distinct subtypes operate in serial or parallel cortical circuits *in vivo*. Lastly, to directly examine whether they participate in sensorimotor integration downstream of LP, we selectively measure the voltage dynamics of LP-recipient subpopulations using an intersectional approach. Together, our work uncovers the real-time, millisecond-scale dynamics of targeted cortical subtypes in navigating animals, as they discriminate between external visual motion and the visual flow predicted by their own actions.

Disclosures: M. Kannan: None. **G. Vasan:** None. **P. Devaraju:** None. **K. McCluskey:** None. **A. Kang:** None.

Presentation Number: NANO65.03

Topic: I.04. Physiological Methods

Support: Alana Fellowship
NIH R21EY028381-01
HHMI
Lisa Yang
Ashar Aziz
K. Lisa Yang and Hock E. Tan Center for Molecular Therapeutics at MIT
John Doerr

Title: Imaging the voltage of neurons distributed across entire brains of larval zebrafish

Authors: *Z. WANG[1], J. ZHANG[1], P. SYMVOULIDIS[1], W. GUO[2], C. ZHANG[1], D. DENG[1], M. A. WILSON[3], E. S. BOYDEN[1];
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Abstract: Neurons interact in networks distributed throughout the brain. Although much effort has focused on whole-brain calcium imaging, recent advances in genetically encoded voltage indicators (GEVIs) raise the possibility of imaging voltage of neurons distributed across brains. To achieve this, a microscope must image at high volumetric rate and signal-to-noise ratio. We present a remote scanning light-sheet microscope capable of imaging GEVI-expressing neurons distributed throughout entire brains of larval zebrafish at a volumetric rate of 200.8 Hz. We measured voltage of ~1/3 of the neurons of the brain, distributed throughout. We observed that neurons firing at different times during a sequence were located at different brain locations, for sequences elicited by a visual stimulus, which mapped onto locations throughout the optic tectum, as well as during stimulus-independent bursts, which mapped onto locations in the cerebellum and medulla. Whole-brain voltage imaging may open up frontiers in the fundamental operation of neural systems.

Disclosures: Z. Wang: None. **J. Zhang:** None. **P. Symvoulidis:** None. **W. Guo:** None. **C. zhang:** None. **D. Deng:** None. **M.A. Wilson:** None. **E.S. Boyden:** None.

Presentation Number: NANO65.04

Topic: I.04. Physiological Methods

Support: HHMI to MJS

Title: Long-term, in vivo voltage imaging to decipher dopamine-mediated learning algorithms in Drosophila

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Abstract: To survive in dynamic environments, animals also act anticipatorily on sensory cues that precede important future events, such as the indications of food, mates, or predators. Nervous systems generate these vital predictions through learning and regulate future learning. Dopamine neurons (DANs), in this predictive learning process, provide essential teaching signals that convey the values predicted by sensory cues (prediction) and the discrepancy between expected and received outcomes (prediction error). However, the underlying neural mechanisms by which the dopamine signals compute and utilize predictions in learning remain obscure. To investigate this, we utilize the Drosophila dopamine system as a model due to its functional conservation and relative simplicity compared to mammalian counterparts. We created an innovative time-lapse voltage imaging technique to overcome the longstanding technical difficulty of accurately, long-term monitoring neural activity in live flies. This novel approach enables us, for the first time, to record neural dynamics in behaving flies across many weeks and with millisecond-scale temporal precision. Notably, by tracking spiking dynamics in DANs throughout the entire learning and memory process, we uncover the existence of predictive dopamine signals, which encode the interplay between innate and learned sensory values. These dopamine-driven teaching signals collectively regulate memory formation and expression within interconnected short- and long-term memory units of the Drosophila Mushroom Body. A computational model, constrained by the Mushroom Body connectome and our neural spiking data, explains how dopamine signals incorporate innate and learned values to regulate memory storage, extinction, and the interaction between short- and long-term memory traces.In summary, our research reveals that flies achieve flexible learning through dopamine-mediated integration of innate and learned valences in the mushroom body. This combined physiological and anatomical mechanism could potentially represent a general means by which ecological information controls learning and memory in various species and brain structures that rely on dopaminergic signaling.

Disclosures: C. Huang: None. **M.J. Schnitzer:** None.

Presentation Number: NANO65.05

Topic: I.04. Physiological Methods

Support: R01 MH101198
RO1 NS099137
RO1 NS090930
R01 MH105427
U54 HD87101
1I01BX001524-01A1

Title: Inhibition tunes hippocampal memory-encoding sequences

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Abstract: Hippocampal spiking sequences encode external sensory cues and link them across short time gaps between them, tracking the passage of our experiences. We previously described hippocampal pyramidal sequences composed of ‘odor-cells’ encoding specific olfactory cues, followed by odor-specific ‘time-cells’ retaining information on the identity and timing of the

preceding cue throughout an ensuing delay period (Taxidis et al., Neuron, 2020). But what is the role of inhibition by parvalbumin- (PV) or somatostatin-expressing (SST) interneurons in shaping such sensory and memory/time representations? We pioneered longitudinal, high-frequency voltage imaging *in vivo*, using the ASAP3 genetically encoded voltage indicator, on the CA1 hippocampal region of PV-Cre and SST-Cre mice. Imaging took place while mice performed an odor-cued delayed non-match-to-sample task (DNMS), shown to yield odor-specific pyramidal sequences during the odor cues and ensuing delays. Mice were imaged during untrained, passive exposure to DNMS trials as well as after training, during DNMS performance. We recorded action potentials and subthreshold membrane dynamics from PV and SST cells, following the same cells across multiple days or before and after training. Roughly half of PV and SST cells had a firing field during the odor cue presentation. Unlike pyramidal cells, these interneurons were not odor-specific, and practically no cells encoded delay timepoints, yielding no time-cell sequences after the odor cue. At odor onset, interneurons exhibited a distinct hyperpolarization which briefly reset their intracellular theta phase and coordinated their rebound odor-spiking into synchronous theta-cycles across cells and trials. Such pronounced and fine-timed inhibition should silence many pyramidal neurons during odor cues. Indeed, using *in vivo* two-photon calcium imaging in transgenic mice under the same setup, we found that most pyramidal cells were inhibited throughout odor presentation. Surprisingly, optogenetic silencing of either PV or SST interneurons throughout odor delivery, combined with electrophysiological (Neuropixel) recordings in CA1, did not increase the number of pyramidal cells activated by the odor. Instead, it reshuffled the pyramidal ensembles that responded to the odor cues, so that a new set of odor cells emerged. Collectively, our findings reveal fine-timed and non-cue-specific PV/SST inhibition during odor cues, which controls the precise ensembles of pyramidal cells that will encode the cue and will initiate the ensuing memory-encoding spiking sequence.

Disclosures: J. Taxidis: None.

Presentation Number: NANO65.06

Topic: I.04. Physiological Methods

Support: BWF-CASI
JPB

Title: Control and watch the brain in action: Toward the physical basis of learning and memory

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Abstract: Learning has been associated with modifications of synaptic and circuit properties, but the precise changes storing information in mammals have remained largely unclear. We combined genetically targeted voltage imaging with targeted optogenetic activation and silencing of pre- and post-synaptic neurons to study the mechanisms underlying hippocampal behavioral timescale plasticity. In mice navigating a virtual-reality environment, targeted optogenetic activation of individual CA1 cells at specific places induced stable representations of these places in the targeted cells. Optical elicitation, recording, and modulation of synaptic transmission in behaving mice revealed that activity in presynaptic CA2/3 cells was required for the induction of plasticity in CA1 and, furthermore, that during induction of these place fields in

single CA1 cells, synaptic input from CA2/3 onto these same cells was potentiated. We further all-optically resolved inhibitory synaptic transmission and synaptic plasticity between cholecystokinin-expressing inhibitory neurons and individual CA1 cells during behavior. These results reveal synaptic implementation of hippocampal behavioral timescale plasticity and define a methodology to resolve synaptic plasticity during learning and memory in behaving mammals.

Disclosures: L. Fan: None.

Presentation Number: NANO65.07

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

Support: NIH grant R01-NS126043
R01MH117042
Brain Research Foundation Scientific Innovations Award
Harvard Brain Science Initiative
Vannevar Bush Faculty Fellowship

Title: Dendritic voltage dynamics: from biophysics to information processing

Authors: P. PARK[1], B. LEE[1], X. WU[1], D. WONG-CAMPOS[1], D. G. ITKIS[1], ***A. COHEN**[2]; [1]Chem. and Chem. Biol., Harvard Univ., Cambridge, MA; [2]Harvard Univ., Cambridge, MA

Abstract: The voltage dynamics in distal dendrites of pyramidal cells are critical to dendritic integration and synaptic plasticity, but these dynamics have been difficult to measure *in vivo*. We developed molecular, optical, and computational tools to map bioelectrical dynamics throughout the dendritic trees of CA1 pyramidal cells in behaving mice. Most subthreshold and spike-related dynamics were highly correlated across branches of similar branch-order, and we only rarely observed spike-like events which were localized to a small portion of the dendritic tree. Nonlinear dendritic excitations played an important role in regulating spike back-propagation and in triggering complex spikes, but we did not observe signatures of nonlinear excitations during dendritic integration. These results imply that dendritic nonlinearities may be primarily involved in shaping learning rules, rather than in rapid information processing.

Disclosures: P. Park: None. **B. Lee:** None. **X. Wu:** None. **D. Wong-Campos:** None. **D.G. Itkis:** None. **A. Cohen:** None.